NOVEL METHODS OF FUMIGATING AUSTRALIAN WILDFLOWERS

THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

BY

MURUGAPPAN MUHUNTHAN
B. Sc. (Agriculture)

SCHOOL OF THE BUILT ENVIRONMENT
VICTORIA UNIVERSITY – AUSTRALIA.

Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary educational institution.

(M.Muhunthan)

ABSTRACT

For the last ten years, the Australian wildflower export trade has been expanding rapidly and it is expected to continue to do so. Quarantine restrictions imposed by importing countries render insect-free flowers and foliage mandatory. Flower exporters have a number of options, currently fumigating just prior to export is a major one. At present methyl bromide or Pestigas®-Insectigas® (mixtures of pyrethrum in carbon dioxide and dichlorvos in carbon dioxide respectively) combinations are available to exporters. However, methyl bromide will be phased out by the year 2005. On the other hand, combined fumigants are not highly toxic to certain active or developmental stages of a number of insect pests. Hence, a study was conducted to determine an efficacious alternative fumigant or fumigant mixture for the post-harvest disinfestation of wildflowers that are destined for the export market.

One strand of the research is quite fundamental in that it elucidates the biology and reproductive behaviour of the leaf rolling moth, *Strepsicrates ejectana* Walker, a species of moth that has not been previously studied. A second strand is very much of an applied nature in that it develops new protocols for the use of phosphine and a mixture of pyrethrum and phosphine to disinfest wildflowers.

It was observed that a moth native to Australia *S. ejectana* infests plantations of Grampian thryptomene, *Thryptomene calycina*. Although the insect is commercially important, previous studies of the species have been quite cursory. As part of this research the biology and reproductive behaviour of this moth have been studied. Of particular importance are observations on the life-cycle of the insect which clearly illustrates that populations of *S. ejectana* consist of two distinct cohorts. In one cohort the eggs are laid in mid-summer, and the eggs of the other cohort are laid in mid-winter.

The larval stages last for 38 to 52 days at 22°C during which they undergo six instar stages. The third to fifth instar larvae are the most destructive stages that survive for 17

to 22 days. The larvae can be reared in a modified lightbrown apple moth dietary medium from the third instar onwards. Of these stages, mature larvae (fourth to sixth instar stages) are difficult to kill by fumigation, presumably due to their living patterns and metabolism. The larvae of this insect construct compact webbing shelters in which they live. Hence, there are possibilities that fumigant may or may not enter into these shelters and act upon the larvae.

Substantial progress has also been made on establishing alternative fumigants to methyl bromide. Preliminary laboratory fumigation experiments indicated that carbon disulphide is highly toxic to all the exposed insect pests. However, the fumigated flowers and foliage showed phytotoxic symptoms at all the concentrations and exposure times investigated. The vase lives of the flowers are much more important for marketability and in view of these results further fumigations with carbon disulphide were not conducted. Preliminary laboratory fumigations using phosphine as an alternative fumigant did not give complete control of the exposed insect pests. However, by increasing the exposure time rather than the concentration of phosphine, it was possible to increase the mortality of the exposed insect pests. Furthermore, phosphine is not phytotoxic to the exposed wildflowers and foliage. This prompted the hypothesis that phosphine may be used to fumigate wildflowers.

Large scale fumigations using a Pestigas®-Phosfume® (two per cent phosphine in carbon dioxide) combination conducted under conditions met in commercial fumigations with an exposure period of 4.5 to 6 h did not give a complete mortality of all the exposed insect pests. These fumigations killed all the exposed larvae and adults of the green peach aphid, *Myzus persicae* (Sulzer). However, some larvae of *S. ejectana* and the lightbrown apple moth, *Epiphyas postvittana* (Walker) were severely affected and some eggs of the two-spotted mite, *Tetranychus urticae* (Koch) survived. Increasing the exposure time to 14 to 18 h killed all the exposed insects in both their active and developmental stages. However, a small number of active stages of *S. ejectana* and *E. postvittana* and eggs of *T. urticae* and *E. postvittana* were not killed if the concentration of phosphine or temperature were not maintained at 0.5 $g.m^{-3}$ and 20°C respectively.

The vase life of the flowers was not reduced significantly under any of the fumigation regimes investigated.

Although fumigations with phosphine alone are efficacious, it was hypothesised that they may be improved by synergising phosphine with pyrethrum. The hypothesis was tested by carrying out large scale fumigation trials. Large scale fumigation trials that mimiced commercial conditions. It was observed that the presence of pyrethrum lowered the temperature required to obtain significant or complete mortality of the insects studied. This has a beneficial effect on the vase life of the flowers that are fumigated.

As a result of carrying out the research, possible protocols for fumigating cut flowers using phosphine and a mixture of pyrethrum and phosphine have been established. As part of this work procedures for breeding *S. ejectana* in the laboratory were developed so that immature and adult stages of the insect could be available for commercial-scale fumigations. The laboratory experiments also enabled the life-cycle of *S. ejectana* to be studied under controlled conditions. Moreover, this research provided information on: phytotoxicity of Phosfume® and Pestigas®+Phosfume® combinations at various concentrations, exposure times and temperatures to four different species of wildflowers and carnation, distribution of phosphine in a 27 m^3 chamber during exposure time, distribution of temperature in a 27 m^3 chamber and the importance of temperature for the postharvest disinfestation of wildflowers of insects.

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Introduction

Chapter 1

Introduction

The global floriculture trade has undergone remarkable changes during the last ten years due to increase in the disposable incomes of consumers in wealthy developed nations and the surging hospitality industry. Also, flowers are used increasingly for home decorations and gift arrangements. Hence, the floriculture industry has established a year-round production base. At present it is worth $A30 to 35 billion per annum globally and it is expanding at an annual rate of 15 to 20 per cent. In many of the highly populated countries where the availability of land is scarce, local supply often falls short of demand and the shortfall is being filled by imports. In addition to this, very high production costs, especially those associated with the heating of glasshouses in the U.S. and Europe have increased the importation of cut flowers into these regions in recent years. Thirty per cent of the world demand is supplied by importation. During the period 1987-88 Europe imported 81.2 per cent and Germany imported 39 per cent of its domestic consumption (Puller *et al.* 1993).

Australian flower exports rose to $A25 million during 1991-92 from $A3 million during 1984-85 and they were expected to grow to $A100 million by the year 2000. Currently, the consumption trends of traditional cut flowers such as carnations, roses, lilies and chrysanthemums are declining, while the demand for Australian wildflowers is rising due to their diversity, colour, fragrance and longevity. At present a number[1] of breeding programs has been initiated in every state to domesticate some of the 32,000 wildflower species which have export market potential, with the intention of quintupling exports in the near future.

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[1] In this thesis 'number' is taken as being a singular noun, although at times this usage leads to inept sounding expressions.

The seasonal differences between the northern and southern hemispheres, a vast amount of fertile land favoured with reliable water supplies and myriad wildflower species offer Australia ample opportunities for expanding its export trade in the near future.

Like all other plants, wildflowers are prone to infestation by a range of insect pests and disease causing pathogens. During a visit to commercial flower farm in the Grampians, a range of mountains in Victoria in 1994, the candidate observed that an entire field of Grampian thryptomene, *Thryptomene calycina* was devastated by an unrecognised insect pest. These plants are grown extensively on a large scale, mainly for export markets and some for domestic consumption. During 1995-96 the growers' incomes were severely reduced by a sudden outbreak of this pest. Samples were collected and the insect pest was identified as the leaf rolling moth, *Strepsicrates ejectana,* Walker, a native pest of Australia. An extensive literature search indicated that, except for a short description of the morphology of adults, nothing was recorded of the species' life cycle, taxonomy or biology. In order to elucidate the life cycle and biology of the insect, field collections were conducted on a monthly basis from 1995-97. Concurrently, the species was reared in the laboratory and in glass-houses.

Insecticidal dipping and removal by hand has been used and continues to be used for the postharvest disinfestation of this insect and other insects that attacks flowers. However, it does not give a complete kill and it is labour intensive. As a result, exporters are turning towards fumigation. Postharvest disinfestation is the last resort for pest control, and field control is imperative in order to save crops from complete destruction, and to ease the pressure on postharvest treatments. Fundamental studies of the biology and life cycle of *S. ejectana* would help growers to identify the ideal time for combating this pest.

In addition to the leaf rolling moth, the lightbrown apple moth, *Epiphyas postvittana,* Walker, the two-spotted mite, *Tetranychus urticae,* Koch, the green peach aphid, *Myzus persicae*, Sulzer and other minor arthropods such as the European earwig, *Forficula auricualria*, Linnaeus and the sugar ant, *Camponotus consobrinus,* (Erichson) also cause

problems in harvesting, handling and marketing. Presence of insects is a severe impediment to exports, and under the International Plant Protection Convention Act (IPPCA), importing countries demand insect-free flower consignments into their countries. All these pests are classified as quarantine pests and the importing countries prevent their entry by imposing stringent quarantine regulations and by requiring zero insect infestations in the imported commodities. If the importing countries detect any live or severely affected (moribund) or injured insects in a consignment of flowers the entire consignment is rejected. It may then be fumigated with methyl bromide, which is expensive and the costs are incurred by the flower exporters. In addition, the fumigation process in the importing countries reduces the vase life of the flowers, reduces their prices and sometimes renders them unmarketable. If the infestations are very high, the entire consignment is incinerated.

Research findings show that 32.5 per cent (Anon. 1988) of the flower consignments exported to Japan are fumigated at their point of entry, either because of the presence of live or pesticide affected insect pests (Table 1.1). This represents a cost of $A700,000/annum to Australian exporters (Anon. 1993-94). On the other hand, Australia also imports a range of cut flowers including roses and carnations from South Africa, Kenya, Israel, India and some European countries. This trade carries with it the risk of introducing exotic pests, most of which are polyphagous, into Australia. One such example is the serpentine leaf miner, *Liriomyza trifolii*, (Riley) which feeds on tomato, celery, cucumber, lettuce, ornamentals, and other glass house crops (Mortimer and Powell, 1984). Hence, an efficient fumigation protocol is important to counteract accidentally imported insect pests.

Exporters of cut and wildflowers have a number of disinfestation options available to them. These include:

- Fumigants - currently only methyl bromide is registered for the postharvest disinfestation of flowers and it is used by a limited number of exporters.

Table 1.1 Plant quarantine inspection data (number of stalks) for various cut and wildflowers imports into Japan from Australia (Anon. 1988).

Type of flowers/foliage	Number inspected ('000's)	Number disinfested				Number destroyed ('000's)
		hydrogen cyanide ('000's)	Methyl bromide ('000's)	Total ('000's)	Disinfestation rate (%)	
Orchid	79,077	10,558	17,586	28,144	35.5	48
Fern	28,114	167	2,457	2,624	9.3	48
Chrysanthemum	15,827	118	14,849	14,968	94.5	2
Carnation	6,990	399	1,206	1,605	22.9	21
Anthurium	5,815	213	198	411	7.0	2
Bear grass	4,911	6	0	6	0.1	0
Gladiolus	3,442	25	2,713	2,738	79.5	0
Freesia	2,996	43	7	50	1.6	5
Nerine	1,839	20	1	21	1.1	0
Daisy	1,677	98	258	356	21.2	0
Lily	1,673	46	8	54	3.2	0
Leucadendron	663	35	42	77	11.6	1
Ornithogalum	546	52	21	73	13.3	1
Rose	332	22	25	47	14.1	0
Chamelaucium	298	15	32	47	15.7	0
Calla	277	18	5	23	8.3	0
Gypsophila	216	33	52	85	39.3	1
Lycoris	167	114	42	156	93.4	0
Leucospermum	166	13	83	94	56.6	1
Protea	134	78	1	79	58.9	0
Brodiea	53	12	7	19	35.8	2
Others[a]	7,203	260	849	1,109	15.4	12
Total	162,416	12,345	40,443	52,788	(32.5)[b]	144

[a] - Includes 200 unspecified flowers/foliage materials.

[b] - Total flowers and foliage disinfested as a percentage of total flowers and foliage inspected.

- Aerosols - a combination of Pestigas® (4g/kg of pyrethrum synergised with 20g/kg of piperonyl butoxide) and Insectigas® (five per cent of dichlorvos in carbon dioxide as a

carrier gas) is used by a small number of exporters. However, because of the higher import tariffs, limited production and higher production tax imposed by various countries on methyl bromide, most exporters are adopting this combined treatment.

- Insecticidal dipping - currently deltamethrin (Cislin10®) has been registered for insecticidal dipping and this is being used extensively by exporters.

- Removal by hand - a traditional method, still being used by a small number of exporters; however, this method is not widespread because of high labour costs.

- Hot water treatment - rarely used, and applicable to only certain species of flowers.

- Cool temperature treatment - not widespread due to the requirements of long exposure periods.

Among these, fumigation is the least time consuming and an effective way to disinfest a range of insect pests and some disease causing organisms. Also, fumigation appears to be the only practical way of disinfesting heavily infested consignments. Fumigants are chemicals which, under a given temperature and pressure, can exist in the gaseous state in sufficient concentration to be lethal to a given pest organism (Bond 1984). Fumigation is a technique that is widely used for the postharvest disinfestation of a range of commodities. From the point of view of postharvest sanitation of wildflowers, fumigation is versatile and valuable. An advantage of using fumigants for the postharvest disinfestation of a range of commodities is that insects are unlikely to avoid lethal concentrations of fumigant in well conducted fumigations.

As mentioned earlier, flower exporters fumigate their produce immediately prior to export with methyl bromide or combined treatment of Pestigas®-Insectigas®, and the fumigation can be completed in two to four hours of exposure time. Methyl bromide controls a wide range of insect pests and their developmental stages without severely affecting the vase life

of many species of flowers. However, under the Montreal Protocol, methyl bromide has been classified as a controlled substance due to its ozone depleting potential. Hence, the signatory countries are bound to restrict the use of methyl bromide. The use of methyl bromide will be reduced to 1991 levels by the year 2000 and subsequently phased out from the market by the year 2005. However, developing countries are permitted to use methyl bromide until 2015. At present there appear to be few satisfactory alternatives to methyl bromide for the efficient disinfestation of wildflowers except the Pestigas®-Insectigas® combined treatment. Also, this treatment is not effective against certain insect pests and their developmental stages, particularly larvae and pupae of the leaf rolling moth, the lightbrown apple moth and young eggs of two-spotted mites. Hence, the wildflower industry requires extensive research to determine new ways of disinfesting insect pests with efficient and environmentally friendly fumigant/fumigant mixtures that do not affect the vase life of the fumigated wildflowers.

Phosphine is an important fumigant that is currently used extensively throughout the world for disinfestation of a wide range of commodities, including some perishables. A major impediment to using phosphine is that relatively long exposure times are required to achieve complete kills of insect infestations. Longer exposure times are not conducive from the viewpoint of both flowers, and foliage (the longer the exposure time, the shorter the vase life) and exporters (time factor). The exposure time can be reduced by increasing the temperature to a certain extent. Also, there is a possibility that the combined treatment using pyrethrum and phosphine may reduce the exposure time and/or temperature requirements.

In general, higher temperatures* accelerate the metabolic rates of insect pests and increase the distribution of fumigant chemicals by increasing molecule diffusion.

*Temperature requirements may vary with species and stages of an insect, and it can be assumed that 18°C or more is sufficient to result in a sufficiently high metabolism of an insect.

Also, there is a possibility of applying pyrethrum to potentiate the toxic action of phosphine.

Pyrethrum is a nerve poison, that agitates the insects and this also increases the metabolism, subsequently, these insects absorb more toxic fumigants. Studies are necessary to determine how the exposure time can be reduced in order to avoid the deterioration of the vase life of the fumigated flowers without compromising the complete kill of insect pests. The overall objective of this work is to devise effective, scientifically well founded and environmentally safe ways of disinfesting Australian wildflowers.

Postharvest disinfestation is the last resort for pest control, and field control is imperative in order to save crops from complete destruction, and to ease the pressure on postharvest treatments. Fundamental studies of the biology and life cycle of *S. ejectana* would help growers to identify the ideal time for combating this pest.

Chapter - 2

Literature review

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Literature review

2.1 Importance of the wildflower industry

The horticultural industry is Australia's third largest export earning agricultural industry, after meat and wheat, with an estimated gross value production (GVP) of $A3.35 billion (McGeoch 1995). Of this, the ornamental flower industry alone is worth $A950 million per annum at farm gate value (cut flowers are worth $A250 million per annum and nursery products are valued at $A700 million per annum, Barry 1995) and this makes a significant contribution to the Australian economy. The demand for flowers throughout the world, especially in Western Europe, North America and Japan, is increasing as disposable incomes increase (Table 2.1). Moreover, as land is used increasingly for commercial and industrial purposes its availability for agriculture is shrinking and the agricultural work force in some countries, particularly Japan, is declining. Further to this, the surging hospitality industry throughout the world, particularly in Asia, and the consequent demand for floriculture products offers the Australian industry a growth option. In addition to this, recent international agreements on freeing world trade will offer more advantages to the Australian floriculture industry.

The world floriculture trade is presently worth $A30 to 35 billion/year, and it is expanding at an annual rate of 15 to 20 per cent (Anon. 1995a, Johnson 1996). The value of Australian floriculture exports reached $A25 million in 1991 to 92 from $A3 million in 1984 to 85 and exports are expected to grow up to $A100 million by the year 2000 (McGeoch 1995). Of the total export of flowers, wildflowers constituted 90 per cent in

1993 (Fuller 1995). Japan (40 per cent), U.S.A. and Germany are the most important markets for Australian flower exports, but Australian flowers account for only 2.5 per cent of the total importation into Japan (Tydens 1995). The growing markets for Australian flowers are Malaysia, Singapore and Taiwan, and potential markets include Indonesia, Korea, China and the Middle East (Carson 1995). In the context of the floriculture industry, Australia has an abundance of fertile land, sufficient water, high year-round light and a close proximity to Asian markets. It is estimated that there are more than 32,000 species of wildflowers still to be identified and commercially exploited. At present, 12,000 species of wildflowers have been identified in Western Australia alone (Wolkinsky 1995), and of these only 200 species are presently used for export purposes.

Table 2.1 World flower consumption (Anon. 1995a).

Country	Population (m)	Per capita flower consumption ($A)	Floriculture consumption ($A m)
Norway	4.2	146	613.2
Switzerland	6.7	126	844.2
Germany	79.5	88	699.6
Denmark	5.1	84	428.4
Sweden	8.6	79	679.4
Austria	7.7	78	600.6
Italy	57.7	74	4269.8
Holland	15.0	70	1050.0
France	56.5	58	3277.0
Belgium	9.9	56	554.4
USA	250.0	50	12,500.0
Japan	124.0	44	5456.0
Greece	10.0	32	320.0
Britain	57.4	28	1664.6
Spain	38.9	26	1011.4

It is apparent from the literature that the world floriculture trade is burgeoning, however, the Australian share of the market has not increased significantly over the past decade. The present exports constitute less than 1 per cent (0.8 per cent) of the total world

floriculture market. The Australian floriculture markets are being seized by other countries. South Africa, South America and Israel are particularly major competitors. Barry (1995) reported that Israel exports more Australian wildflowers than does Australia. Every year $A29 million worth of Australian kangaroo paw, wax flower and banksias are being marketed by Israel in Europe and the U.S.A. The main reason is the Australian floriculture industry that has great potential has mainly focused on the domestic market due to lack of information and greater distances from these countries. There are enormous opportunities in overseas markets to be gained by promoting Australia's unique flora. As mentioned earlier, Australia is blessed with enormous natural resources in its native plants and flowers (Moody 1996). Indeed it has a largest gene pool in the world, and it has only just started to realise the significance of these genetic assets. The industry, through groups such as the Flower Export Council of Australia (FECA) is developing new initiatives such as a national flower competition for native Australian flowers, better promotion in importing countries, research projects for varietal improvements through breeding, better postharvest disinfestation and handling. These outcomes have been delivered towards enhancing exports and awareness of Australian wildflowers throughout the world.

Australia's geographic location in the Southern Hemisphere offers unique advantages over other major flower producing and exporting countries. The summer in the Southern Hemisphere coincides with winter in the Northern Hemisphere which facilitates increased exports to European countries. Furthermore, Australia is provided with diverse climatic regions ranging from temperate zones in the south to tropical in the north. Significant irrigation facilities in the semi-arid regions towards the centre of the continent have enabled the production of a diverse and expanding range of wildflowers throughout the year. The varied climates of Australia give many species an extended flowering season and a longer period of availability. Australia has the potential to produce flowers that can supply the world's growing demand with unusual and distinctive flowers and plants. With its unique genetic resource, research that offers uniform productivity, high yield and high quality and promotion, the Australian wildflower industry would be provided with all the necessary criteria for a better future.

2.2 Reasons for the need to eliminate methyl bromide

Importers of cut flowers do not tolerate live insects in consignments of flowers they receive. For this reason flowers must be disinfested before they leave exporting countries. Methyl bromide has been used extensively as a fumigant for a long period of time (at least 60 years, Taylor, 1994) throughout the world by the agricultural, forestry, food processing and transportation groups. It has been used at facilities such as ports, railways and airports, where fumigation of imported commodities is common (Sheen 1996). Methyl bromide provides a broad spectrum of fumigant action and it has been considered to be an ideal chemical for pest and disease control in floriculture, horticulture and quarantine situations. Moreover, methyl bromide is effective against a wide range of insect pests and disease-causing pathogens in soils, stored grains, buildings and in quarantine (Anon. 1995c).

The protective ozone layer in the stratosphere prevents potentially harmful ultraviolet rays from reaching the earth. The ultraviolet rays are responsible for certain cancers in humans and animals, hence, ozone is an important component of the global atmosphere. This ozone layer began to thin in the Antarctic region in the early 1980s and the ozone hole continues to expand. The Montreal Protocol, which was set up in 1987, established a framework for reducing and completely phasing out chlorofluro-carbons (CFCs), and compounds containing bromine, and switching to more environmentally friendly substances by the year 2005. In addition, the American Council of Governmental and Industrial Hygienists (ACGIH) classified methyl bromide as a substance identified by other sources as a suspected or confirmed human carcinogen (Banks, 1994).

In response to this, the Clean Air Act (CAA) was passed, that declares that any product with an Ozone Depleting Potential of 0.2 or more will be eliminated from use in the near future. Bromine, present in methyl bromide, is a potential ozone depletor, with an Ozone Depleting Potential of 0.7 (Taylor 1994) and most of the chemical found in the stratosphere is now thought to originate from man-made sources. However Mix (1992)

reports that in the ozone layer the main sources of bromine are methyl bromide released from the ocean water, bio-mass burning and to a lesser extent commercially manufactured methyl bromide. In November 1992, methyl bromide was added to the Montreal Protocol's list of controlled substances because it is estimated to be responsible for 10 per cent of global ozone depletion. The production and supply of methyl bromide will reduce rapidly, and subsequently manufacture and export will cease by the year 2005. Of the total 150 signatory countries, thirty developed countries including Australia, New Zealand, Japan, the United States, and Western Europe, agreed that the use of methyl bromide will be entirely banned by the year 2005. In the interim, there will be reduction of 25 per cent by the year 2001.

About 66,000 t of methyl bromide were used worldwide during 1990 (Table 2.2). Eighty per cent of this was used for soil fumigation (Anon. 1995c). Australia imported a total of 860 t of methyl bromide during 1991, of which 20 per cent was used for quarantine and pre-shipment purposes (Anon. 1995c). During 1992, the total methyl bromide used was 804 tonnes. Of this 164 t were used for quarantine purposes and 145.5 t were used for floriculture. There was a substantial increase in demand for methyl bromide in Australia during 1994, resulting in a total purchase of 1100 t. However, the 1995 Australian imports of methyl bromide were limited to 689 t (Anon. 1995c).

Table 2.2 clearly illustrates the need for an alternative fumigant, because the demand for fumigants was certainly increasing steadily. Comparatively large amounts of methyl bromide are used for soil fumigation than for quarantine or structure or chemical intermediaries. However, use of methyl bromide for various fumigation purposes is enormous and demand is growing steadily although heavy taxes are being imposed.

Table 2.2 Global sales of methyl bromide (x1000 t)-adapted from Watson *et al.* (1992).

Year	Soil	Quarantine/Commodity	Structural	Chemical intermediates	Total
1984	30.4	9.0	2.2	4.0	45.6
1985	34.0	7.5	2.3	4.5	48.3
1986	36.1	8.3	2.0	4.0	50.4
1987	41.3	8.7	2.9	2.7	55.6
1988	45.1	8.0	3.6	3.8	60.5
1989	47.5	8.9	3.6	2.5	62.5
1990	51.3	8.4	3.2	3.7	66.6

2.3 *Alternative fumigants*

Given the increasing demands for fumigants, and the adverse environmental effects caused by methyl bromide there is a search for alternative fumigants. Desirable attributes of an alternative fumigant include:

- highly toxic to target insects and it would kill them quickly;
- non-residual in that no, or minimal residues would remain in the fumigated commodities and it would be easy to air-off or purge;
- easy to apply;
- safe to the applicator and to the environment;
- an aggressive penetrator and to diffuse rapidly;
- economical;
- a low fire hazard;
- low in its phytotoxicity and no adverse effect on germination; and
- chemically inert with respect to metals.

Based on their biochemical action, fumigants are broadly classified as anaesthetic, narcotic or respiratory inhibitors. Hence, it is vital to understand the mode of action and toxicological properties of fumigants to obtain maximum efficiency in fumigation processes for killing target organisms. The effective use of existing fumigants and the development of new fumigants and/or fumigant mixtures is now essential because of concern over toxicological and environmental hazards that have forced several fumigants to be restricted or removed from the market (Banks 1994). Similarly, Bond (1984) stated that fumigants such as acrylonitrile, carbon tetrachloride, ethylene dibromide, ethylene dichloride, ethylene oxide, methallyl chloride, sulphuryl fluoride and trichloroethylene had been phased out from the market due to their carcinogenic effects, residues, ineffectiveness or environmental contamination. Hence, knowledge of the toxicological properties of fumigants will help in devising safe and effective control approaches, and to avoid the development of resistance of insect pests to fumigants.

In brief, various factors that influence the success of how well fumigants kill insects depend on the:

- interaction of the fumigants and oxygen on the insects;
- differential permeability of the tracheal system at differential oxygen tension; and
- relative ability of absorption of the fumigants by the insects.

At present, low boiling point fumigants such as methyl bromide (CH_3Br), phosphine (PH_3) and comparatively high boiling point compounds such as liquid fumigants (carbon disulfide (CS_2), ethylene dibromide ($BrCH_2CH_2Br$), ethylene dichloride ($ClCH_2CH_2Cl$) and carbon tetrachloride (CCl_4) and aerosols such as dichlorvos (CCl_2=CHO.PO.$(OCH_3)_2$), and pyrethrum are used for postharvest fumigation of a wide range of agricultural commodities. Lindgren *et al.* (1958) stated that phosphine is an efficient insect killing fumigant. Hansen and Hara (1994) averred that phosphine requires a range of exposure times (usually longer, due to its slow acting nature) to kill all the insects compared to other fumigants.

Methyl bromide (CH_3Br) is one of the common fumigants effective against a range of insect pests. It can be used for low temperature fumigations due to its low boiling point (it boils at 4.5°C). However, the molecular weight of methyl bromide is 94.95, thus it is almost three times heavier than air, hence, stratification in an enclosed space is quite possible. Methyl bromide is nonflammable in nature (Davidson and Peairs 1966). Apparently, methyl bromide can be applied economically and efficiently under a wide range of conditions without producing excessive residues in commodities (Bond 1980). However, there are possibilities that repeated fumigations on the same commodity may leave a substantial residue of inorganic bromide (Bond 1980).

Table 2.3 Important properties of some major fumigants used for postharvest disinfestation of commodities (Adapted from Monro 1961a).

Name	Molecular weight	Boiling Point (°C)	Flammability by volume in air (%)	Remarks
Methyl bromide	94.9	3.6	Nonflammable	General fumigant
Phosphine	34.04	-87.4	1.79	General fumigant; good penetrating ability
Ethylene oxide	44.1	10.7	3 to 80	Controls micro-organisms as well as insects
Hydrogen cyanide	27.0	26.0	6 to 41	General fumigant; poor penetrating ability
Ethylene dibromid	187.9	131.0	Nonflammable	Mainly spot fumigant admixed with other fumigants
Carbon tetrachlorid	153.8	77.0	Nonflammable	Used mainly in mixtures with flammable compounds to Reduce fire hazard and aid distribution
Carbon disulfide	76.1	46.3	1.25 to 44	Grain fumigant; usually mixed with nonflammable compounds
Chloropicrin	164.4	112.0	Nonflammable	Powerful tear gas; has been used as grain and spot fumigant
Ethylene dichloride	98.9	83.9	6 to 16	Usually mixed with carbon tetrachloride
Dichlorvos	221.0	120 at 0.14 mm	Nonflammable	Good only in free spaces; very toxic to most stored product insect

Dichlorvos (DDVP) is recommended as a postharvest disinfestation treatment for wildflowers and foliage (Seaton and Joyce 1993). However, Hamlen and Henley (1979) report that DDVP is phytotoxic to the foliage of some plants namely *Aphelandra squarrosa* Nees., Florida ruffle fern, *Nephrolepis exaltata* (L.) Schott and *Gynura procumbens* (Lour.) Merrill. Amos and Evans (1979) found that dichlorvos is effective

against a wide range of stored products insect pests, especially flying insects. Similarly, Banks (1994) indicates that dichlorvos is highly toxic to most insect pests.

Carbon disulfide (CS_2) is a pale yellowish liquid, highly flammable in nature, and heavier than water and well established as a fumigant of both soil and enclosed spaces (Davidson and Peairs 1966). One of the characteristic features of this fumigant is, that at room temperature it rapidly vaporises, and its vapour is more than 2.5 times denser than air. One of the inherent characteristics of carbon disulfide is that it is explosive in nature and this poses problems in storage and handling (Price, 1985). Hence, it is formulated in mixtures with nonflammable ingredients for fumigation of various agricultural commodities. Generally, carbon disulfide penetrates well into the commodities, but relatively large dosages are required to kill insects.

Hydrogen cyanide (HCN) is one of the most toxic insect fumigants. The use of hydrogen cyanide is important in treating growing plants and trees, citrus and other fruits and dried fruits (Monro 1961a). Because of the high degree of sorption at atmospheric pressure, hydrogen cyanide does not penetrate well into some materials (Bond *et al.* 1969).

Previous studies by Hamlen and Henley (1979) and Lindgren *et al.* (1958) have shown that fumigants have a range of qualities in controlling insect pests and that fumigants may be phytotoxic to the treated foliage. Hence, it is important to choose a suitable fumigant/fumigant mixture that could control all target insect pests without any phytotoxic effects on the treated flowers.

In brief, among these fumigants, phosphine seems to have most of the desirable properties of an alternative fumigant to methyl bromide. Although, phosphine is a slow acting fumigant, it is highly toxic to a range of insect pests and their developmental stages. Also, it is an aggressive penetrator, it has low or negligible sorbent properties and comparatively low concentrations are sufficient for a complete kill of a range of insect pests and their developmental stages.

Table 2.4 Some important properties of four fumigants (adapted from Bond 1983).

Properties	Phosphine	Methyl bromide	Ethylene dibromide	Ethylene dichloride
Commercial formulation	Tablets, sachets, gas in cylinders	Liquid in cans and Cylinders	Liquid	Liquid
Solubility in water (g/100mL)	Slight	1.3 at 25°C	0.43 at 30°C	0.87 at 20°C
Penetration	Excellent	Very good	Poor	poor
Sorption	Negligible	Some	High	high
Desorption and aeration	Rapid	Fairly rapid	Slow	slow
Toxicity to insects	High	high	High	low
Rate of kill	Slow	Rapid	Slow	slow
Effect on germination	almost negligible	Variable	Variable	little
Adverse reaction	Corrodes copper	Combines with sulfur to Create odours	-	-

2.4 Important properties of methyl bromide and phosphine

At atmospheric pressure, methyl bromide is a colourless liquid with a boiling point of 4.5°C. It is stable, non-flammable, corrosive to aluminium, magnesium, and their alloys (Worthing and Walker 1987a,b). It is a potent insecticide with some acaricidal properties, and it is useful for the fumigation of plants and plant products in stores. Moreover, methyl bromide's broad spectrum of activity against nematodes, soil-borne pathogens, insects and weeds render it an ideal soil fumigant (Taylor 1994). In addition, methyl bromide fumigation is used for vegetables, cut flowers, bulbs, corms and root stock materials, possibly due to its short exposure time requirements (Taylor 1994). It can be assumed that due to its rapid action, short exposure periods are sufficient, hence, methyl bromide does not appear to be highly phytotoxic.

Unlike phosphine, methyl bromide is effective over a wide range of temperatures and it has been used successfully in cold climates i.e. -5 to -10°C (Bond 1983). On the other hand,

methyl bromide is not a highly and aggressive penetrator like phosphine, hence, recirculation (Taylor 1994) or mixing with carbon dioxide (Calderon and Carmi 1973) are essential for better penetration.

Sorption is one of the major mechanisms that reduce the fumigant concentration in enclosed spaces. Of all fumigants, phosphine appears to be adsorbed the least, depending on the commodity fumigated (Graver and Annis 1994, Monro 1961a and Weller *et al.* 1995). However, Leesch and Gillenwater (1976) tested the toxicity and sorption of phosphine and methyl bromide on larvae of the pecan weevil, *Curculio caryae,* (Horn.) and to pecans respectively. They found that methyl bromide with a concentration of 32 mg/L and an exposure of 24 h killed all the larvae that were present inside the nuts with exit holes. However, 80 and 112 mg/L of methyl bromide were necessary at 27°C and 15°C respectively at 24 h of exposure to kill the larvae inside the nuts without exit holes. Phosphine, with an exposure time of 96 h and a concentration of 2.8 mg/L at 15°C and 27°C did not give complete control of the larvae. They found that 95 per cent of the applied phosphate was sorbed by the pecans within 24 h of exposure, however, in the case of methyl bromide only 60 per cent was sorbed. On the other hand, the phosphine in the empty container showed little or no decrease in the concentration during the 96 h exposure time. A 48-h aeration forced out virtually all of the phosphine and only a trace amount of phosphine (from 2000 ppm to 0.0012 ppm) was present in the nutmeats. They concluded that sorption of phosphine by the pecans could be the apparent reason for the failure of phosphine to control the larvae.

The toxicity of phosphine is strongly insect species specific. Muthu *et al.* (1970) compared the toxicity of phosphine, ethylene dibromide and methyl bromide to the mould mite *Tyrophagus putrescentiae* (Schrank) at 27±1°C and concluded that ethylene dibromide was more highly toxic to the mite species than phosphine and methyl bromide. At the median lethal dose level it was 46 and 17 times more toxic than methyl bromide and phosphine respectively to *T. putrescentiae.*

Phosphine appears to be highly toxic to all stages of insect pests, including the more tolerant developmental stages, namely eggs and pupae, in which the metabolic rates are low and subsequent uptake of fumigant gas is also very small. For example, Rajendran and Muthu (1989), claimed that on a weight and per mole basis phosphine is highly toxic to eggs of the rust-red flour beetle, *Tribolium castaneum,* (Herbst) compared to other fumigants, including methyl bromide. Younger eggs of most insect species are more tolerant to methyl bromide and ethylene bromide than to phosphine.

2.5 *Inherent qualities of phosphine*

We have observed that phosphine appears to be a possible alternative to methyl bromide for fumigating cut flowers. Its properties will now be examined in more detail. Phosphine has gained world wide acceptance as an efficient and effective fumigant for disinfesting a range of commodities due to its lethality to a range of insect pests, and their developmental stages, most of the mite species, some disease-causing organisms, and vertebrates including humans. The absence of residues and the possibility of repeated fumigations if required without significant dangers of cumulative residues render phosphine a fumigant to be seriously considered for fumigating wildflowers. Although phosphine has been used over a long period of time and it has several advantages over other fumigants, the properties of phosphine are poorly understood. From the available data it is apparent that a precise comparison of susceptibility of a species to phosphine is not possible, because of a wide range of concentrations used, the exposure periods used and the life cycle of the insect species studied.

2.5.1 Physical properties

Phosphine is colourless and odourless in nature. However, due to the presence of impurities, namely diphosphoric acid (P_2H_4) it has an objectionable garlic or calcium

carbide or impure acetylene odour (Graver and Annis 1994). Also, they indicated that the odour is often greatly reduced by its adsorption onto the commodity leaving phosphine without a smell, hence, the absence of smell does not always mean that a fumigation enclosure is free of phosphine. The density of phosphine is similar to that of air, ca. 1.5307 kg/m^3, cf air = 1.2929 kg/m^3 under standard temperature and pressure. The molecular weight of phosphine is 34.04, hence, phosphine distributes uniformly without layering or stratification in the chamber. Phosphine is a spontaneously inflammable gas due to the presence of traces of other hydrides of phosphorous with an explosion limit of 26.1 to 27.1 mg/L (Worthing and Walker 1987). Moreover, Monro (1961a) indicated that flammability of phosphine is induced at or above 100°C and at reduced pressures, particularly in dry atmospheres.

The boiling point of fumigant chemicals generally increase with increasing molecular weight and the boiling point of phosphine is extremely low, ca -87.4°C, which is associated with its low molecular weight. The very low boiling point of phosphine (relative to ambient temperature) means it will not condense at atmospheric pressure. Also, the molecular weight of a fumigant influences its diffusive capacity which is an important criterion for a successful fumigation. This is encapsulated by Graham's law which may be stated as "under comparable conditions, the relative speeds of diffusion of gases are inversely proportional to the square root of their relative densities". Diffusion is an important factor in the penetration process into commodities, and the lower the molecular weight, the greater the rate of diffusion of fumigant gases; diffusion tends to carry fumigant molecules into interstitial spaces.

The excellent penetration and diffusive capacity of phosphine aid in the penetration not only through the spiracles of insects, but also through the cuticles. Leesch *et al.* (1982), who conducted fumigation trials to determine the penetration capacity of phosphine in dates. They found that phosphine penetrates and diffuses up to 4.23 m in closely and uniformly packed dates. Also, Muller (1996) found that phosphine penetrates inside the polyethylene vials to depths of 0.9 to 1.8 m.

The degree of solubility of fumigants in water varies significantly (Dieterich *et al.* 1967). The extent of phosphine adsorption also depends on the water content of the commodity and higher water content leads to higher residue deposition on the fumigated commodity. Gases which are distinctly polar and which are in contact with a liquid exhibit strong attractive forces between their molecules and the molecules of the liquid. The amount of gas dissolved in water decreases with increasing temperature. A high solubility of a fumigant gas in water is disadvantageous in the fumigation process, in which high toxic concentrations accumulate in the treated commodities, especially commodities that contain substantial amounts of water (flowers, foliage and fruits). Slightly and low water soluble fumigants will remain in the gas phase longer than more highly water-soluble gases. Phosphine is sparingly soluble in water and non-soluble in oils, and it is highly volatile in nature.

2.5.2 Chemical properties

One of the disadvantages of using phosphine is that under certain conditions phosphine reacts and causes corrosion of precious metals, copper and copper alloys such as brass (Monro 1961a). For this reaction to take place the presence of moisture is essential, moreover the reaction is accelerated by air that contains salt. Phosphine combined with moisture forms a mild acid called phosphoric acid. This acid can react with copper and other metals resulting in corrosion. Hence, it is highly likely that phosphine would damage electrical equipment containing copper and copper alloys. Due to its reaction with copper, a hypothesis could be posed that copper-containing compounds such as cytochrome oxidase could be the target site for the phosphine action on insects (Al-Hakkah 1989 and Chefurka *et al.* 1976).

2.5.3 Oxidative properties

During normal fumigation (pure phosphine/air mixture) apparently there is no oxidation, hence, it is stable (Robinson and Bond 1970). However, there are possibilities for atmospheric oxidation of phosphine, once it is absorbed on to surfaces or in the presence of biological materials. The chemical nature of phosphine residues in the presence of air are lower oxy-acids of phosphorus that are eventually converted into orthophosphate (Robinson and Bond 1970).

$$2PH_3 + O_2 \rightarrow P_2H_4 + H_2O.$$

(Diphosphine)

$$P_2H_4 + H_2O + O_2 \rightarrow 2H_3PO_2.$$

(Hypophosphite)

$$2H_3PO_2 + O_2 \rightarrow 2H_3PO_3.$$

(Phosphite)

$$2H_3PO_3 + O_2 \rightarrow 2H_3PO_4.$$

(Orthophosphate)

2.5.4 Physiological effects of phosphine

2.5.4.1 On higher animals

The primary effects of phosphine toxicity are apathy, sleepiness, respiratory distress and disturbed circulatory regulation. The secondary symptoms include anoxemic convulsions as a result of primary effects (Monro 1961a).

2.5.4.2 On insects

Bond *et al.* 1969 reported that the phosphine toxicity symptoms include: (depending upon exposure) slight repetitive tremors of the labial palps leading to leg tremors, knock-down, loss of coordination of muscle activity, bursts of tremors spaced by long periods of exhaustion, body convulsions and complete paralysis. This progresses to death. However, narcotised insects are observed to recover after aeration without any symptoms and developed into normal insects (no physical disabilities).

Phosphine toxicity causes irreversible damage, and once established the symptoms never regress (Bond *et al.* 1969 and Bond and Upitis 1973). If the phosphine fumigation is concluded while symptoms are developing, the symptoms' manifestations are frozen at that level. In some cases a delay in development has been reported e.g. cadelle, *Tenebroides mauritanicus,* (Linnaeus) (Bond and Upitis 1973).

2.5.4.3 On germination

In general, phosphine fumigation does not affect the germination and growth of seeds. Younis *et al.* (1989), reported that germination and seedling growth of onions (*Allium cepa,* L.), fumigated with phosphine at concentrations of 7 or 14 mg/L for two to four weeks at 6 per cent moisture content, were not affected. Moreover, Natarajan and Bagyaraj (1984) found that phosphine had no adverse effect on germination of the seeds of blackgram and field beans.

2.6 General characteristics

One of the peculiarities of phosphine is that it exerts its toxicity to insects quite differently from that of other fumigants. Phosphine generally does not produce a normal dose-mortality relationship. Several authors have indicated that higher concentrations of phosphine do not necessarily produce higher mortalities to a range of insect species (Querashie *et al.* 1965 and Winks 1985). At high concentrations, phosphine reduces or prevents the respiration of insects due to its narcotising and subsequent paralysing effects. Phosphine has been reported to achieve a 100 per cent mortality of heavy infestations in a range of commodities and insects fumigated at high temperatures and longer exposure times with low dose rates.

Phosphine is a respiratory poison (Nakakita *et al.* 1974 and Price 1980) and it attacks vital respiratory components, namely cytochrome oxidase (Robinson and Bond 1970). This was confirmed by the findings of Chefurka *et al.* (1976). They studied the mode and site of action of phosphine on the granary weevil, houseflies, and mouse liver. They found that phosphine acts on the respiratory chain of mitochondria, and indicated that phosphine is a

specific non-competitive inhibitor of only one site in the respiratory chain called cytochrome oxidase. Phosphine inhibits the respiration of mitochondria in the ion-pumping state (i.e. respiration stimulated by ion uptake) and this may lead to death of an insect. Furthermore, Ca^{++} and K^{+} uptake are sensitive to higher concentrations of phosphine (Chefurka *et al.* 1976).

Oxygen is found to be essential for the absorption, and subsequent toxicity of phosphine on insects (Bond *et al.* 1969 and Bond *et al.* 1967). In anoxic conditions, where oxygen is not available, phosphine is not toxic due to the fact that no appreciable amount of phosphine is absorbed by the insects. This was substantiated by the findings of Bond *et al.* (1969). They examined insects under depressed or inhibited respiratory levels by treating them with cyanides, and found that phosphine was not absorbed in appreciable amounts.

Absorption of phosphine varies considerably among insect species. Some insect species became saturated within five hours of exposure and absorbed no more phosphine, while some insect species continuously absorb it at a slow rate for 24 h or longer. Bond and Upitis (1973) observed that the uptake of phosphine by the adults of the confused flour beetle, *Tribolium confusum* Jacquelin du Val was higher than that of the granary weevil, *Sitophilus granarius* (Linnaeus) adults. The *T. confusum* adults absorbed 5.5 μm/g of phosphine that were exposed to a phosphine concentration of 0.117 mg/L. However, the adults of *S. granarius* absorbed lesser amounts (2.8 μm/g) at a phosphine concentration of 0.118 mg/L.

Due to insect control failures and consequent insect survival in commercial fumigations, it would be desirable that fumigants have a sterilising property or other effects which directly or indirectly affects the reproduction of the survivors. Phosphine, in general, inhibits the reproduction or multiplication of certain stored product insects but to some extent this depends on the dose rate and developmental stage exposed. Howe (1973) reported a reduction of fecundity for the adults of *S. granarius* that had been fumigated at larval or pupal stages. Similarly, Al-Hakkah *et al.* (1985) found that adults of the tropical

warehouse moth, *Ephestia cautella* (Walker) had reduced fecundity and fertility when fumigated at the pupal stage.

In general, the toxicity of phosphine is low during exposures of up to 5 or 6 h, and increases first rapidly and then more slowly as exposure time is increased (Bell and Glanville 1973). This was further confirmed by Lindgren *et al.* (1958), Reynolds *et al.* (1967), Brown *et al.* (1969) and Howe (1973). Moreover, Price (1984) found that insects absorb more phosphine during the first few hours of the exposure period. However, he pointed out that immediately after this, the insects may be either knocked down or narcotised depending on the phosphine concentration. In both cases there will not be any further uptake of phosphine.

Although, there is considerable information of phosphine toxicity on a wide range of insect pests, there is a paucity of information on the toxicity of phosphine to micro-organisms. Dharmaputra *et al.* (1993) found that phosphine (concentration of 2.1 g/t and an exposure period of 5 days) reduces the total population of all the species of storage fungi that commonly attack soybean meal (17 species including *Aspergillus candidus* and *Penicillum citrinum).* However, the population increased during subsequent storage. On the other hand, Hocking (1991) found that phosphine was in general lethal only to growing fungi, and that it had less effect on the mycelium and dormant spores.

2.7 Phosphine: Exposure time vs dose rate

There is myriad of research findings on the toxicity of phosphine at various exposure times and concentrations to a range of stored product insect pests. The results of these experiments suggest that the full potential of phosphine could be achieved only if the exposure times are long enough (Barker 1969a, Bell 1979, Bell and Glanville 1973, Bengston 1972, Bond *et al.* 1969, Bond *et al.*1977, Hocking and Banks 1992, Howe 1973, Howe 1974, Kashi 1982, Kenaga 1961, Lindgren *et al.* 1958, Monro 1961a, Muller 1995, Querashi *et al.* 1965, Reynolds *et al.* 1967, Roth 1973, Seo *et al.* 1979, Vincent and

Lindgren 1972a, Wainman *et al.* 1975 and Zettler *et al.* 1986). The extent to which the exposure time can be reduced by increasing the dose of phosphine is limited, because exposing insects to high concentrations can cause narcosis in the target insects or insects may enter a state called protective stupefaction (Bond and Monro 1967 and Winks 1985).

In a narcotic state, the insects are more or less inactive, and their metabolic rates are low so that absorption rates of toxic gases and their reaction with them are reduced. During narcosis insects seldom respire, this is evidenced by the findings of Edwards (1953). He found that insects can withstand oxygen deficient ambient atmospheres, and some insects can live anaerobically for up to two days without injury, and that on their readmission to air the oxygen debt would be repaid. Also, there is a possibility that the increased concentration of phosphine affects the uptake of phosphine by preventing the respiratory activity of insects. At increased dosages, the biological system may be found to turn over greater amounts of phosphine, or the presence of a permeability barrier which is present in the tracheae of the insects would restrict the entry of phosphine (Chaudry and Price 1990).

Essentially, exposure time is an important factor in the toxicity of fumigants to insects, specifically by influencing;

- the dosage factor (uptake of fumigants from the surrounding gas).
- the response factor-time for the absorbed fumigant to elicit a response-to-death (Winks 1982).

Time as a dosage factor, and time as a response factor begin to be important immediately and simultaneously after an insect has come into contact with a fumigant and they cannot be separated (Hewlett 1974).

Research indicates that phosphine is highly toxic to insects even at low concentrations provided sufficient exposure time is maintained. For example, a 95 per cent mortality of the adults of cigarette or tobacco beetle, *Lasioderma serricorne* (Fabricius) can be

achieved with a concentration of 0.005 mg/L of phosphine and an exposure period of 72 h at 21.1°C. If the phosphine concentration is further reduced to 0.0021 mg/L, 95 per cent kill can still be achieved by increasing the exposure time to 144 h (Vincent and Lindgren 1977). Likewise, Nakakita *et al.* (1974) investigated the response of adult maize weevil, *Sitophilus zeamais* Motschulsky, to a range of phosphine concentrations (5 to 20000 ppm) and exposure times (12, 18 and 24 h) at a temperature of 25°C. They found that a concentration of 50 ppm phosphine and exposure periods of 12 and 18 h produced higher mortality (59 per cent) than higher concentrations used (5000 ppm). The higher concentration (5000 ppm) resulted in 44 per cent mortality. On the other hand, 24 h fumigation gave 100 per cent mortality at concentrations ranging from 25 to 1000 ppm, but concentrations up to 20,000 ppm did not give complete mortality. It was conjectured that phosphine suppresses the oxygen uptake at higher concentrations.

Higher concentrations of phosphine paralyse the spiracular muscles and prevent active respiration, which in turn reduces the availability of oxygen essential for the reaction of phosphine (Bond *et al.* 1967, Jones 1938, Kashi and Chefurka 1976). Furthermore, Kashi (1981) indicates that in high concentrations, the molecules of phosphine that gain quick access to the nervous system may act to paralyse the insects. Thereby, further uptake of phosphine by the insect will cease, consequently, a complete kill of target insect pests will not be achieved.

Although the uptake of phosphine by insects does increase with increasing concentration, the rate is not directly proportional to the degree of change in concentration, and its effectiveness is mainly determined by the length of the exposure time. This was substantiated by the findings of Lindgren and Vincent (1966). They found that an exposure time of 2 h with a concentration of 6.5 mg/L does not give a complete kill of a range of stored product insects including *T. sternale* and *T. confusum.* However, longer exposure time of 16 h with a concentration of 0.048 mg/L was effective in giving a complete kill. Likewise, Hawkes (1973) recommended that complete control of the first instar larvae of fall army worm, *Spodoptera littoralis* (Biosdual) can be achieved with a

phosphine concentration of 1.5 mg/L and an exposure of 24 h. However, the concentration can be halved (0.75 mg/L) if the exposure is increased to 48 h. Similarly, in his experiments on *S. granarius* with a concentration between 0.1 mg/L to 0.95 mg/L of phosphine, Reichmuth (1994) found that in general the speed of uptake or the slope of the uptake curve increased with increasing concentration. But, the uptake slowed rapidly once the concentration reached 0.95 mg/L. The above mentioned facts clearly demonstrate that the increase in exposure time may stimulate phosphine uptake and, is in contrast to increasing concentration, which stimulates the active exclusion of phosphine.

Winks (1985) found that at an exposure period of 1.8 h at a temperature of 25°C and with a relative humidity of 70 per cent a concentration of only 0.19 mg/L of phosphine is sufficient for 99 per cent kill of *T. castaneum* adults. A concentration of 48.3 mg/L phosphine at the above mentioned conditions narcotised 99 per cent of the *T. castaneum* adults within 0.05 h of exposure. The insects that are more susceptible to narcosis have the greater chance of survival because the absorption and subsequent toxicity were insignificant for narcotised insects.

Likewise, Kashi (1982) claimed that mortality increased with increasing exposure time but not with increasing concentration. A phosphine concentration of 1.96 mg/L with an exposure of 22 to 24 min killed 2 and 6 per cent respectively of *T. castaneum* and *T. confusum* adults. However, increasing the exposure time to 40 to 45 min with similar phosphine concentrations raised the mortality rate of *T. castaneum* to 65 per cent and of *T. confusum* to 24 per cent. Moreover, a phosphine concentration of 1.97 mg/L of phosphine for 12 min of exposure period killed 1 per cent of the adults of the rice weevil, *Sitophilus oryzae* (Linnaeus). Increasing the exposure period to 16 min increased the mortality rate to 5 per cent.

Concentration-time products required for 99 per cent kill of different ages of the same insect species vary considerably. Winks (1982) examined the toxicity of phosphine to different ages of *T. castaneum* (CTC-4 strain) adults at 25°C and 70 per cent relative

humidity with an exposure time of 6 h. His recommendations for 99 per cent mortality of different ages of adults are as follows (in concentration time products basis):

- for 1 to 3 days old - 0.455 mg h/L of phosphine;
- for 20 to 22 days old - 0.398 mg h/L of phosphine; and
- for 45 to 50 days old - 0.475 mg h/L of phosphine.

There is a paucity of research on the insecticidal efficacy of phosphine or any other fumigants to insect pests of wildflowers. Development of an efficient postharvest disinfestation techniques applied to wildflowers either with standard phosphine (one per cent phosphine in nitrogen as a carrier gas) or Phosfume® (2 per cent of phosphine in carbon dioxide as a carrier gas), will not only benefit the burgeoning wildflower industry to a great extent, but the work will be a contribution to fumigation technology in general.

2.7.1 Resistant strains

If the exposure time is inadequate, the chances of insects developing resistance towards a fumigant, especially to phosphine, are high. Winks and Ryan (1992) reported that a number of insect pests have developed phosphine resistance including *R. dominica* and *S. oryzae*. These resistant insect strains can be controlled with phosphine only if adequate phosphine concentrations are maintained for an adequate exposure period. Generally, resistant strains and their developmental stages require higher dosages of phosphine and exposure times than susceptible strains for a complete kill (Price and Mills 1988, Tyler *et al.* 1983). For example, Monro *et al.* (1972) observed that the resistant strains of *S. granarius* are tolerant of high concentrations (13.3 mg/L of phosphine) and it requires 78 h to obtain a complete control. On the other hand, normal strains of *S. granarius* can be controlled with a concentration of 0.61 mg/L and an exposure time of 27 h.

The rates of uptake of toxic fumigant by the susceptible strains were generally higher than the resistant strains. Chaudry and Price (1990) found that, the susceptible strains of lesser grain borer, *Rhyzopertha dominica* Fabricius absorbed much more (33.36±1.71 μg phosphine/g of insect) than the resistant strains (1.87±0.27 μg/g of insect). The mortality rates are lower or nil (0 to 5 per cent) for phosphine resistant *R. dominica* strains after 20 h of exposure with a concentration of 0.1 mg/L of phosphine. However, increasing the exposure time to 72 h with the same phosphine concentration gave 100 per cent mortality. The susceptible strain required only 0.02 mg/L of phosphine with 48 h of exposure for 100 per cent mortality.

2.7.2 Diapause larvae

Due to their lower metabolic rates, diapause larvae are generally more tolerant of phosphine fumigation than the active stages. Again complete control of these stages can only be achieved by increasing the exposure time rather than the concentration of phosphine. Bell (1979) found that diapause larvae of tobacco moth, *Ephestia elutella* (Hübner) are difficult to control at 10 h of exposure time with a concentration of 0.35 mg/L of phosphine at 20°C (only 9.5 per cent mortality). However, 97.5 per cent mortality was achieved under similar conditions when the exposure time was increased to 40 hours. On the other hand, Bell and Glenville (1973) recommended that diapause larvae of *E. elutella* could be controlled with a lower concentration of phosphine (0.02 mg/L) and an exposure period of 20 h maintained at a temperature of 20°C and 70 per cent relative humidity. However, increasing the concentration to either 0.27 or 0.75 mg/L from 0.2 mg/L and reducing the exposure time to 6 h from 20 h did not give complete control.

Sometimes, it is difficult for the fumigant to reach the sites that insects inhabit, because of their different living patterns. In the case of *T. granarium*, in addition to its tolerance to phosphine, its refuge seeking behaviour and existence of a special type of recurrent dormancy in the larval stage makes it difficult for fumigants to diffuse to it, and act upon it. However, Bell and Wilson (1995) in their fumigation killed 99.7 per cent of these

phosphine-resistant strains by exposing them for 96 h to a phosphine concentration of 0.54±0.05 mg/L at 20°C. On the other hand, a 32-h of exposure under similar conditions killed only 10.5 per cent of these larvae which had been held in crevices for 16 weeks at 20°C.

Importantly, in the case of phosphine toxicity the conventional Haber's concentration-time product (CTP) rule does not apply. High tolerance is manifested when the exposure times are too short, whereby phosphine cannot exert its full toxic potential on the insects. The CTP rule states that over a certain range of concentration and exposure time a specified CTP will give a constant level of kill regardless of the concentration chosen to achieve this dose. The response follows the relationship $\mathbf{C^n \times T = K}$.

Where; **C** is concentration, (g/m^3 or mg/L).

n is a toxicity index.

T is time, (hours).

K is a constant for mortality, that is species specific.

The toxicity index, n, varies with fumigant, species, developmental stage and strain. Moreover, environmental factors such as temperature, relative humidity and food also substantially influence the toxicity index. The dose absorbed is a function of the concentration, C, of the fumigant in the surrounding atmosphere and the time, T, to which the insect is exposed to the fumigant. The response to the fumigant is not always immediate and requires a certain time for a biochemical reaction to occur which will bring about the death, knock down/narcosis or other responses.

2.8 Impact of temperature on the response of insects to fumigants

The most important environmental factor influencing the action of fumigants on insects is temperature. Reasons for this include:

- the physical sorption of gas by a commodity or container increases at low temperatures i.e. less than 10°C (Monro 1961b). Consequently, the concentration and exposure time of fumigant required to kill a given developmental stage of an insect decreases with increasing temperature (Bell and Knight 1992, Cox *et al.* (1984), Lindgren and Vincent 1966 and Vincent and Lindgren 1975) because proportionally more fumigant is available to exert its toxicity on the insects;

- lower temperatures reduce the rate of diffusion of fumigant gases (Rauscher *et al.* 1972 and Sinclair and Lindgren 1958) resulting in longer exposure times. Similarly, Berk (1964) suggests that lower temperatures tend to accelerate "stratification" or "layering" of fumigants at the bottom of the container, because of a lower rate of diffusion in cool air than in warm air. In general, warm air accelerates the dispersion of fumigant molecules; and

- lower temperatures reduce the metabolic rate of insects which in turn increases their tolerance to a fumigant by reducing their uptake of toxic gas. As a general rule, most postharvest insect pests require temperature between 15 to 30°C for growth and reproduction, and in this temperature range insects are active and take up more toxic materials. Below or above this temperature development is likely to slow and it eventually stops.

A defined temperature range is imperative for the growth and development of an insect, and above or below this range there will not be a normal growth and development. Fields (1992) classified the temperature regime into three categories namely optimum (25 to 33°C) where the maximum rate of development occurs, sub optimum (13 to 22°C or 33 to 35°C) where development slows, and lethal (5 to -15°C or 50 to 60°C). At optimum temperature the growth and development of insects would be optimum, hence, the resulting metabolism and fumigant uptake would also be high. An ultimate aim of this research is to

develop commercially viable fumigation strategies that are compatible with the logistics of the Australian wildflower export industry. In arriving at such strategies it is necessary to have a thorough knowledge of the effects of various temperatures on the effectiveness of phosphine, and information obtained from the literature are summarised as follows:

- Price (1985) observed that by increasing the temperature from 20°C to 30°C, the phosphine uptake of *R. dominica* increased from 0.35 mg/g of insects to 1 mg/g of insects.

- At 25°C, Wainman *et al.* (1975) found that the concentrations of phosphine required for a complete kill of *S. granarius* are less (8 mg h/L) than at the lower temperature of 15°C in which a concentration-time product of 150 mg h/L of phosphine is imperative. Also, the exposure time required at 25°C is half (8 days) the exposure time at 15°C (16 days).

- Hole *et al.* (1976) observed that at higher temperatures (25 to 30°C) the exposure period required for control of a range of stored product insect pests including *S. oryzae* and saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) became markedly shorter. At 10°C and 15°C an exposure time of 2 to 16 days and a concentration of 0.017 to 1.5 mg/L of phosphine are crucial for a complete disinfestation of the exposed insect pests. On the other hand, they found that at 25°C an exposure time of 2 to 10 days and concentrations of 0.012 to 1.4 mg/L of phosphine are sufficient for 100 per cent kill. However, only 1 to 4 days of exposure, and concentrations of 0.046 to 0.36 mg/L of phosphine is adequate to kill all the exposed stored product insect pests at 30°C.

- Vincent and Lindgren (1977) found that eggs of *L. serricorne* require exposure to 1.206 mg/L of phosphine for 72 h at 4.4°C temperature for 95 per cent mortality. However, the concentration of phosphine required for the same kill reduced to 0.009 mg/L at 21.1°C.

- Bell (1977a) indicated that at 10°C phosphine was lethal to insect pests, provided sufficient exposure time and concentration of phosphine were maintained. He observed that *E. elutella* larvae were killed completely at temperatures of 10°C to 30°C, and at 10°C a concentration-time product of 8.2 mg h/L of phosphine and an exposure of 93 h resulted in 99 per cent mortality. However, at 30°C a CTP of 5.1 mg h/L for an exposure of 55 h are sufficient for 99 per cent kill.

- Hawkes (1973) investigated the effects of two different temperatures on the response of *S. littoralis* to phosphine with a concentration-time product (CTP) range of 36 mg h/L to 288 mg h/L. The author established that at 15°C none of the treatments gave 100 per cent mortality, even after 72 h preconditioning of larvae at 20°C and 48 h fumigation with a concentration-time product (CTP) of 288 mg h/L. However, at 20°C phosphine was completely effective after pre-conditioning for 72 h at 20°C and fumigation for 48 hours with all CTPs used. Also, he showed that at 20°C for 24 h fumigation with the CTP of 36 mg hr/L or more was sufficient for a complete kill. These results indicate that the tolerance shown by the insect pests was higher at lower temperatures. However, all these insects can be killed by increasing the temperature to a certain extent. Likewise, it is postulated that the highly tolerant stages such as diapause larvae, eggs and pupae can be killed by increasing the temperature.

- Muller (1994) found that a combination of low levels of phosphine (65 to 100 ppm), higher temperatures (32 to 37°C) and 4 to 6 per cent of carbon dioxide fumigation for 24 h killed all the exposed stages of the Angoumois grain moth, *Sitotroga cerealella,* (Olivier), *T. castaneum*, the warehouse beetle, *Trogoderma variabile,* Ballion and *S. oryzae*.

- Vincent and Lindgren (1975) showed that the effectiveness of phosphine against adults of *T. variabile* increases with increased temperature. Complete kill of this adults was achieved with a phosphine concentration of 2 mg/L at 15.6°C, however, at 26.7°C, only 0.2 mg/L of phosphine was sufficient for complete mortality.

- Jalil *et al.* (1970) found that at 24°C the concentration of phosphine required for 90 per cent kill of the mould mite, *Tyrophagus putrescentiae* (Schrank) adults reduced to 50 per cent (70.4 mg/L) compared with at 20°C when a concentration of 146 mg/L of phosphine is required.

- Bowley and Bell (1981) fumigated eggs and mobile stages (larvae and adults) of the seed mite, *Tyrophagus longior* (Gervais), flour mite, *Acarus siro,* Linnaeus and grain storage mite, *Glycyphagus destructor* (Schrank) using methyl bromide, phosphine, methyl chloroform, ethyl formate, ethylene oxide, methallyl chloride, ethyl bromide, carbon tetrachloride, ethylene dibromide, ethylene dichloride, methyl formate and acrylonitrile. At 10°C, complete control of exposed insects was possible only if the exposure time was seven weeks, and the exposure time requirement at 15°C was reduced to less than half (three weeks). The exposure time required for a complete kill of exposed insect pests was further reduced to two weeks at 20°C.

- Barker (1969a) emphasised that concentration of phosphine required to kill 50 per cent of the adult *Cryptolestes ferruginueus,* (Steph.) and *Cryptolestes turticus,* (Grour.) (1 to 5 days old) at 24°C are less (0.059 and 0.47 mg/L of phosphine respectively) than at 19°C (0.108 and 0.78 mg/L of phosphine respectively) with an exposure of 5 days. Also, the author found that the eggs of *C. ferruginueus* (0 to 24 h old) require 4 mg/L of phosphine and 24 h of exposure at 19°C for 50 per cent kill. On the other hand, at 24°C, a concentration of 2.5 mg/L of phosphine and 24 h of exposure were sufficient to kill 50 per cent of the exposed eggs.

- Ho and Winks (1995) studied the effects of 2 different temperatures on eggs and adults of the booklouse, *Liposcelis bostrichophila* (Badonnel) and psocids, *Liposcelis entomophila* Enderlein with a phosphine concentration of 1.5 $g.m^{-3}$. They concluded that, at 25°C or more an exposure period of 7 days were sufficient to kill all the eggs and adults exposed. However, at 15°C, the exposure period requirement increased to 10 days for complete mortality.

- Bell and Wilson (1995) investigated the toxicity of phosphine to resistant khapra beetle, *Trogoderam granarium* Everts, larvae under a range of temperatures, concentrations, and exposure times. They concluded that complete kill of *T. granarium* was possible with an exposure time of 5.7 days (4 to 7 days) and 0.13±0.01 mg/L of phosphine if the temperature was 25°C. However, at 20°C a higher concentration of phosphine (0.54±0.05 mg/L) and longer exposure time (7.1 days, range of 6 to 10 days) were essential for a complete kill of *T. granarium*.

- Roth and Kennedy (1972) noticed that as the temperature increased from 5.6-6.7°C to 32.2-35°C, the exposure time reduced from 24 h to 6 h with a slightly higher concentration of phosphine (0.812 mg/l at 5.6 or 6.7°C and 0.872 mg/l at 32.5 or 35°C) for a 100 per cent kill of the cotton boll weevil, *Anthonomous grandis,* Boheman.

As might be expected, in general phosphine resistant strains necessitate higher fumigation temperature than that required to kill the susceptible strains. Winks and Ryan (1992), investigated the insecticidal efficacy of phosphine at fixed concentrations (0.47 to 2 $g.m^{-3}$) exposure times (two to four days) and temperatures (15 and 25°C) to phosphine-susceptible strains of *O. surinamensis, T. castaneum, C. ferrugineus* and the lesser grain borer, *Rhyzopertha dominica* (F.) and phosphine resistant-strains of *R. dominica* and *S. oryzae.* They found that phosphine susceptible-strains could be controlled at these concentrations investigated. The exposure time required at 25°C, was two days, while at 15°C the time required was doubled (to kill the phosphine-susceptible strains). However, the resistant strains of *R. dominica* and *S. oryzae* survived when exposed to a phosphine concentration of 2 $g.m^{-3}$ and an exposure of 14 days at 15°C. On the other hand, at 25°C all the exposed insects were killed with a concentration of 0.15 $g.m^{-3}$ and an exposure of 8 days.

Due to their poikilothermal nature, active or developmental stages of insects do not respond instantaneously to rises in temperature. Hence, preconditioning of insects for certain periods at the desired temperatures is necessary. Hawkes (1973) found that eggs of

one to two day old *S. littoralis* were tolerant of phosphine with a concentration-time product of 144 mg hr/L (24 h of exposure and a phosphine concentration of 6 mg/L). However, when the eggs were preconditioned at 20°C for 72 h before fumigation 100 per cent mortality was achieved with a concentration-time product of 36 mg hr/L (1.5 mg/L of phosphine). On the other hand, 48 and 72 h of preconditioning at 15°C did not produce a complete kill at a CTP of 288 mg hr/L (6 mg/L) of phosphine.

In brief, the metabolic processes of different species and their developmental stages vary greatly and they depend on temperature. At higher temperatures, when the development and metabolic processes are rapid, the toxic action is also rapid. At lower temperatures, the metabolic rate is slow and the toxic action of fumigant also declines to effectively zero. Conversely, fumigants are more efficient at higher temperatures.

2.9 *Toxicity of phosphine to immature stages of insect pests*

Relatively inactive developmental stages, such as eggs and pupae, have comparatively lower metabolic rates than the active stages such as larvae or adults (Ho and Winks 1995). Also, among these stages, both young eggs and young pupae are more tolerant to fumigation than their mature counterparts. In addition, these stages are protected by the egg chorion or pupal case, which provide impediments to the fumigant reaching the target sites and acting upon them. Although fumigants, such as phosphine, can easily penetrate these barriers, it is clear that the concentration and exposure time that give complete mortalities of active stages will not necessarily give the same results for the inactive stages. These stages are highly tolerant of the concentrations and exposure times that would kill all the active stages and this necessitates certain modifications to the exposure time or concentration or temperature.

The tolerance of developmental stages to phosphine can be regarded as a common phenomenon and it is beyond doubt linked with lower metabolic rates during development. Moreover, distribution of air to all parts of the larval or adult insect body is achieved by the tracheal system. However, eggs and pupae rely entirely on cutaneous diffusion for their gas exchange (Edwards 1953). Penetration of gas into eggs is through the egg-shell, and the rate depends on the variation between the inner and outer tensions of the diffusive gases, the permeability of the protective chorion and number and size of pores (Edwards 1953). Diffusion within the cell depends on the diffusion distance, the tension differential between gas space and embryonic tissues and embryonic metabolic gradient. The final diffusion rate depends upon the variation of embryonic surface and on the size, heterogeneity and activity of the tissues (Edwards 1953).

The findings (toxicity of phosphine to eggs and pupae) of various authors are given below:

- Barker (1969b) observed that 0 to 24-h-old eggs of *C. turticus and C. ferrugineus* were highly resistant to phosphine when exposed for 5 days at a temperature of 24°C. He emphasised that 0.03 to 0.076 mg/L of phosphine with an exposure time of 5 days at 24°C is sufficient to kill 50 per cent of the adults (22 to 26 days old) of *C. ferrugineus* and *C. turticus*, however, eggs (0 to 24 h) of these species necessitate 2.5 mg/L of phosphine under similar conditions.

- Vincent and Lindgren (1972) reported that a small number of 0 to 1 day old eggs of Dermestide namely, *Trogoderma glabrum* (Herbst), *T. sternale* and *T. variabile* survived an exposure of 24 h to a concentration of 6 mg/L of phosphine fumigated at a temperature of 21.2°C, however, all mature eggs and other stages exposed at the above mentioned conditions were killed. Also, Lindgren and Vincent (1966), found that one day old eggs of *T. confusum* were more tolerant than two to six day old eggs (Table 2.5).

Table 2.5 Concentration Time Product requirement of phosphine for a 99 per cent kill of *T. confusum* eggs of different ages with an exposure period of 16 h at 26.7°C (Lindgren and Vincent 1966).

Age (days)	Concentration Time Product (mg h/L)
1	17.9
2	7.2
3	2.56
4	3.04
5	1.76
6	0.96

- Vincent and Lindgren (1977) observed that 0 to 24 h old eggs of *L. serricorne* were more tolerant of phosphine than other stages regardless of temperature or exposure time.

- Bell and Wilson (1995), found that 0 to 24 h old eggs of *T. granarium* are more highly tolerant of phosphine fumigation than four to five days old eggs. They found that a phosphine concentration of 0.28±0.01 g/m^3 at 25°C and 70 per cent relative humidity at 20 h of exposure time killed 95 per cent of the exposed four to five days old eggs, where this fumigation gave only 13 per cent kill of 0 to 24 h old eggs. However, increasing the exposure time to 72 h killed 90 per cent of these younger eggs.

- Bond (1980) identified that one of the reasons for tolerance of eggs and pupae to phosphine compared with larvae or adults is that the uptake of toxic gases by the developmental stages is significantly lower. In his trials using phosphine concentrations of 0.14 to 1.69 mg/L and an exposure of five hours to various stages of *T. castaneum,* he found that at 0.55 mg/L of phosphine, the uptake and mortality of larvae were 115 μ grams/g of insects and 93 per cent respectively. However, the young pupae absorbed 22 μ grams/g of insect and the mortality was 18 per cent. Similarly, Nakakita and Winks (1981) found that the pupae of *T. castaneum* required substantially

higher CTP than larvae of the same species. Furthermore, of these stages, younger ones of a particular stage were comparatively more tolerant than their mature counterparts as can be gleaned from Table 2.6.

Table 2.6 Dosages (mg h/L) of phosphine required to kill 99.9 per cent of different stages of *T. castaneum* at 25°C, 60 per cent relative humidity with a 6 h exposure period (adapted from Nakakita and Winks 1981).

Stage	$LD_{99.9}$ (mg h/L)
15 day larvae	0.52
20 day larvae	0.29
early pupae	11.94
late pupae	1.12

The level of tolerance to phosphine by eggs and pupae varies widely from species to species. This was confirmed by the work of Hole *et al.* (1976) (Table 2.7). They found that the pupae of *S. oryzae, S. zeamais, S. granarius* and *C. ferrugineus* were more tolerant of phosphine than the eggs (0.91 to 1.65 mg/L of phosphine with an exposure of 2 days at 25°C for 100 per cent mortality) (Table 2.7 and 2.8). However, eggs of *R. dominica, L. serricorne, T. granarium* and *T. castaneum* were particularly tolerant of phosphine (requiring 0.18 to 0.32 mg/L of phosphine and an exposure of 2 days at 25°C for 100 per cent mortality) than the pupae. Moreover, they found that found that at 25^0C an exposure period of 7 to 8 days and a phosphine concentration of 3 mg/L were essential to kill all the pupae of *S. granarius* and *S. oryzae*. However, at 30°C, the exposure tine required for a complete kill was reduced to half (four days) and only 0.7 mg/L of phosphine was needed.

Table 2.7 Minimum mean concentration required to achieve selected levels of mortality in stored product beetle cultures with different lengths of exposure to phosphine at 10°C (adapted from Hole *et al.* 1976).

Exposure (days)	**Range of concentrations tested (mg/L)**	**Species**	**Most tolerant stage**	**Concentrations (mg/L) for levels of mortality (%)**			
				90	99	99.9	100
2	0.07 - 0.32	*Ptinus tectus*	egg and pupa	*	-	-	-
		Trogoderma granarium	egg	0.24	-	-	-
		Sitophilus granarius	pupa	0.13	-	-	-
4	0.90 - 0.58	*S. granarius*	pupa	0.09	-	-	-
		T. granarium	egg	0.17	-	-	-
		P. tectus	pupa	0.09	-	-	-
8		*T. granarium* Diap. Larvae	larva	*	-	-	-
		S. granarius	pupa	0.09	0.72	-	-
		P. tectus	egg	0.09	0.19	-	0.39
		T. granarium	-	-	-	-	0.09
16	0.10 - 0.76	*S. granarius*	pupa	0.10	0.19	-	-
		T. granarium Diap. Larvae	larva	-	0.10	-	-
		P. tectus	-	-	-	-	0.10

*- Mortality less than 90 per cent at highest CT product tested.

Table 2.8 Minimum mean concentration required to achieve selected levels of mortality in stored product beetle cultures with different lengths of exposure to phosphine at 25°C (adapted from Hole *et al.* 1976).

Exposure (days)	Range of concentrations tested (mg/L)	Species	Most tolerant stage	Concentrations (mg/L) for levels of mortality (%)			
				90	99	99.9	100
2	0.054 - 1.60	*Sitophilus oryzae*	pupa	0.83	-	-	-
	0.054 - 2.96	*Sitophilus zeamais*	pupa	0.4	1.6	-	-
	0.054 - 2.96	*Sitophilus granarius*	pupa	0.26	0.79	-	-
	0.044 - 0.26	*Caryedon serratus*	pupa	0.044	-	-	-
	0.054 - 2.96	*Acanthoscelides obtectus*	pupa	0.054	0.22	0.79	2.96
	0.044 - 2.96	*Lasioderma serricorne*	egg	0.054	0.22	0.79	1.60
	0.054 - 1.60	*Trogoderma granarium with diap. Larvae*	egg	0.083	-	-	0.79
	0.044 - 0.79	*Ptinus tectus*	pupa	-	0.083	0.18	0.4
	0.027 - 0.16	*Cryptolestes pusillus*	egg	0.027	0.073	-	0.16
	0.027 - 0.26	*Tribolium castaneum*	egg	0.027	-	0.73	0.16
	0.015 - 0.16	*Oryzaephilus surinamensis*	egg	-	-	0.015	0.027
4	0.044 - 1.65	*S. granarius*	pupa	0.044	0.36	0.91	1.65
	0.044 - 1.65	*S. oryzae*	pupa	0.044	0.086	0.91	1.65
	0.044 - 1.65	*S. zeamais*	pupa	0.044	0.19	0.36	0.91
	0.044 - 0.32	*C. serratus*	pupa	0.044	0.086	-	-
	0.044 - 1.65	*A. obtectus*	egg	0.044	0.19	-	0.32
	0.044 - 1.65	. *granarium* with diap. Larv	egg	-	0.044	-	0.32
	0.044 - 1.65	*L. serricorne*	egg	0.044	0.052	0.085	0.32
	0.026 - 1.65	*R.. dominica*	egg	0.026	0.085	0.097	0.18
	0.044 - 0.086	*Cryptolestes ferrugineus*	pupa	0.044	0.086	-	-
	0.026 - 0.091	*P. tectus*	pupa	0.026	-	0.044	0.086
	0.026 - 0.18	*T. castaneum*	egg	0.026	-	0.046	0.085
	0.015 - 0.18	*C. pusillus*	egg	0.026	0.046	-	0.085
	0.026 - 0.18	*O. surinamensis*	-	-	-	-	0.026
7	0.022 - 0.74	*S. oryzae*	pupa	0.022	0.042	0.16	0.32
	0.022 - 0.74	*S. granarius*	pupa	0.022	0.041	0.081	0.32
	0.042 - 0.74	*L. serricorne*	egg	-	0.041	0.081	0.32
	0.042 - 0.18	*C. serratus**	pupa	-	0.042	0.084	0.18
	0.013 - 0.74	*A. obtectus*	egg	-	0.013	-	0.081

Exposure (days)	Range of concentrations tested (mg/L)	Species	Most tolerant stage	Concentrations (mg/L) for levels of mortality (%)			
	0.013 - 0.74	. *granarius* with diap. Larv	egg	0.013	-	0.022	0.045
	0.013 - 0.74	*S. zeamais*	pupa	0.013	-	-	0.042
	0.042 only	*C. ferrugineus*	-	-	-	-	0.041
	0.013 - 0.16	*T. castaneum*	egg	-	0.013	-	0.041
	0.013 - 0.74	*P. tectus*	egg	-	-	0.013	0.041
	0.013 - 0.74	*R. dominica*	egg	-	-	0.013	0.022
	0.013 - 0.16	*C. pusillus*	-	-	-	-	0.013
	0.013 - 0.042	*O. surinamensis*	-	-	-	-	0.013
10	0.02 - 0.26	*S. oryzae*	pupa	-	-	0.020	0.13
	0.2 - 0.26	*S. granarius*	egg	-	0.02	-	0.038
	0.038 only	*C. serratus*	-	-	-	-	0.038
	0.012 - 0.26	*L. serricorne*	egg	-	0.012	-	0.020
	0.012 - 0.26	*S. zeamais*	pupa	-	0.012	-	0.20
	0.012 - 0.26	*A. obtectus*	-	-	-	-	0.12
	0.012 - 0.02	*T. castaneum*	pupa	-	-	-	0.12
	0.012 - 0.26	*P. tectus*	-	-	-	-	0.12
	0.012 - 0.26	*R. dominica*	-	-	-	-	0.12

*- mortality less than 90 per cent at highest CT product tested.

Al-Hakkah *et al.* (1985) found that two day old pupae of *E. cautella* were more resistant to phosphine than 3 to 4 days old or 6 to 7 days old that were exposed to a phosphine concentration of 0.014 mg/L for 24 h of exposure at room temperature. Moreover, they found that the percentage emerging from these sub-lethal doses of phosphine-fumigated pupae were 86 (2 days old), 76 (3 to 4 days old) and 13 per cent (6 to 7 days old pupae). However, most of these emerged adults were malformed. The rate of malformation was higher for 6 to 7 day old pupae (58 per cent) than 2-day-old pupae (28 per cent).

Although information is scarce on the response of male or female individuals of a particular species to phosphine fumigation, there is a possibility that the male or female of a particular species would be more or less tolerant. Winks and Waterford (1986) observed in their fumigation trials using phosphine on phosphine resistant strains of *T. castaneum* adults that females are more susceptible than male adults. A concentration-time product of

2.19 mg hr/L (0.01 mg/L) of phosphine is necessary for 99 per cent mortality of males at 25°C and 60 per cent relative humidity. However, only 1.44 mg hr/L of phosphine is sufficient to control 99 per cent of the female population under similar temperature and exposure time as experimented with male adults. Al-Hakkah (1989) suggests that the target site of phosphine is an enzyme, hence, the stages or sex or insect species that have higher enzyme activity are more susceptible to phosphine fumigation. Moreover, he found that acetylcholinestrase enzyme activity was higher among the males of all developmental stages of *E. cautella*. A 24 h old female adult's acetylcholinestrase activity was recorded as 0.407+0.90 µmol/mL/min. Whereas, in males the activity was 0.530+0.140 µmol/mL/min. This suggests that enzyme activity varies among sexes and different insect species; hence, this may influence the toxicity of fumigants.

2.10 Insecticidal efficacy of pyrethrum

Although phosphine may be used as a stand-alone fumigant, it is often used with synergising agents such as pyrethrum. Pyrethrum, with or without piperonyl butoxide as a synergising agent, has been used widely for many years to control insect pests that attack stored products. Lloyd and Hewlett, (1958) studied its efficacy against bruchid pests of stored products, namely the bean weevil, *Acanthoscelides obtectus* (Say). Potter (1935) studied its effect against *P. interpunctella* and Weaving (1970) examined its effectiveness against bruchid beetles of stored pulses namely *A. obtectus,* Mexican bean weevil, *Zabrotes subfasciatus,* Boheman and *C. chinensis*. Pyrethrums are extracted from the flowers of chrysanthemum, *Pyrethrum cinerariaefolium* Vis and these flowers contain six insecticidal esters namely; cinerin I (cin. I), jasmolin I (jas. I), pyrethrin I (pyr. I), cinerin II (cin. II), jasmolin II (jas. II) and pyrethrin II (pyr. II) (Tsuda *et al.*, 1972). Each of these esters has different insecticidal properties (Bunk, 1996).

Pyrethrums are highly toxic to a range of insect pests and fish, however, they have a low or zero toxicity to mammals and they are rapidly degraded in the stomach to harmless metabolites by hydrolysis of the ester (Carter *et al.* 1975, Chandler 1952 and Miskus and Anderws 1972). In the environment their degradation is promoted by sunlight (Nash 1954) and air (Chen and Cassida 1969). Chen and Cassida (1969) and Miskus and Andrew (1972) have reported that pyrethrum is stable when exposed to atmosphere in darkness, and there have been no reports of loss through evaporation. Because of these properties and higher costs, the use of pyrethrum in agricultural pest control has been somewhat limited (Miskus and Andrews 1972).

Pyrethrums are practically insoluble in water, but they are readily soluble in organic solvents such as alcohols, chlorinated hydrocarbons, nitromethane and kerosene. Their mode of action is that of a non-systemic insecticide with contact action causing a rapid paralysis or knockdown, with death occurring at a later stage (Worthing and Walker 1987b). In their extensive histological examination on the nervous system of insects poisoned with pyrethrum with and without various synergists, Hartzell and Scudder (1942) found that pyrethrum acts on the peripheral nervous system of an insect. Due to its rapid degradation, pyrethrum is generally synergised with piperonyl butoxide (the technical product containing 80 per cent of pure compound (3,4-deoxymethylene-6-propyl benzyl) (butyl) diethylene glycol ether), (Mallis 1982). Piperonyl butoxide, or PBO, inhibits the detoxification of pyrethrum by the insects by blocking the enzyme that detoxifies the pyrethrum and stabilise it in thin films. (Dove 1947).

Generally, the response of various insect species to different fumigant chemicals varies considerably (Carter *et al.* 1975). There is evidence that the toxicity of pyrethrum is also insect species specific. Carter *et al.* (1975) found that pyrethrum synergised with piperonyl butoxide is far more effective against *L. serricorne* than the closely related drugstore beetle, *Stegobium paniceum,* (Linnaeus). Moreover, Amos *et al.* (1978) and Amos and Evans (1979) found that pyrethrins synergised with piperonyl butoxide reduced the population and prevented the breeding of *O. surinamensis*, *E. figulilella* and *P.*

interpunctella adults that commonly infest sultanas. They concluded that pyrethrin treatments were highly effective in protecting the sultanas from these insect infestations and maintaining the degree of contamination to low levels. Similarly, Lloyd and Hewlett (1958) found that two common insect pests of stored products, namely *A. obtectus* and *C. chinensis*, were highly susceptible to pyrethrin or pyrethrin-piperonyl butoxide mixtures in oil.

Lloyd (1973) showed the synergistic effects of synthetic pyrethroids with fumigants such as phosphine, metham sodium and dichlorvos. A number of storage insect pests, namely *S. granarius, T. castaneum, O. surinamensis, L. serricorne* and *S. paniceum* was used as bioassays. In his studies he found that the synthetic pyrethroids reduce the resistivity of insect pests by their knock down effects, and reduce the exposure time thereby reducing the cost and chemicals. Apparently, the synergist effect is greatly influenced by the test insect, formulation, pyrethrum-synergistic ratio, method of administration and choice of either kill or knock down as the measured response. On the other hand, Pricket and Ratcliff (1977), found that insecticide resistant and susceptible strains of *T. castaneum* avoided pyrethrum treated surfaces due to the repellency action of pyrethrum. However, pyrethrum/pyrethroid insecticides combined with a fumigant accelerate the mortality rate of insect pests (Wood and Wood 1991). They reported that *Protea* flowers fumigated with dichlorvos for 16 h resulted in 91.6% mortality of insect pests. When protea flowers were treated in an enclosed chamber for 2 h with a combination of pyrethrin and dichlorvos, both propelled by carbon dioxide, the combination was more efficacious than either of the insecticides alone.

2.11 *Phytotoxicity*

Of all horticultural produce, flowers and foliage are the most perishable due to their high respiration rates. Hence, they are prone to rapid deterioration and are highly susceptible to damage. This necessitates taking the utmost care during postharvest handling. Generally, respiration of the flowers and foliage increases exponentially with increasing temperature.

The higher respiration rate usually increases the senescence and growth. Hence, the vase life of flowers can be maintained by appropriate temperature maintenance.

However, use of fumigants to control insect pests of cut and wildflowers without causing damage to them requires a delicate balance, because most of the fumigants are effective at higher temperatures, similarly insects are active at higher temperature. However, as mentioned earlier, higher temperatures are inimical to life of the flowers. Moreover, fumigant concentration also accelerates phytotoxicity. As mentioned earlier, the vase life of flowers is important as it determines the price and marketability. Slight damage renders the flowers unmarketable and it greatly reduces their prices to such a level that they are unprofitable.

Use of various fumigants (carbon disulfide, ethylene dibromide, phosphine, ethylene dichloride, dichlorvos, hydrogen cyanide, ethylene oxide, carbon tetrachloride and chloropicrin) for postharvest control has been well established for a range of commodities except flowers and foliage. However, the use of phosphine and methyl bromide for the control of major insect pests with a primary focus on phytotoxicity has been reported by a small number of researchers. These studies are typified by the work of Weller *et al.* (1995) using methyl bromide and phosphine on rice flowers, *Ozothamnus disomifolius*, flannel flowers, *Actinotus helianthis*, Kangaroo paw, *Anigozanthus sp*, King protea, *Protea cynaroides* and Banksia, *B. coccinea*. Karuanratne *et al.* (1997) studied the effects of phosphine on King protea, Tulip, Kangaroo paw and Geraldton wax flowers.

Research findings indicate that methyl bromide is non toxic to flowers at lower fumigation temperatures. Powell (1979) reported that combining methyl bromide fumigation (at a concentration of 102 mg.h/L at 5°C or 75 mg.h/L at 10°C) with cold storage (at 0 to 2°C for 1 to 4 days) was effective against the eggs of *S. littorialis* on chrysanthemum. This CTP did not cause any damage to the chrysanthemum cuttings. Moreover, Heyler and Ledieu (1989), found that methyl bromide at concentrations of 10.5 to 13.5 g/m^3 for 4 to 5 h of exposure at 14°C killed all the larvae of leaf miner, *Liriomyza trifolii* Burgess without

considerably affecting the vase life of the chrysanthemum cuttings. However, a similar exposure time and concentration at 16°C damaged the cuttings considerably. As mentioned earlier, to be effective phosphine fumigation requires temperatures higher than this. Hence, there are possibilities either exposed flowers or foliage or both may be affected by the phosphine fumigations due to higher temperatures.

Phytotoxic effects of phosphine to various fruits have been investigated by several authors. Hatton and Cubbedge (1986) found that phosphine-treated grapefruit showed accelerated rind injury, this proceeds to scald and rind breakdown that includes aging and pitting. However, Windeguth *et al.* (1976) point out that grapefruit and avocado fumigated with phosphine at a concentration of 600 ppm and exposures of 6, 12 or 24 h did not produce any symptoms of phytotoxicity.

This literature survey has established that phosphine may be a useful alternative to methyl bromide. It is clear that there may be a conflict between phytotoxicity and insecticidal efficacy, and that the window of opportunity may be narrowed by the need for phosphine to be applied at a temperature about 18°C or more. As a result of carrying out the literature survey, the effectiveness of phosphine against immature insects must also be carefully studied. Finally, it is clear that phosphine fumigations will take longer than those carried out using methyl bromide, and this will impact on the logistics of the cut flower export industry.

Chapter - 3

Lifecycle and biology of the leaf rolling moth, *Strepsicrates ejectana* Walker (Tortricidae:Lepidoptera)

Chapter 3

Life cycle and biology of the leaf rolling moth, *Strepsicrates ejectana* Walker (Tortricidae : Lepidoptera)

3.1 Scope of the work on life cycle and biology

Australian wildflowers of the genus *Thryptomene* are cultivated for local and export markets, notably for Japan and the USA. There are large plantations of Grampians thryptomene, *Thryptomene calycina* (Lindl.), in the Wimmera District of Victoria close to the Grampians. During the latter part of 1994, while on a field visit to the Austbloom Pty. Ltd. plantation at Laharum (in the Grampians), patches of *T. calycina* bushes with symptoms of yellowing and drying of foliage were observed. Closer examination revealed that some of the bushes were heavily infested with different stages of larvae. Each larva had constructed a webbing shelter using 3 to 90 leaves (depending on the larval instar stage); hence, the ability of the plant to photosynthesise was reduced considerably. This can lead to stunted growth or the death of the plant due to defoliation.

Bunches of *T. calycina* were dipped in a solution of the insecticide formulation Ambush® (permethrin) prior to export, but some of the larvae within the shelters survived the treatment. Also the shelters detracted from the quality of the bunch and sometimes had to be removed by hand to make bunches saleable.

The larvae feed on the epidermis of the leaves, and the affected leaves become yellow and they ultimately turn brown. Once the leaves become yellowish, the larvae move within the webbing shelter and feed on new leaves, or if the webbing shelter is completely consumed they construct new webbing shelters. A single larva can

Figure 3.1 An uninfested *T. calycina* plant at Austbloom, Laharam, Vicoria.

Figure 3.2 An unifested *T. calycina* plant with flowers.

completely defoliate a branch during its life cycle. Hence, damage caused by the larvae is economically significant. Thus the larvae present both a postharvest and a field infestation problem. The presence of larvae, which have survived treatments, has caused rejections of *T. calycina* consignments in Japan. Some larvae were reared to adults, which were identified by the Division of Entomology, Commonwealth Scientific and Industrial Research Organisation (CSIRO), Canberra as being those of the leaf rolling moth, *Strepsicrates ejectana*, Walker (Tortricidae:Lepidoptera) a native insect of Australia. An extensive literature search was carried out to determine the existing state of knowledge of the life-cycle and biology of this insect. This would help in field control of the insect, and subsequently in postharvest disinfestation. Nielson *et al.* (1996) have reported that early publications of Walker indicate that this species has been known by five names, viz *S. ejectana, S. servilisana, S. infimana, S. saxana* and *S. ligniferana.* Of these "*ejectana*" is given priority. Meyrick (1881) gave some superficial information on the morphology of the adults and larvae of this species, but no details of the morphology of other stages have been reported. The literature is completely devoid of any information on the life-cycle and biology of *S. ejectana*. Since this information may be crucial to developing an integrated pest management strategy that includes postharvest fumigation for this insect pest, field and laboratory studies on this species were initiated as part of this doctoral research.

During 1994 and 1995 the number of *Thryptomene* bushes in the Austbloom plantation attacked by *S. ejectana* increased. Some were largely defoliated and died. Heavy infestations were observed in 1996. Similar problems were reported and it was observed that other plantations in the district were also infested by this insect pest. However, wild *T. calycina* in the Grampians National Park showed no signs of attack by *S. ejectana*. This may have been because wild *T. calycina* grow into large bushes and are surrounded by other shrubs and trees which provide protection for small birds which could feed on the larvae. In the commercial plantations there was no such protection from predatory birds.

Some exporters have tried using postharvest treatments with a combination of the aerosols Pestigas® and Insectigas® which is effective against some insect pests of cut

flowers but fails to kill all *S. ejectana* larvae in their shelters. The only registered postharvest treatment that has been reported to be successful is fumigation with methyl bromide.

As pointed out above, before the commencement of the research reported in this thesis, the scientific literature was completely devoid of information on the life-cycle biology of *S. ejectana*, and morphological descriptions of the species were extremely sketchy. Series of both laboratory experiments and field observations were therefore established to determine the following biological features of the species:

- the number of larval instar stages;
- data on the life cycle, i.e. the number of days required for each stage to develop at two temperatures, hence, to determine the total time required to complete the life cycle from eggs to adults;
- establish the number of generations per year; and
- the biology as it relates to feeding and sheltering.

The successful completion of this research has necessarily entailed developing suitable laboratory procedures for rearing and studying populations of *S. ejectana*, such as the selection of an artificial diet for rearing the larvae.

3.2 *Materials and Methods*

The above objectives were met by studying *S. ejectana* reared on an artificial feeding medium under controlled laboratory conditions. Glass-house studies were also carried out by rearing the species on potted *T. calycina* plants at the Institute for Horticultural Development, Knoxfield, Victoria. These laboratory and glass-house experiments

complemented field studies carried out at the Austbloom plantations in the Grampians. The field studies enabled the effects of external factors, especially temperature and rainfall, on the growth and development of *S. ejectana*. Mass rearing of the species in the laboratory also served the pragmatic purpose of supplying the various developmental stages of this insect for fumigation studies that were carried out on a commercial scale, also as part of this doctoral research.

3.2.1 Field studies

3.2.1.1 Environment

Field studies on the behaviour and life cycle of this insect were conducted from October 1994 to June 1997 on insects collected from Austbloom, at Laharum in the South Wimmera, Victoria. Horsham is located at a latitude of 36° 43' south and a longitude of 142° 12' east. The annual rainfall recorded in 1995 was 510 mm and the highest rainfall was recorded during June. A relative humidity range of 45 to 85 per cent was recorded during the same period. The highest relative humidity was recorded during June when it was 85 per cent and the lowest, 45 per cent, was recorded during January.

3.2.1.2 Temperature measurement

The day and night temperatures of the sampling site were obtained by installing 4 "T" type thermocouples among the canopies of *Thryptomene* plants. The thermocouples were connected to a Data Taker® 505 and measurements were taken on an hourly basis from September 1994 to April 1997. The program and other procedures of using Data Taker®505 were the same as described in section 3.2.2.2. Approximately every month

the data were down loaded onto a computer and the batteries of the Data Taker®505 were recharged for 14 to 16 h.

3.2.1.3 *Collection of samples for laboratory studies*

Leaf and webbing shelters of *S. ejectana* larvae and all other stages were collected randomly every month from September 1994 to September 1997, at the Austbloom plantation. This property is 250 h, mainly growing *T. calycina* with smaller areas allocated to other Australian native species including geraldton wax and *Eucalyptus spp.*

Sections of *T. calycina* stems (200 to 270 mm long) were dislocated from the top of the bushes using a pair of secateurs. The selected samples were transferred into a number of 2 to 3 L polyethylene containers (20 to 40 stems per bucket) and these buckets were covered with a *terylene* gauze cloth that was secured with a rubber band. The stems on which *S. ejectana* were living were transported to the Institute for Horticultural Development, Knoxfield, Victoria, for observation and breeding of the insects, during which a temperature of 19±1°C was maintained. Samples were assessed within 48 h of removal from the plants.

3.2.1.4 *Pupating behaviour*

The number of pupae gathered from the field-collected samples was very small compared with the number of larvae collected. It was hypothesised that pupation may take place in the soil or on the outside of the webbing shelters where there is a possibility that pupae may have been dispersed somewhat by either rain or wind. Therefore the top soil near to the heavily infested bushes was collected on the same occasions and sieved using a fine mesh sieve to see if any pupae could be collected.

3.2.1.5 *Collection of adults*

The presence of adult moths amongst the foliage and in the surrounding environment was observed each month during the morning, around midday and in the evening at this property. Initially the area was observed for any activity (flying, feeding or resting) and if any adults were sighted they were captured using a nylon mesh net fitted with a long handle (length of 1000 mm). Adult moths in the foliage were examined by slightly disturbing the plants, and the flying adults were captured by throwing the net over them. The captured adults were immediately transferred individually into 50 mL lidded plastic tubes without damaging their wings or appendages. If the adults were collected for rearing, they were (2 to 3 individuals) transferred immediately into 200 mL lidded polyethylene cups to which 1 to 2 mL of 10 per cent honey solution had been added. The lids of the cups were perforated using a fine needle (5 to 7 tiny holes) to allow air to diffuse into the cups.

Once transported to the laboratory, the sex of the moths was determined based on morphological parameters (morphological parameters are discussed in detail in Section 3.3.2.4) and they were released into cages that contained potted *T. calycina* plants. These plants were kept in a glass-house with a temperature of 19±1°C and a relative humidity of 50 to 55 per cent. A photoperiod of 12 h light and 12 h dark was maintained using five 15W halogen lamps that were located 3000 to 3500 mm from the canopy at different positions in the glass-house.

3.2.1.6 *Assessment of field collected samples*

The webbing shelters were removed from the stems by hand or by using a pair of scissors, and the shelters were opened using sterilised forceps and/or needles without damaging the larvae. Once a webbing shelter was opened, the larva was separated from its shelter, and the instar stage was determined based on the colour of the head capsule and body length using a suitable measuring scale. The larvae were then transferred into

a number of plastic Petrie dishes (9 to 12 mm diameter) that were fitted with perforated lids and the stages of the larvae were confirmed visually under a microscope. However, the larvae (especially the fourth to sixth instar) were difficult to handle as they were very mobile and they exhibited twitching movements whilst they were being touched with a brush, hence, these larvae were handled using a pair of forceps gently without damaging them. Once a larva had been removed from its webbing shelter, any head capsules inside the shelter were searched for.

Once the larval stages had been established they were immediately transferred either into the modified lightbrown apple moth medium or on to potted *T. calycina* plants for rearing, or they were used immediately in the large-scale fumigation experiments described in Chapter 4.

The number of eggs present in the collected samples was established by examining the upper and lower surfaces of the first 30 to 40 leaves from the top of the stems, both using the naked eye and under a microscope. Occasionally all other leaves were observed for the presence of any eggs. The ages of the eggs were determined based on the external appearance as young (one to three days old), medium age eggs (four to seven days old) and mature eggs (eight days old and above). The parameters used for identifying the age of eggs are described in detail in Section 3.3.2.4. These were incubated at a controlled temperature of 19°C and 40 to 45 per cent relative humidity, in 30 mL lidded polyethylene cups to which water soaked absorbent cotton wool had been added.

3.2.1.7 Alternative hosts

Possible alternative hosts for these larvae or adults were investigated by observing nearby plants in the field. Apparently the *T. calycina* is a plant that is rich in essential oil; hence, similar plants such as *Eucalyptus* were observed on a monthly schedule for the presence of this insect.

3.2.1.8 Parasites and predators of S. ejectana

The field-collected samples were observed under a microscope and by the naked eye for the presence of any moulds on the webbing shelters or leaves and the effect of moulds on the growth and development of larvae and pupae. The samples infested with mould were maintained individually in 30 mL plastic containers at a controlled temperature of 19°C and 70 to 75 per cent relative humidity.

3.2.1.9 Other insect pests obtained from T. calycina

All the samples collected from Horsham were observed thoroughly by naked eye for the presence of any other insect species. If any insects were found they were separated, identified and recorded.

3.2.2 Laboratory studies

3.2.2.1 Controlled conditions

Three different 20 m^3 controlled temperature rooms held at 19°C (70 to 75 per cent relative humidity), 22°C (60 to 65 per cent relative humidity) and 25°C (40 to 45 per cent relative humidity) were used in the study of the life cycle and biology of *S. ejectana*. The walls of these controlled temperature rooms were permanently fitted with 12 metal racks, each of which was 330 mm wide and separated in height by 400 mm. This gave sufficient space to facilitate the mass rearing of the various developmental stages of the insect. These rooms were artificially lit using six 15W fluorescent lamps to give a photoperiod of 12 h light and 12 h dark.

The 19°C and 22°C rooms were fitted with a refrigeration system with defrost coil heaters to maintain the temperature at the desired level. Thermostats were fitted on the rear walls below the heating system and sensors were fitted on the front wall of the room. The 25°C room was fitted with an air conditioner (Sanyo Microcomputer®) and an electric fan heater (Goldair®). The sensors of the thermostatic controller (that can be adjusted from 0 to 30°C) were placed as in the other rooms. The spatial variation of temperature in these rooms was typically 0.30°C (range of 0.25 to 0.50°C).

A further room, held at 4°C, was of 22 m^3 capacity was fitted with an air conditioner to maintain the temperature constantly at this level. The sensors and thermostat (that can be adjusted from 0 to 10°C) were placed on the rear and front walls of the rooms respectively. A similar photoperiod as mentioned above was maintained. A 30 m^3 glass-house with a constant temperature of 19±1°C and 50 to 55 per cent relative humidity was used for rearing eggs, first and second instar larvae and adults. This glass-house was fitted with heating and cooling systems. The photoperiod of this glass-house was extended to 12 h light and 12 h dark (late evenings and early mornings) during winter and early spring by fitting five 15W halogentated lamps. These lights were placed 3000 to 3500 mm from the canopy of the plants.

A colony of *S. ejectana* was established at the Institute for Horticultural Development, Knoxfield, initially by infesting the potted *T. calycina* plants or a modified lightbrown apple moth medium with fifth and sixth instar larvae that had been collected from the field at Horsham. The strategy of using the readily available late instar larvae, adopted during the initial stages of the research, was aimed at providing information on the likelihood of development of all stages of larvae under these conditions.

To reduce over-populating the plants that may have led to their complete defoliation and death, only a limited number of larvae (10 to 15 larvae per plant) were placed on each plant. The selected larvae were placed along with their webbing shelters among the branches of the host plant, rather than amongst the foliage. This procedure was adopted because it had been observed that placing the webbing shelters amongst the foliage induced the adjacent leaves to yellow, turn brown and ultimately to be shed.

Hence, the larvae could use the leaves in the shelters until they became dry, they then moved to fresh leaves of the plant. These larvae were allowed to develop into adults, and once the adults began to lay eggs, the life cycle studies were initiated.

3.2.2.2 Temperature measurement

The temperature of the glass-house was measured by installing three "T" type thermocouples in the canopy of the *Thryptomene* plant inside the metal cage. These thermocouples were connected to a Data Taker 505® supplied by Data Tronics and was connected to a portable computer while programming and unloading the data. The data logger operated under the software called De Terminal® supplied by Data Tronics, Melbourne, Australia. The temperatures were measured at hourly intervals for three months while the adults, eggs and first instar larvae were being observed.

3.2.2.3 Life cycle studies

The time required for *S. ejectana* to complete its life cycle from egg to adult was studied in the laboratory at two temperatures. Signs of courtship and oviposition were scanned for in the glass-house on a daily schedule in the mornings (8:00 to 9:00) and evenings (16:00 to 17:00) except Saturdays and Sundays, with minimum or nil disturbance to the adults. If any eggs were sighted on the leaves or stems (on which they were laid) they were colour-coded using permanent fine-tip markers without damaging the leaves or eggs. A particular colour represented a particular day the egg was laid or observed.

Eggs - Initially, the eggs were classified as young eggs (0 to 24 h), medium age (two to seven days) and mature eggs (24 to 48 h before hatching or eight days and more) based on age and external appearance (discussed in detail in Section 3.3.2.4). The survival

rates and times taken for egg to hatch duration of eggs whilst on plants and once they had been removed from plants were investigated on potted *T. calycina* plants and on lightbrown apple moth medium. The plants were maintained in a glass-house with a controlled temperature of 19±1°C and 50 to 55 per cent relative humidity. The studies on the diet medium were conducted at a controlled temperature of 19°C and 70 to 75 per cent relative humidity.

Survival of eggs on the medium – This experiment was carried out to investigate the ability of eggs to hatch once it was removed from the plant, leaf intact. Also, the observations would give an indication on the survival rate of first instar larvae on the artificial medium or on cotton wool pads.

A series of ten eggs of different ages (zero to one day, two to seven days and eight days and above) was collected by cutting the leaves complete with their peduncles using a sharp edged razor blade, without damaging the eggs or leaves. All these stages of eggs were immediately transferred individually into 30 mL lidded polyethylene containers to which had been added either a 15 to 18 g piece of modified lightbrown apple moth medium or an absorbent cotton wool pad (3 to 4 mm thickness) that had been soaked in water. The dimensions of the medium were: length-25 to 27 mm, width-10 to 12 mm and thickness-4 to 6 mm.

The eggs were placed facing upwards on top of this medium or on the cotton wool as this would maintain the eggs as laid in a natural way. The lids were perforated (two to three holes per lid) using a fine needle to facilitate air movement and reduce condensation. Adding more than three holes would possibly cause the medium and the leaves to dry rapidly, and this is not conducive to the development of eggs. These were maintained in two different temperatures and relative humidities. They were:

- 19°C and 70 to 75 per cent relative humidity; and

- 22°C and 60 to 65 per cent relative humidity.

A photoperiod of 12 h light and 12 h dark were provided during this experiment.
It was observed that once the leaves were dislocated from the plants both the mature and young eggs failed to hatch. This may be because:

- the eggs may be highly sensitive, hence, unable to survive (irrespective of age) once they are removed from plants.

Larvae - As mentioned earlier, due to the paucity of information on this insect, it was decided to find a suitable artificial medium on which to rear all the larval instar stages and/or to determine the instar stages that are most likely to survive on the medium. Modified lightbrown apple moth medium was selected, because both the lightbrown apple moth and the leaf rolling moth are from the same family Tortricidae. Although, these two insects are from the same family, the biology and host species are entirely different, hence these experiments were designed to determine the suitability of using this artificial diet for this larvae. This medium was developed by Shorey and Hale (1965), using haricot beans instead of navy beans, to rear lightbrown apple moth larvae which is a most widespread, polyphagous insect pest. In order to rear the larvae of *S. ejectana* to the pupal stage they were placed on the medium, both in their shelters and when they were exposed (without shelters).

Larvae reared with and without webbing shelters - One to twenty-four-hour old larvae (20 in number) were removed from the leaves of *T. calycina* using a fine camel hair brush. Second to sixth instar larvae construct webbing shelters using more than three leaves, hence, they were removed from their webbing shelters using forceps and/or needles without damaging them. These separated larvae were transferred individually into different 30 mL lidded polyethylene containers. The lids of the containers were perforated with two to three fine holes using a needle.

Initially the medium (Appendix 4) was poured hot into polyethylene food containers (61 x 40 x 25 mm) and allowed to cool and set (*ca.* 93 to 110 g and a thickness of 10 to 14 mm) under a laminar floor chamber. This medium was cut into slices of 10 to 12 mm squares using a sterilised blunt knife. These slices were transferred individually

into 30 mL containers using forceps. Rearing conditions were observed regularly and recorded; if any condensation or drying was observed larvae were transferred into new containers.

Group rearing: The feasibility of rearing various instar stages in large containers (10 to 12 larvae per container) similar to lightbrown apple moths was investigated using first to sixth instar larvae with and without their webbing shelters. Feeding and sheltering patterns of early instars, especially first instars, were different from other stages. Hence, this study would gives an indication on the most suitable instar stages that can be reared on the medium in groups, and this will reduce the handling time and economic use of resources (reduce the number of cups and amount of medium required for rearing).

Various polyethylene food containers of 61 x 40 x 25 mm were added with 93 to 110 g of the lightbrown apple moth medium. Grooves (in a pattern of 9 to 12 mm squares) were made in the diet using a sterilised blunt knife so that it was divided into 180 to 200 pieces to provide refuge for the moth larvae. A gap of 1 to 1.5 mm between grooves was maintained to facilitate the movement of larvae under or among the grooves of the medium. The group rearing was carried out by placing 10 to 12 larvae (various instar stages) with and without their webbing shelters in these rectangular containers. The lids of the containers were covered with a paper tissue, to absorb condensed moisture thereby reducing the likelihood of fungi developing. The tissue was changed at intervals of seven to nine days. The developmental times of the larvae were investigated at two different controlled temperatures and relative humidities. They were:

- 22°C and 60 to 65 per cent relative humidity; and

- 25°C and 40 to 45 per cent relative humidity.

Pupating behaviour - Pupating behaviour of *S. ejectana* was studied in the following ways, using sixth instar larvae that were in the final larval stage. They were:

- on potted *T. calycina* plants that were heavily infested with sixth instar larvae (40 to 50 larvae per plant). Fresh foliage in these plants was sparse because most of the foliage was occupied by the larval webbing shelters; and

- on potted *T. calycina* plants that were infested with only 10 to 15 sixth instar larvae and on which there was ample fresh foliage.

Both these were maintained at a constant temperature of 19°C and 40 to 45 per cent relative humidity.

- on the feeding medium (10 to 12 sixth instar larvae were placed in different 90 × 90 × 180 mm polyethylene food containers), maintained at a controlled temperature of 22°C and 60 to 65 per cent relative humidity; and

- in containers (90 × 90 × 180 mm) with lids, that were filled with *T. calycina* (15 to 20 branches with foliage) heavily infested with late sixth instar larvae. These larvae were maintained in a controlled temperature condition of ca. 25°C and 60 to 65 per cent relative humidity.

Once these larvae became pupae, they were removed without damaging them from their anchorages using sterilised forceps and transferred into different 200 mL lidded polyethylene cups. Typically the pupae were spongy in texture for up to 24 h after pupation; hence, pupae were prone to damage quiet easily while handling during this period. The pupal case sclerotised within 48 h after pupation. Hence, the pupae were removed after this period or once the colour changed to red or brown (immediately after pupation females were generally reddish and males were greenish brown in colour). To each cup 10 to 12 pupae were added that were sexed based on size, colour and number of abdominal segments. The sex of these pupae was determined visually using the naked eye and under a microscope. The lengths of the pupae were measured using a standard scale of 0 to 150 mm length. Generally male and female pupae were maintained in separate cups to study the duration of the pupal stage.

The effect of two different temperatures on the duration of pupal stage was studied by placing male and female (each 25 pupae) in controlled temperature rooms of:

- 22°C and 60 to 65 per cent relative humidity; and
- 25°C and 40 to 45 per cent relative humidity.

Ten pupae (48 h following pupation) were placed in different 300 ml polyethylene cups. The lids of the cups were perforated using a fine needle (7 to 9 holes per cup). The condition of pupae was examined regularly.

Adults - Initially, the female moths were identified while they were ovipositing on the plants, these were captured using an aspirator and transferred into different transparent plastic tubes. Female moths were distinguished by identifying the morphological differences in the wings, thorax and abdomen by visually using a microscope and by naked eye. The length of the body was measured using a standard scale of 0 to 150 mm. The special markings on the wings and abdomen and their sizes were recorded. These similar criteria were used for determining sex in the field-collected and laboratory-reared adults.

The sex differentiation of the adults that emerged from the laboratory culture was made 24 h after emergence, under a laminar floor chamber. This procedure was adopted because immediately after emergence the adults are less active, their wings are not dry and it is difficult to observe distinguishing characteristics. Moreover, there are possibilities that the wings may be damaged whilst being handled at this stage.

Emerged adults (six males and six females) were transferred into different 200 mL lidded polyethylene cups using an aspirator. The lids were perforated (five to seven holes per lid) using a fine needle for air circulation into the cups. These adults were fed with 10 per cent honey solution (2 to 4 mL per cup), to which antioxidants had been added. The honey solution was soaked in absorbent cotton wool pad and placed in

small plastic lids. The method of honey solution preparation is discussed in detail in Appendix 2.

The honey solution was replenished at four to five day intervals without disturbing the adults. It was observed that a large number of ants, attracted by the honey solution, entered these cups and they were found feeding on the adult moths. In order to prevent the entry of ants, the cups (five to seven cups per tray) were placed in rectangular plastic trays (450 × 600 × 60 mm). These trays were partially filled with 750 to 900 mL of tap water in which 1 to 2 g of detergent (Pyroneg®) was added.

The egg laying behaviour, capacity and number of days required to lay eggs were studied at two different controlled temperature conditions, namely,

- 22°C and 60 to 65 per cent relative humidity; and

- 25°C and 40 to 45 per cent relative humidity.

The cups were observed regularly for eggs three to four days following the introduction of adults. The condition of the adult moths was monitored two days following the introduction of adults and observed daily except Saturdays and Sundays, until the adults were dead.

Reproduction - The egg laying behaviour, number of days required to lay eggs following emergence and number of eggs laid by adult moths were studied using potted *T. calycina* plants obtained from Pine Wood Nursery, Melbourne. A glass-house maintained at a constant temperature of 19±1°C and 50 to 55 per cent relative humidity was used for culturing these plants. These plants were housed individually in iron framed cages (174 x 174 x 270 mm), where the top and sides of which were fitted with muslin gauze-cloth. Watering was carried out once every two to three days without disturbing the adults and the drained water was collected by placing a plastic tray (450 x 600 x 50 mm width, length and height respectively) under the pots. The collected

drained water was disposed of promptly to prevent the drowning of adults and to reduce the growth of algae.

The plants were fertilised at intervals of 15 to 20 days using a slow release fertiliser, namely Osmocote® (5 to 7 g per plant). Each cage was supplied with 12 male and 12 female adults of the same age (24 to 48 h old). These moths were fed with 10 per cent honey solution soaked into an absorbent cotton wool pad and placed in small cup (five to seven cups per cage). The honey solution was replenished every three to five days on a fresh cotton pad because after this time the honey solution had become fermented or had dried out.

3.2.2.4 Determination of instar stages

To determine the number of larval instars of *S. ejectana*, shortly after hatching (1 to 2 h), fifty individuals were transferred individually into different 30 mL lidded polyethylene cups. These larvae were handled using a fine camelhair brush. These cups were filled with 18 to 20 g (thickness of 3 to 4 mm) of light-brown apple moth medium (LBAM) diet. All cups containing the insects were incubated at a controlled temperature of 22°C and 60 to 65 per cent relative humidity.

The containers (30 mL) were firstly observed by naked eye, and then under the microscope near to the larval webbings because it is in this region that the head capsules are likely to be located. Observations were made daily except on Saturdays and on Sundays. If any head capsules were found they were removed using a blunt needle or camelhair brush that were slightly wetted to improve their adhesion, since they were readily dispersed by slight disturbances in the air. Once it was established that an instar stage was completed (determined based on size of the larva and shelter), but the head capsule could not be found, the larva was transferred into a new container to which the diet had been added. Later, the container that previously housed the larva was immersed into a 750 mL glass beaker filled with 500 to 600 mL of cold tap water,

hence, if there was any head capsule it would float on the surface of water. These were removed using a fine camel hair brush and transferred in a manner similar to the other head capsules as mentioned above.

Head capsules of the first and second instar larvae were also collected from the first 12 to 30 leaves of potted *T. calycina* plants by observing them two to three days after emergence of larvae and continuously for up to 16 days. They were collected using a wetted fine camelhair brush and transferred into 70 mL lidded long plastic tubes. These tubes were filled with 70 per cent ethanol (40 to 50 mL per tube) and labelled. For all other stages, head capsules were sought five to seven days after the beginning of a new instar stage. If no head capsules were found after seven days, they were again sought after 9 to 12 days. This is because some individuals may require longer time to complete a particular instar stage.

The collected head capsules were transferred from the tubes into glass Petrie dishes just before taking measurements. The length and width of these head capsules were measured using a microscope fitted with a graduated eye-piece. These were measured within 24 h of removal. While measuring the head capsules, the lengths of larval instar stages were also measured using a standard scale of 0 to 150 mm length. Other characteristics recorded were the colour of head capsule, thorax, abdomen and other distinctive markings on the larvae.

3.2.2.5 Cool hardiness of eggs, larvae and pupae

The tolerance of eggs (laid on potted *T. calycina* plants that were maintained in a glass-house at a temperature of 19±1°C), fifth and sixth instar larvae (collected from the field in Horsham) and pupae (collected from the larvae that were reared in a controlled temperature room in medium) to a low temperature was investigated by placing each of these stages at a controlled temperature environment at 4°C. Due to lack of availability

the responses of other larval instar (one to fourth) stages and adults to this temperature were not be able to be investigated.

Eggs - Young and mature eggs, together with the leaves on which they had been laid, were collected from *T. calycina* and immediately placed onto moistened absorbent cotton wool pads. The surfaces of the leaves onto which the eggs had been laid were facing upwards. Three to four leaves with eggs were transferred into different lidded 30 mL polyethylene containers, to which had been added 15 to 18 g of modified lightbrown apple moth medium. These procedures were carried out at 21±1°C, if they were transferred immediately into an environment with a temperature of 4°C, there is a possibility of significant condensation of moisture vapour in the leaves and medium. Condensation may lead to decay of both leaves and eggs. Hence, these were first transferred into an environment at 14°C for 3 to 4 h (preconditioning) and later transferred to a controlled temperature of 4°C. The condition of the eggs was observed daily except on Saturdays and Sundays. Two to four days after placing them at this temperature, 30 per cent of the containers showed heavy condensation on the lids and sides, hence, the eggs were transferred into new containers containing fresh medium.

Larvae - The adaptability of larvae to lower temperatures was investigated by three different methods, namely:

Method-1: The branches that contained webbing shelters were trimmed, and larvae and their webbing shelters were transferred into lidded polyethylene containers of 90 x 90 x 180 mm. These containers were partially filled with 95 to 100 g of modified lightbrown apple moth medium. Ten to twelve larvae were placed in each container. Once the larvae were ready to be inoculated onto the medium, they were initially preconditioned for 3 to 4 h at 14°C and then transferred into controlled temperature room of 4°C.

Method-2: The larvae were removed from their webbing shelters with minimum disturbance and without any damage to them whilst using forceps. They were

transferred individually into 30 mL lidded rectangular polyethylene containers to which 18 to 20 g of modified lightbrown apple moth medium (length of 3 to 4 mm with a thickness of 3 to 5 mm) had been added. These larvae were immediately transferred to the controlled temperature room maintained at 4°C without preconditioning them at 14°C. During the first 48 to 72 h the larvae were observed to be moving around the lid and sides of the containers. It was only after this time that the larvae began to construct webbing shelters. This experiment is aimed to indicate whether plunging the larvae, without webbing shelters, into a temperature of 4°C from 20°C has any effect on their ability to construct webbing shelters, and to complete their life cycle in general.

Method-3: The larvae were removed from their webbing shelters, transferred individually into 30 mL lidded polyethylene plastic cups as described in the previous paragraph. In this experiment the larvae were maintained at a controlled temperature of 19°C and 70 to 75 per cent relative humidity for 72 h before placing them at a controlled temperature of 4°C. This aids the larvae to occupy the medium (90 per cent of these larvae settled in the medium by constructing shelters) before being transferred to a temperature of 4°C and later these were transferred into 4°C controlled temperature room.

To reduce the disturbance to the larvae, conditions were assessed at every three to four day interval. The feeding pattern was studied by observing the feeding holes in the medium and the amount of fresh faeces present on the medium, again at intervals of three to four days. The faeces were removed once the regular observations were completed.

Pupae - The young and mature pupae were collected from the medium maintained in a controlled temperature room of 22°C and 60 to 65 per cent relative humidity. Eighteen pupae (12 female and 6 male) were transferred into different lidded 200 mL polyethylene cups. The lids of these cups were perforated (five to seven holes per lid) using a fine needle to facilitate air circulation inside the cups. These were maintained at a controlled temperature room of 4°C and the conditions of pupae were observed daily except on Saturdays and Sundays.

3.2.2.6 Resistance to moulds and predators

The pathogens and predators that attack larvae and pupae were investigated under laboratory conditions. Fungi that were collected from the field among the *T. calycina* were introduced into containers (61 x 40 x 25 mm) that were filled with LBAM medium and to which had been added 20 to 25 third and fourth instar larvae. These containers with slightly dampened paper tissues on their lids were maintained in a controlled temperature of 22°C and 60 to 65 per cent relative humidity. Once mould was established, these were inoculated into different containers as follows;

Larvae (third and fourth instars) with and without their webbing shelters (20 of each instar) were placed in 30 mL lidded polyethylene containers and in 61 × 40 × 25 mm rectangular polyethylene containers to which had been added LBAM medium. Similarly, these moulds were introduced in the foliage of potted *T. calycina* plants that had various instar stages and pupae.

Predator: All stages of the predatory mite *P. persimilis* obtained from Bio Protection, Queensland, Australia were introduced into the containers (20 to 25 individuals per container) with a similar dimension to those described above. These containers housed various larval instar stages (third to sixth instars) and these were maintained under conditions similar to those mentioned above. Similarly, pupae of known age (three to nine days old) were maintained in different 200 mL lidded polyethylene containers that had been inoculated with a similar number of *P. persimilis* eggs and larvae.

3.2.2.7 Alternative hosts

The alternative hosts for these larvae were identified by placing third to sixth instar stages (each 10 in number) and adults on bean, capsicum and strawberry plants in cages

that were maintained in a glass-house. The conditions of these stages were observed regularly.

3.3 Results

3.3.1 Field studies

3.3.1.1 Number of different stages of *S. ejectana* found in the collected samples

The numbers of eggs, larvae, pupae and adults present in the samples that were collected from Horsham (Aust Flora Pacific Pty Ltd) during various seasons of the year are given in Table 3.1. Percentages of different larval instar stages collected during various months of the year are given in Table 3.2. Generally, in most of the monthly collections more than one larval instar stage was recorded except on three occasions. In April, May and June (late autumn and early winter) only one larval instar was collected. This is likely to have been due to the adverse weather conditions that are not conducive for the growth and development of all stages, especially in late June 1995 and mid April 1996, where only sixth instar larvae were obtained. More than four instar stages had been found in the samples that were collected during early January 1996, mid-September 1996, mid-October 1996, early November and early February 1997.

Empty shelters were intercepted in all the samples collected, this indicates that larva moved to new shelters once all the green materials were eaten up.

A small number of adults was captured during most of the year, except during winter (June, July and August) and sometimes, early spring (September). The reason for the absence of adults during winter or early spring would be either their activity was low or they were not alive during these periods. In the field the adult *S. ejectana* were mostly seen resting on foliage of plants during the morning and at midday, or occasionally flying around the bushes (1500 to 2000 mm above the ground). Even a slight disturbance of the bushes caused these moths to take flight. Adult moths became active and began flying during twilight on hot sunny days.

Table 3.1 Numbers of eggs, larvae, pupae and adults present in the field-collected samples from Horsham for the period of September - 1994 to February - 1997.

Date	Eggs	Larvae (instar stages)						Total larvae	Pupae	Empty shelters		Adults
		1st	2nd	3rd	4th	5th	6th			Small	Large	
8/9/94	6	0	120	60	0	0	0	180	0	12	3	0
10/94	0	0	60	140	0	0	0	200	0	40	6	0
11/94	0	0	20	40	74	12	0	146	3	9	32	2
8/12/94	0	0	0	0	120	40	0	160	0	45	16	9
1/95	2	0	0	0	0	60	74	134	0	21	65	6
2/95	0	12	67	25	0	0	0	104	2	6	40	7
3/95	0	0	0	70	20	0	0	90	0	48	14	5
4/95	0	0	0	0	15	90	0	105	1	69	18	3
2/5/95	0	0	0	0	20	50	0	70	1	24	72	5
22/6/95	0	0	0	0	0	0	20	20	2	36	120	0
7/95	12	0	0	0	0	14	83	97	0	81	97	0
17/8/95	0	50	250	0	0	0	0	300	0	7	180	0
9/95	0	0	20	160	40	0	0	220	1	54	63	3
10/10/95	0	0	0	20	350	0	0	370	0	0	7	12
11/95	0	0	0	0	10	200	160	370	2	0	40	6
14/12/95	0	0	0	0	0	100	120	220	0	0	90	7
9/1/96	0	160	40	100	50	100	0	450	0	12	37	7
8/2/96	0	0	0	0	150	300	0	450	0	34	70	12
14/3/96	0	0	0	0	0	30	40	70	0	0	520	8
15/4/96	0	0	0	0	0	4	0	4	0	0	400	10
8/5/96	9	3	0	0	0	2	0	14	0	3	325	9
13/6/96	0	27	24	13	0	0	0	64	0	25	244	0
23/7/96*	0	342 (218)	306 (144)	69 (60)	0	0	0	717	0	110	119	0
22/8/96*	0	0	72 (54)	52 (41)	0	0	0	124	0	148	148	0
16/9/96*	0	0	12 (12)	125 (117)	19	9	5	170	0	36	25	0
14/10/96*	0	0	16 (4)	237 (21)	58 (14)	227 (27)	122	760	0	29	18	0
6/11/96	3	0	12	85	125	174	165	561	0	31	11	3
10/12/96	0	0	0	56	30	66	114	266	0	37	118	7
1/97	4	12	101	0	0	0	0	113	2	3	16	4
5/2/97	0	0	19	52	45	27	9	152	1	6	15	5

* - Diapause or quiescent stage. ( ) - indicated number of diapause larvae.

Table 3.2 Percentage of different stages of larvae collected during different seasons of the year.

Date	Larval percentage (instar stages)					
	First instar	Second instar	Third instar	Fourth instar	Fifth instar	Sixth instar
8/9/94	0.0	67.0	33.0	0.0	0.0	0.0
10/94	0.0	30.0	70.0	0.0	0.0	0.0
11/94	0.0	14.0	27.0	50.0	9.0	0.0
8/12/94	0.0	0.0	0.0	75.0	25.0	0.0
1/95	1.5	0.0	0.0	0.0	45.5	53.0
2/95	11.0	64.0	25.0	0.0	0.0	0.0
3/95	0.0	0.0	77.0	23.0	0.0	0.0
4/95	0.0	0.0	0.0	14.0	86.0	0.0
2/5/95	0.0	0.0	0.0	29.0	71.0	0.0
23/6/95	0.0	0.0	0.0	0.0	0.0	100.0
7/95	0.0	0.0	0.0	0.0	14.0	86.0
21/8/95	17.0	83.0	0.0	0.0	0.0	0.0
9/95	0.0	9.0	72.0	19.0	0.0	0.0
12/10/95	0.0	0.0	4.0	96.0	0.0	0.0
11/95	0.0	0.0	0.0	3.0	54.0	43.0
15/12/95	0.0	0.0	0.0	0.0	45.0	55.0
4/1/96	36.0	9.0	22.0	11.0	22.0	0.0
12/2/96	0.0	0.0	0.0	33.0	67.0	0.0
14/3/96	0.0	0.0	0.0	0.0	43.0	57.0
15/4/96	0.0	0.0	0.0	0.0	100.0	0.0
8/5/96	60.0	0.0	0.0	0.0	40.0	0.0
13/6/96	43.0	37.0	20.0	0.0	0.0	0.0
23/7/96	48.0	42.0	10.0	0.0	0.0	0.0
22/8/96	0.0	58.0	42.0	0.0	0.0	0.0
16/9/96	0.0	7.0	74.0	11.0	5.0	3.0
14/10/96	0.0	2.0	31.0	21.0	30.0	16.0
8/11/96	0.0	2.0	15.0	22.0	32.0	29.0
10/12/96	0.0	0.0	21.0	11.0	25.0	43.0
5/2/97	0.0	12.5	34.0	30.0	17.5	6.0

For development and multiplication a certain temperature is essential, and during mid-late winter (7/96 and 8/96) and early and mid spring (9/96 and 10/96), where the ambient temperature was low, most of the larvae entered into a diapause or quiescent stage. Diapause larvae can be easily identified by their external appearance, they are

dark green or black in colour, and they also become short and lean. The abdomen is more hairy and the posterior end is round. These larvae are mostly inactive and immobile. Most of the mid winter diapause or quiescent larvae died (16 per cent survival rate) whereas late winter and early autumn larvae had a survival rate of 70 per cent.

Survival rates among various diapause larval instars were considerably different. All the second instar larvae died within five to seven days after being transferred into a controlled temperature room of 22°C. It was also noted that once fifth to sixth instar diapause larvae were transferred into this controlled temperature room ca. 22°C most of the larvae (60 per cent) survived and developed into normal pupae, but they took a longer period to pupate. Similarly, 40 per cent of the third and fourth instar larvae survived and developed into normal pupae. The numbers of days required for pupation of third, fourth, fifth and sixth instar larvae were 45, 27, 18 and 9 days respectively.

The samples collected from the field, and observations made on the potted plants indicated that all these larvae live individually in shelters. Similarly, in the artificial medium it was observed that larvae live individually in shelters.

Once the temperature of the glass-house at Institute for Horticultural Development exceeded 40°C for 4 days (during December 1996) all the larvae, pupae and adults that were housed in cages were killed, however, the *T. calycina* plant survived. This clearly indicates that higher temperature is lethal to larvae or any other stages. Similarly, it is evident from the cold hardiness studies (discussed in Section 3.3.2.9) that prolonged low temperatures are not conducive to the growth and development of the stages exposed. The temperature during various seasons of the year in the Grampians fluctuates considerably (Table 3.4). Most of the mid and late winter overnight temperatures fell to less than zero, however, the day temperatures were above 12°C. Also, the webbing shelters give them some protection to *S. ejectana* from the adverse weather conditions. It is possible that the shelters retain some of the heat of respiration of the larvae. Hence, it can be concluded those lower temperatures (in this case 4°C or less) for prolonged periods are only lethal to *S. ejectana*.

The results presented in these two tables starkly portray for the first time the life-cycle of *S. ejectana* in the field. The data on the numbers of the various developmental stages of the larvae clearly show that two generations of *S. ejectana* develop each year. Each generation is separated by a period of about six months. This becomes evident when considering the data presented in Table 3.1 which shows that in September 1994 only second and third instar larvae predominated. One month later second and third instar larvae were again found, but this time third instar larvae predominate; this strongly suggests that the second instar larvae observed in September have matured somewhat. In November there were still no first or sixth instar larvae, but the second and third instar larvae from the previous month have developed so that fourth instar larvae are the most abundant. This remains the case in December but by January 1995 the larvae have matured. It is seen from the Table 3.2 that in January 1995 no first instar larvae were observed, but by February a new generation of *S. ejectana* was developing. From these data it seems that there is a six month difference in the generations. A periodicity of this order is confirmed by observing that the peaks in the number of first instar larvae appears in August 1995, January 1996, July 1996 and again in January 1997.

The efforts exerted to find pupae are described in Section 3.3.1.2. At this point it is sufficient to note that in the field they proved extremely hard to find.

The abundance of adult *S. ejectana* contrasts sharply with that of the larvae. The larvae appear twice per year in two sharply defined cohorts, whereas the adults are essentially aestival; they are fairly uniformly abundant for about the warmest seven months of the year. It would seen that the adult females lay their eggs for a limited duration to ensure that sufficiently large cohorts of the insects are available for mating in high summer and mid-autumn. This hypothesis is strongly supported by the data on the maturation of larvae results of lab studies on the life-cycle of *S. ejectana* which indicate that adults lay their eggs has a period of less than about 14 days.

After they have been harvested, cut flowers may be subjected to conditions that are more uniform than those experienced in the field because they are likely to be kept in

the buildings, shipping containers, transport vehicles and so on. In some cases the conditions under which the plants are housed may be less hostile to their associated pests than those encountered in the field. Indeed, the conditions may resemble that of the lab. For these reasons the data collected on the life-cycle of *S. ejectana* under laboratory conditions may be particularly useful in devising postharvest pest management strategies for the moth.

The insects in the field experience conditions that are quite different from those that may be experienced after their host plants have been harvested. There may be significant diurnal changes in temperature, an average of 16.6°C at Horsham in January. Short term fluctuations in temperature can result in extreme conditions being experienced by the pest. In January the maximum ambient temperature exceeds 37.4°C one day in seven, whilst the minimum temperature is less than 9.9°C one day in seven. On average the temperature in June falls to less than 0.7°C on about four days.

The insects not only have to survive these extremes, but they also need to adopt successful breeding strategies. A study of the behaviour of the insect in the field is likely to indicate at which interventions are most likely to be successful in controlling the pest.

3.3.1.2 A comparison of the life-cycle of *S. ejectana* in the field and in the laboratory.

The conditions under which *S. ejectana* develop in the field and those under which they develop in the laboratory are quite different. (However, the field and laboratory data may be usefully compared, and as a result it may be possible to develop pest control procedures). It has been noted in Section 3.3.3.1 that there are two generations of moth per year. One of them appears to commence with the laying of eggs in summer and the other in winter. Because the biannual cohorts is very distinct it appears that the laying of eggs occurs within a short period of the emergence of adults. This supposition is confirmed by the observations made in the laboratory that females began to mate within

three to five days of their emerging, and that they stop laying eggs after they are about 14 days old. The insects complete their life-cycle much more rapidly in the laboratory than in the field, but the conditions within the laboratory are close to optimum. The average minimum temperature during the winter is 4.3°C and the average maximum is 14.4°C, much lower than the 19°C of the laboratory. Over the summer months the average daily minimum and maximum temperatures are 13.5°C and 29.2°C. It would be reasonable to hypothesise that development takes longer for those populations that have to survive winter. There is some, if not entirely compelling evidence this is indeed the case as can be seen in Table 3.3.

Table 3.3 *S. ejectana's* development during 1994 to 1996.

Period	Time to develop	Notes
9/94 to 1/95	4 months	Observations begin with second instars.
1/95 to 6/95	5 months	Time includes one month of winter.
8/95 to 12/95	4 months	Development occur over warmer months.
1/96 to 3/96	2 months	Development occurs during the warmer months.
5/96 to 12/96	7 months	Development takes place during the winter months.

The shortest development time, two months, occurs over the summer months, whereas the cohort that take the longest time encompasses the coolest months.

In the field, the pests are subjected to seasonal changes in the climate, as well as the vagarious of the diurnal variations in the weather. Insects reared in the laboratory were maintained under constant and controlled conditions in which the photoperiod was 12 h that corresponds to the equinoxes.

3.3.1.3 Implications for pest control

S. ejectana was first reported to be a pest of thryptomene by Williams (1996), and it was this observation motivated that study. The prophylactic action of reducing the populations of pest insects in the field is an integral component of a pest management strategy. If properly carried out it can make postharvest treatments less onerous. From the observations made in this study it would appear that the population of *S. ejectana* could be reduced if regular (perhaps weekly) observations were made of the adult moths. As soon as they were observed a program of insecticidal spraying should be commenced aimed at reducing the numbers of adult females during the time that they are particularly fecund. If this were done in early summer, say, one could expect to see no further adults until perhaps early autumn, when there would be just a few. If these were also sprayed in a timely manner the number of immatures could be further reduced and ultimately the pest may become insignificant in the cultivated plantations of thryptomene.

The number of eggs and pupae present among the samples was very low. A small number of eggs was found among the samples during early May 1996 and early November 1996. However, these eggs failed to hatch under laboratory conditions (19°C and 70 to 75 per cent relative humidity).

Samples that were collected during December, February and January 1997 were infested with meat ants that were feeding on the larvae of *S. ejectana*. In the field, it was observed that ants enter the webbing shelters and drag out the larvae whereupon they consume them on the webbing shelters or on the foliage. Fourth and fifth instar larvae were mostly found to be attacked by the ant and it took 0.5 to 1 h to devour the larvae completely. Adult jumping spiders were also found attacking larvae (mostly third and fourth instars) by entering the webbing shelters and sucking the body fluids of the larvae. Only the head and outer skin remained after they complete their feeding and that takes 5 to 7 h.

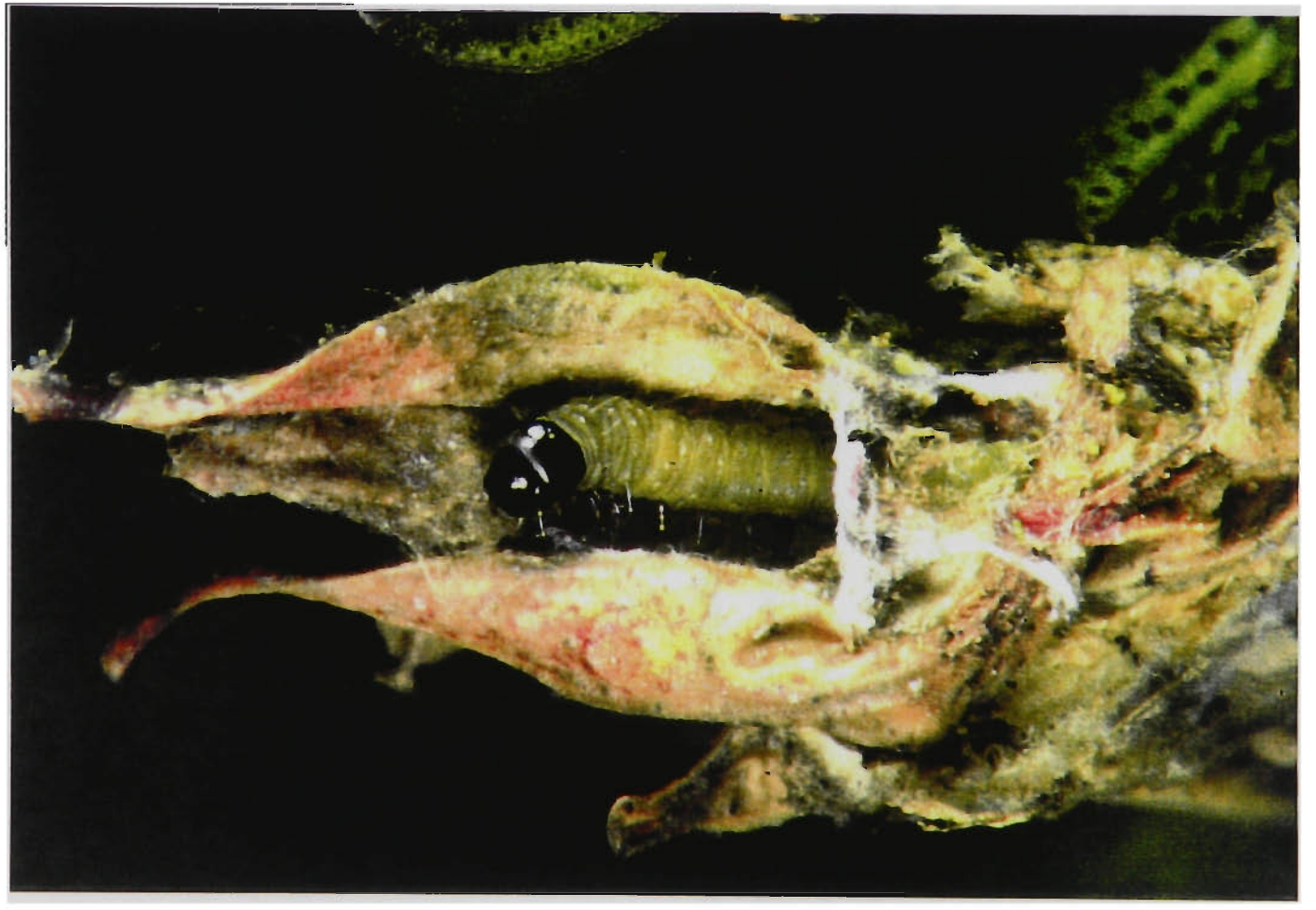


Figure 3.3 A larva of *S. ejectana* in the webbing shelter.

Survival rate of field-collected (Horsham) larvae that were reared in potted T. calycina plants: Highest survival rates (85 per cent) of second and third instar larvae, and 100 per cent survival rate for all other instar stages were recorded for those individuals that were transported from Horsham to the Institute for Horticultural Development. These larvae were removed from their webbing shelters and transferred directly onto foliage of potted *T. calycina* plants within one hour following removal. However, survival rates of the first instar larvae were low (30 per cent); probably they were damaged whilst being handled or they were unable to find a suitable leaf in the potted *T. calycina* plant.

All the larvae that were transferred with their webbing shelters onto plants survived and developed into normal pupae. Most of the larvae (third to sixth instar) that were placed on plants with their webbing shelters remained in their shelters until they became dry, whereupon they moved to nearby branches of the plant and constructed new webbing shelters. However, 10 to 20 per cent of the third instar larvae moved away from their shelters two to three days after placement and constructed new webbing shelters on plants.

3.3.1.4 Pupae and pupation

Only a small number of pupae was collected during early May, late June, early November 1995 and early February 1997. All these pupae survived and developed into adults when they were maintained at a temperature of 25°C and 40 to 45 per cent relative humidity. Compared with the number of larvae collected, the number of pupae recorded was much lower, perhaps due to the method of pupation in which the pupae may be dislocated from the webbing shelters by wind or rain and short duration of the pupal stages. Also, random observations among the plants rarely indicated the presence of pupae.

Collection and assessment of top soil near to the bushes indicated that there were no pupae or pupal cases found among the samples. This suggests that the pupal case might be blown away by the wind. Observations made in the laboratory suggest that most of the pupation took place on the plants themselves, but the exact mode depends on the availability of foliage.

3.3.1.5 Egg laying behaviour of adults

Observation of field-collected samples clearly show that adult *S. ejectana* lay eggs not continuously or throughout the year. This was evident from the presence of eggs only at different times of the year. Moreover, studies conducted at the Institute for Horticultural Development, Knoxfield indicate that adult moths did not require a specific period to lay eggs. Once they were released on plants they start laying eggs after five to seven days irrespective of the season of the year. Bursts of eggs were laid once the adults reached the egg laying stage. Therefore, overlapping generations within a year are possible.

3.3.1.6 Temperature

Temperature measurements obtained during various periods of the year are given in Table 3.4. It is clear from this table that temperature differences between different seasons of the year are considerable. During mid and late winter (July and August) the minimum temperature plunges to sub zero. During this period the growth and development of *S. ejectana* was affected significantly. However, a small percentage of the larval stages survived and pupated later in the year. Similarly, summer temperatures of 38 to 40°C are not uncommon: however, these high temperatures were not lethal to all stages of the insect, perhaps because the overnight temperatures fell to a range of 18 to 20°C.

Table 3.4 Temperatures at Austflora Pacific Pty during the period of sampling (1996 to 1997).

Month	Average temperature (°C)		Maximum temperature (°C)		Minimum temperature (°C)	
1996	In the bushes	Ambient	In the bushes	Ambient	In the bushes	Ambient
March	20.5	20.9	23.7	23.9	16.7	18.5
April	23.5	23.7	25.7	26.0	19.6	20.2
May	12.2	12.2	15.6	15.7	5.7	6.3
June	11.5	11.7	14.5	14.8	9.0	10.0
July	9.3	9.2	12.4	12.9	-2.0	-3.0
August	8.9	9.1	11.5	12.0	-3.9	-4.0
September	10.51	11.01	13.56	14.04	2.2	3.6
October	14.7	15.1	16.5	16.9	3.2	4.0
November	15.3	16.0	18.9	19.4	11.6	12.2
December	17.3	17.9	21.7	21.6	12.6	13.7
1997January	23.6	24.1	25.9	26.2	17.7	18.5
February	23.9	24.1	28.0	27.3	18.3	19.3
March	19.3	19.5	22.8	23.7	15.6	16.4
April	22.0	22.9	24.9	25.7	18.9	19.0
May	9.1	9.3	14.6	15.4	9.9	10.0
June	9.5	9.8	15.4	15.7	-3.1	-.2.7
July	8.1	8.1	14.2	15.0	-4.1	-3.9

3.3.1.7 Other insects

Numbers of other insects intercepted in the samples collected from the field are given in Table 3.5. Most frequently, larvae of geometrid, adults of sugar ants, and spiders and collembola were recorded. Geometrid larvae were found in small numbers in the field-collected samples. They seldom construct webbing shelters, but were found feeding on foliage of *T. calycina*. Hence, they were vulnerable to the vagaries of the weather and/or birds or other predators, thus, their population was limited. Once they were transferred into LBAM rearing medium, the method used to rear larvae of *S. ejectana*, not a single larva survived. All these larvae were dead within three to five days of their being transferred; however, if they reared in *T. calycina* foliage most of them pupated and became normal adults. Adults of collembolla were found among the old and dried webbing shelters that were abandoned by the *S. ejectana* larvae (mostly

Table 3.5 Number of other insects and fungi found among the samples collected from Horsham.

Date	Spiders (Adults)	Geometrid (Larvae)	Sugar ants (Adults)	Collembolla (Adults)	Fungus mould
8/9/94	12	3	14	2	0
10/94	4	2	16	12	3
11/94	9	3	11	14	2
8/12/94	7	2	3	10	0
1/95	5	4	9	7	0
2/95	6	3	18	24	0
3/95	5	14	21	41	0
4/95	7	6	14	36	2
2/5/95	9	4	12	20	12
23/6/95	7	0	2	27	14
7/95	5	0	3	9	6
21/8/95	4	0	16	3	15
9/95	9	5	18	4	4
12/10/95	6	0	0	6	9
11/95	7	1	12	12	0
15/12/95	12	1	0	24	0
4/1/96	7	2	0	33	0
12/2/96	4	4	2	41	0
14/3/96	2	0	11	54	0
15/4/96	5	0	3	30	0
8/5/96	12	1	0	27	14
13/6/96	4	2	0	32	9
23/7/96	6	3	0	12	21
22/8/96	9	0	12	2	23
16/9/96	12	13	14	-	7
14/10/96	10	3	7	11	0
8/11/96	9	0	12	21	0
10/12/96	12	0	0	18	0
1/96	10	6	7	12	0
5/2/97	4	0	0	45	0
3/97	11	4	14	9	0
4/97	2	3	7	11	12
5/97	6	11	9	4	9
6/97	9	15	14	7	5

the sixth instar larval shelters). Collembolla generally live in groups (10 to 12 individuals per shelter). Sugar ants were found feeding on flowers or just moving

among the foliage of *T. calycina*. A higher number of individuals was intercepted especially during warmer months. On the other hand, spiders were recorded from all the field collected samples (throughout the year), and they have been found living individually in vacant larval webbing shelters.

Other insects intercepted include-large numbers of larvae and adults of the ornate aphid, *Myzus ornatus* Laing - on 22/8/96 and 16/9/96, small numbers of the soft brown scale, *Coccus hesperidum* Linnaeus - on 8/11/96 the common hover fly larvae, *Melangyna viridiceps* (Macquart)-on 22/8/96, the shield bug, *Poecilotoma perconfusca* - on 16/10/96 and the meat ant, *Iridomyrmex purpureus* (F. Smith).

3.3.2 Alternative hosts

All those plants that were adjacent to the field of *T. calycina* were free from *S. ejectana*. Even during dry months or after harvesting periods, where availability of *T. calycina* foliage was less, the remaining larvae prefer to live on *T. calycina* rather on other plants especially *Eucalyptus*. During adverse periods, the larva undergoes a diapause stage.

3.3.3 Laboratory studies

3.3.3.1 Adaptability

Survival rate of eggs on the rearing medium - All the young and mature eggs of *S. ejectana* placed in the rearing medium and on cotton wool failed to hatch. The leaves that were maintained in the 22°C controlled temperature room showed slight wilting within 48 h. This was followed by browning and drying of leaves within 96 h. Also, most of the eggs became yellow within 48 h and then they turned to brown in 72 h, indicating that they were dead.

Survival rate of eggs on plants - Seven days following emergence of adults, eggs were found on the *T. calycina* plants. Generally eggs were laid singly if there were plenty of

young leaves. The eggs were laid near to the middle of upper surface of the leaves (Figure 3.4). Also, very rarely eggs were observed on the lower surface of leaves. The highest number of eggs was recorded 9 to 12 days following emergence of adults and number of eggs laid declines gradually thereafter. Generally, adult moths were found to lay eggs for 15 to 18 days. When there were 12 to 16 adult moths (both sexes) caged with a *T. calycina* plant, eggs were found only on leaves five to seven down the branches from the terminal leaf buds. When more moths (24 to 27 individuals) were present, eggs were found also on other leaves-up to 36 leaves down from the terminal leaf bud, presumably because all the most suitable leaves already supported eggs.

In the field and glass-house it was observed that the adult moths prefer to lay eggs singly on young leaves, presumably due to the longevity of leaves. In one instance, nine eggs were laid on a single leaf (18th leaf from top) next to each other, and they had the appearance of a mass of eggs (Figure 3.5), when a large number of adults (36 individuals of both sexes) were reared in a single cage. The eggs were firmly attached to leaves, and this prevented them from being carried away by heavy winds or other disturbances.

Survival rate of larvae on the medium - The adaptability of various instar larvae with and without their webbing shelters that were reared either singly or in groups is given in Table 3.6.

Table 3.6 Survival rate (per cent) of various larval instar stages that were reared in modified LBAMedium - with and without their webbing shelters in two types of containers.

Instar stage	Individual rearing.				Group rearing.			
	With shelter		Without shelter		With shelter		Without shelter	
	Live (%)	Dead (%)	Live (%)	Dead (%)	Live (%)	Dead (%)	Live (%)	Dead (%)
First	20.0	80.0	0.0	100.0	0.0	100.0	0.0	100.0
Second	50.0	50.0	0.0	100.0	30.0	70.0	0.0	100.0
Third	90.0	10.0	75.0	25.0	75.0	25.0	20.0	80.0
Fourth	95.0	5.0	90.0	10.0	95.0	5.0	70.0	30.0
Fifth	100.0	0.0	80.0	20.0	100.0	0.0	75.0	25.0
Sixth	100.0	0.0	80.0	20.0	100.0	0.0	75.0	25.0

Figure 3.4 Single egg of *S. ejectana* on the upper surface of the *T. calycina* leaf

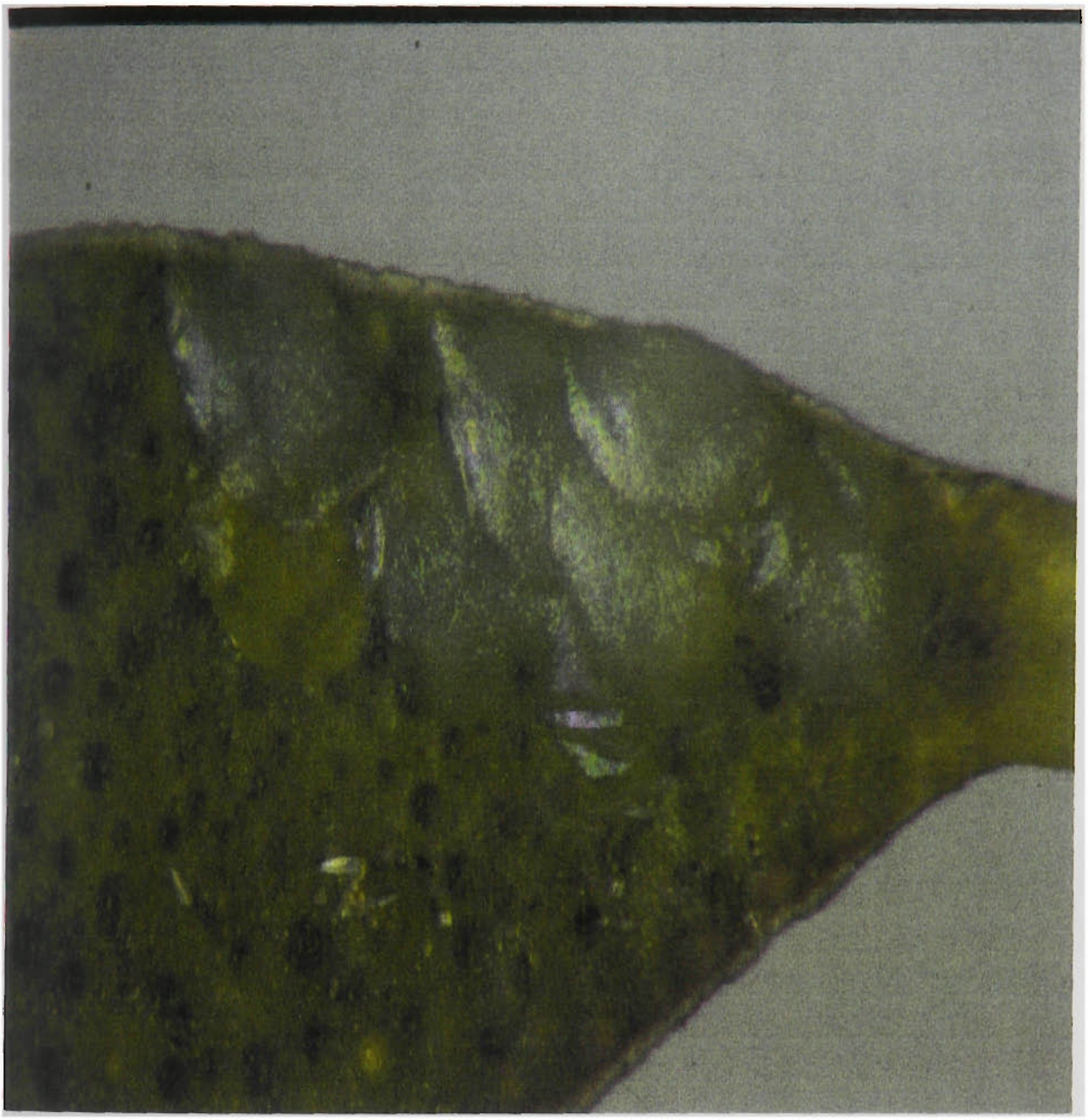


Figure 3.5 Mass of *S. ejectana's* eggs on the upper surface of the *T.calycina* leaf

The diet proved acceptable to the larvae that were successfully reared to pupal and adult stages. Survival rates of first and second instar larvae in this diet were very low, and all the larvae without webbing shelters died two to three days after being placed on it. However, it is possible to rear the larvae of *S. ejectana* from third instar onwards. The duration of various stages of this insect were determined by observing when they changed from one stage to another.

First instar larvae - When newly hatched first instar larvae were transferred (without their shelters) to the LBAM diet (in both 30 mL and 61 x 40 x 25 mm rectangular containers) all of them died within 48 to 60 h. Most of the larvae (75 per cent) moved towards the lid 1 to 2 h after having been transferred, after which they settled on the lids or the top side-wall of the container. Some (25 per cent) remained on the medium for 24 to 48 h, later they moved towards the lid and settled on it. However, most of these larvae (70 per cent) moved towards the medium (48 to 60 h following transfer) and were found dead on top of the medium or on the bottom of the cups. The remaining larvae were found dead on sides or lid of the container. When these larvae were transferred individually with the leaf shelter to the LBAM diet in 30 mL cups, 20 per cent survived and developed into later larval instars and eventually into adult moths. However, not a single larva that was transferred with its leaf shelter survived that was reared in groups; all these larvae were dead within four to six days following placement.

It was observed that the larvae that survived were feeding on the leaves for the first five to six days after being transferred, and by this time most of these larvae had reached the second instar stage. Second instar larvae are comparatively more tolerant than first instar stages, hence, they can survive on the artificial diet to a certain extent. Once the leaves began to dry or became depleted as a food source, the larvae moved to the sides of the medium, constructed shelter and began to feed on the medium. Most of the surviving second instar larvae (90 per cent) preferred to live on the sides of the medium that was adjacent to the wall of the cup. The remaining larvae tunnelled into the medium and constructed shelters underneath the medium.

Second instar larvae - Similarly, when second instar larvae were transferred to the modified LBAM diet without their shelters (both in individual and group rearing) they all died. However, most of those (50 per cent) in shelters that were reared individually, survived and developed into pupae. On the other hand only 30 per cent of the larvae with their webbing shelters reared in groups survived and developed into adults. These larvae remained in the webbing shelters for the first three to five days, and once the shelters begun to dry they moved out from them. These larvae generally selected sites in the medium within 2 to 3 h following emergence from their shelters. A small number of larvae (25 per cent) settled beneath the medium.

Third instar larvae - Third instar larvae were, however, able to survive and develop on the modified LBAM diet without having their leaf shelters (75 per cent survival) when they were placed individually in 30 mL cups. These larvae settled and fed in a manner similar to that of first and second instar larvae. However, only 20 per cent of the larvae that were reared in-groups (10 to 12 larvae per container) survived. It was observed that these larvae were unable to construct shelters among the grooves of the medium, presumably due to difficulties in manoeuvring either due to the compact nature of medium and/or small groves. On the other hand, the survival rate was higher (75 per cent) once these were placed with their webbing shelters (group rearing) and 90 per cent survival rate was recorded for the larvae that were reared individually. Furthermore, in most of the shelters condensation was observed, 48 to 60 h following placement, but this did not affect the growth of the larvae. These shelters began to dry seven to nine days after their placement; however, the larvae remained in their shelters even after shelters had dried.

Observations made on the medium indicated that the larvae fed on the surface of the medium (evidenced by feeding holes) that were directly beneath the shelters. These larvae rarely moved out from their shelters to the grooves or beneath the medium. However, in the 30 mL cups, if they were placed without webbing shelters, all the larvae moved towards the lid and remained there for 24 to 30 h. Later the surviving larvae moved towards the sides of the medium (between two pieces) or rarely underneath the medium. On the other hand, only 40 per cent of the larvae that were reared in-groups

moved toward the sides of the container and settled there. Later (24 to 30 h) they moved into the grooves of the medium and settled on it.

Fourth instar larvae - Fourth instar larvae that were placed in medium without webbing shelters moved towards the lids of the containers 10 to 20 min after their placement and stayed there for 24 to 48 h. Later the surviving larvae moved towards the medium and constructed webbing shelters using silken threads on the sides of the medium. A large proportion (90 per cent) of fourth instar larvae that were placed onto the feeding medium without their webbing shelters (individual rearing) survived and developed into normal pupae. Similarly, 70 per cent of the larvae that were group reared survived and developed into normal pupae. Most of these larvae (70 per cent) constructed shelters in between grooves of the medium, generally near to the bottom of the containers. Some larvae (30 per cent) tunnelled into the medium and settled beneath the medium. Similarly, 95 per cent of the larvae that were placed with their webbing shelters (both individual and group rearing) survived, and all these larvae remained in their webbing shelters and fed on the medium that was beneath the shelters.

Fifth and sixth instar larvae - The highest survival rates were observed for the fifth and sixth instar larvae that were reared individually when they were placed on the feeding medium with or without their shelters (100 and 80 per cent respectively). Likewise, 75 and 100 per cent survival rates were recorded for larvae reared in groups without and with their webbing shelters. These larvae completed their live cycles in a similar way to larvae that were reared on potted *T. calycina* plants.

Behaviour of larvae on plants; first instar larvae - Those larvae that were left on the plants moved to terminal leaf buds (first two to four leaves from top) where they built shelters in the middle of an individual leaf using silken threads secreted from their mouth parts, and they fed on the epidermis of the young leaves. Generally, a single larva feeds on a single leaf and the affected leaf shows slight browning in the middle. However, the damage is superficial and most of the affected leaves remain on the plants without becoming completely dried or dropping off the plants. Once a larva had

completed feeding on a leaf, it moved to new leaves that were within seven to nine leaves from the top of the plant, i.e. to semi-mature leaves.

Second instar larvae - In potted plants (height of 550 to 750 mm), second instar larvae construct webbing shelters using 3 to 25 individual leaves, usually 12 to 18 leaves below from top of the plant. These leaves are semi mature in nature. Feeding starts from the innermost first leaf of the webbing shelters and proceeds to the outer layer of leaves. The outermost 5 to 7 leaves remain without being fed on, and these leaves turn to yellow and brown thus acting as a protective barrier to the larvae from rainwater and direct sunlight. It was observed from the samples collected in the field during seasonal rains that even heavy and/or continuous rainfall did not reach beyond the first three to five layers of the webbing shelters. Moreover, the webbing shelters protect the larvae from desiccation during periods of hot weather.

Occasionally, second instar larvae were found feeding on flowers, by joining three to seven flowers that were adjacent to each other by using silken threads secreted from their mouth parts. The affected flowers change their colour to yellow then brown and they are then shed from the plants.

As the larvae mature, the number of leaves used for the webbing shelter increases. The leaves were arranged in layers, hence, the larvae can move within the shelter.

Third and fourth instar larvae - Third and fourth instar larvae use 40 to 90 leaves to construct webbing shelters and consume up to 20 to 30 leaves. These stages generally use a single stem to construct shelters, however, under abnormal circumstances (during dry periods or if the plants have sparse leaves) it uses two to three adjacent stems for constructing webbing shelters.

Fifth and sixth instar larvae - Fifth and sixth instar stages mostly use two to three adjacent small branches, depending on the number of leaves present to construct webbing shelters. The leaves of these branches are firmly attached together by silken threads that are secreted from the mouth-parts of the larvae, and the construction

process is rapid, taking 5 to 7 h. However, a small percentage of larvae use a single stem (mature leaves) to construct webbing shelters. These stages are voracious feeders that consume 40 to 50 leaves.

Pupation on the medium - One to two days before pupation, the larvae stop feeding and undergo morphological changes. The anterior end becomes more round and the posterior end becomes narrower. Most of the mature sixth instar larvae (90 per cent), that were reared both in groups and individually, 24 to 48 h before pupation, moved towards the top or sides of the container to pupate. The remaining 10 per cent pupated among the grooves or underneath the medium. Pupae were mostly attached horizontally to the lids or between two layers of tissue that were attached to the lid. Occasionally pupation also occurs on the outer surface of tissues, hanging vertically from the posterior end or attached horizontally on the tissue. The pupae were immobile, however, if prodded they showed a wriggling movement of the abdominal segments.

The pupae were soft and light reddish (females) or greenish (males) for the first 24 to 30 h after pupation and rapidly changed their colour (females are reddish and males are reddish brown in colour) and sclerotised within 30 to 48 h. As they mature, the colour of their eyes changes to brown or red, and later becomes black (48 to 72 h before hatching). The wing pads begin to develop at the same time as the body colour changes. The colour change begins from the head and spreads towards the abdomen and 24 h before hatching the pupal colour changes completely to brownish black. All the pupae on the lids survived and developed into adults, but only 30 per cent of the pupae that were present underneath the medium survived. Most of them were trapped under the medium and unable to hatch; some emerged but were unable to fly because they had damaged their wings.

On heavily populated plants: Pupation generally took place just outside the webbing shelters, i.e. the pupae were hanging half inside the shelters or just outside attached to the outer surface of the shelter. The glass-house studies showed that most of the pupation happens just outside the webbing shelters (70 per cent), half inside and half

outside or outside (20 per cent) and attached to the surface of the webbing shelters. Only a small number (10 per cent) of pupae was found inside the webbing shelters.

On sparsely populated plants: The larvae that were reared on sparsely populated plants moved towards the terminal leaves of the plant (20 to 40 leaves from top) two to three days before pupation and used three to five young leaves to construct webbing shelters. The larvae were encircled by a thin cocoon-like covering and they pupated inside this cocoon. This acts as a protective barrier against the vagaries of the weather. Once emerged, the adult can easily break through this cocoon and fly away.

Adults - The bodies of newly emerged adult *S. ejectana* are soft and pliable in nature and remained inactive for the first 2 to 3 h after emergence (unable to fly, resting on the bottom of the cages or cups). They began walking some six hours after emergence and began to fly 9 to 12 h following emergence. The adult moths remained active up to 21 days, and fed both nectar from the flowers and honey solution. Adults that were maintained in potted *T. calycina* plants lived longer (16 to 21 days) than those were maintained in cups (9 to 12 days) or Petrie dishes (11 to 13 days). Except for the adults that were maintained on potted plants, all other adults were found loosing their wing scales six days after emergence.

In the glass-house the activity of adults appeared to be similar throughout the day, probably due to the constant temperature. These moths were resting on the side of the nets or moving energetically from one end of the cage to the other, except during oviposition or feeding. Courtship mostly took place on the side of the nets and rarely on foliage. In the cups and Petrie dishes the adults were found resting on the lids or top sides. When *S. ejectana* adults were handled for breeding in a similar way (in 200mL cups) to that used for culturing LBAM, they died within nine to twelve days of emergence without laying eggs on the ribbed sides of the cups, as do LBAM adults. On the other hand, eggs were observed (three to seven per Petrie dish) when they were maintained in glass Petrie dishes of 10 to 12 mm diameter, but, none of these eggs survived. However, the strategy of caging the moths with a *T. calycina* plant proved successful.

Three to five days after being caged the moths were observed mating, remaining joined for *ca.* 30 min. Matings were observed to take place only in the morning (8:00 to 11:00). The moths spent most of their time on the gauze sides of the cages and most matings took place in this situation. Adult moths were also sighted on the bottom of the cages only while feeding on honey solution. Egg laying was observed on several occasions, and it usually took place during the morning. A female ready to lay would move onto a selected leaf and settle there for 35 to 40 min to carry out the egg laying process.

3.3.3.2 Determination of larval instar stages

Collections of larval head capsules showed that the larvae undergo six instar stages, and the size differences between instars as indicated by head capsule measurements are given in Table 3.7. The body lengths of these different instar stages are given in Table 3.8. First instar larvae are pale yellow in colour with light brown heads, the mean length of the head capsule is 0.126 mm and the mean length of whole body is 1.5 mm. The duration of the first instar larvae is four to six days at 19°C. However, at 22°C the duration was shortened to three to four days. First instar larvae, immediately after emergence are slightly immobile and become fully active within 6 to 9 h after emergence.

The abdomen of the second instar larvae is yellow in colour, and the head capsule is dark brown with a length of 0.2 to 0.23 mm. The length of body is 2.3 to 2.7 mm. Generally larvae require 5 to 7 days and 4 to 6 days at 22°C and 25°C respectively to complete this stage. Third instar larvae are 5.2 to 5.7 mm long and creamy yellow in colour, the head capsules are 0.29 to 0.31 mm in size, and the colour of the head capsule changes to black from dark brown as they mature. However, the colour of the abdomen varies, depending on the type of food consumed. Those larvae that feed on the plants appear to be greener than those feeding on the medium that are creamy yellow, which

Table 3.7 Dimensions of head capsules of various larval instars of *S. ejectana* (n=25).

Larval stage	Length of head capsule, centre dorsal measurement (mm).			Length of head capsule, lateral measurement (mm).		
	Mean	Range	Standard deviation	Mean	Range	Standard deviation
First instar	1.26	1.1-1.5	0.15	1.49	1.3-1.7	0.14
Second instar	2.14	2.0-2.3	0.1	2.45	2.4-2.5	0.05
Third instar	2.98	2.9-3.1	0.07	3.45	3.3-2.6	0.1
Forth instar	3.66	3.5-4.0	0.2	4.2	4.0-4.6	0.22
Fifth instar						
a) male	4.32	4.3-4.35	0.011	4.96	4.92-4.99	0.017
b) female	4.38	4.35-4.4	0.009	5.02	4.97-5.04	0.015
Sixth instar						
a) male	4.86	4.83-4.89	0.013	5.54	5.51-5.56	0.1
b) female	4.92	4.9-4.95	0.013	5.6	5.4-5.9	0.022

Table 3.8 Body lengths of different stages of *S. ejectana.* (n=50).

Stage	Mean length (mm)	Range (mm)	Standard deviation	Remarks
Eggs	0.098	0.097 - 0.099	0.006	Transparent and change to creamy yellow
Larvae				
a)1st instar	1.5	1.4 - 1.6	0.009	Head light brown, abdomen pale yellow
b)2nd instar	2.5	2.3 - 2.7	0.01	Head brown, abdomen yellow
c)3rd instar	5.5	5.2 - 5.7	0.012	Head dark brown, abdomen creamy yellow
d)4th instar	8.5	8.2 - 8.7	0.011	Head black, abdomen creamy yellow
e)5th instar				
a) Male	13.5	12.0 - 14.0	0.01	Head black, abdomen green or creamy
b) Female	15.5	15.0 - 16.0	0.008	Head black, abdomen slight reddish or greenish
f)6th instar				
a)Male	17.1	16.2 - 18.3	0.081	Head black, abdomen green or yellow
b)Female	19.4	18.2 - 21.2	0.096	Head black, abdomen reddish or yellow
Pupae				
a) Male	8.0	7.9 - 8.2	0.006	Pale red with dark red eyes and change to brown
b)Female	9.8	8.7 - 9.1	0.009	Red with dark red eyes and change to brown
Adults				
a)Male	10.8	10.0 - 11.0	0.08	
b)Female	11.2	11.0 - 12.0	0.19	

correspond to the colour of the medium. The head capsules of the fourth instar larvae are a blackish colour and their width is 0.35 to 0.4 mm. The abdomen is generally creamy yellow or green in colour and the length is 8.2 to 8.7 mm. Similarly, as the larvae grow the colour (both abdomen and head capsule) changes and abdominal length increases. Also, number of days required to complete instar stage increases as the development progresses.

The sex of these larvae of *S. ejectana* can be determined from the fifth instar stage onwards. The female larvae tend to have a more reddish colour and males are greenish in colour. The body lengths of fifth instar male and female larvae were 12 to 14 mm and 15 to 16 mm respectively. Similarly, the lengths of the head capsules of male and female larvae differ slightly. The head capsules of males are smaller (4.3 to 4.35 mm) than those of females (4.35 to 4.4 mm). Sixth instar male (Fig 3.6) larvae are yellowish or greenish, 16 mm long and shorter than female larvae. Females (Fig 3.7) are 18 mm long and reddish in colour. As the larvae matured the head capsule becomes black and shiny.

3.3.3.3 Life cycle studies

Generally *S. ejectana* can complete its life cycle within 63 to 91 days in a temperature range of 19 to 22°C (Table 3.9). Eggs and adults were reared at a constant temperature of 19.0±1°C, all other stages were reared at 22°C.

Table 3.9 Duration's of larval and pupal stages of *S. ejectana* at 22°C and 60 to 65 per cent r.h. and eggs and adult moths at 19±1°C (glasshouse).

Stage	Mean (days)	Standard deviation	Range (days)
Eggs	6.7	1.08	5-9
Larvae	41.56	4.07	38 - 52
Pupae	9.86	1.07	8-12
Adults	16.92	1.7	15-21
Total	75.04	7.92	66 - 94

Hence, if the environmental conditions are suitable, three to four generations within a year are quiet possible. Of all the stages eggs require a comparatively shorter time (5 to 9 days) at 19.0°C to become larvae.

Larval stages require a longer time to become pupae. Damage caused by the larvae to *T. calycina* plants is very significant as a result of continuous feeding for 35 to 52 days. The adult moths live up to 21 days and begin to lay eggs five to seven days following their emergence.

The number of days required to complete the larval stages varies slightly at the two temperatures. At 22°C, 38 to 52 days (Table 3.10) are necessary for completion of these stages (from second to sixth instar stage). On the other hand, only 35 to 47 days are sufficient for completion of these stages at 25°C (Table 3.11). Similarly pupae require 8 to 12 days to become adults at 22°C and this time is reduced to 7 to 9 days at 25°C.

Table 3.10 Duration of various instars at 22°C and 60 to 65 per cent r.h (at controlled temperature conditions).

Instar stage	Mean (days)	Standard deviation	Range (days)
First	3.76	0.76	3 - 4
Second	5.9	0.83	5 - 7
Third	8.04	1.0	6 - 9
Fourth	8.85	0.91	7 - 10
Fifth	9.47	0.6	9 - 11
Sixth	9.3	0.73	8 - 11
Total	41.56	4.07	38 - 52
Pupae	10.8	0.9	8 - 12

Table 3.11 Duration of various instars at 25°C and 40 to 45 per cent r.h (at controlled temperature conditions).

Instar stage	Mean (days)	Standard deviation	Average (days)
First	3.76	0.76	3 - 4
Second	4.89	0.73	4 - 6
Third	6.57	0.9	5 - 8
Fourth	6.63	0.83	6 - 8
Fifth	9.21	0.97	9 - 11
Sixth	8.78	0.63	8 - 10
Total	39.84	4.82	35 - 47
Pupae	8.03	0.95	7 - 9

Figure 3.6 A male *S. ejectana* larva

Figure 3.7 A female *S. ejectana* larva .

3.3.3.4 Morphology

Eggs - Newly laid eggs are ellipsoid in shape, with a length of 0.98 to 0.99 mm (Figure 3.4) and they are transparent. As they develop, the eggs become a yellowish creamy colour and their surfaces become raised slightly. The mature eggs are shiny and their colour changes to reddish yellow 24 to 48 h before hatching. Once the larvae have emerged from the eggs, the damaged shells usually adhere on the leaves. No injury or oviposition marks were observed on the leaves.

Pupae - The male pupae of *S. ejectana* tended to be smaller (8 mm) than the females (8.4 mm) and light red in colour, (Figure 3.8). A period of 8 to 12 days is required for the pupae to become adults at a constant temperature of 22°C and 60 to 65 per cent relative humidity. However, at 25°C and 40 to 45 per cent relative humidity, seven to nine days are sufficient.

Adults - Adult male *S. ejectana* are smaller (1.08 mm) than the females (1.12 mm) and lighter grey in colour (Figure 3.9 and 3.10). The males had faint spot markings on their forewings whereas these markings were larger and much darker in the females. The forewings are slightly dilated posteriorly, light ashy-grey with a broad dark suffused central streak. The latter are better defined and darker in the female. The male has a tuft of long raised scale on the basal one-third of its anterior margin. The tuft may often be concealed under the folded margin. Its hind wings are dark grey.

3.3.3.5 Feeding pattern

The growth rate of larvae is positively correlated with the amount of food consumed. It was not possible to establish the feeding pattern of the larvae in shelters, but in the medium it was observed that the larvae feed in bursts. The larvae feed continuously for 0.5 to 1 h at any time of the day (night and/or day). Similarly, the adults were also found to feed on the honey solution or flowers in bursts, rather than continuously.

Fig 3.8: Male (right) and female (left) pupae of *S. ejectana*.

Figure 3.9 Adults of male (right) and female (left) *S. ejectana*.

Figure 3.10 A female adult of *S. ejectana*.

3.3.3.6 Alternative hosts

The larvae that were maintained on other plants (beans, strawberries and capsicums) did not construct webbing shelters, nor did they feed. They moved away from the plants onto the bottom of the cages for the first 6 to 9 h and later they settled in top or sides of the cages. Most of these larvae died of starvation within seven to twelve days.

3.3.3.7 The effects of moulding of the webbing shelters on larvae

Fungal activity is usually associated with conditions of high relative humidity that may represent a hostile climate to some insect species. For this reason, the effects of fungi in the local environment on the development of *S. ejectana* was investigated. Occasionally, during spring and autumn periods some field-collected samples were covered with mould, but the larvae were unaffected. The laboratory studies conducted by inoculating mould on shelters indicate that the intensity of moulding was higher for those larvae that were maintained in a controlled temperature room at 19°C than those at 22°C or 25°C. Twenty per cent of the third instar larval shelters that were maintained at 19°C were affected by this mould, however, not a single larva (fourth to sixth instar) was affected. In general the mould spread to the first three to five leaves of the webbing shelter. Hence, it did not reach the sites of larvae, however, due to the lower compactness of third larval instar shelters, a small proportion of the larvae was affected and eventually killed. This mould generally encircles the larvae and the affected larvae became mummified and changed their colour and it took three to six days for the mould to kill the larvae. In other cases the shelters could have acted as a protective barrier against this mould, hence most of the larvae survived.

On the other hand, 90 per cent of the third to sixth instar larvae that were placed without webbing shelters at a temperature of 19°C and 70 to 75 per cent relative humidity in both individually and group rearing were affected by this mould. Most of these affected larvae died within three to five days following infestation. However,

some fifth and sixth instar larvae took seven to nine days to be killed by these moulds. Those larvae that were maintained at 22 and 25°C were less affected, (40 and 30 per cent respectively of the larvae died as the result of moulding) presumably due to the comparatively higher temperature and low relative humidity. The spread of mould was more rapid in large containers (61 x 40 x 25 mm, group rearing) than in small containers (30 mL, individual rearing). Moreover, moulding was accelerated by damp conditions. In the plants, that were maintained in a glass-house, the fungi developed sparsely on terminal leaves, presumably because conditions were not conducive for their development. Hence, due to the lower infestation, not a single larva was affected.

Mouldings also observed in the laboratory-reared larvae that were not artificially inoculated with these fungi. These mould infestations were reduced considerably by placing a tissue in the lids or the containers. This tissue needs to be changed once it becomes damp or once in four to five day intervals. Moreover, these infestations were reduced to a certain extent by spraying 70 per cent ethanol on lids and the surrounding environment and allowing the ethanol to evaporate.

3.3.3.8 Predatory mites, Phytoseiulus persimilis

P. persimilis infestations were severe in both the containers (individual and group rearing) to which the medium had been added, and at all temperatures (19, 22 and 25°C) investigated. Comparatively higher numbers of infestations were observed in group rearing at 25 and 22°C than at 19°C as these mites fare well at higher temperatures. These mites multiplied rapidly and feed on the larvae, pupae and adults. A container (10 to 12 larvae of any instar stage) can be consumed by these mites within three to five days of infestation. Generally, groups (15 to 30 individuals) of mites were found attacking an individual; a larva or adult can be completely devoured (except for the larval head capsule and adult wings) within three to four days following infestation. Similarly, pupae can be completely devoured by these mites (except the pupal case) within five to seven days following infestation. Once, the mites devour the insects,

they eat the medium which disintegrates, and is transformed into dust within three to four days.

These mites prefer damp conditions, and opening the containers once in three to four days reduced the relative humidity and facilitated fresh air circulation. Moreover, placing a tissue on the lids absorbs the condensed moisture and reduces the multiplication of predatory mites. The intensity of this mite attack on the larvae that were placed with their webbing shelters was inconsiderable. As it was mentioned earlier, damp conditions are essential for their survival. Although, most of these shelters contained condensed water vapour, only 10 per cent of the second to fourth instar larvae were affected.

3.3.3.9 Cold hardiness

The field temperature during night at Horsham during winter months (most of the mid winter) plunges to sub-zero, but the day temperatures reach 10 to 15°C. During this period most of the larvae undergo diapause and other stages were not sighted. Hence, in order to investigate the tolerance to continuous lower temperatures by various stages of this insect, they were exposed to a constant temperature of 4°C. As mentioned earlier, the field temperature was not constant, hence, some stages can tolerate this condition. However, it can be assumed that the survival rate and development at a constant sub-optimum temperature would be entirely different.

Eggs – The leaves carrying young eggs placed on the medium were in good condition for the first 10 days, but none of the eggs hatched. The leaves began to change colour from day 11 onwards and condensation was observed in some of the cups (20 per cent). Most of the (70 per cent) eggs showed yellowing, as would live eggs, three to four days after placement. This suggests that these eggs may have been developing, but they failed to hatch. These eggs remained unchanged (without any morphological changes) for 15 to 20 days, after this period these eggs became yellowish brown and eventually desiccated.

All the mature eggs remained as they were, without any colour or apparent physiological changes. However, at the end of seven days most of the eggs turned brown and began to shrink.

***Larvae, Method 1*:** Results obtained from these studies are presented in Table 3.12. The data from this table suggest that the larvae that remained in their webbing shelters were active for longer periods than those larvae that were without webbing shelters. These larvae were active for seven to eight days (fifth instar larvae) seven to nine days (sixth instar larvae), and after this period they became less active. Both fifth and sixth instar stages responded in almost the same way at this temperature. The webbing shelters could have acted as a thermal barrier that helped to retain some heat of respiration within the shelter. Samples collected from Horsham during a rainy period also showed that most of the rain water reached only the first three outer leaves of the webbing shelters, hence, the larvae were protected from the rain water by the webbing shelters. This might have helped the larvae to remain alive and active for longer. However, those larvae of *S. ejectana* that were kept at 4°C did not develop any further. Although these larvae were dead within 15 to 20 days of placing them at this temperature, their external appearance remained similar to other healthy larvae for up to 40 days i.e. they exhibited no change in colour or shape. However, once these were transferred from 4°C to a 22°C controlled temperature room, all the larvae showed signs of shrinking (within 48 to 72 h of placing them), and 24 h following this the body fluids of the larvae begun to ooze from their abdomens. The head and thorax segments were unaffected and remained intact.

Faeces observed for the first seven days from these larvae indicate that either the already digested (might have eaten while in the normal temperature) food material were excreted or these were feeding for at least the first two or three days. The amount of excrement observed gradually reduced, and this indicates either that only some of the larvae were feeding for the first few days or that feeding reduced as time progressed, or simply that feeding stopped rather abruptly, but the food took a while to be digested and excreted. It should be noted that, in the fumigation trials, it was noted that severely

affected and affected larvae continued to excrete faeces for one to three days even though they did not feed.

Method 2: All the larvae that were maintained without webbing shelters died earlier (7 to 15 days) than the larvae that were kept with their webbing shelters (15 to 20 days). Less than half of the larvae (40 per cent) constructed webbing shelters on the sides of the medium and a very few were found beneath the medium. Twenty per cent of the larvae remained on top of the medium without constructing shelters. These larvae became inactive within four to nine days and died earlier (within seven to nine days) following placement. The rest of the larvae were found unsettled (on the sides or lids of the container). Generally, larvae were active for first four to nine days and they became less active after this period. Although, excrement was observed for the first three days, there was no sign of feeding on the medium as evidenced by the absence of nibbling. This suggests that all the larvae cease feeding soon after being placed in this temperature.

Method 3: These larvae took 5 to 12 days for complete mortality to occur, and only a small number of larvae (15 per cent) constructed webbing shelters on the side of the medium. Twenty five per cent of the larvae were found on the medium and rest were found on the lids sides of the cups. These larvae were active for a shorter period (three to six days). Most of the (75 per cent) fifth instar larvae were dead within three to four days of placing at this temperature and remaining larvae were dead within five to twelve days. Excrement was observed for the first two days, however, absence of feeding signs on the medium suggests that they seldom fed during this period. The results indicate that larvae were unable to carry out their normal functions at this temperature.

Pupae - Only three (two females and a male) mature, nine days old, pupae hatched 4 days after placement, and all others were still in the pupal stage for up to 15 days at this temperature. The behaviour of these adults that emerged was similar to other normal

Table 3.12 Response of fifth and sixth instar larvae to 4°C in a controlled temperature room.

Method	Insect status (active) days						Time taken for mortality (days)			Faces
	Fifth instar			Sixth instar			Average	S .D	Range	
	Average	S.De	Range	Average	S.D	Range				
1) webbing shelters	7.2	0.41	7-8	7.5	0.82	7-9	17.7	1.52	15-20	first 7 days
2)medium and pre-treated	4.85*	1.59	4-9	-	-		8.55	2.37	7-15	first 3 days
3) medium and directly on cool room	3.65*	1.08	3-6	-	-		6.5	2.01	5-12	first 2 days

*- for both fifth and sixth instar larvae

adults and completed their life-cycle similar to other adults. The colour or shape of unhatched pupae remained the same during this exposure period. Fifteen days after these pupae were transferred to the controlled temperature room of 22°C, nine pupae hatched and developed into normal adults. All the unhatched pupae changed its colour to black within 60 to 72 h, shrank and failed to hatch.

3.4 Discussion

Laboratory experiments show that the larvae can be reared successfully in modified lightbrown apple moth medium. Although the success rate for first instar larvae in this medium was very low, it is possible to rear from second or third instar larvae. In the medium there are possibilities for moulding or predatory mite attacks, that may completely destroy the culture. These infestations can be reduced to a certain extent by taking precautionary measures such as circulating air in the containers and placing a tissue on the lid. However, in the plants fungus moulding was much less and predatory mite infestation was virtually nil, presumably due to the unfavourable environmental conditions. In the laboratory experiments (moulding and predatory mite attack were induced by creating favourable conditions for them) 19°C control temperature condition is favourable for moulding and 22, and 25°C are favourable for predatory mite attack. It was observed that the larvae were less affected if they remained within their webbing shelters (fourth to sixth instar stages). This may be due to the compact nature of larval webbing shelters. Due to its less compact nature of webbing shelters, third instar larvae are vulnerable to both fungus mould and *P. persimilis* attacks.

Temperature has a significant effect on metabolism and physiological function of most of the growth stages of insects (Scriber and Slansky, 1981). The majority of the insects are ectothermic (produce insufficient metabolic heat to elevate body temperature), hence, they rely on external thermal energy (Casey, 1993). The larvae and pupae that were maintained in a constant temperature of 4°C failed to develop further and died within 20 to 25 days and during this period all these stages ceased growth and

development. Also, experiments conducted at temperatures of 19, 22 and 25°C indicate that as the temperature increases the time required for development is reduced. This was further substantiated by the samples obtained from Horsham. Larval numbers were higher and growth rate was most rapid during the warmer months than the cooler months. However, high temperatures (40°C and above) are lethal to all stages.

This fact was further substantiated by the findings of Common (1990). He indicated that in general, growth of larvae mainly depends on temperature, relative humidity and other physical factors in the environment. Below or above certain temperatures feeding and growth ceases. Such suspension of activities temporarily leads to quiescent stages. Samples collected during mid and late winter from Horsham, where the field temperature occasionally goes down to sub zero during night and the day length is nine or ten hours, might have induce diapause condition. Samples collected from the field suggest that all the first to fourth instar larvae had died, some third and fourth instar had survived and most of the fifth and second instars survived. This indicates that mature larval stages can withstand these adverse weather conditions to a certain extent. Moreover, Common (1990) suggests that those insects that pass through two or more generations per year may become quiescent under unfavourable environmental conditions. The life-cycle studies of *S. ejectana* confirm that this insect undergoes three to four generations per year. This would provide more efficient way of surviving through temporary adverse environmental conditions.

The larvae undergo six instar stages and except first instar larvae all other instar stages construct webbing shelters using leaves. The first instar larvae construct shelters using silken thread in young single leaves. Pupation generally took place in thin cocoon with in a thin webbing shelter and under the abnormal (drought or lesser foliage) conditions pupation also possible just outside the webbing shelters. The adults are active mainly during warm weather periods. Eggs are laid in burst, once an adult moth is ready to lay eggs, hence, overlapping generations in an year are highly possible.

Chapter - 4

Large scale fumigation trials using Phosfume®

Chapter 4

Large scale fumigation trials using Phosfume®

4.1 Objectives of the experiment

One of the principal hypothesis to be tested in this work is that phosphine can be used commercially to disinfest flowers. The choice of phosphine was made, not only after having completed a detailed literature search, but also after conducting some preliminary experiments described in Appendix I. The experiments aimed to evaluate the phytotoxicity of phosphine and carbon disulphide, and to determine typical concentration and time required if phosphine is used to disinfest wildflowers. The preliminary experiments showed:

- carbon disulfide is highly toxic to insects, flowers and foliage; and
- although phosphine fumigations did not result in complete mortality, it is observed that increasing the exposure time subsequently results in mortality, without causing severe damage to the exposed flowers or foliage.

For these reasons it was decided to reject the hypothesis that carbon disulfide might be an alternative to methyl bromide in commercial fumigations. The hypothesis that phosphine might be a practical alternative was retained, even though exposure times longer than those presently used by industry might be required. To test this latter hypothesis under commercial conditions, a series of large-scale field trials was conducted.

As part of the research presented in this thesis, a large range of protocols for breeding several species of insects was developed. Methods for assessing the effects of fumigation on all stages of insect development were also developed and refined as part of this research. These are reported in this chapter.

Conditions in the laboratory and large scale fumigations differ in the degree of control that can be achieved over them: laboratory experiments can usually be reasonably well controlled, whereas commercial scale experiments are subject to numerous vagaries. For example, the temperature in a commercial chamber is influenced by the external ambient conditions, and the concentration of the fumigant may be locally diminished as a result of leakage of air from outside. For these reasons it is necessary to monitor key variables that are likely to affect the efficacy of a fumigation throughout each treatment.

Large-scale fumigation trials were designed to investigate the insecticidal efficacy of phosphine at various concentrations and at various exposure times (short: 4.5 to 6 h and long: 14 to 18 h) to a range of insect pests and their developmental stages, which commonly infest wild and cut flowers. The experiments were conducted in three different chambers (at various locations in Victoria) used for commercial and experimental fumigations.

4.2 Materials and methods

4.2.1 Bioassay insects used in large scale fumigations

4.2.1.1 The leaf rolling moth, Strepsicrates ejectana

Eggs - The life cycle and biology studies conducted in the laboratory indicate that once the eggs were removed from plants, even with the leaves intact with their stem, the survival rate was zero. The lethality of phosphine to eggs was not therefore investigated.

Larvae - Leaf rolling moth larvae (second to sixth instar stages) were collected randomly together with their webbing shelters by cutting the stems (50 to 60 mm below the webbings) using secateurs. Collections were made every month from a commercial

T. calycina plantation at Horsham, Victoria. The larvae were transferred to a laboratory at Knoxfield, Victoria in various 2 to 4 L polyethylene containers (50 to 70 webbing shelters per container). These containers were covered with muslin cloth tops secured with rubber bands. The collected shelters were trimmed and transferred into rectangular, lidded polyethylene containers (10 to 12 larvae with shelters per container) with dimensions of 90 x 90 x 180 mm. The modified lightbrown apple moth medium was used to line the bottom of the container (2 to 3 mm thickness) with grooves (0.7 to 0.9 mm width) in which the larvae might take refuge. The lids were each fitted with a tissue to absorb the condensed moisture to avoid moulding, and this tissue also acted as an anchorage for the pupae. Just before pupation, the mature larvae move towards the lids and pupate on this tissue.

The larvae were maintained at a controlled temperature of 22°C, and a relative humidity of 60 to 65 per cent. The conditions of the larvae were observed on a daily basis. If moulding or predatory mite attacks were observed (using a microscope or the naked eye) on the medium, the larvae were transferred into new containers filled with fresh medium, and the infested medium was destroyed. If the larvae were infested with the mites or mould these were disposed of by autoclaving them.

Pupae - Once the larvae reached the late sixth instar stage (just 24 to 48 h prior to pupation) most of them (80 to 90 per cent) migrated towards the lid or side of the container and pupated at these sites. Once the larvae became pupae they were allowed to remain in that position for another 24 h, because immediately after pupation the pupae were soft, hence, they were prone to be damaged easily. They require a certain time (24 to 48 h) to become sclerotised. As the pupae became sclerotised they changed from light red to a deeper red in colour (females) or brown (males), after which 10 pupae were transferred into lidded 200 mL polyethylene cups. These were maintained in a controlled temperature room of 25°C and 40 to 45 per cent relative humidity. Batches of pupae were allocated to different age groups, one to three days old pupae were classified as young pupae, and four days or above were classified as mature pupae.

Two to three hours before fumigation, the pupae (young and mature) were transferred separately into different lidded 200 mL polyethylene containers (1 to 10 pupae per container) based on the age (days) by visual identification and transferred to the fumigation site. The excess pupae were kept under cool conditions (4°C) for the purpose of maintaining the colony for the fumigation experiments to be described in this chapter. However, the pupae that were maintained under these conditions for more than 12 days were not used in the experiments because of their lower survival rate which may have given rise to misleading results if they were used in fumigations.

4.2.1.2 The lightbrown apple moth, Epiphyas postvittana

The eggs, larvae, pupae and adults of the lightbrown apple moth were obtained by starting a culture using egg masses of known ages that had been maintained at a constant temperature of 4°C. These were obtained from the Institute for Horticultural Development, Knoxfield, Victoria.

Eggs - The egg masses (young eggs of one to three days old) were transferred into lidded rectangular polyethylene containers with dimensions of 90 x 90 x 180 mm. These eggs were laid on the side-wall of the 200 mL cups and the cups were cut longitudinally (5 to 7 mm width and 50 to 60 mm length). Each container was supplied with 10 to 15 egg masses and each mass contained 25 to 30 live eggs. A tissue was placed under the lid to absorb condensed moisture, and these containers were maintained in a controlled temperature room of 25°C, and 40 to 45 per cent relative humidity.

Larvae - Once the egg masses changed their colour from green to brown (five to seven days), they were transferred onto an artificial diet, eponymously designated the modified lightbrown apple moth medium. Each container was filled with 95 to 100 g of this medium (2 to 3 mm thickness), fitted with a lid and tissue and the medium was cut in to rectangular pieces (5 to 7 mm x 3 to 5 mm) using a sterilised blunt knife to

provide refuges for the larvae. The tissue paper not only provided the mature larvae a suitable place for pupation, but it also absorbed condensed moisture thereby preventing moulding. Moreover, this tissue reduced the likelihood of infestation by the predatory mite *P. persimilis*. The larvae were kept in three controlled temperature rooms maintained at 19, 22 and 25°C. The lower temperature (19°C) reduced the growth rate and the high temperatures accelerated the growth rate, thus ensuring a continuous supply of all instar stages was maintained at all times.

Once the larvae had reached the fourth instar stage, only 20 to 25 larvae per container were retained to avoid over crowding and competition. The rest of the larvae were transferred to new containers. The medium and larvae sometimes became infested with predatory mites; to avoid this, the tissue was changed regularly, and the container had to be opened regularly to circulate fresh air. If the medium and larvae were infested with the predatory mites they were disposed of by autoclaving.

Pupae - Just before pupation, the mature sixth instar larvae migrate towards the lids of the containers, and to pupate on the tissue or on the lid itself. Immediately after pupation they were green in colour and delicate in nature, hence they were prone to damage whilst being handled. They were therefore allowed to harden during which time their colour changed from green to brown, a process that takes 24 to 48 h. Once these pupae were ready to handle, they were removed carefully from the surfaces using a pair of forceps. These pupae were used either for fumigation or for breeding purposes. For breeding purposes, the pupae were sexed and transferred into 200 mL plastic cups (six male and six female pupae per cup) fitted with lids. These were maintained at a controlled temperature of 25°C and 40 to 45 per cent relative humidity and these pupae generally take 10 to 12 days to emerge at this temperature. Sometimes these pupae were attacked by common sugar ants *C. consobrinus*. To prevent the attack by common sugar ants the cups (12 to 15) were placed in a plastic tray (450 x 300 x 70 mm) that was filled with 500 to 600 mL of water to which disinfectant had been added.

Two to three hours before fumigation mature and young pupae were separated and transferred into different similar dimension cups as described in the previous paragraph. Each cup was provided with 10 pupae and these cups were covered with a muslin cloth that was secured with a rubber band.

Adults - Sometimes a small number of pupae failed to hatch because they were damaged whilst they were being handled or they were attacked by predatory mites. Hence, all the containers were checked once the pupae emerged, and the gender ratio of males to females was maintained one to one (six males and six females). Sex differentiation of adults was achieved based on morphological characteristics; females are generally larger than males, and males have distinctive markings on the base of their wings - markings which are absent in females. Once adults emerged, an aspirator was used to transfer the adults into 200 mL grooved plastic cups fitted with lids. The egg laying capacity was affected if the adults were maintained in smooth plastic cups.

Each lid was perforated with 8 to 10 fine holes made by a needle to facilitate air circulation. The adults were fed with 10 per cent honey solution (3 to 5 mL per cup) soaked in absorbent cotton wool and placed in small plastic cups. This was replaced with fresh honey solution at intervals of three to four days. The adults began laying eggs six to seven days after their emergence and continued to lay up to 14 to 15 days. The eggs were laid in masses in the grooves and sometimes on the lids of the cups. Once, the adults completed laying eggs, adults were removed and disposed of carefully by autoclaving them.

The cups that were used for maintaining adults were cut into 15 to 18 longitudinal pieces (7 to 10 mm), using a pair of scissors each piece containing one or two egg masses. Immediately after they had been laid the eggs were green, and their colour changed to brown five to seven days after laying (two to three days before hatching). Only green eggs (one to three days old) were stored to maintain the colony, and these were (50 to 70 pieces of cup on which eggs had been laid) placed in sealable plastic bags and stored in a cool room maintained at 4°C. For fumigation purposes, eggs of one to seven days old were used and these were placed in 90 x 90 x 180 mm

rectangular polyethylene containers (10 to 15 pieces per container). All the eggs that were kept in the cool room (4°C) for 20 days, and those that were more than 20 days old were destroyed, because their lower survival rate may yield unreliable results if used in fumigation experiments. Various larval instar stages were obtained by incubating large batches of eggs at intervals of 15 to 20 days and maintaining them at three different temperatures.

The eggs, larvae, pupae and adults were examined regularly, and just before the large scale fumigation experiments were performed. Any discoloured or deformed individuals resulting from the infestation of predatory mites or moulding or damaged while handling were discarded. Only healthy individuals were used for fumigation. Sometimes, a small number of eggs failed to hatch, perhaps because they were unfertilised. It is impossible to identify whether the eggs were fertilised or not until the larvae had emerged. Hence, while fumigating eggs, the batches of eggs for fumigation and control were chosen from the same cup, and the control eggs provided an idea of the fertilised status of the young eggs. However, the fertilised status of mature eggs can be easily distinguished from the well formed larval head that can be observed through the chorion when viewed under a microscope.

For fumigation purposes young (one to seven days old) and mature (eight days and above) adults were used. These adults were transferred into different 200 mL polyethylene cups (10 adults per cup) that were covered with *Terylene* cloth and secured with a rubber band.

4.2.1.3 The two-spotted mite, Tetranychus urticae

Various life stages (eggs, larvae, and adults) of two-spotted mites were obtained from a large scale export-oriented rose plantation near Officer, Victoria. These were immediately transferred onto two to three leaf stage haricot bean seedlings (12 to 15 days old) at the Institute for Horticultural Development, Knoxfield, Victoria. To maintain this colony haricot bean seedlings were established by planting them in

batches in seven to nine days intervals. Haricot bean seeds (75 to 80 seeds per tray) were planted at 20 x 30 mm intervals, and 18 to 20 mm deep in rectangular perforated plastic trays with dimension of 140 x 110 x 45 mm. This container was filled with sterilised potting mix called Defender®.

The seedlings were maintained closely spaced, and this facilitated easy movement of larvae, and adults from one seedling to another, also this helps to maintain the *T. urticae* population growth. A 150 x 120 x 20 mm tray was placed under the seedling tray to collect excess water and this water was disposed of promptly. Immediately after the sowing of seeds, water was sprayed sparingly and repeated every three days or whenever signs of drying of the upper surface (0.5 to 1 mm) of the soil was observed. This method of watering reduced microbial attacks on the seeds and reduced attacks of fungus gnats, *Bradysia coprophila*.

Frequently, a large number of fungus gnats was observed among the seedlings. The adults, as well as competing with the *T. urticae* for space, also act as a virus vector. Adult fungus gnats were captured by hanging a yellow sticky tape near to the seedling tray and this sticky trap was replaced after intervals of 12 to 15 days. The trays were treated with 1.5 g chlorpyrifos (14 per cent w/w) to avoid other soil-dwelling insect infestations. Once the seedlings reached two to three leaf stage, the infested leaves (one to two heavily infested leaves per row of seedlings) were transplanted randomly among the leaves of the seedlings. The culture was maintained in a controlled temperature room of 22°C and 60 to 65 per cent relative humidity. The photoperiod was extended by using a timer in the late evenings and early mornings (18:6 light:dark) by fitting two fluorescent lights (15W) 750 mm above the plant canopy. The mites were introduced into new seedlings every 20 to 22 days and the old culture was destroyed by autoclaving them. Beans were planted at intervals of 10 to 12 days to maintain the colony.

One week before fumigation experiment was carried out, heavily infested middle bean leaves (generally the middle leaves have a balanced population of all stages) were introduced into the bean seedlings planted individually in small plastic pots (400 to 500

mL capacity). These were maintained at the same controlled temperature conditions as mentioned in the previous paragraph.

Under unfavourable environmental conditions (lower temperature and short daylight) *T. urticae* undergoes a diapause stage, where metabolism and growth rates are very low. These conditions may lead to lower uptake of toxic chemicals consequently, complete mortality of all the exposed individuals may not be achieved. Hence, the response of diapause larvae to various fumigation conditions was studied. Diapause conditions were induced by reducing the photoperiod (8:16 light:dark hours), reducing the temperature to 19°C and increasing the population by inoculating more highly infested leaves into the seedlings. This latter strategy helped to increase the diapause stage by enhancing competition. A separate culture of this colony was maintained under these conditions.

Bean plants infested with mites (a large number infested young and mature eggs, nymphs, adults and diapause stage) were fumigated in plastic cone shape cages, the bases of which were 240 × 240 mm, and the tops of which were 450 × 450 mm. The cages were 700 mm high. The top and sides of the cages were fitted with muslin cloth with dimensions of 400 × 400 mm and 650 × 400 mm respectively.

4.2.1.4 The green peach aphid, Myzus persicae

The larvae and adults of green peach aphids were obtained from the Institute for Horticultural Development, Knoxfield, Victoria and reared in cabbage and capsicum seedlings. These were maintained in a glass house at a temperature of 19±1°C and a relative humidity of 40 to 45 per cent. These seedlings were planted in perforated plastic containers (300 to 500 mL) filled with sterilised potting mix and were placed on rectangular metal trays to collect water (dimension of 540 x 174 x 60 mm). Each tray was supplied with 8 pots (two to three seedlings per container). The seedlings were

placed in different iron framed muslin gauze covered rectangular cages (450 x 600 mm) to prevent predation by parasites and predators.

Due to the heavy infestation of aphids most seedlings showed yellowing of their middle and lower leaves within five to seven days of inoculation. Moreover, once the population of aphids had developed there was a greater possibility of the plants being attacked by sooty mould; infestations by ants were also high because of the honeydew secreted by the aphids. Hence, the infested leaves were transferred into new seedlings at intervals of 12 to 14 days, and old cultures were destroyed. Furthermore, this reduced over-crowding of aphids and prevented the development of apterous aphids. Heavy infestations of braconid parasitic wasps, *Aphidius matricariae* Haliday were observed in the colony, the adults of which lay eggs mainly on larvae; the infested larvae mummified and subsequently died. To prevent serious depletion of the aphids these adult wasps were captured by hanging one or two yellow sticky traps among the foliage. This strategy reduced the population of parasitic wasps considerably. Traps were changed every 15 to 20 days.

Two to three hours before large scale fumigation trials were carried out, four to six well established cabbage or capsicum plants with sufficient populations of all stages of aphids were transferred into muslin clothed topped and side plastic cages similar to those used for *T. urticae* for transportation to the site of the field trial.

4.2.1.5 The ornate aphid, Myzus ornatus Laing

The nymphal and adult stages of ornate aphids were obtained from large scale *T. calycina* plantations located near Horsham, Victoria. These were introduced onto potted *T. calycina* plants. These plants were housed in a galvanised iron cage (600 x 600 x 900 mm) fitted with *Terylene* gauze sides and tops. The plants were watered daily except at the weekend, and a rectangular plastic tray (120 x 70 mm) was placed underneath the pots to collect drained water. The plants, along with the aphids, were

maintained in a glass house with a temperature of 19±1°C and a relative humidity of 40 to 45 per cent.

The aphids secrete a honey solution and this attracts *C. consobrinus*. Sooty mould infestations were observed once the aphid population became high. Hence, the population of aphids was controlled by maintaining only a limited number of individuals (200 to 250 larvae and 150 to 180 adults) by removing excess aphids once a week using a fine camel hair brush. This strategy reduced the sugar ant and shooty mould infestations considerably. No parasitic wasp attacks were observed in these cages so the original population was presumed to be free of these parasites.

Due to their specific growing conditions and their limited availability in Melbourne it proved difficult to maintain more than six *T. calycina* plants. Hence, alternative hosts were sought. Although, the literature suggests that these aphids infest potato and strawberries, the infestations and multiplication rates on these plants that were maintained at the same environmental conditions were minimal. Outlined below are the procedures that were followed to rear this aphid. Potato seedlings (variety Desire) and strawberry runners (varieties Pajaro, Selva and Parker) grown in perforated plastic pots were obtained from the Institute for Horticultural Development, Knoxfield, Victoria. These plants were housed under similar conditions to the *T. calycina* plants; larvae and adults (70 to 90 individuals per plant) of this aphid were introduced from *T. calycina* plants to potato and strawberries using a fine camel hairbrush. The plants were fertilised (Osmocoat®) and watered.

4.2.1.6 The cabbage aphid, Brevicoryne brassicae (Linnaeus)

Larvae and adult cabbage aphids *B. brassicae* were collected from cauliflowers growing at Knoxfield, and introduced to Chinese cabbage and cabbage seedlings. Initially, the Chinese cabbage seeds (20 to 25 seeds per tray) were planted in perforated plastic rectangular seedlings trays (dimensions of 295 x 525 mm). Once these seedlings reached the 2 to 3 leaf stage they were transplanted individually in 20 mm

diameter plastic pots. The Chinese cabbages were initially housed individually in 310 x 310 x 450 mm square wooden cages of which the sides and top were fitted with *terylene* gauze cloth. However, to increase the multiplication and free movement of aphids among the seedlings, these seedlings were transferred to a large cage (5700 x 410 x 5400 mm) in which the side and top consisted of *terylene* gauze cloth and the door was made of transparent glass.

Cabbage seedlings were obtained from the Institute for Horticultural Development, Knoxfield, and maintained under conditions similar to those of the Chinese cabbage. These cabbages were placed in a glasshouse at a controlled temperature of 19±1°C and 50 to 55 per cent relative humidity. It was observed that the growth and multiplication of the aphids were higher in the Chinese cabbage than the cabbage seedlings. Hence, growing these aphids on cabbage seedlings was abandoned. In a similar way to the other populations of aphids reared as part of these experiments these aphids were also infested with braconid parasitic wasps, *Diaeretiella rapae* (M'Intosh). Hence, similar preventative methods as mentioned above were carried out.

4.2.1.7 The European earwig, Forficula auricularia

The young stages and adults of the European earwig were collected from among the flower petals of *Protea cynaroides* at a large-scale commercial plantation in Gembrook, Victoria. Earwigs were removed from the petals by using forceps without damaging the earwigs' appendages or bodies. Also, earwigs were collected from among the abandoned boxes or beneath plant pots at Knoxfield, Victoria. The adults (10 to 12 individuals) were transferred into 100 mm diameter and 40 mm high circular polyethylene containers fitted with lids and to which sultanas or fresh apple had been added. A 75 to 90 mm diameter orifice was made on the centre of lids using a drill and this was covered with a *terylene* gauze cloth to promote air circulation. The feed often became infested with microbes, hence, they were transferred into new containers with new feed added every seven to nine days. Moreover, cannibalism among individuals

was also noticed, hence, individuals of similar ages (five to seven individuals per container) were maintained in containers, with sufficient food.

Five to seven pieces (20 to 27 mm x 30 to 35 mm) of rectangular corrugated cardboard were placed on the bottom of the container to provide refuges for these individuals. These were maintained at a controlled temperature room of 25°C and 40 to 45 per cent relative humidity. Due to the specie's inclination for darkness, this latter condition was induced by placing two to three containers in a rectangular card board box (600 x 750 x 400 mm), with perforations for air circulation.

4.2.1.8 The meat ant, Iridomyrmex purpureus (F. Smith)

Meat ants *I. purpureus* were collected from a *Thryptomene* plantation, near Horsham, Victoria, by setting pitfall traps. A small amount of absorbent cotton wool that was soaked in 10 per cent honey or concentrated sugar solution was placed in 200 mL wide mouthed glass jar (dimension of 75 mm high and 60 mm diameter). The opening of this jar was lined with a thin layer of Fluon® (an aqueous dispersion of polytetra fluoro ethylene) supplied by Ajax Chemicals, Melbourne, Australia. A 75 to 77 mm x 60 to 63 mm hole was made on the ground where the activity of the ants was high, or near to a nest and the jar was buried in this hole. The ants were attracted by the honey solution and fell into this jar, but they were unable to escape because of the presence of Fluon® on the opening of jar.

These ants were transported to the Institute for Horticultural Development, Knoxfield, Victoria and they were kept in a cool room maintained at 4°C to reduce their activity. These ants are highly active and highly aggressive at normal room temperatures. Once the ants became inactive (after some 48 to 72 h at the cool temperature), 50 to 60 adults were transferred into 700 mL glass jars (90 mm diameter narrowing to 70 mm at the top and 125 mm high) using a pair of forceps without damaging them. The container was added with 25 to 30 g of sand collected from the nest. The ants were fed with a 10 per cent honey solution and sultanas (5 to 15 sultanas per jar) and the food was replenished

every 5 to 7 days. The honey solution (5 to 7 mL) was soaked in a small absorbent cotton wool pad that was kept in small plastic cups.

The lids of the jars were made of plastic and a hole (20 mm) was drilled through the middle and this hole was covered with a square wire mesh (22 x 22 mm) to promote the circulation of air. The openings of these jars were coated with a thin layer of Fluon® to prevent the movement of ants on the lids. These ants were kept in either a 4°C cool room or at a controlled temperature room at 14°C. Forty-eight to seventy-two hours before they were fumigated, batches of adults were transferred to a higher temperature of 25°C and 40 to 45 per cent relative humidity. The ants were kept for only 18 to 20 days (4°C) or 10 to 12 days (14°C) at these conditions. Beyond this time all were destroyed, because keeping them for a longer time was observed, in separate experiments, to result in their mortality within a further 7 to 10 days at 22°C naturally.

4.2.1.9 The soft brown scale, Coccus hesperidum Linnaeus

Soft brown scale *C. hesperidum* were collected from the stems of *T. calycina* plants at Horsham, Victoria, and transported to the Institute for Horticulture Development, Knoxfield. They were inoculated into potted *T. calycina,* strawberry runners (variety Silvan) and navy bean seedlings that were housed in different galvanised iron cages (700 x 700 x 750 mm). The cage sides and tops were fitted with *terylene* gauze and the base was formed from a single iron plate. Infested plants were maintained in a glasshouse at a constant temperature of 19±1°C, and 40 to 45 per cent relative humidity. The plants were watered daily except on Saturdays and Sundays, and the excess water was collected by placing a 450 x 250 mm rectangular tray beneath the pots.

The larvae and adults suck the sap from the stem, hence, heavily infested plants die quickly. To avoid this only a limited population of 50 to 70 larvae and adults was maintained. The excess individuals were removed using a pair of forceps and disposed of carefully by autoclaving them. Two to three hours before fumigation experiments were begun, stems with a healthy population of larvae and adults were selected and

removed from the plant (*Thryptomene*) using secateurs. If bean plants were used in the experiments, the entire plants were used, because bean plants are highly sensitive. If branches are cut-off from the plants, they tend to wilt rapidly. Also, whole strawberry plants were used for fumigation, because they were plentiful and easy to infest.

4.2.1.10 The predatory mite, Phytoseiulus persimilis

All stages of predatory mites *P. persimilis* were obtained from Bio Protection, Queensland, and were introduced into highly populated (40 to 50 individuals) fifth and sixth instar lightbrown apple moth larvae. The larvae of lightbrown apple moths were reared in exactly the same way as those used in the fumigation experiments, which is discussed in Section 4.2.1.2. The predatory mites prefer damp conditions; that was created by maintaining the culture in a controlled temperature room of 19°C and 70 to 75 per cent relative humidity. Once the predatory mites had been introduced into the medium the containers were tightly sealed and kept unopened for the first 15 to 20 days, thus inducing damp conditions. In order to avoid contaminating other cultures, the mites were kept separately and the populations were limited by destroying excess culture. Excess individuals were placed in plastic bags, and then disposed of carefully by autoclaving them.

4.2.1.11 Other insects

The adults of jumping spiders, nymphs and adults of Rutherglen bugs, *Nysius vinitor* Bergroth and adults of collembola were obtained from the foliage of *T. calycina*, and they were collected simultaneously with samples of *S. ejectana*. These were fumigated in their natural habitat within 24 to 48 h of collection.

4.2.1.12 Control insects

Two to twelve hours before fumigation, insects were selected randomly from the culture and divided equally into two batches. One batch was used for control and other

was used for fumigation. Both batches were maintained under identical conditions, save for the time one batch was being fumigated. During fumigations the control insects were maintained in control temperature rooms with a temperature range of 18 to 24°C. The conditions of these insects were assessed regularly and maintained until they complete their life cycle.

4.2.2 *Large scale fumigation trials*

4.2.2.1 *Fumigation chambers*

Large scale fumigation trials were conducted in three chambers, each with a different volume. The three chambers used may be specified briefly as:

- a cylindrical chamber with a volume of 900 L made of steel and coated with grey paint. This chamber is located at the Keith Turnbull Research Institute (KTRI), Frankston, Victoria.

- two chambers, each with an internal volume of 27 m^3 were used. They were converted shipping containers that were fabricated from steel, and their exterior surfaces were painted white to reflect solar radiation. They were located on the properties of commercial flower exporters at Gembrook and Emerald in the Dandenong Ranges, Victoria.

The chamber at Frankston is used for various experimental purposes (fumigating citrus or sultanas using either phosphine or dichlorvos), and the chambers at Emerald and Gembrook are used exclusively for commercial purposes (fumigating wild and cutflowers using either methyl bromide or Pestigas®-Insectigas® combinations), save for when they were used for the experiments described in this work.

4.2.2.2 27 m^3 modified shipping containers

Shipping containers at two locations namely Emerald and Gembrook, were used for the commercial scale fumigation experiments. The shipping container located at Emerald was fabricated from steel and with an internal volume of 27 m^3 (5,850 x 2,420 x 2,060 mm length, width and height respectively) coated with white paint to better reflect solar radiation and was used for most of the fumigations (Figure 4.1). It is presently located at Emerald, although some of the initial experiments were carried out when the chamber was located at Keysborough, an outer suburb of Melbourne. The interior of the chamber was lined with reinforced plastic and thermally insulated, except for the door located at the front of the container. The shipping containers were originally refrigerated, hence their construction resulted in their being thermally insulated. The chamber was resting on iron slabs (four corners of the chamber), 300 mm from the ground and a permanent shed was erected (900 mm above chamber) using corrugated iron sheets to protect the chamber from direct solar radiation and rain.

The door was fabricated from steel plate fitted with neoprene rubber seals. It hung from chains attached to a movable gantry mounted on top of the chamber and it was arranged so that the door could be moved completely to one side of the fumigation chamber. This allowed easy access to trolleys on which the flowers to be fumigated were placed. During fumigation the door was held in place by four steel lugs attached to bolts welded to the front of the chamber.

The cylinders containing the fumigants were housed in a cubicle (1400 x 500 x 1000 mm length, width and height respectively) attached to the back of the chamber. The fumigants were delivered into the chamber from the cylinders through a solenoid valve that was connected to a stainless steel pipe. This pipe was attached to the sidewall of the chamber, through a hole made on the back wall.

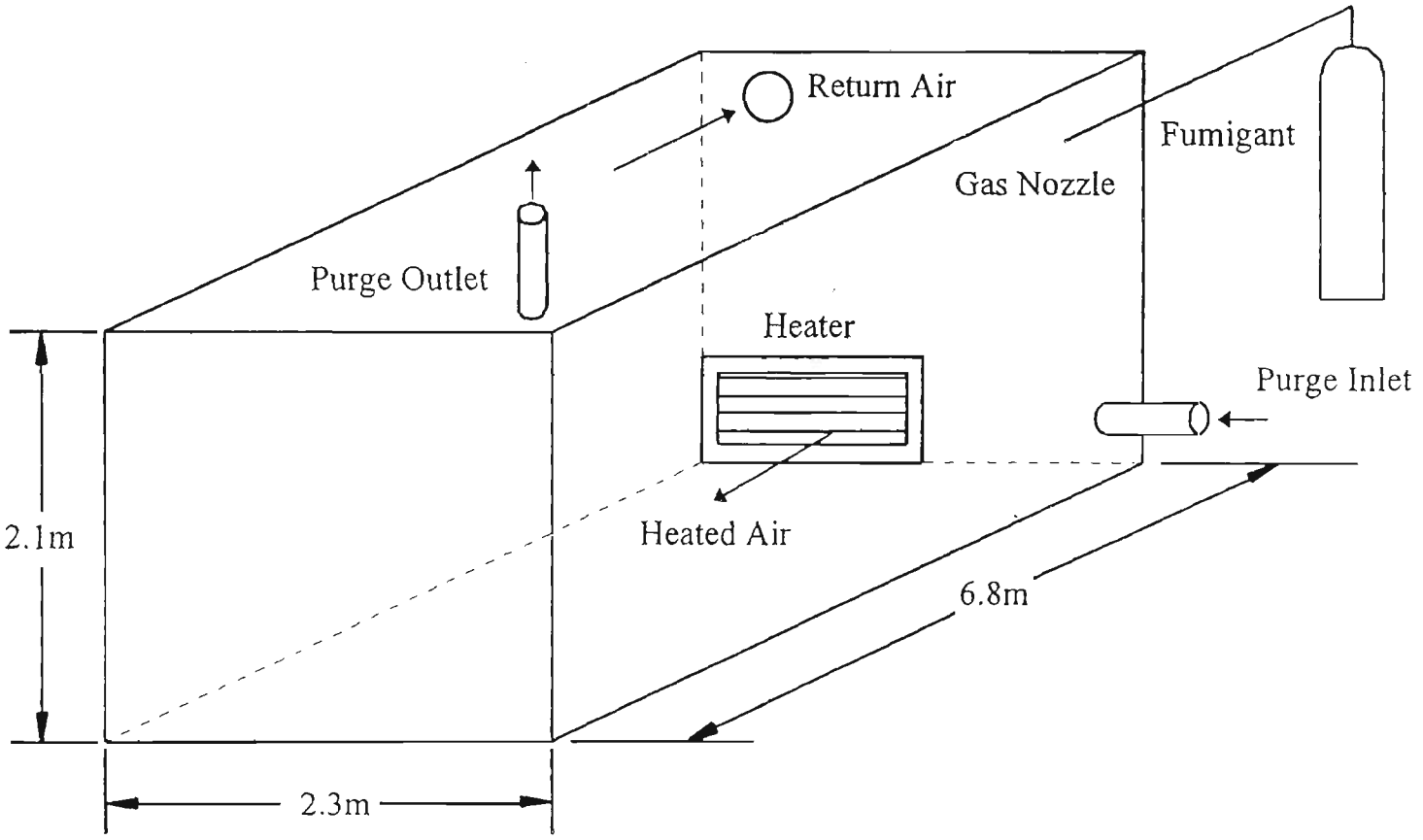


Figure 4.1 The $27m^3$ fumigation chamber at Emerald used for fumigation trials.

4.2.3 Fumigant

4.2.3.1 Phosfume®

Phosfume® (2 per cent phosphine mixed with carbon dioxide as a carrier gas) was supplied in a standard "C" type cylinder (70 kg tare weight and 40 kg net weight) by British Oxygen Gases (BOC), Preston, Victoria (formerly Commonwealth Industrial Gases) and was used for all the fumigation trials.

4.2.3.2 Fumigant delivery system - Emerald

Fumigant aerosols or gases were released into the chamber through a stainless steel nozzle attached to aluminium piping. The nozzle was placed 1.95 m above the floor on one of the side walls of the chamber, 3.8 m from the door. The aluminium pipe which delivered gas to the nozzle passed through a hole in the back wall of the chamber where it was connected to a solenoid valve operated by a timer. This was linked to a solenoid valve and flexible stainless steel pipe fitted with nuts for attachment to gas bottles. The solenoid regulated the gas input into the chamber, that was originally set up for commercial use with Pestigas® and Insectigas®, cylinders of which were connected to the flexible pipes. The commercial fumigation process involved a 5-sec burst of Pestigas®, followed 10 min later with a 10-sec burst of Insectigas®. To introduce Phosfume® into the chamber the Insectigas® cylinder was disconnected and replaced by a cylinder of Phosfume® in the same manner as was Insectigas®. At the back of the chamber there was a circular observation port so that it was possible to view the operation of the nozzle whilst the gas was being introduced into the chamber.

The gas delivery system was connected to an adjustable timer (1 to 10 sec) and by pressing the timer the required amount of gas was delivered in to the system. The amount of fumigant delivered into the chamber was controlled by placing the cylinder

on a digital scale (capacity of 150 kg). The mass of gas delivered was recorded. The active ingredient delivered while using a predetermined amount of Phosfume® was studied during different seasons of the year, and at different weights of the cylinder and are discussed in Chapter 8, Section 8.3.

The chamber used at Gembrook (Figure 4.2) was similar to the one located at Emerald, and described above except:

- it has a somewhat more sophisticated ducting system with a powerful fan used to purge the chamber of phosphine;
- the fumigant delivery system in located on the rear wall of the chamber, on the left hand side viewed from the front; and
- the floor was made from grooved pressed steel, which was covered by galvanised iron sheets to facilitate movement of trolleys of flowers.

4.2.4 Fumigation procedure

The fumigations were carried out either overnight, starting at 14:00 to 17:00 and terminating the following morning at 8:00 to 9:00 (long exposure, 14 h or more) or during the day, beginning at 8:00 or 9:00 and completed at 12:00 or 15:00 (short exposure, 4.5 to 6 h). These times fitted in with the logistics of the export of wildflowers.

Most of the fumigation experiments at Emerald were conducted using one or two trolleys (2,700 x 1,200 x 900 mm length, width and height respectively) on to which were placed 4 to 6 plastic buckets (20 L capacity). These buckets were filled with 7 to 9 L of tap water. These trolleys were made up of steel fitted with tyres for easy manoeuvring and each has two tiers. The distance between upper and lower tier was 1000 mm,

Figure 4.2 The $27m^3$ fumigation chamber at Gembrook used for fumigation trials.

hence, buckets filled with flower bunches could be easily placed on both these tiers. Flowers and foliage (five to seven bunches per bucket) were then placed in the buckets and transferred into both tiers of the trolley. The tiers of the trolleys were fabricated from a metal mesh with a very large open area, thus allowing fumigants and other gases to circulate with very little resistance.

The fumigations at Gembrook were conducted under slightly different, but commercially important, operating conditions. The difference was that the entire chamber was filled with flowers and foliage in order to maximise the throughput of floricultural produce (Figure 4.3). The upper and lower tier of metal trolleys (2,500 x 1,100 x 1,400 mm length, width and height respectively) were loaded with flower bunches in plastic buckets (15 L capacity) filled with 5 to 7 L of tap water. The tiers, or shelves of the trolleys used at Gembrook were fabricated from plates of iron. The biota were placed in the chamber as follows:

- the larvae of leaf rolling moths were placed among the flowers with their webbing shelters, or at different locations in the chamber along with modified lightbrown apple moth medium and with their webbing shelters. The pupae were placed in 200 mL polyethylene cups (7 to 10 pupae per cup); covered by a *terylene* cloth secured with a rubber band;

- the ligthbrown apple moth larvae were fumigated along with their medium in rectangular containers placed at various locations in the chamber. The pupae were placed in 200 mL plastic cups and covered on top with *terylene* cloth. The mature eggs and adults were fumigated in 200 mL polyethylene cups covered with a muslin gauze. The young eggs were fumigated by placing pieces of cups that contained eggs in rectangular polyethylene containers (dimension of 90 × 90 × 180 mm); and

- the two-spotted mites were housed in rectangular plastic cages with dimensions of 250 x 250 x 450 mm (length, width and height respectively) which were covered with *terylene* cloth on the top and sides (240 x 400 mm). Three to four cages of two-spotted mites were used in the fumigations, each cage housed with three to

Figure 4.3 Disposition of experimental flowers on a trolley.

five bean plants (18 to 20 days old) with on which populations of two-spotted mites were established.

- Aphids (green peach and cabbage aphids) were fumigated similarly as mentioned above. For ornate aphids, one or two small stems that contain 50 to 250 individuals (both adults and larvae) were cut from *T. calycina* plants and placed in rectangular polyethylene containers with dimensions of 90 × 90 × 180 mm and covered with a muslin cloth.

- The European earwigs were fumigated in rectangular polyethylene containers of 61 x 40 x 25 mm (10 to 12 earwigs per container) to which five or six pieces of corrugated cardboard (dimensions typically 15 x 25 mm) had been added. The rectangular containers were covered with a muslin cloth top secured by a rubber band.

- A 700 mL wide-mouthed lidded glass jar was used to house the meat ants and each jar contained 75 to 100 adults. The jar openings (2 mm) were coated with a thin layer of Fluon® to prevent the ants from escaping. These were fumigated with and without lids that were fitted with a wire mesh.

- All other insects (spiders and Rutherglen bugs) were fumigated along with the *T. calycina* foliage.

Because the larvae of the leaf rolling moth *S. ejectana*, have caused rejections at Japanese ports, this species was targeted in several of the initial large scale experiments. Green peach aphids were also studied in the initial experiments because of their ready availability. However, as the experimental techniques for rearing or trapping the remaining species were developed the responses of the full range of insect species described above were studied. The insects were placed in different tiers of the trolley (10 to 120 mm and/or 1,100 to 1,200 mm from the floor), and at various locations (at four corners and in the centre) of the chamber to determine whether or not there was any effect of the spatial distribution of phosphine concentration. Once the

flowers and insects were transferred into the chamber, it was made air tight by closing the door and vent, and the heater and fan were then switched on.

The fumigants were admitted once the chamber was heated to a temperature of 16°C or above and the temperature in the chamber was obtained through a portable lap-top computer that was connected to the Data Taker®. Phosfume® was added into the chamber after placing the cylinder on a digital weighing scale (capacity of 0 to 150 kg) by adjusting the timer to 1 to 10 sec of multiple bursts. Sometimes while delivering fumigants (if the required amount of Phosfume® was more than 1.5 kg) the chamber became pressurised as indicated by the walls of the chamber expanding outwards, with associated creaking sounds. This is undesirable because this may lead to the chamber losing its gas-tightness. To reduce the magnitude of the pressure rise, the fumigant was delivered in multiple bursts of 5 to 7 sec duration with intervals of 10 to 15 sec between each burst. Occasionally the vent was opened for 2 to 3 sec to reduce the pressure, if the chamber became pressurised.

4.2.5 Keith Turnbull Research Institute, Frankston

4.2.5.1 Fumigation chamber

A third series of experiments, which may be regarded as triplicating the set obtained in the commercial systems, was carried out in an intermediate scale (900 L) fumigation chamber (Figure 4.4). The chamber is located at the Keith Turnbull Research Institute located at Frankston, Victoria. It consists of a cylindrical metal tank with a length of 1350 mm and an internal diameter of 910 mm. The chamber is affixed with bolts to concrete slab and it is housed in a shed and the chamber is fitted with doors at either end. A removable sliding basket (1250 mm long and 850 mm high) was fitted 365 mm below from a sampling port on the top of the chamber and 200 mm above from the bottom of the chamber. This basket narrows to a width of 530 mm to the base in order to facilitate the movement.

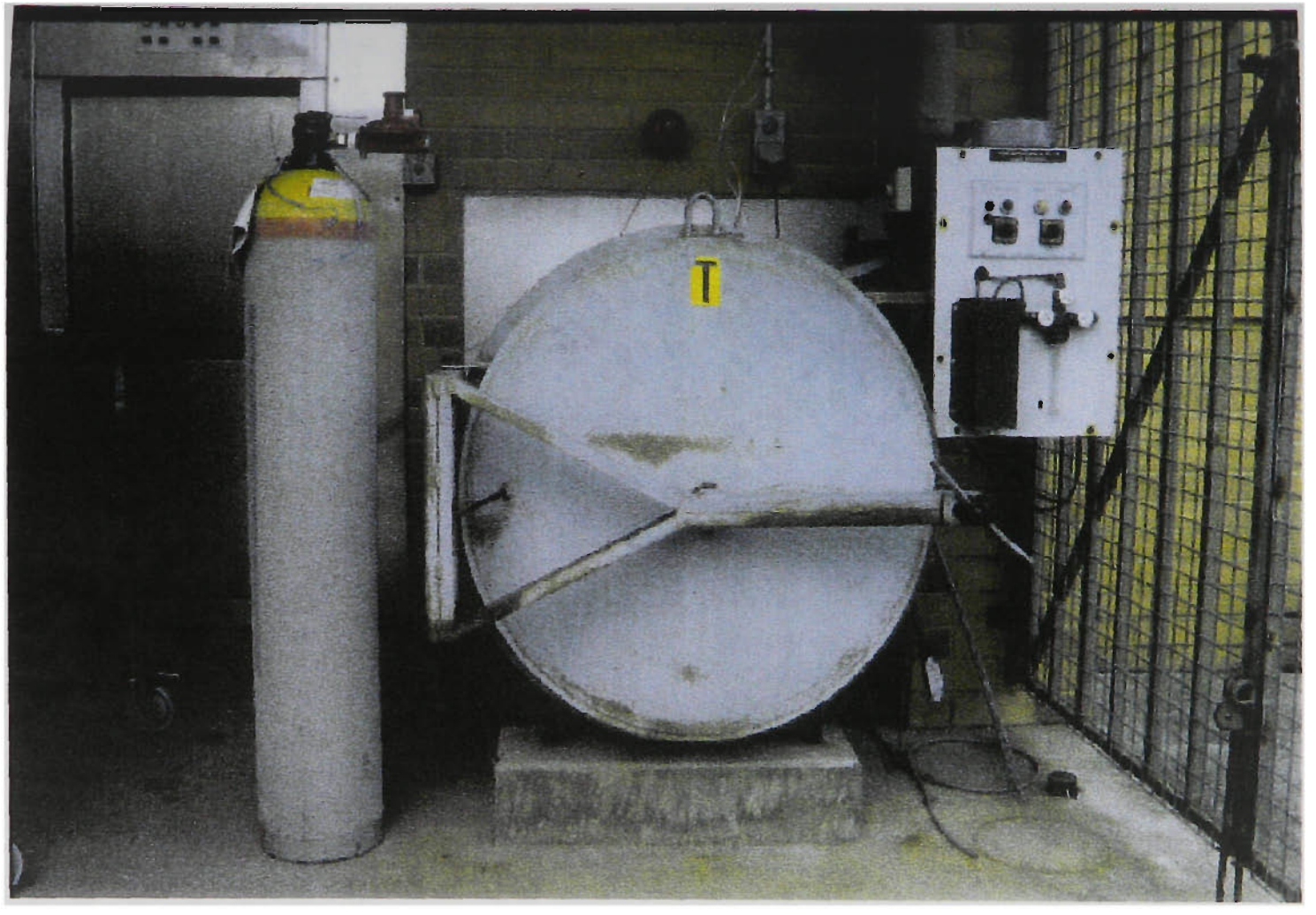


Figure 4.4 The 900L fumigation chamber at Frankston, Victoria used for fumigation trials

The fumigant was delivered and sampled through a 20 mm internal diameter sampling port that was located on top of the chamber, and which was 200 mm from the front door. A 1kW heater supplied by Helios Electric, Melbourne, controlled by a thermostat that can be set at temperatures between 0°C and 40°C was installed on the bottom of the chamber, 620 mm away from the front door of the chamber. The sensor of the thermostat was located just below the sliding basket (320 mm from the door), hence, this can be moved to any location that is deemed to be representative of the entire chamber. The power for the heater was drawn from the junction box (250V) which was installed next to the heater. The power switches for circulating and exhausting the fumigant were fitted externally 500 mm away from the chamber.

Mixing of fumigants were carried out by installing a fan next to the junction box. Moreover, a 120 mm internal diameter iron pipe was attached to the bottom of the chamber to remove the fumigants while purging. These purged fumigants were delivered into the atmosphere through a 100 mm internal diameter iron pipe with its open end located 100 m above from the ground level.

4.2.5.2 Fumigation and gas sampling

The fumigant was delivered into the chamber by fitting a 2 mm internal diameter semi-flexible nylon tube (length of 750 mm) through the gas sampling port. This tube was inserted on to a 20 mm threaded nut and this was fitted to the sampling port and the fumigants were delivered 60 to 75 mm above the bioassay insects. The bioassay insects, plants and recorders were placed on the sliding basket fabricated from steel mesh. The steel mesh does not impede the movement of the fumigant through the chamber. Once the insects have been placed in the chamber, the chamber was made air tight by closing the door and purging vent. The chamber was preheated to a desired temperature by switching on the heater for 10 to 15 min prior to the delivery of fumigant. Warning signs were pasted on both sides of the chamber, just before the fumigants were added into the chamber.

Phosfume® was delivered into the chamber from the cylinder through a regulator (supplied by British Oxygen Gases, Sydney, Australia) for predetermined times (generally, the regulator delivers 2 g of Phosfume® per minute). A semi flexible 2 mm internal diameter nylon tube was fitted to the regulator and this tube has a metal cap with threads on one end. This enabled it to be connected to the tube that was connected to the gas sampling port. The phosphine concentration in the chamber was measured 7 to 10 min following release of Phosfume®. A 7 mm internal diameter flexible polyethylene tube was fitted to the metal tap of the gas delivery line. A Dräger® tube can be easily inserted into this tube while sampling.

The gas samples were obtained through a gas nylon tube which was fitted to the sampling port of the chamber. Initially a used Dräger® tube was used to calibrate the nylon tube and the polyethylene tube; once these were calibrated, a new Dräger® tube was used for measuring the concentration.

4.2.6 Post fumigation assessment of insects

4.2.6.1 All stages

The age of eggs (young and mature), larvae (various instar stages), pupae (early and late) and adults of all fumigated insects were determined based on either the stage of development or age adjudged by external appearance. The criteria used for identifying different insects and their developmental stages are summarised in Table 4.1 and 4.2.

Fumigated insects were transported to the Institute for Horticultural Development, Knoxfield within 1 to 1.5 h of the completion of the fumigation and during this period a constant temperature of 19°C was maintained. Assessments were made at a temperature range of 20±1°C. The first assessment was made 2 to 3 h following fumigation and second assessment was made four to five hours after the first assessment. All the fumigated insects were observed carefully for another 48 h at 24-h intervals. The live and severely affected individuals were separated from the dead ones

and transferred separately into different containers. Assessments were continued at 24-h intervals until complete mortality of all the exposed insect pests and their developmental stages was attained.

Table 4.1 Criteria used for determining the stage of *T. urticae*.

Insect pest	Body colour
1 to 3 days old eggs	Transparent
4 to 5 days old eggs	Creamy yellow
6 days old eggs	yellow with two red spots
Larvae	Green
Adults	Yellow

The fumigated insects were classified as live, affected, severely affected and dead based on their response to external mechanical and light stimuli. Mechanical stimuli were provided by gently prodding the insects, using a needle or forceps, and light stimuli were provided by a 15W-halogen lamp. In the case of live, affected or severely affected larvae or adults the numbers of days taken to attain complete mortality were recorded. During this period behavioural, feeding patterns and morphological and physiological changes were observed and recorded. The fumigated insects were classified as dead, severely affected, affected and live based on these following criteria:

Table 4.2 Criteria used for determining stage of the fumigated lepidopteran insect pests.

Insect species and stage	Head capsule		Body	
	Colour	Size (mm)	Colour	Size (mm)
Strepsicrates ejectana				
Larvae: Second instar	Brown	-	Green	2.3-2.7
Third instar	Brown	-	Green	5.2-5.7
Fourth instar	Brownish black	-	Green	8.2-8.7
Fifth instar	Black	-	greenish yellow or red	12.0-16.0
Sixth instar	Black	-	yellow or red	16.2-21.2
1 to 3 days old pupae – Male	-	-	Brown	7.9-8.2
- Female	-	-	reddish brown	8.7-9.1
4 to 6 days old pupae – Male	-	-	Brown	7.9-8.2
- Female	-	-	Red	8.7-9.1
7 days old pupae - Male	-	-	dark brown	7.9-8.2
-Female	-	-	Red	8.7-9.1
Epiphyas postvittana				
Eggs: 1 to 3 days old	-	-	Green	-
4 to 7 days old	-	-	yellowish brown	-
More than 7 days old	-	-	dark brown	-
Larvae: First instar	Greenish yellow	0.2	greenish yellow	2.0
Second instar	Yellow	0.3	Yellowish	4.0
Third instar	Brown	0.4 - 0.5	Green	5.0 - 6.0
Fourth instar	Brown	0.6 - 0.8	greenish yellow	7.0 - 8.0
Fifth instar - Male	Yellowish brown	0.8 - 1.0	Green	10.0 - 15.0
Sixth instar - Male	Yellowish brown	1.1 - 1.4	Green	18.0-21.0
1 to 3 days old pupae – Male	-	-	greenish yellow	9.0-10.5
- Female	-	-	greenish yellow	11.5-126
4 to 7 days old pupae – Male	-	-	Brown	9.0-10.5
- Female	-	-	Brown	11.5-126

Dead - These insects neither moved spontaneously, nor responded by reflex movements when slightly prodded with a needle or forceps under a light source. Generally, dead larvae and adults change their colour from green to brown three to four days after fumigation, and they subsequently turn completely black. The time taken for these changes to take place varies and this depends on the developmental stage of insect, and

the phosphine concentration during exposure, the temperature during exposure and the duration of the exposure.

Severely affected - These were moribund in that they exhibited slightly moving mouth parts and appendages, or gave slight jerks whilst they being subject to prodding. However, there is no independent movement of either mouth parts or legs. These insects normally lie on their backs or sides, as if they are in a paralytic stage.

Affected - These insects exhibit spontaneous movements of their mouth parts and/or appendages, and they are able to move slowly whilst being prodded. Affected larvae or adults lay in a ventral position in a manner that is similar to healthy individuals. A small number of larvae was observed to begin feeding within 24 to 48 h of fumigation, and then to cease.

Live - Such individuals can move, feed and behave in a manner that is similar to the control insects and can complete their life-cycle. Importantly, the developmental stages proceed to the next stage of development.

The status of the fumigated developmental stages (eggs and pupae) could not be identified immediately after fumigation. Hence, these were incubated for 14 to 16 days or until they changed colour and shape, indicating that they were dead. That is well above the normal time required to hatch. The individuals that survived were closely monitored for their behavioural and physiological conditions and were compared with the control batch.

4.2.6.2 Eggs

The mature eggs (four days and older) were fumigated in cups (200 mL polyethylene); immediately after fumigation, these cups were cut into 5 to 7 mm pieces; each of these pieces contained 1 to 2 egg masses. The fertility status of mature eggs can be easily verified by observing them under a microscope when a developed larval head can be easily seen under the chorion. However, it is impossible to determine the condition of the young eggs.

The young eggs (one to three days old) were transferred into 90 × 90 × 180 mm polyethylene containers with lids. The mature eggs (four to nine days old) were transferred into modified lightbrown apple moth medium and incubated similarly to the normal culture. The conditions of eggs were assessed regularly and compared with the control eggs. The growth pattern and reproductive capacity of the emerged larvae, pupae and adults and also, F_1 generation were observed and compared with the unfumigated batch.

4.2.6.3 Pupae

Most of the normal live pupae show wriggling movements of the last three to four or most of the segments while prodded or occasionally without prodding. The pupae were assessed under a microscope for any wriggling movements of their abdomens by prodding them with a blunt object. Those pupae that showed wriggling movements while prodded at the first and second assessment, were classified as live and these were maintained separately from other pupae. These were sexed and transferred into 200 mL lidded polyethylene plastic cups (1 to 12 pupae per container, depending on availability) and maintained in a controlled temperature room of 22°C and 60 to 65 per cent relative humidity.

The condition of pupae was assessed regularly until they hatched or changed their colour to black and/or shape (disfigured) ca for 15 to 18 days. Generally, the live pupae change their colour gradually from their heads to then abdomens 24 to 48 h before hatching, and dead pupae change their colour rapidly to black in 3 to 5 days after fumigation this is followed by drying and shrinking. The pupae were classified as

Live - can fully emerge from pupal case, able to release their wings and survive normally for at least two days.

Affected - can fully emerge from pupal case, but unable to fly and they died within two days of emergence.

Severely affected - half emerged, unable to release their wings from the pupal case and died 24 to 48 hours following emergence.

Dead - no emergence of adults and pupae changed their colour and/or shape.

The emerged adults were transferred to various lidded polyethylene plastic cups (200 mL) and were fed with 10 per cent honey solution (2 to 3 mL) soaked in absorbent cotton wool that was placed in the cups. Perforations (9 to 12 tiny holes) were made on the lids using a fine needle to circulate air in the cups. However, these adults were often attacked by *C. consobrinus* that were attracted by the honey solution. Hence, these cups (10 to 12) were placed in rectangular plastic tray (450 x 250 mm) filled with water (500 to 650 mL) to which a detergent (1 to 2 g of Pyronex®) had been added and this prevented the ants from reaching the cups. If a large number of adults emerged, six males and six females were maintained in each cup and if less than 12 adults emerged from a fumigated batch all the emerged adults were maintained in a single cup and the numbers of males and females that emerged were recorded. The egg laying capacity, time required to lay eggs and longevity of the emerged adults were noted and compared with the unfumigated batch.

The eggs laid by these adults were reared and the growth pattern and reproduction capacity of the F_1 generation was observed. Once these observations were completed all progeny of fumigated insects were disposed of thoroughly to avoid any chances of their developing resistance to phosphine fumigations.

4.2.6.4 Maintenance of fumigated insects

All fumigated insects were transferred back to the fumigated medium or cups or containers once the assessments were completed, and maintained for up to four or five days. The rationale behind this is that once the flowers were fumigated, they were packed into cardboard boxes that were either perforated or non-perforated and transported to their destinations. This normally takes two to four days to reach the quarantine point of the importing countries. Hence, the insects were kept continuously with the fumigated foliage, and similar conditions to these were created in these trials by placing the fumigated insects with their medium. However, four to five days after fumigation these were transferred into new medium or leaves, to avoid any microbial attacks or moulding, since these were highly susceptible to these attacks due to their lower immunity. The different methods used were as follows;

- Larvae and pupae of the leaf rolling moth, the lightbrown apple moth, larvae and adults of three species of aphids, adults of the meat ant and the European earwig were maintained at controlled temperature of 22°C and 60 to 65 per cent relative humidity.

- Eggs, larvae and adults of the two-spotted mite and eggs of the lightbrown apple moth were held at 25°C and 40 to 45 per cent relative humidity.

The insects (fumigated and control) were monitored daily except on Saturdays and Sundays, however, if fumigations were conducted on Thursdays or Fridays, assessments were made on Saturdays and Sundays. The dead insects were removed from the

medium 4 to 5 days following fumigation to avoid moulding and microbial infestations, and transferred to new medium.

The conditions of these dead insects were observed for reactions such as colour change, and oozing of body fluids. Four to five days following fumigation, all the remaining live or affected or severely affected larvae of the lightbrown apple moth and leaf rolling moth were transferred into individual 30 mL lidded polyethylene cups in which 25 to 27 g of fresh modified lightbrown apple moth medium had been placed. This gave ideal conditions for feeding and sheltering of these individuals since they were highly susceptible to microbial or attack by predators. Perforations were made on the lids using a fine needle to facilitate air circulation and prevent condensation. Similarly, the affected or severely affected *T. urticae* or *Myzus spp* were transferred onto new plants or leaves four to five days after fumigation.

4.2.6.5 Larvae of the leaf rolling moth and the lightbrown apple moth

Larvae that appeared to be dead in the initial assessment were transferred into separate fumigated medium based on the instar stages and further assessments were made until they changed colour to black or began oozing fluid. The reason for this is that sometimes phosphine narcotised the insects and larvae, or adults may appear dead for up to 24 h, hence, it is imperative to monitor the conditions continuously for at least this time. The severely affected, affected and live larvae were transferred immediately after assessment into the fumigated medium, separately based on instar stages, in lidded containers. A tissue was added to the lids to prevent condensation.

4.2.6.6 The two-spotted mite

Eggs - Generally the middle leaves of the bean plants (2 to 3 leaves from the top) contained a well balanced proportion of all stages, however, young or mature leaves mainly contained either more eggs or more adults. Hence, in the initial experiments the

middle leaves were removed from the fumigated plants and the numbers of larvae and adults present were assessed under a microscope. To obtain an estimate of numbers exposed during fumigation the number of individuals present in the middle leaves were counted and multiplied by the number of leaves on the bean plants and divided by two, to compensate for the unbalanced populations. In the later experiments (all the long exposure time ie. 14 h or more) all the leaves (top, middle and bottom) were observed and results were recorded. Moreover, the response of eggs to fumigants was investigated randomly by selecting 500 to 700 eggs from all leaves and incubating them at 25°C and 40 to 45 per cent relative humidity.

It is impossible to assess the condition of the fumigated individuals on the bean plant itself due to their smaller size. Removing of mites from the plants necessitates extra time due to their minuscule size. Hence, the individual leaves were cut off from the bean seedlings at the base of the stem using a pair of scissors. While cutting, there are possibilities that air may enter to the phloem and block the water uptake. This accelerates the drying of leaves making it difficult to judge the effects of fumigation, since fresh conditions of leaves were conducive for the development of eggs. Hence, the stems were immersed in water while cutting. The bean leaves were highly sensitive and started drying three to four hours after removal from the plant if not properly treated. Hence, the following method was followed to incubate the eggs.

- the leaves were transferred into various lidded 10 or 11 mm plastic Petrie dishes to which two or three 9 or 10 mm diameter Whatman 55 qualitative filter papers soaked in water, or a layer (2 to 3 mm thickness) of moist absorbent cotton wool had been added. A 18 to 20 mm diameter hole was made on the centre of the Petrie dish (lid) using a drill and this was covered with a 20 to 22 mm square metal wire mesh to assist air circulation. The moist filter paper or cotton wool kept the bean leaves in fresh condition and also provided the necessary humidity for the eggs. These were replenished regularly with fresh water without wetting the leaves or eggs. The condition of the eggs was assessed regularly (at a 2 to 3 days interval) and the emerged larvae were transferred using a fine camel hair brush into uninfested bean seedlings that were planted in pots. These were housed in plastic cages (500 × 400 ×

200 mm) fitted with muslin gauze cloth. A similar temperature (controlled temperature of 20°C), relative humidity (60 to 65 per cent) and photoperiod (16 h light and 8 h dark) were maintained to these fumigated and control insects. The growth pattern and multiplication of the emerged larvae were observed and compared with the unfumigated insects.

Larvae and adults - The conditions of fumigated larvae and adults were assessed under a microscope using a light source (15W) and the insects were mechanically stimulated by means of a needle. The leaves (2 to 3) were then transferred into a polyethylene food containers (90 × 90 × 180 mm) without lids to which water soaked absorbent cotton wool had been added. The containers were held at 25°C and 40 to 45 per cent relative humidity. The insects were assessed again three to four hours after the first assessment and the results were confirmed with the third assessment (24 h after the second assessment). The affected or severely affected larvae and adults were maintained with the same leaves placed in Petrie dishes (similar to those used for eggs) and the conditions were assessed regularly.

4.3 *Statistical analysis of results*

The relationship between the proportion of dead individuals (eggs, larvae, pupae and adults) and the following explanatory variables were examined: date, mass of Phosfume® added (kg), initial phosphine concentration ($g.m^{-3}$), final phosphine concentration ($g.m^{-3}$), exposure time (h), temperature (°C) and relative humidity (per cent). To do this, Pearson correlation coefficients were first calculated. Then a model with a binomial distribution and a logit link function was used to express the proportion dead in terms of the explanatory variables. A stepwise procedure was used, in which only variables making a significant contribution to the model were included. Models were fitted using the statistical package Genstat 5. Results of the statistical analysis are presented in Appendix 3.

4.4 Results

4.4.1 The leaf rolling moth, Strepsicrates ejectana

4.4.1.1 Larvae

The responses of various larval instars of *S. ejectana* to phosphine fumigations, conducted at Emerald, with different concentrations, exposure times and temperature ranges are presented in Table 4.3. Due to their smaller size and the fact that they are found in very young buds it is difficult to collect first instar larvae so they were not exposed in these fumigations. All the second instar and most of the third fourth, fifth and sixth instar larvae were dead when assessed (2 to 3 h after being fumigated). These larvae might have been killed during the fumigations.

However, a small number of individuals was severely affected in most of the trials if one or more of the variables (temperature, exposure time or final phosphine concentration) were not maintained at sufficient levels. These variables are interrelated, hence, from a practical point of view, it is difficult to distinguish which variables significantly influence the mortality of the exposed insect pests. Results were analysed extensively, and it was hypothesised that certain criteria had to be satisfied to obtain a complete morality of the exposed larvae. It was surmised that an average temperature of 20°C or more, an exposure time of 15 h or more and a final phosphine concentration of 0.25 $g.m^{-3}$ or more are necessary to achieve maximum mortality of all the exposed larvae. Failure to meet even one criterion did not give a complete mortality of the exposed larvae. In these circumstances a small number of larvae was severely affected and these individuals took various times (24 to 168 h) to die. The quarantine regulations of most of the countries require that products be free of insect pests and the presence of even a single severely affected or live larva could result in rejection of an entire consignment.

Table 4.3 Toxicity of phosphine at various concentrations, exposure times and temperature to second to sixth instar larvae of *S. ejectana* fumigated in a 27 m^3 chamber at Emerald and a 900 L chamber at Frankston.

Date	hosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Leaf rolling moth *Strepsicrates ejectana*			
		Initial	Final			Live	S. aff	Dead	Total
30/9/96	1.74	0.25	0.15	16.0	18.4 (15.5-20.8)	0	3	64	67
30/9/96	0	0	0	0	22.0	65	0	2	67
18/12/96	1.0	0.41	0.12	16.0	17.1	0	18	10	28
18/12/96	0	0	0	0	22.0	27	0	0	27
30/12/96	1.8	0.43	0.36	16.0	18.4 (17.6-19.9)	0	6	32	38
30/12/96	0	0	0	0	22.0	37	0	1	38
14/1/97	1.98	1.09	0.61	16.0	25.5 (22.9-28.9)	0	0	23	23
14/1/97	0	0	0	0	22.0	23	0	0	23
23/1/97	2.36	1.2	0.94	15.0	18.2 (16.9-20.7)	0	7	20	27
23/1/97	0	0	0	0	22.0	25	0	2	27
10/2/97	27	0.98	0.85	15.5	18.6 (17.8-20.6)	0	2	36	38
10/2/97	0	0	0	0	22.0	37	0	1	38
12/2/97	27	0.94	0.55	17.0	23.9 (18.1-34.8)	0	2	23	25
12/2/97	0	0	0	0	22.0	25	0	0	25
17/3/97	30	0.64	0.43	16.0	16.3 (11.8-24.1)	0	4	11	15
17/3/97	0	0	0	0	22.0	15	0	0	15
20/3/97	25	1.08	0.97	16.0	16.9 (14.3-21.6)	0	3	9	12
20/3/97	0	0	0	0	22.0	12	0	0	12
24/3/97	23	0.78	0.57	17.0	17.4 (15.3-24.1)	0	1	5	6
24/3/97	0	0	0	0	22.0	6	0	0	6

Results of the statistical analysis of the experiments are described in detail in Appendix 3.

The results are grouped into three classes, namely: **Good** (achieving a 100 per cent mortality), **Marginal** (achieving 90 to 99 per cent mortality) and **Poor** (achieving 89 per cent or less mortality). The number of larvae severely affected increases if one or more of the criteria are not met. Also, the times required for the severely affected larvae to die vary significantly and this is directly related to the fumigation conditions. The time taken by the severely affected larvae to die is an important factor in postharvest disinfestation, as the flower consignment requires minimum of 48 h to

reach the importing country. Hence, those severely affected larvae that die within 48 h following fumigation are unlikely to cause any quarantine problems. The Temperature-Time trajectories are presented in Appendix 4.

Complete mortality of all the larval instar stages was obtained with an initial phosphine concentration of 1.09 $g.m^{-3}$ and an exposure period of 16 h at an average temperature of 25.5°C (range of 22.9 to 28.9°C). The responses of larvae that were fumigated in a 900 L chamber at Frankston and a 27 m^3 chamber at Emerald were similar. In all the experiments control mortalities were considerably less they may have resulted from natural causes, on augmented by handling.

Analysis of results

The nature of the experiments was such that they were difficult to replicate. This is because they were carried out under commercial conditions and it was difficult to control the temperature-time trajectory and the concentration-time trajectory of the phosphine. Hence, if a floriculturist or exporter wishes to be confident that a fumigation is to be successful, it was hypothesised that the following criteria should be met:

- Exposure time: 15 h or more.
- Final phosphine concentration: 0.25 $g.m^{-3}$ or more.
- Temperature: 20°C or more (only average temperature was taken into consideration, because temperature distribution pattern varies considerably. Detailed analysis of temperature distribution on an hourly basis is presented in Appendix 4).

One out of two fumigations gave a complete mortality of all the exposed larvae (Table 4.4). Only two out of twenty five larvae (eight per cent) were severely affected as a result of the 12/2/97 fumigation, where all the fumigation criteria are above the required level. However, it should be noted that one larva died 72 h following fumigation and other died 96 h following fumigation.

Table 4.4 The fumigations with phosphine that satisfy the criteria.

	Number of larvae severely affected	Number of larvae fumigated
Good		
14/1/97	-	23
Marginal		
12/2/97	2 (8%)	25

Of all the fumigations that did not satisfy the fumigation criteria, not a single fumigation gave a complete mortality of the exposed larvae. Two out of eight fumigations are marginal, where only a small number of larvae were severely affected. Only one or two larvae were still severely affected 72 h following fumigation, and these larvae died 72 to 96 h following fumigation. On the other hand, six fumigations were poor.

Table 4.5 The fumigations with phosphine that did not satisfy the criteria.

	Number of larvae severely affected	Number of larvae fumigated
Marginal		
30/9/96	3 (4.4%)	67
10/2/97	2 (5.2%)	38
Poor		
18/12/96	18 (64.0%)	28
30/12/96	6 (18.7%)	32
23/1/97	7 (25.9%)	27
17/3/97	4 (26.7%)	15
20/3/97	3 (25.0%)	12
24/3/97	1 (16.0%)	6

( ) – indicates per cent severely affected.

Table 4.6 indicates the number of days required for the phosphine fumigated severely affected larvae to die. Most of these larvae died within 48 h after fumigation and only one or two larvae were still severely affected up to five days later. The severely affected third instar larvae died one day following fumigation and fourth to sixth instar stages required longer times for mortality (two to five days). Among these larvae all the fourth and fifth instar larvae had died within 48 h and only sixth instar larvae were still severely affected for up to 5 days.

Table 4.6 Number of days required for the severely affected larvae that were fumigated with various concentrations of phosphine, exposure times and temperatures to attain complete mortality.

Date	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	No. of larvae severely affected assessment time after fumigation (h)					No. of days for complete mortality	Instar stage
	Initial	Final			3	24	48	72	96		
30/9/96	0.25	0.15	16.0	18.4(15.5-20.8)	3	1	-	-	-	1	III and V
18/12/96	0.41	0.12	16.0	17.1	18	14	7	2	-	3	VI and V
30/12/96	0.43	0.36	16.0	18.4(17.6-19.9)	6	5	3	1	1	5	III and VI
23/1/97	1.2	0.94	15.0	18.2(16.9-20.7)	7	7	4	1	1	5	VI
10/2/97	0.98	0.85	15.5	18.6(17.8-20.6)	2	2	1	1	1	5	V
12/2/97	0.94	0.55	17.0	23.9(18.1-34.8)	2	2	2	1	-	4	IV and V
17/3/97	0.64	0.43	16.0	16.3(11.8-24.1)	4	4	1	1	-	4	V
20/3/97	1.08	0.97	16.0	16.9(14.3-21.6)	3	1	1	1	-	4	V and VI
24/3/97	0.78	0.57	17.0	17.4(15.3-24.1)	1	1	1	1	-	4	IV

At the end of fumigation some of the exposed larvae had moved out (mostly fourth and fifth instar stages) from their webbing shelters and they were found on the medium or among the foliage. Higher mortality percentages were recorded for the larvae that were found on the medium, i.e. those exposed directly to phosphine. A comparatively higher number of larvae that remained inside their shelters were severely affected. However, a major proportion of these larvae died suggesting that phosphine penetrated well in to the shelters and acted on the target sites of the larvae.

The statistical analysis presented in section A3.4.1 of Appendix 3 points to the importance of temperature in determining the mortality of the larvae of *S. ejectana*.

4.4.1.2 Pupae

The results obtained from the phosphine fumigated young and mature pupae of both sexes of *S. ejectana* at various concentrations and exposure times are presented in Table 4.7. Fumigations conducted on 20/3/97 and 24/3/97, did not give a complete mortality of all the pupae exposed. Fumigation conducted on 12/2/97 killed all the exposed pupae. Except for this fumigation (12/2/97), in all other fumigations a small number of pupae were severely affected. Adults that emerged from these severely affected pupae were often abnormal. The affected adults emerged fully from the pupal cases, but being unable to fly, they were found resting on the bottom of the cups. These adults did not show any response to prodding and died within 24 to 36 h after emergence. The live adults that emerged from the fumigated pupae behaved similarly to the unfumigated adults i.e. feeding, flying and reproducing.

Table 4.7 Toxicity of phosphine at various concentrations, exposure times and temperatures to pupae of *S. ejectana* fumigated in a 27 m^3 chamber at Emerald.

Date	Phosfume® (kg)	Phosphine concentration gm-3		Exposure time (h)	Temperature (°C)	R.H (%)	Leaf rolling moth *Strepsicrates ejectana*					
		Initial	Final				Pupae					
							Live		S. affected		Dead	
							Male	Female	Male	Female	Male	Female
17/3/97	0.020	0.64	0.43	16.0	16.3 (11.8-24.1)	85.3 (44.8-90.3)	-	-	-	3	-	4
17/3/97	0	0	0	0	25.0	45.0	0	7	0	0	0	0
20/3/97	0.025	1.08	0.97	16.0	16.9 (14.3-21.6)	88.5 (71.8-95.2)	1	1	1	0	3	5
20/3/97	0	0	0	0	25.0	45.0	5	6	0	0	0	0
12/2/97	27	0.94	0.55	17.0	23.9 (18.1-34.8)	84.4 (53.7-93.5)	0	0	0	0	5	4
12/2/97	0	0	0	0	25.0	45.0	5	4	0	0	0	0
24/3/97	0.023	0.78	0.57	17.0	17.4 (15.3-24.1)	85.8 (50.1-93.4)	1	2	2	0	0	4
24/3/97	0	0	0	0	25.0	45.0	3	6	0	0	0	0

Data portrayed in Table 4.7 indicate that both males and females responded similarly to phosphine fumigations. The number of days required for the fumigated surviving pupae to emerge is given in Table 4.8. The fumigated surviving or severely affected pupae took three to five days longer to emerge than the unfumigated pupae, that were held at 25°C and 40 to 45 per cent relative humidity. The data from this table indicate that phosphine could have delayed or arrested the development to a certain extent.

Table 4.8 Number of days required for the fumigated and control pupae of *S. ejectana* to hatch.

Date	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Number of days required to emerge		
	Initial	Final			1 to 3 days*	4 to 6 days*	> 7 days*
17/3/97	0.64	0.43	16.0	16.3(11.8-24.1)	13 – 15	-	-
17/3/97	0	0	0	25.0	10 – 12	6 – 8	3 - 4
20/3/97	1.08	0.97	16.0	16.9(14.3-21.6)	13 – 15	9 – 10	-
20/3/97	0	0	0	25.0	10 – 12	6 – 8	3 - 4
24/3/97	0.78	0.57	17.0	17.4(15.3-24.1)	13 – 16	8 – 10	-
24/3/97	0	0	0	25.0	10 – 12	6 – 8	3 - 4

*- age of eggs (in days).

The percentage of pupae of different ages that survived, and that were exposed to different concentrations of phosphine, exposure times and temperatures are given in Table 4.9. The results show that the survival rate was higher among the young pupae of one to three days old than the mature pupae.

Table 4.9 Percentage of different age pupae of *S. ejectana* emerging after being fumigated under a range of concentrations, times and temperatures in a 27 m^3 chamber at Emerald.

Date	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Per cent of pupae hatched		
	Initial	Final			1 - 3 day old	4 - 6 days old	> 7 days old
17/3/97	0.64	0.43	16.0	16.3 (11.8-24.1)	100.0	0.0	0.0
20/3/97	1.08	0.97	16.0	16.9 (14.3-21.6)	100.0	75.0	20.0
24/3/97	0.78	0.57	17.0	17.4 (15.3-24.1)	100.0	50.0	0.0
Control	0	0	0	25.0	95.0	95.00	100.00

4.4.2 The lightbrown apple moth, *Epiphyas postvittana*.

4.4.2.1 Eggs

The results of fumigating young and mature eggs of *E. postvittana* with phosphine are presented in Table 4.10. Phosphine concentrations of 0.48 to 1.04 $g.m^{-3}$, exposure periods of 15 to 17.25 h and temperatures of 17.5 to 23.9°C were investigated. A large number of young and mature eggs survived in all the fumigation trials except in one fumigation (12/2/97), where in this particular fumigation the average temperature was 23.9°C. It is clear from this table, in this particular fumigation except for temperature all other variables were almost the same. Hence, it can be assumed that the comparatively higher temperature may have contributed to the complete mortality of all the exposed eggs (12/2/97).

Table 4.10 Toxicity of phosphine at various concentrations, exposure times and temperatures to the eggs of *E. postvittana* that were fumigated in a 900 L chamber at Frankston.

Date	Phosfume® (g)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	RH (%)	Lightbrown apple moth *Epiphyas postvittana*		
		Initial	Final				Live	Dead	Total
12/2/97	25	0.94	0.55	17.0	23.9(18.1-34.8)	84.4(53.7-93.5)	0	120	120
12/2/97	0	0	0	0	25.0	45.0	117	3	120
26/5/97	27	1.0	0.84	17.25	17.5(16.0-19.1)	90.6(85.3-97.0)	120	80	200
26/5/97	0	0	0	0	25.0	45.0	198	2	200
3/6/97	26	1.04	0.48	16.0	18.3(16.7-19.9)	80.2(67.0-87.4)	210	90	300
3/6/97	0	0	0	0	25.0	45.0	294	6	300
15/7/97	20	0.86	0.48	16.5	17.7(14.9-20.5)	-	180	220	400
15/7/97	0	0	0	0	25.0	45.0	396	4	400
8/7/97	0.16	0.48	0.08	15.0	17.5(15.0-19.8)	-	120	60	180
8/7/97	0	0	0	0	25.0	45.0	708	12	720

The emerged larvae completed their life-cycle in a way similar to the larvae emerged from the control batch. These results suggest that the eggs were highly tolerant to the

above mentioned fumigation conditions. Increasing the temperature may reduce the chances of survival of eggs. However, increasing the temperature beyond 20°C may not be conducive to an acceptable vase life of the exposed flowers. Some mortality of the control experiments was observed, which could be either due to handling or as a result of natural death.

Fumigation criteria

Temperature: 23.9°C or more

Exposure time: 17 h or more

Phosphine concentration: 0.55 $g.m^{-3}$ or more.

One fumigation that satisfied the criteria gave a complete mortality of all the exposed eggs (Table 4.11).

Table 4.11 The fumigations with phosphine that satisfy the criteria.

	Number of eggs survived	Total number of eggs fumigated
Good		
12/2/97	-	120

All the four fumigations that did not satisfy the criteria resulted in a large number of eggs surviving. The number of eggs that survived increased with the reduction of one or more variables especially temperature and/or exposure time.

Table 4.12 The fumigations with phosphine that did not satisfy the criteria.

	Number of eggs survived	Total number of eggs fumigated
Poor		
26/5/97	120(60%)	200
3/6/97	210(70%)	300
15/7/97	180(45%)	400
8/7/97	120(66%)	180

The colour and external appearance of dead fumigated and live unfumigated eggs were similar for the first three days. Hence, it was difficult to determine whether the fumigated eggs were alive or dead for the first three days after fumigation. The colour of dead young fumigated eggs changed abruptly to dark brown from green, and they then proceeded to dry or shrink on their upper surfaces four to six days following fumigation. Similarly, the fumigated dead mature eggs showed desiccation and drying symptoms, three to five days following fumigation.

Times required for the surviving fumigated and unfumigated eggs to emerge are presented in Table 4.13. Data from this table clearly illustrate that there was a substantial delay in hatching of eggs surviving fumigation compared with the control eggs. This was more pronounced in young eggs of one to three day old which it took four to seven days more to hatch than the unfumigated eggs. Likewise, the four to six day old eggs required two to three days longer than their unfumigated counterparts. The reproductive or egg laying capacity of adults that emerged from this batch was not affected. Also, there was no difference between the number of eggs laid and the number hatched from the fumigated eggs and unfumigated ones.

Table 4.13 Number of days required for the fumigated eggs of *E. postvittana* to hatch when maintained at a controlled temperature of 25°C.

Date	Phosphine concentration (gm^{-3})		Exposure time (h)	Temperature (°C)	Number of days required to hatch		
	Initial	Final			1 - 3 days*	4 - 6 days*	> 7 days*
26/5/97	1.0	0.84	17.25	17.5(16.0-19.1)	12 – 15	7 – 9	-
3/6/97	1.04	0.48	16.0	18.3(16.7-19.9)	14 – 16	7 – 8	-
15/7/97	0.86	0.48	16.5	17.7(14.9-20.5)	13 – 15	6 – 8	-
8/7/97	0.48	0.08	15.0	17.5(15.6-19.8)	11 – 13	6 – 8	-
Control	0	0	0	22.0	7 – 9	4 – 6	0

*- age of egg fumigated.

4.4.2.2 Larvae

The mortality rate of first and second larval instar of *E. postvittana* following fumigations at different concentrations of phosphine (0.33 to 1.32 $g.m^{-3}$), exposure times (15.5 to 18.0 h) and temperatures (13.3 to 23.9°C) in a 900 L and 27 m^3 chamber are given in Table 4.14. The results show that the first instar larvae are highly susceptible to phosphine fumigation and complete mortality of all the exposed larvae was achieved in all the fumigations. Similar to first instar larvae, second instar larvae are also susceptible to phosphine fumigation, where complete mortality was achieved in all the fumigations. The reasons for the complete mortality of both these stages could possibly be due to their metabolism that may make them vulnerable to phosphine fumigation. It may be also simply be due to their living pattern (feeding and sheltering on the surface or on the grooves of the medium, hence, they were directly exposed to a lethal dose of phosphine).

Table 4.14 Toxicity of phosphine to first and second instar larvae of *E. postvittana* fumigated with various concentrations, exposure times and temperatures in a 900 L and 27 m^3 (Emerald) chamber.

Date	Phosfume® (Kg)	Phosphine oncentration gm^{-3}		Exposure time (h)	Temperature (°C)	RH (%)	Lightbrown apple moth *Epiphyas postvittana*		
		Initial	Final				Live	Dead	Total
30/9/96	1.74	0.33	0.13	16.0	18.4(15.5-20.8)	-	0	50	50
30/9/96	0	0	0	0	22.0	65.0	50	0	50
30/12/96	1.8	0.43	0.36	16.0	18.4(17.6-19.9)	-	0	57	57
30/12/96	0	0	0	0	22.0	65.0	57	2	59
14/1/97	1.98	1.09	0.61	16.0	25.5(22.9-28.9)	-	0	126	126
14/1/97	0	0	0	0	22.0	65.0	122	4	126
18/1/97	2.36	1.2	0.94	15.0	18.2(16.9-20.7)	-	0	96	96
18/1/97	0	0	0	0	22.0	65.0	95	1	96
26/2/97	4.64	0.97	0.61	16.0	18.9(18.1-19.9)	-	0	65	65
26/2/97	0	0	0	0	22.0	65.0	63	2	65
10/2/97	27*	0.98	0.85	15.5	18.6(17.8-20.6)	-	0	66	66
10/2/97	0	0	0	0	22.0	65.0	66	0	66
12/2/97	27*	0.94	0.55	17.0	23.9(18.1-34.8)	84.4(53.7-93.5)	0	45	45
12/2/97	0	0	0	0	22.0	65.0	45	0	45
17/3/97	30*	0.64	0.43	16.0	16.3(11.8-24.1)	85.3(44.8-90.3)	0	54	54
17/3/97	0	0	0	0	22.0	65.0	54	2	56
20/3/97	25*	1.08	0.97	16.0	16.9(14.3-21.6)	88.5(71.8-95.2)	0	25	25
20/3/97	0	0	0	0	22.0	65.0	24	1	25
24/3/97	23*	0.78	0.57	17.0	17.4(15.3-24.1)	85.8(50.1-93.4)	0	33	33
24/3/97	0	0	0	0	22.0	65.0	33	0	33
9/4/97	30*	1.32	0.48	16.25	21.1(18.8-25.5)	90.5(44.8-96.4)	0	50	50
9/4/97	0	0	0	0	22.0	65.0	50	0	50
14/4/97	25*	1.01	0.76	17.0	18.9(18.1-19.5)	91.2(79.1-93.4)	0	48	48
14/4/97	0	0	0	0	22.0	65.0	48	0	48
21/4/97	27*	1.22	0.41	16.0	19.4(17.2-25.3)	87.3(60.3-92.8)	0	60	60
21/4/97	0	0	0	0	22.0	65.0	56	4	60
29/4/97	33*	1.14	0.87	16.0	18.4(13.9-26.6)	91.7(62.3-96.4)	0	40	40
29/4/97	0	0	0	0	22.0	65.0	40	0	40
5/5/97	33*	0.83	0.76	18.0	18.3(16.4-21.9)	83.4(66.1-94.6)	0	40	40
26/5/97	27*	1.0	0.84	17.25	17.5(16.0-19.1)	90.6(85.3-97.0)	0	36	36
3/6/97	26*	1.04	0.48	16.0	18.3(16.7-19.9)	80.2(67.0-87.4)	0	45	45
3/6/97	0	0	0	0	22.0	65.0	45	3	48
15/7/97	20*	0.86	0.48	16.5	17.7(14.9-20.5)	-	0	67	67

Third instar larvae - The results of the phosphine fumigations at various concentrations, exposure times and temperatures for the third instar larvae are presented in Table 4.15. Data from this table clearly illustrate that all the exposed larvae were killed in most of the fumigations and a small number was severely affected. This indicates that the third instar larvae are slightly tolerant to phosphine fumigations than that of the first and second instar larvae.

Table 4.15 Toxicity of phosphine to third instar larvae of *E. postvittana* fumigated with various concentrations, exposure times and temperatures in a 900 L chamber.

Date	hosfume® (min)	Phosphine Concentration gm^{-3}		Exposure time (h)	Temperature (°C)	RH (%)	Lightbrown apple moth *Epiphyas postvittana*			
		Initial	Final				Live	S. aff	Dead	Total
10/2/97	27	0.98	0.85	15.5	18.6(17.8-20.6)	-	0	0	18	18
10/2/97	0	0	0	0	22.0	65.0	18	0	0	18
12/2/97	27	0.94	0.55	17.0	23.9(18.1-34.8)	84.4(53.7-93.5	0	0	25	25
12/2/97	0	0	0	0	22.0	65.0	25	0	0	25
17/3/97	30	0.64	0.43	16.0	16.3(11.8-24.1)	5.3(44.8-90.3)	0	3	24	27
17/3/97	0	0	0	0	22.0	65.0	27	0	0	27
20/3/97	25	1.08	0.97	16.0	16.9(14.3-21.6)	8.5(71.8-95.2)	0	2	29	31
24/3/97	23	0.78	0.57	17.0	17.4(15.3-24.1)	5.8(50.1-93.4)	0	1	22	23
24/3/97	0	0	0	0	22.0	65.0	23	0	0	23
9/4/97	30	1.32	0.48	16.25	21.1(18.8-25.5)	0.5(44.8-96.4)	0	0	24	24
9/4/97	0	0	0	0	22.0	65.0	24	0	0	24
14/4/97	25	1.01	0.76	17.0	18.9(18.1-19.5)	1.2(79.1-93.4)	0	0	16	16
14/4/97	0	0	0	0	22.0	65.0	14	0	2	16
21/4/97	27	1.22	0.41	16.0	19.4(17.2-25.3)	7.3(60.3-92.8)	0	0	15	15
21/4/97	0	0	0	0	22.0	65.0	15	0	0	15
29/4/97	33	1.14	0.87	16.0	18.4(13.9-26.6)	1.7(62.3-96.4)	0	0	20	20
29/4/97	0	0	0	0	22.0	65.0	20	0	0	20
5/5/97	33	0.83	0.76	18.0	18.3(16.4-21.9)	3.4(66.1-94.6)	0	0	15	15
5/5/97	0	0	0	0	22.0	65.0	15	0	0	15
26/5/97	27	1.0	0.84	17.25	17.5(16.0-19.1)	0.6(85.3-97.0)	0	0	40	40
26/5/97	0	0	0	0	22.0	65.0	39	0	1	40
3/6/97	26	1.04	0.48	16.0	18.3(16.7-19.9)	0.2(67.0-87.4)	0	0	18	18
3/6/97	0	0	0	0	22.0	65.0	18	0	0	18
15/7/97	20	0.86	0.48	16.5	17.7(14.9-20.5)	-	0	0	20	20
15/7/97	0	0	0	0	22.0	65.0	20	0	0	20

The results obtained from the fumigation trials conducted at Gembrook are presented in the Table 4.16. In these fumigation trials complete mortality of all the exposed third instar larvae was achieved.

Table 4.16 Toxicity of phosphine to third instar larvae of *E. postvittana* fumigated with various concentrations, exposure times and temperatures in a 27 m^3 chamber located at Gembrook.

Date	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Lightbrown apple moth *Epiphyas postvittana*		
		Initial	Final			Live	Dead	Total
30/9/96	1.74	0.33	0.13	16.0	18.4 (15.5-20.8)	0	39	39
30/9/96	0	0	0	0	22.0	36	3	39
30/12/96	1.8	0.43	0.36	16.0	18.4 (17.6-19.9)	0	45	45
30/12/96	0	0	0	0	22.0	43	2	45
14/1/97	1.98	1.09	0.61	16.0	25.5(22.9-28.9)	0	24	24
14/1/97	0	0	0	0	22.0	23	1	24
23/1/97	2.36	1.2	0.94	15.0	18.2 (16.9-20.7)	0	16	16
23/1/97	0	0	0	0	22.0	16	0	16
26/2/97	4.64	0.97	0.61	16.0	18.9(18.1-19.9)	0	21	21
26/2/97	0	0	0	0	22.0	20	1	21

Fumigation criteria

Temperature: 17.5°C or more.

Exposure time: 15 h or more.

Final phosphine concentration: 0.13 $g.m^{-3}$ or more.

All the 15 fumigations that satisfied the criteria gave a complete control of all the exposed third instar larvae (Table 4.17).

Table 4.17 The fumigations with phosphine that satisfy the criteria.

	Number of larvae severely affected	Number of larvae fumigated
Good		
30/9/96	-	39
30/12/96	-	45
14/1/97	-	24
23/1/97	-	16
26/2/97	-	21
10/2/97	-	18
12/2/97	-	25
9/4/97	-	24
14/4/97	-	16
21/4/97	-	15
29/4/97	-	20
5/5/97	-	15
26/5/97	-	40
3/6/97	-	18
15/7/97	-	20

All the three fumigations that did not satisfy the criteria did not give a complete mortality of all the exposed larvae (Table 4.18). A small number of individuals were severely affected once the temperature was not maintained sufficiently. The number of larvae severely affected increased with decreasing temperature. This is evident by the fumigation conducted on 17/3/97, where the temperature was 16.3°C and 12.5% of the larvae were severely affected. However, only 4.5% of the larvae were severely affected when fumigated at a temperature of 17.4°C.

Table 4.18 The fumigations with phosphine that did not satisfy the criteria.

	Number of larvae severely affected	Total number of larvae fumigated
Marginal		
20/3/97	2 (6.9%)	31
24/3/97	1(4.5%)	22
Poor		
17/3/97	3 (12.5%)	27

Table 4.19 shows the number of days required for the severely affected third instar larvae to die. Data from this table indicate that all these severely affected larvae were dead 3 to 72 h following fumigation.

Table 4.19 Number of days required for complete mortality of the severely affected larvae that were fumigated in a 900 L chamber at Frankston.

Date	Phosphine concentration gm^{-3}		No. of larvae severely affected assessment after fumigation (h)				No. of days for complete mortality
	Initial	Final	3	24	48	72	
24/3/97	0.78	0.57	1	1	1	0	2
9/4/97	1.32	0.48	3	1	1	1	3

Fourth instar larvae

Insecticidal efficacy of phosphine to fourth instar larvae at various concentrations, exposure times and temperatures are presented in Table 4.20. These larvae responded in a similar way to third instar larvae, i.e. a small number of larvae were severely affected in some fumigations.

Table 4.20 Toxicity of phosphine to fourth instar larvae of *E. postvittana* fumigated with various concentrations, exposure times and temperatures in a 900 L chamber.

Date	Phosfume® min	Phosphine Concentration gm $^{-3}$		Exposure time (h)	Temperature (°C)	RH (%)	Lightbrown apple moth *Epiphyas postvittana*			
		Initial	Final				Live	S. aff	Dead	Total
12/2/97	27	0.94	0.55	17.0	23.9(18.1-34.8)	84.4(53.7-93.5)	0	0	24	24
12/2/97	0	0	0	0	22.0	65.0	24	0	0	24
17/3/97	30	0.64	0.43	16.0	16.3(11.8-24.1)	85.3(44.8-90.3)	0	7	32	39
17/3/97	0	0	0	0	22.0	65.0	37	0	2	39
20/3/97	25	1.08	0.97	16.0	16.9(14.3-21.6)	88.5(71.8-95.2)	0	9	12	21
20/3/97	0	0	0	0	22.0	65.0	20	0	1	21
24/3/97	23	0.78	0.57	17.0	17.4(15.3-24.1)	85.8(50.1-93.4)	0	2	18	20
24/3/97	0	0	0	0	22.0	65.0	20	0	0	20
9/4/97	30	1.32	0.48	16.25	21.1(18.8-25.5)	90.5(44.8-96.4)	0	0	33	33
9/4/97	0	0	0	0	22.0	65.0	30	0	3	33
14/4/97	25	1.01	0.76	17.0	18.9(18.1-19.5)	91.2(79.1-93.4)	0	0	18	18
14/4/97	0	0	0	0	22.0	65.0	18	0	0	18
21/4/97	27	1.22	0.41	16.0	19.4(17.2-25.3)	87.3(60.3-92.8)	0	0	20	20
21/4/97	0	0	0	0	22.0	65.0	20	0	0	20
29/4/97	33	1.14	0.87	16.0	18.4(13.9-26.6)	91.7(62.3-96.4)	0	0	27	27
29/4/97	0	0	0	0	22.0	65.0	25	0	2	27
5/5/97	33	0.83	0.76	18.0	18.3(16.4-21.9)	83.4(66.1-94.6)	0	0	20	20
5/5/97	0	0	0	0	22.0	65.0	20	0	0	20
26/5/97	27	1.0	0.84	17.25	17.5(16.0-19.1)	90.6(85.3-97.0)	0	0	20	20
26/5/97	0	0	0	0	22.0	65.0	20	0	0	20
3/6/97	26	1.04	0.48	16.0	18.3(16.7-19.9)	80.2(67.0-87.4)	0	0	12	12
3/6/97	0	0	0	0	22.0	65.0	12	0	0	12
15/7/97	20	0.86	0.48	16.5	17.7(14.9-20.5)	-	0	0	15	15
15/7/97	0	0	0	0	22.0	65.0	0	0	15	15

The number of days required for the severely affected larvae to die is given in Table 4.21. Most of these severely affected larvae died within 24 h following fumigation and only a single larva was still severely affected for a longer period (168 h).

Table 4.21 Time required for the severely affected larvae to die.

Date	Phosphine (gm^{-3})		No. of larvae severely affected assessment after fumigation (h)						No. of days for complete mortality
			3	24	48	72	96	168	
17/3/97	0.64	0.43	7	2	1	1	1	1	6
20/3/97	1.08	0.97	9	3	1	1	1	1	7
24/3/97	1.00	0.84	2	2	1	-	-	-	1

The results of the phosphine fumigations of fourth instar larvae of *E. postvittana* in a $27m^3$ chamber at Emerald are given in Table 4.22. Fumigations were conducted with a phosphine concentrations of 0.33 to 1.2 $g.m^{-3}$, exposure times of 15 to 16 h and temperatures of 18.2 to 25.5°C. All fumigations except two fumigations (fumigated on 30/9/96 and 23/1/97) gave a complete mortality of all the exposed fourth instar larvae. In these particular fumigations only single larva was severely affected at the end of the fumigation. The severely affected larva died within 48 hours following fumigation.

Table 4.22 Toxicity of phosphine to fourth instar larvae of *E. postvittana* fumigated with various concentrations, exposure times and temperatures in a 27 m^3 chamber at Emerald.

Date	Phosfume® (kg)	Phosphine Concentration gm		Exposure time (h)	Temperature (°C)	Lightbrown apple moth *Epiphyas postvittana*			
		Initial	Final			Live	S.aff	Dead	Total
30/9/96	1.74	0.33	0.13	16.0	18.4 (15.5-20.8)	0	1	20	21
30/9/96	0	0	0	0	22.0	21	0	0	21
0/12/96	1.8	0.43	0.36	16.0	18.5 (17.6-19.9)	0	0	24	24
14/1/97	1.98	1.09	0.61	16.0	25.5(22.9-28.9)	0	0	33	33
14/1/97	0	0	0	0	22.0	33	0	0	33
23/1/97	2.36	1.2	0.94	15.0	18.2 (16.9-20.7)	0	1	41	42
23/1/97	0	0	0	0	22.0	42	0	0	42
26/2/97	4.64	0.97	0.61	16.0	18.9(18.1-19.9)	0	0	54	54

Fumigation criteria

Temperature: 17.5°C or more

Exposure time: 16 h or more

Final phosphine concentration: 0.36 $g.m^{-3}$ or more

Twelve fumigations that were conducted with the criteria gave a complete mortality of all the exposed fourth instar larvae (Table 4.23).

Table 4.23 The fumigations with phosphine that satisfy the criteria.

	Number of larvae severely affected	Total number of larvae fumigated
Good		
12/2/97	-	24
9/4/97	-	33
14/4/97	-	18
21/4/97	-	20
29/4/97	-	27
5/5/97	-	20
26/5/97	-	20
3/6/97	-	12
15/7/97	-	15
30/12/96	-	24
14/1/97	-	33
26/2/97	-	54

The Table 4.24 clearly indicates that all the five fumigations that did not satisfy the criteria did not give complete mortality of the exposed larvae.

Table 4.24 The fumigations with phosphine that did not satisfy the criteria.

	Number of larvae severely affected	Total number of larvae fumigated
Marginal		
30/9/96	1(4.8%)	21
23/1/97	1(2.4%)	42
Poor		
17/3/97	7(18.0%)	39
20/3/97	9(43.0%)	21
24/3/97	2(10.0%)	20

Fifth instar larvae

The results of the various fumigation trials to fifth instar larvae of *E. postvittana* conducted at different concentrations of phosphine, exposure times and temperatures are

presented in Table 4.25. A small number of larvae were severely affected in some fumigations and some fumigations gave a complete mortality of all the exposed larvae.

Table 4.25 Toxicity of phosphine to fifth instar larvae of *E. postvittana* fumigated with various concentrations, exposure times and temperatures in a 900 L chamber.

Date	Phosfume® Min	Phosphine Concentration gm^{-3}		Exposure time (h)	Temperature (°C)	RH (%)	Lightbrown apple moth *Epiphyas postvittana*			
		Initial	Final				Live	S. aff	Dead	Total
10/2/97	27	0.98	0.85	15.5	18.6(17.8-20.6)	-	0	3	16	19
10/2/97	0	0	0	0	22.0	65.0	19	0	0	19
12/2/97	27	0.94	0.55	17.0	23.9(18.1-34.8)	84.4(53.7-93.5)	0	0	15	15
12/2097	0	0	0	0	22.0	65.0	15	0	0	15
17/3/97	30	0.64	0.43	16.0	16.3(11.8-24.1)	85.3(44.8-90.3)	0	1	29	30
17/3/97	0	0	0	0	22.0	65.0	29	0	1	30
20/3/97	25	1.08	0.97	16.0	16.9(14.3-21.6)	88.5(71.8-95.2)	0	7	21	28
20/3/97	0	0	0	0	22.0	65.0	28	0	0	28
24/3/97	23	0.78	0.57	17.0	17.4(15.3-24.1)	85.8(50.1-93.4)	0	4	27	31
24/3/97	0	0	0	0	22.0	65.0	31	0	0	31
9/4/97	30	1.32	0.48	16.25	21.1(18.8-25.5)	90.5(44.8-96.4)	0	0	24	24
9/4/97	0	0	0	0	22.0	65.0	24	0	0	24
14/4/97	25	1.01	0.76	17.0	18.9(18.1-19.5)	91.2(79.1-93.4)	0	7	37	44
14/4/97	0	0	0	0	22.0	65.0	41	0	3	44
21/4/97	27	1.22	0.41	16.0	19.4(17.2-25.3)	87.3(60.3-92.8)	0	7	20	27
21/4/97	0	0	0	0	22.0	65.0	25	0	2	27
29/4/97	33	1.14	0.87	16.0	18.4(13.9-26.6)	91.7(62.3-96.4)	0	5	32	37
29/4/97	0	0	0	0	22.0	65.0	36	0	1	37
5/5/97	33	0.83	0.76	18.0	18.3(16.4-21.9)	83.4(66.1-94.6)	0	2	14	16
5/5/97	0	0	0	0	22.0	65.0	16	0	0	16
26/5/97	27	1.0	0.84	17.25	17.5(16.0-19.1)	90.6(85.3-97.0)	0	0	10	10
26/5/97	0	0	0	0	22.0	65.0	10	0	0	10
3/6/97	26	1.04	0.48	16.0	18.3(16.7-19.9)	80.2(67.0-87.4)	0	3	15	18
3/6/97	0	0	0	0	22.0	65.0	18	0	0	18
15/7/97	20	0.86	0.48	16.5	17.7(14.9-20.5)	-	0	2	20	22

Results of fumigating fifth instar larvae of *E. postvittana* in a 27 m^3 chamber are given in the Table 4.26. Fumigations were conducted with phosphine concentrations of 0.33 to 1.2 $g.m^{-3}$, exposure times of 15 to 16 h and temperatures of 18.2 to 25.5°C. A small number of larvae were severely affected in some fumigations and other fumigations gave a complete control all the exposed larvae.

Table 4.26 Toxicity of phosphine to fifth instar larvae of *E. postvittana* fumigated with various concentrations, exposure times and temperatures in a 27 m^3 chamber (Emerald).

Date	Phosfume (kg)	Phosphine concentration gm-3		Exposure time (h)	Temperature (°C)	Lightbrown apple moth *Epiphyas postvittana*			
		Initial	Final			Live	S.aff	Dead	Total
30/9/96	1.74	0.33	0.13	16.0	18.4(15.5-20.8)	0	3	47	50
30/9/96	0	0	0	0	22.0	49	0	1	50
30/12/96	1.8	0.43	0.36	16.0	18.4(17.6-19.9)	0	2	43	45
30/12/96	0	0	0	0	22.0	45	0	0	45
14/1/97	1.98	1.09	0.61	16.0	25.5(22.9-28.9)	0	0	14	14
14/1/97	0	0	0	0	22.0	18	0	0	18
23/1/97	2.36	1.2	0.94	15.0	18.2(16.9-20.7)	0	4	26	30
23/1/97	0	0	0	0	22.0	30	0	0	30
26/2/97	4.64	0.97	0.61	16.0	18.9(18.1-19.9)	0	0	51	51

Fumigation criteria

Temperature: 21.1°C or more

Exposure time: 16 h or more

Final phosphine concentration: 0.48 $g.m^{-3}$ or more

It is clear from the table that all the three fumigations that satisfied the criteria gave a complete mortality of all the exposed larvae (Table 4.27).

Table 4.27 The fumigations with phosphine that satisfy the criteria.

	Number of larvae severely affected	Number fumigated
Good		
12/2/97	-	15
9/4/97	-	24
14/1/97	-	14

It can be seen from the Table 4.28 that two out of fourteen fumigations that did not satisfy the criteria gave a complete mortality of all the exposed larvae due to reasons unknown. Also, four fumigations were marginal where a small number of individuals were severely affected. All these severely affected larvae were dead within 72 h following fumigations. Eight fumigations that did not satisfy the criteria were of poor and a number of larvae were severely affected. These larvae took 24 to 168 h for complete mortality.

Table 4.28 The fumigations with phosphine that did not satisfy the criteria.

	Number of larvae severely affected	Number fumigated
Good		
26/5/97	-	10
26/2/97	-	51
Marginal		
17/3/97	1(3.3%)	30
15/7/97	2(9.0%)	22
30/9/96	3(6.0%)	50
30/12/96	2(4.4%)	45
Poor		
5/5/97	2(12.5%)	16
10/2/97	3(15.8%)	19
20/3/97	7(25.0%)	28
24/3/97	4(12.9%)	31
14/4/97	7(16.0%)	44
21/4/97	7(25.9%)	27
29/4/97	5(14.0%)	37
3/6/97	3(16.6%)	18
23/1/97	4(13.3%)	30

Number of days required for the severely affected larvae to die is given in Table 4.29. Generally most of the larvae were found dead 24 to 48 h after fumigation. Only a single larva was found still severely affected beyond this time. A period of two to five days was observed to be essential for complete mortality of all the severely affected larvae.

Table 4.29 Number of days required for complete mortality of the severely affected larvae.

Date	Phosfume®		No. of larvae severely affected assessment after fumigation (h)						No. of days for complete mortality
	Initial	Final	3	24	48	72	96	120	
10/2/97	0.98	0.85	3	2	2	2	-	-	3
17/3/97	0.64	0.43	1	1	1	1	1	1	5
20/3/97	1.08	0.97	7	1	1	1	1	-	4
24/3/97	0.78	0.57	4	2	2	1	1	-	4
14/4/97	1.01	0.76	7	7	6	1	-	-	3
21/4/97	1.22	0.41	7	7	7	5	2	1	5
29/4/97	1.14	0.87	5	5	3	2	1	1	5
5/5/97	0.83	0.76	2	2	2	1	-	-	3
3/6/97	1.04	0.48	3	1	1	-	-	-	2
15/7/97	0.86	0.48	1	1	1	-	-	-	2

The number of days required for complete mortality of the severely affected fifth instar larvae are given in Table 4.30. Similar to other instar stages most of the severely affected fifth instar larvae were dead within 3 to 24 h following fumigation. Only a single larva was still severely affected for 48 h.

Table 4.30 Number of days required for the severely affected larvae to die.

Date	Phosphine concentration gm^{-3}		No. of larvae severely affected assessment after fumigation (h)			No. of days for complete mortality
	Initial	Final	3	24	48	
30/9/96	0.33	0.13	3	1	1	2
30/12/96	0.43	0.36	2	2	-	1
14/1/97	1.09	0.61	4	1	1	2
23/1/97	1.2	0.94	4	1	1	2

Sixth instar larvae

The mortality rate of the phosphine fumigated sixth instar larvae of *E. postvittana* with various concentrations, exposure times and temperatures are presented in Table 4.31. This table clearly illustrates that a number of larvae were severely affected in all the trials.

Table 4.31 Toxicity of phosphine to sixth instar larvae of the *E. postvittana* fumigated with various concentrations, exposure times and temperatures in a 900 L chamber.

Date	Phosfume (min)	Phosphine Concentration gm^{-3}		Exposure time (h)	Temperature (°C)	RH (%)	Lightbrown apple moth *Epiphyas postvittana*			
		Initial	Final				Live	S. aff	Dead	Total
10/2/97	27	0.98	0.85	15.5	18.6 (17.8-20.6)	-	0	7	4	11
10/2/97	0	0	0	0	22.0	65.0	11	0	0	11
12/2/97	27	0.94	0.55	17.0	23.9 (18.1-34.8)	84.4(53.7-93.5)	0	2	14	16
12/2/97	0	0	0	0	22.0	65.0	16	0	0	16
17/3/97	30	0.64	0.43	16.0	16.3 (11.8-24.1)	85.3(44.8-90.3)	0	14	33	47
17/3/97	0	0	0	0	22.0	65.0	45	0	2	47
20/3/97	25	1.08	0.97	16.0	16.9 (14.3-21.6)	88.5(71.8-95.2)	0	29	14	43
20/3/97	0	0	0	0	22.0	65.0	43	0	0	43
24/3/97	23	0.78	0.57	17.0	17.4 (15.3-24.1)	85.8(50.1-93.4)	0	3	16	19
24/3/97	0	0	0	0	22.0	65.0	17	0	2	19
9/4/97	30	1.32	0.48	16.25	21.1(18.8-25.5)	90.5(44.8-96.4)	0	1	20	21
9/4/97	0	0	0	0	22.0	65.0	21	0	0	21
14/4/97	25	1.01	0.76	17.0	18.9(18.1-19.5)	91.2(79.1-93.4)	0	2	20	22
14/4/97	0	0	0	0	22.0	65.0	22	0	0	22
21/4/97	27	1.22	0.41	16.0	19.4(17.2-25.3)	87.3(60.3-92.8)	0	4	15	19
21/4/97	0	0	0	0	22.0	65.0	19	0	0	19
29/4/97	33	1.14	0.87	16.0	18.4(13.9-26.6)	91.7(62.3-96.4)	0	13	45	58
29/4/97	0	0	0	0	22.0	65.0	57	0	1	58
5/5/97	33	0.83	0.76	18.0	18.3(16.4-21.9)	83.4(66.1-94.6)	0	2	20	22
5/5/97	0	0	0	0	22.0	65.0	22	0	0	22
26/5/97	27	1.0	0.84	17.25	17.5(16.0-19.1)	90.6(85.3-97.0)	0	4	18	22
26/5/97	0	0	0	0	22.0	65.0	22	0	0	22
3/6/97	26	1.04	0.48	16.0	18.3(16.7-19.9)	80.2(67.0-87.4)	0	7	7	14
3/6/97	0	0	0	0	22.0	65.0	14	0	0	14

The proportion of larvae that was severely affected was comparatively higher from the 16 h or less fumigations than the larvae that were fumigated for 17 h or more. These results suggest that the sixth instar larvae are more tolerant than younger larvae presumably due to their lower metabolism.

Number of days required for the complete mortality of the severely affected larvae is given in Table 4.32. Most of these larvae died 3 to 48 h after fumigation and only a single larva was still severely affected for 72 to 120 h.

Table 4.32 Number of days required for complete mortality of the severely affected larvae.

Date	Phosphine concentration gm^{-3}		No. of larvae severely affected assessment after fumigation (h)						No. of days for complete mortality
	Initial	Final	3	24	48	72	96	120	
10/2/97	0.98	0.85	7	6	1	1	1		4
12/2/97	0.94	0.55	2	1	1	-	-	-	2
17/3/97	0.64	0.43	1	1	1	1	-	-	3
20/3/97	1.08	0.97	29	9	2	1	-	-	3
24/3/97	0.78	0.57	3	1	1	1	-	-	3
9/4/97	1.32	0.48	3	2	2	1	1	-	4
14/4/97	1.01	0.76	3	3	2	1	1	1	5
21/4/97	1.22	0.41	4	4	2	1	1	-	4
29/4/97	1.14	0.87	13	10	4	1	1	-	4
5/5/97	0.83	0.76	4	1	1	1	-	-	3
26/5/97	1.0	0.84	2	1	1	1	1	-	4
3/6/97	1.04	0.48	10	3	1	1	1	-	4
15/7/97	0.86	0.48	3	2	1	1	-	-	3

A small number of sixth instar larvae was severely affected and all others were dead in all the fumigations (Table 4.33) that were conducted at Emerald. All the control individuals survived and completed their life cycle.

Table 4.33 Toxicity of phosphine to sixth instar larvae of *E. postvittana* fumigated with various concentrations, exposure times and temperatures in a 27 m^3 chamber at Emerald

Date	Phosfume (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Lightbrown apple moth *Epiphyas postvittana*			
		Initial	Final			Live	S.aff	Dead	Total
30/9/96	1.74	0.33	0.13	16.0	18.4(15.5-20.8)	0	3	46	49
30/9/96	0	0	0	0	22.0	49	0	0	49
30/12/96	1.8	0.43	0.36	16.0	18.4(17.6-19.9)	0	2	52	54
30/12/96	0	0	0	0	22.0	54	0	0	54
14/1/97	1.98	1.09	0.61	16.0	25.5(22.9-28.9)	0	3	30	33
14/1/97	0	0	0	0	22.0	33	0	0	33
23/1/97	2.36	1.2	0.94	15.0	18.2(16.9-20.7)	0	4	27	31
23/1/97	0	0	0	0	22.0	31	0	0	31

Number of days required for the severely affected sixth instar larvae to die is given in Table 4.34. Unlike the fifth or sixth instar larvae that were fumigated in a 900 L chamber, these severely affected larvae were dead 3 to 72 h following fumigation.

Table 4.34 Number of days required for complete mortality of the severely affected larvae.

Date	Phosphine concentration gm^{-3}		No. of larvae severely affected assessment after fumigation (h)				No. of days for complete mortality
	Initial	Final	3	24	48	72	
30/9/96	0.33	0.13	3	1	1	1	3
30/12/96	0.43	0.36	2	2	2	2	3
14/1/97	1.09	0.61	3	2	1	-	2
23/1/97	1.2	0.94	4	2	2	-	2

The results obtained from the 27 m^3 chamber at Gembrook are presented in Table 4.35. Similar to other trials, a number of larvae were severely affected in both fumigations.

Table 4.35 Toxicity of phosphine to sixth instar larvae of *E. postvittana* fumigated with various concentrations, exposure times and temperatures in a 27 m^3 chamber at Gembrook.

Date	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Lightbrown apple moth *Epiphyas postvittana*			
		Initial	Final			Live	S.aff	Dead	Total
18/12/96	1.0	0.41	0.12	16.0	17.1	0	29	19	48
18/12/96	0	0	0	0	22.0	47	0	1	48
8/7/97	0.16	0.48	0.08	15.0	17.5(15.6-19.8)	0	3	43	46
8/7/97	0	0	0	0	22.0	46	0	0	46

The number of days required for the severely affected sixth instar larvae to die is presented in Table 4.36. These severely affected larvae required 2 to 5 days for complete mortality, however, the majority died 3 to 72 h after fumigation.

Table 4.36 Number of days required for complete mortality of the severely affected larvae.

Date	Phosphine concentration gm^{-3}		No. of larvae severely affected assessment after fumigation (h)				No. of days for complete mortality	Instar stage
	Initial	Final	3	24	48	72		
18/12/96	0.41	0.12	29	21	2	1	11	IV, V and VI
8/7/97	0.48	0.08	3	3	1	1	7	VI

Fumigation criteria

Not a single fumigation (fumigation conditions are outlined below) gave a complete mortality of the exposed sixth instar larvae. A number of larvae were severely affected following completion of experiments and these individuals took 3 to 168 h for complete mortality. A large number of larvae had died within 48 h following fumigations and only a single larva was still severely affected for another 168 h.

Temperature: 16.3 to 25.5°C

Exposure time: 15 to 18 h

Final phosphine concentration: 0.13 to 0.94 $g.m^{-3}$.

It is clear from this Table 4.37 that of the 17 fumigations six were marginal in nature and others were poor. Of all the poor fumigations, 18/12/96 (60.4 per cent severely affected), 10/2/97 (63.7 per cent severely affected) and 20/3/97 (67.4 per cent severely affected) fumigations had large number of severely affected larvae.

Table 4.37 The fumigations with phosphine that did not satisfy the criteria.

	Number of larvae severely affected	Number fumigated
Marginal		
9/4/97	1(4.8%)	21
14/4/97	2(9.1%)	22
5/5/97	2(9.0%)	22
30/9/96	3(6.1%)	49
30/12/96	2(3.7%)	54
14/1/97	3(9.0%)	33
8/7/97	3(6.5%)	46
Poor		
18/12/96	29(60.4%)	48
10/2/97	7(63.7%)	11
12/2/97	2(12.5%)	16
20/3/97	29(67.4%)	43
24/3/97	3(15.8%)	19
17/3/97	14(29.8%)	47
21/4/97	4(21.1%)	19
29/4/97	13(22.4%)	58
26/5/97	4(18.2%)	22
3/6/97	7(50.0%)	14
15/7/97	3(16.6%)	18
23/1/97	4(12.9%)	31

4.4.2.3 Pre-pupae

Toxicity of phosphine to pre-pupae of *E. postvittana* is presented in Table 4.38. In all the fumigations a small number of individuals were severely affected, except in one fumigation (fumigated on 12/2/97) where complete mortality of all the exposed pre-pupae was achieved. Ninety per cent of these pre-pupae were found on the medium and

all these were killed, probably during fumigation. Only those pre-pupae that remained in the medium were severely affected and these were dead 3 to 96 h following fumigation.

Table 4.38 Toxicity of phosphine to the pre-pupae of *E. postvittana* fumigated with various concentrations, exposure times and temperatures in a 900 L chamber.

Date	Phosfume (min)	Phosphine concentration gm^{-3}		Exposure Time (h)	Temperature (°C)	RH (%)	Lightbrown apple moth *Epiphyas postvittana*			
		Initial	Final				Live	S.aff	Dead	Total
10/2/97	27	0.98	0.85	15.5	8.6 (17.8-20.6)	-	0	2	7	9
10/2/97	0	0	0	0	22.0	65.0	9	0	0	9
12/2/97	27	0.94	0.55	17.0	3.9 (18.1-34.8)	84.4(53.7-93.5)	0	0	14	14
12/2/97	0	0	0	0	22.0	65.0	12	0	2	14
17/3/97	30	0.64	0.43	16.0	6.3 (11.8-24.1)	85.3(44.8-90.3)	0	1	6	7
20/3/97	25	1.08	0.97	16.0	6.9 (14.3-21.6)	88.5(71.8-95.2)	0	2	14	16
20/3/97	0	0	0	0	22.0	65.0	16	0	0	16
24/3/97	23	0.78	0.57	17.0	7.4 (15.3-24.1)	85.8(50.1-93.4)	0	1	5	6
24/3/97	0	0	0	0	22.0	65.0	5	0	1	6
14/4/97	25	1.01	0.76	17.0	18.9(18.1-19.5)	91.2(79.1-93.4)	0	1	8	9
14/4/97	0	0	0	0	22.0	65.0	9	0	0	9
29/4/97	33	1.14	0.87	16.0	18.4(13.9-26.6)	91.7(62.3-96.4)	0	2	10	12
29/4/97	0	0	0	0	22.0	65.0	12	0	0	12
5/5/97	33	0.83	0.76	18.0	18.3(16.4-21.9)	83.4(66.1-94.6)	0	3	10	13
5/5/97	0	0	0	0	22.0	65.0	12	0	1	13

Fumigation criteria

Temperature: 23.9°C or more

Exposure time: 17 h or more

Phosphine concentration: 0.55 $g.m^{-3}$ or more

Only one fumigation that satisfy the criteria gave a complete mortality of all the exposed pupae (Table 4.39).

Table 4.39 The fumigations with phosphine that satisfy the criteria

	Number of pre-pupae severely affected	Number fumigated
Good		
12/2/97	-	14

Table 4.40 clearly shows that all the seven fumigations that did not satisfy the fumigation criteria failed to kill all the exposed pupae.

Table 4.40 The fumigations with phosphine that did not satisfy the criteria.

	Number of pre-pupae severely affected	Number fumigated
Poor		
10/2/97	2(22.0%)	9
17/3/97	1(14.3%)	7
20/3/97	2(12.5%)	16
24/3/97	1(16.7%)	6
14/4/97	1(11.1%)	9
29/4/97	2(16.6%)	12
5/5/97	3(23.1%)	13

Data from this table (Table 4.41) indicate that most of these severely affected pre-pupae were dead within 48 h after fumigation, and only a single pre-pupa was still severely affected for 72 to 96 h following fumigation.

Table 4.41 Number of days required for complete mortality of the severely affected pre-pupae.

Date	Phosphine concentration gm^{-3}		No. of larvae severely affected assessment after fumigation (h)					No. of days for complete mortality
	Initial	Final	3	24	48	72	96	
10/2/97	0.98	0.85	2	2	2	0	0	2
17/3/97	0.64	0.43	1	1	1	0	0	2
20/3/97	1.08	0.97	2	2	2	1	1	4
24/3/97	0.78	0.57	1	1	1	0	0	2
14/4/97	1.01	0.76	1	1	1	0	0	2
29/4/97	1.14	0.87	2	1	1	1	0	3
5/5/97	0.83	0.76	3	3	1	1	1	4

4.4.2.4 Pupae

The results of phosphine fumigation on young and mature pupae of both sexes are presented in Table 4.42.

Table 4.42 Toxicity of phosphine to pupae of *E. postvittana* fumigated with various concentrations, exposure times and temperatures in a 900 L chamber at Frankston.

Date	Phosfume® (min)	Phosphine concentration gm-3		Exposure time (h)	Temperature (°C)	Lightbrown apple moth *Epiphyas postvittana*			
		Initial	Final			Live	S. aff	Dead	Total
10/2/97	27	0.98	0.85	15.5	18.6 (17.8-20.6)	0	0	32	32
10/2/97	0	0	0	0	25.0	32	0	0	32
12/2/97	27	0.94	0.55	17.0	23.9 (18.1-34.8)	0	0	29	29
12/2/97	0	0	0	0	25.0	28	0	1	29
17/3/97	30	0.64	0.43	16.0	16.3 (11.8-24.1)	0	6*	21	27
17/3/97	0	0	0	0	25.0	27	0	0	27
20/3/97	25	1.08	0.97	16.0	16.9 (14.3-21.6)	0	2*	32	34
20/3/97	0	0	0	0	25.0	34	0	0	34
24/3/97	23	0.78	0.57	17.0	17.4 (15.3-24.1)	0	0	15	15
24/3/97	0	0	0	0	25.0	14	0	1	15
9/4/97	30	1.32	0.48	16.25	21.1(18.8-25.5)	0	0	22	22
9/4/97	0	0	0	0	25.0	21	0	1	22
14/4/97	25	1.01	0.76	17.0	18.9(18.1-19.5)	0	0	30	30
21/4/97	27	1.22	0.41	16.0	19.4(17.2-25.3)	0	0	19	19
21/4/97	0	0	0	0	25.0	16	0	3	19
29/4/97	33	1.14	0.87	16.0	18.4(13.9-26.6)	0	0	24	24
29/4/97	0	0	0	0	25.0	22	0	2	24
5/5/97	33	0.83	0.76	18.0	18.3(16.4-21.9)	0	0	51	51
5/5/97	0	0	0	0	25.0	50	0	1	51
26/5/97	27	1.0	0.84	17.25	17.5(16.0-19.1)	0	0	36	36
26/5/97	0	0	0	0	25.0	36	0	0	36
3/6/97	26	1.04	0.48	16.0	18.3(16.7-19.9)	0	0	34	34
3/6/97	0	0	0	0	25.0	32	0	2	34
15/7/97	20	0.86	0.48	16.5	17.7(14.9-20.5)	0	0	60	60
15/7/97	0	0	0	0	25.0	60	0	0	60
8/7/97	0.16	0.48	0.08	15.0	17.5(15.6-19.8)	0	0	50	50

* - Died 24 to 48 h after hatching.

A phosphine concentration of 0.48 to 1.32 $g.m^{-3}$ (final concentration of 0.08 to 0.97 $g.m^{-3}$) with an exposure period of 15 to 18 h at a temperature range of 17.4 to 23.9°C was investigated. Immediately after fumigation, and for up to 48 hours, all the fumigated pupae responded in a similar manner to the control pupae while prodding i.e. wriggling movements of abdominal segments. However, this wriggling ceased 48 to 72 h after the end of fumigation. All the dead pupae turned to brownish black four to five days after fumigation, and this proceeded to black accompanied by desiccation and shrinking. However, in two fumigations (conducted on 17/3/97 and 20/3/97) a number of pupae were severely affected, probably due to lower fumigation temperature. The adults from these pupae did not fully emerged and they were unable to release their wings and abdomen from the pupal case. They died within 48 h of their attempted emergence.

Fumigation criteria

Temperature: 17.4°C or more

Exposure time: 15 h or more

Phosphine concentration: 0.08 $g.m^{-3}$ or more

This table clearly illustrates that once all the fumigation criteria are met, complete mortality of all the exposed pupae can be achieve. In all the 12 fumigations that satisfy the criteria, all the pupae exposed were killed.

Table 4.43 The fumigations with phosphine that satisfy the criteria.

	Number of pupae severely affected	Number fumigated
Good		
10/2/97	-	32
12/2/97	-	29
24/3/97	-	15
9/4/97	-	22
14/4/97	-	30
21/4/97	-	19
29/4/97	-	24
5/5/97	-	51

	Number of pupae severely affected	Number fumigated
26/5/97	-	36
3/6/97	-	34
15/7/97	-	60
8/7/97	-	50

The Table 4.44 indicates that one fumigation is marginal and one is of poor, where a number of pupae were survived. The surviving pupae completed their life-cycles similar to the control pupae.

Table 4.44 The fumigations with phosphine that did not satisfy the criteria.

	Number of pupae not killed	Number fumigated
Marginal		
20/3/97	2(6.0%)	34
Poor		
17/3/97	6(22.2%)	27

The proportion of different age male and female pupae that severely affected from different fumigations are given in Table 4.45. The results indicate that one to three-day old pupae of both sexes were more tolerant of phosphine fumigation than their mature counterparts. This table indicates that highest survival rate was obtained from the comparatively lower concentration of phosphine (0.43 $g.m^{-3}$) and lower temperature (16.3°C) fumigations than from the higher concentration of phosphine (0.97 $g.m^{-3}$) and

Table 4.45 Number of fumigated pupae of different sex and age that severely affected.

Date	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Number hatched					
					Male			Female		
	Initial	Final			1 to 3 days		> 4 days	1 to 3 days		> 4 days
					S.aff	Dead	Dead	S.aff	Dead	Dead
17/3/97	0.43	16.0	16.0	16.3(11.8-24.1)	2	4	6	4	4	7
20/3/97	0.97	16.0	16.0	16.9(14.3-21.6)	0	7	9	2	7	9

higher temperature (16.9°C) fumigations. Moreover, the data suggest that female pupae are comparatively more resistant to phosphine fumigation than male pupae. The pupae that survived hatched three to five days later than the control pupae and this could be due to the delaying effects of phosphine.

4.4.2.5 Adults

The responses to fumigation with phosphine of adult *E. postvittana* to various concentrations and exposure times at different temperatures are presented in Table 4.46.

Table 4.46 Toxicity of phosphine to adults of *E. postivttana* fumigated with various concentrations, exposure times and temperatures in a 900 L chamber.

Date	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	RH (%)	ightbrown apple moth *Epiphyas postvittana*		
		Initial	Final				Live	Dead	Total
10/2/97	27	0.98	0.85	15.5	18.6(17.8-20.6)	-	0	15	15
10/2/97	0	0	0	0	22.0	65.0	15	0	15
17/3/97	30	0.64	0.43	16.0	16.3(11.8-24.1)	85.3(44.8-90.3)	0	25	25
17/3/97	0	0	0	0	22.0	65.0	25	0	25
20/3/97	25	1.08	0.97	16.0	16.9(14.3-21.6)	88.5(71.8-95.2)	0	21	21
20/3/97	0	0	0	0	22.0	65.0	21	0	21
24/3/97	23	0.78	0.57	17.0	17.4(15.3-24.1)	85.8(50.1-93.4)	0	18	18
24/3/97	0	0	0	0	22.0	65.0	16	2	18
9/4/97	30	1.32	0.48	16.25	21.1(18.8-25.5)	90.5(44.8-96.4)	0	27	27
9/4/97	0	0	0	0	22.0	65.0	27	0	27
14/4/97	25	1.01	0.76	17.0	18.9(18.1-19.5)	91.2(79.1-93.4)	0	20	20
14/4/97	0	0	0	0	22.0	65.0	20	0	20
21/4/97	27	1.22	0.41	16.0	19.4(17.2-25.3)	87.3(60.3-92.8)	0	18	18
21/4/97	0	0	0	0	22.0	65.0	18	0	18
29/4/97	33	1.14	0.87	16.0	18.4(13.9-26.6)	91.7(62.3-96.4)	0	24	24
29/4/97	0	0	0	0	22.0	65.0	23	1	24
5/5/97	33	0.83	0.76	18.0	18.3(16.4-21.9)	83.4(66.1-94.6)	0	29	29
5/5/97	0	0	0	0	22.0	65.0	29	9	29
3/6/97	26	1.04	0.48	16.0	18.3(16.7-19.9)	80.2(67.0-87.4)	0	30	30
3/6/97	0	0	0	0	22.0	65.0	30	0	30
15/7/97	20	0.86	0.48	16.5	17.7(14.9-20.5)	-	0	25	25

The responses of various ages of adults (2 to 12 days old of both sexes) were similar and all were killed in all the experiments. Hence, it can be concluded that adult moths are of highly sensitive to phosphine fumigation.

4.4.3 The two-spotted mite, Tetranychus urticae

4.4.3.1 Eggs

The stages of eggs were determined 2 to 3 h after fumigation by visual inspection, under a microscope using the colour and shape of the egg as parameters. The young eggs are generally transparent, and as they mature their colour changes to yellow, and subsequently they become creamy yellow 24 to 48 h before hatching. Moreover, the mature eggs can be easily identified by the presence of two red dots on the surface of the chorion. The young and mature eggs were distinguished by putting markings using different coloured fine-tip permanent markers near to the eggs on the leaves without damaging the eggs or leaves.

The results of the phosphine fumigations of eggs (both young and mature) are given in Table 4.47. Fumigations were conducted with phosphine concentrations of 0.25 to 1.2 $g.m^{-3}$, exposure times of 15 to 16 h and temperatures of 18.2 to 25.5°C. In most of the fumigations a number of young eggs (one to three days old) survived and developed into normal adults. However, complete mortality of all the four to six days old eggs was obtained in all the fumigations.

Table 4.47 Toxicity of phosphine to eggs of *T. urticae* fumigated with various concentrations, exposure times and temperatures in a 27 m^3 chamber at Emerald.

Date	Phosfume (kg)	Phosphine Concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Two-spotted mite *Tetranychus urticae*		
		Initial	Final			Live	Dead	Total
30/9/96	1.74	0.25	0.15	16.0	18.4(15.5-20.8)	0	465	465
30/9/96	0	0	0	0	25.0	460	5	465
30/12/96	1.8	0.43	0.36	16.0	18.4(17.6-19.9)	0	754	754
30/12/96	0	0	0	0	25.0	752	2	754
14/1/97	1.98	1.09	0.61	16.0	25.5(22.9-28.9)	5	115	120
14/1/97	0	0	0	0	25.0	118	2	120
23/1/97	2.36	1.2	0.94	15.0	18.2(16.9-20.7)	125	520	645
23/1/97	0	0	0	0	25.0	644	1	645
1/5/97	4.64	0.97	0.61	16.0	18.9(18.1-19.9)	0	270	270
1/5/97	0	0	0	0	25.0	270	0	270

Results obtained in the fumigation trials that were conducted in a 900 L chamber at Gembrooke and Frankston are given in Table 4.48 and 4.49. A small number of young eggs had survived in some fumigations.

Table 4.48 Toxicity of phosphine to eggs of *T. urticae* fumigated with various concentrations, exposure times and temperatures in a 27 m^3 chamber at Gembrook.

Date	Phosfume® kg	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Two-spotted mite *Tetranychus urticae*		
		Initial	Final			Live	Dead	Total
18/12/96	1.0	0.41	0.12	16.0	17.1	127	796	923
18/12/96	0	0	0	0	22.0	918	5	923
8/7/97	0.16	0.48	0.08	15.0	17.5(15.6-19.8)	630	770	1400
8/7/97	0	0	0	0	22.0	1378	22	1400

Table 4.49 Toxicity of phosphine to eggs of *T. urticae* fumigated with various concentrations, exposure times and temperatures in a 900 L chamber at Frankston.

Date	Phosfume ® Kg	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (^0C)	RH (%)	Two-spotted mite *Tetranychus urticae*		
		Initial	Final				Live	Dead	Total
12/2/97	20	0.94	0.55	17.0	23.9 (18.1-34.8)	84.4 (53.7-93.5)	0	184	184
12/2/97	0	0	0	0	22.0	60.0	182	2	184
12/3/97	20	0.42	0.38	15.5	15.7 (14.0-23.0)	85.2 (62.7-90.3)	68	165	233
12/3/97	0	0	0	0	22.0	60.0	230	3	233
17/3/97	30	0.64	0.43	16.0	16.3 (11.8-24.1)	85.3 (44.8-90.3)	46	180	226
17/3/97	0	0	0	0	22.0	60.0	226	0	226
20/3/97	25	1.08	0.97	16.0	16.9 (14.3-21.6)	88.5 (71.8-95.2)	0	220	220
20/3/97	0	0	0	0	22.0	60.0	218	2	220
24/3/97	23	0.78	0.57	17.0	17.4 (15.3-24.1)	85.8 (50.1-93.4)	29	241	270
24/3/97	0	0	0	0	22.0	60.0	270	0	270
9/4/97	30	1.32	0.48	16.25	21.1(18.8-25.5)	90.5(44.8-96.4)	2	358	360
9/4/97	0	0	0	0	22.0	60.0	360	0	360
14/4/97	25	1.01	0.76	17.0	18.9(18.1-19.5)	91.2(79.1-93.4)	3	274	279
14/4/97	0	0	0	0	22.0	60.0	277	2	279
21/4/97	27	1.22	0.41	16.0	19.4(17.2-25.3)	87.3(60.3-92.8)	12	478	490
21/4/97	0	0	0	0	22.0	60.0	488	2	490
29/4/97	33	1.14	0.87	16.0	18.4(13.9-26.6)	91.7(62.3-96.4)	20	580	600
29/4/97	0	0	0	0	22.0	60.0	600	0	600
5/5/97	33	0.83	0.76	18.0	18.3(16.4-21.9)	83.4(66.1-94.6)	0	180	180
5/5/97	0	0	0	0	22.0	60.0	180	0	180
26/5/97	27	1.0	0.84	17.25	17.5(16.0-19.1)	90.6(85.3-97.0)	37	683	720
26/5/97	0	0	0	0	22.0	60.0	720	0	720
3/6/97	26	1.04	0.48	16.0	18.3(16.7-19.9)	80.2(67.0-87.4)	69	531	600
3/6/97	0	0	0	0	22.0	60.0	597	3	600
15/7/97	20	0.86	0.48	16.5	17.7(14.9-20.5)	-	45	245	290
15/7/97	0	0	0	0	22.0	60.0	288	2	290

Fumigation criteria

Exposure time: 16 h or more

Initial phosphine concentration: 0.15 $g.m^{-3}$ or more

Final phosphine concentration: 0.069 $g.m^{-3}$ or more

Temperature: 16.9°C or more

Six out of seven fumigations that satisfy the criteria have produced desired results, where a large number of eggs were exposed and were killed (Table 4.50).

Table 4.50 Number of fumigations fitted into this category.

	Number of eggs survived	Total number of eggs fumigated
Good		
30/9/96	-	465
30/12/96	-	754
1/5/97	-	270
26/2/96	-	103
12/2/97	-	184
5/5/97	-	180
Marginal		
24/3/97	29 (10.0%)	270

In the marginal fumigation a small number of eggs survived and developed into normal larvae. The probable reason for the failure would be the lower initial temperature, where the initial temperature begun with less than 16.9°C (15.0 to 16.9°C) for the first five hours, also during middle of the fumigation the temperature was less than optimal for another 4 h.

Of the 12 fumigations that did not meet the criteria only a single fumigation gave a complete control of all the exposed eggs (Table 4.51). A small number of eggs survived in six fumigations presumably due to higher initial phosphine concentrations. Also, five fumigations yielded poor control of eggs, this is either due to higher initial phosphine concentration or lower average temperature.

Table 4.51 Fumigations that did not meet the criteria

	Number of eggs survived	Total number of eggs fumigated
Good		
20/3/97	-	220
Marginal		
14/1/97	5(4.2%)	120
9/4/97	2(0.5%)	360
14/4/97	3(1.0%)	279
21/4/97	12(2.4%)	490
29/4/97	20(3.3%)	600
26/5/97	37(5.1%)	720
Poor		
12/3/97	68(29.0%)	233
17/3/97	46(20.3%)	226
3/6/97	69(11.5%)	600
15/7/97	45(15.5%)	290
23/1/97	125(19.4%)	645

Table 4.52 shows the number of larvae that emerged at various days after fumigation from the incubated eggs. The time required to hatch and the number of eggs that survived were compared with control eggs by running a parallel colony that was maintained at identical conditions. All the unfumigated eggs hatched three to five days at a constant temperature condition of 25°C and 40 to 45 per cent relative humidity. However, the young eggs surviving phosphine fumigations took 7 to 11 days for emergence. The fumigated eggs that survived began to emerge four days after incubation and the highest numbers emerged five to seven days following fumigation. Not a single egg hatched 11 days following fumigation. However, in the control samples the emergence was uniformly spaced, as one would expect in a normal culture. Of all the eggs fumigated, the order of tolerance in descending order is one day old > two days old > three days old. Eggs of four days old and above were far more susceptible to phosphine fumigation than younger eggs.

Table 4.52 Number of days required for the surviving fumigated eggs to hatch.

Date	Phosphine Concentration gm^{-3}		Exposure time (h)	No. of days required for the surviving eggs for hatching										No. Hatched
	Initial	Final		3	4	5	6	7	8	9	10	11	>12	
14/1/97	1.09	0.61	16.0	0	0	0	1	2	2	0	0	0	0	5
23/1/97	1.2	0.94	15.0	0	10	24	36	40	15	10	0	0	0	125
12/3/97	0.42	0.38	15.5	0	1	1	2	3	2	2	1	0	0	12
17/3/97	0.64	0.43	16.0	0	0	0	0	5	0	2	0	0	0	7
24/3/97	0.78	0.57	17.0	0	5	6	3	7	1	5	2	0	0	29
9/4/97	1.32	0.48	16.25	0	0	2	0	0	0	0	0	0	0	2
14/4/97	1.01	0.76	17.0	0	0	0	3	0	0	0	0	0	0	3
21/4/97	1.22	0.41	16.0	0	0	0	2	5	5	0	0	0	0	12
26/5/97	1.0	0.84	17.25	0	3	5	7	13	7	2	0	0	0	37
3/6/97	1.04	0.48	16.0	0	7	5	7	30	10	10	0	0	0	69
25/6/97	0.47	0.33	18.0	0	0	0	0	0	1	4	0	0	0	5
8/12/96	0.41	0.12	16.0	0	15	12	18	40	12	10	15	5	0	127
8/7/97	0.48	0.08	15.0	5	21	4	250	100	50	100	50	50	0	630

Table 4.53 clearly illustrates that large numbers of one-day old eggs had survived and these developed into normal larvae and completed their life-cycle. However, comparatively small number of two to three days old eggs survived. The observations made on the F_1 generation showed that the reproduction or multiplication was not affected and these were similar to the individuals that emerged from the control eggs. These results indicate that some young eggs are more tolerant to phosphine fumigation than their mature counterparts.

Table 4.53 Number of different age eggs of *T. urticae* hatched that were fumigated with different concentrations of phosphine, exposure times and temperatures.

Date	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Number hatched (age of eggs)			
	Initial	Final			1	2	3	4-6
14/1/97	1.09	0.61	16.0	25.5(22.9-28.9)	5	0	0	0
23/1/97	1.2	0.94	15.0	18.2(16.9-20.7)	96	20	9	0
12/3/97	0.42	0.38	15.5	15.7(14.0-23.0)	54	12	2	0
17/3/97	0.64	0.43	16.0	16.3(11.8-24.1)	30	16	0	0
24/3/97	0.78	0.57	17.0	17.4(15.3-24.1)	29	9	0	0
9/4/97	1.32	0.48	16.25	21.1(18.8-25.5)	2	0	0	0
14/4/97	1.01	0.76	17.0	18.9(18.1-19.5)	3	0	0	0
21/4/97	1.22	0.41	16.0	19.4(17.2-25.3)	10	2	0	0
29/4/97	1.14	0.87	16.0	18.4(13.9-26.6)	16	4	0	0
26/5/97	1.0	0.84	17.25	17.5(16.0-19.1)	30	7	0	0
3/6/97	1.04	0.48	16.0	18.3(16.7-19.9)	50	15	4	0
15/7/97	0.86	0.48	16.5	17.7(14.9-20.5)	41	4	0	0
18/12/96	0.41	0.12	16.0	17.1	110	15	2	0
8/7/97	0.48	0.08	15.0	17.5(15.6-19.8)	519	24	16	9

4.4.3.2 Diapause larval stage

The mortality rate of diapause stages that were exposed to various concentrations of phosphine, exposure times and temperatures are presented in Table 4.54. The results from these fumigation trials indicate that the diapause stages of *T. urticae* are susceptible to the concentrations, exposure times and temperatures that were exposed.

Table 4.54 Toxicity of phosphine to diapause larvae of *T. urticae* fumigated with various concentrations, exposure times and temperatures in a 900 L chamber at Frankston.

Date	Phosfume® Kg	Phosphine Concentration gm^{-3}		Exposure time (h)	Temperature (°C)	RH (%)	Two-spotted mite *Tetranychus urticae*		
		Initial	Final				Live	Dead	Total
12/2/97	20	0.94	0.55	17.0	23.9 (18.1-34.8)	84.4 (53.7-93.5)	0	79	79
12/2/97	0	0	0	0	22.0	60.0	75	4	79
12/3/97	20	0.42	0.38	15.5	15.7 (14.0-23.0)	85.2 (62.7-90.3)	0	63	63
12/3/97	0	0	0	0	22.0	60.0	63	0	63
17/3/97	30	0.64	0.43	16.0	16.3 (11.8-24.1)	85.3 (44.8-90.3)	0	117	117
17/3/97	0	0	0	0	22.0	60.0	115	2	117
20/3/97	25	1.08	0.97	16.0	16.9 (14.3-21.6)	88.5 (71.8-95.2)	0	200	200
20/3/97	0	0	0	0	22.0	60.0	197	3	200
24/3/97	23	0.78	0.57	17.0	17.4 (15.3-24.1)	85.8 (50.1-93.4)	0	60	60
24/3/97	0	0	0	0	22.0	60.0	60	0	60
9/4/97	30	1.32	0.48	16.25	21.1(18.8-25.5)	90.5(44.8-96.4)	0	70	70
9/4/97	0	0	0	0	22.0	60.0	70	0	70
14/4/97	25	1.01	0.76	17.0	18.9(18.1-19.5)	91.2(79.1-93.4)	0	180	180
14/4/97	0	0	0	0	22.0	60.0	178	2	180
21/4/97	27	1.22	0.41	16.0	19.4(17.2-25.3)	87.3(60.3-92.8)	0	65	65
21/4/97	0	0	0	0	22.0	60.0	65	0	65
29/4/97	33	1.14	0.87	16.0	18.4(13.9-26.6)	91.7(62.3-96.4)	0	120	120
29/4/97	0	0	0	0	22.0	60.0	120	0	120
5/5/97	33	0.83	0.76	18.0	18.3(16.4-21.9)	83.4(66.1-94.6)	0	135	135
5/5/97	0	0	0	0	22.0	60.0	135	0	135
26/5/97	27	1.0	0.84	17.25	17.5(16.0-19.1)	90.6(85.3-97.0)	0	270	270
26/5/97	0	0	0	0	22.0	60.0	270	0	270
3/6/97	26	1.04	0.48	16.0	18.3(16.7-19.9)	80.2(67.0-87.4)	0	60	60
3/6/97	0	0	0	0	22.0	60.0	60	0	60
15/7/97	20	0.86	0.48	16.5	17.7(14.9-20.5)	-	0	15	15

4.4.3.3 Larvae

Table 4.55 indicates the effects of various concentrations of phosphine, exposure times and temperatures on the exposed larvae of *T. urticae*. Some fumigations gave a

complete mortality of all the exposed larvae and in some fumigations a small number were severely affected.

Table 4.55 Toxicity of phosphine to larvae of *T. urticae* fumigated with various concentrations, exposure times and temperatures at Gembrook and Emerald.

Date	Phosfume (kg)	Phosphine oncentration gm^{-3}		Exposure Time (h)	Temperature (°C)	R.H (%)	Two-spotted mite *Tetranychus urticae*			
		Initial	Final				Live	S. aff	Dead	Total
30/9/96	1.74	0.25	0.15	16.0	18.4(15.5-20.8)	-	0	2*	425	427
30/9/96	0	0	0	0	22.0	40.0	425	0	2	427
18/12/96	1.0	0.41	0.12	16.0	17.1	-	0	3*	947	947
18/12/96	0	0	0	0	22.0	40.0	940	0	7	947
8/7/97	0.16	0.48	0.08	15.0	17.5(15.6-19.8)	-	0	5*	72	72
8/7/97	0	0	0	0	22.0	40.0	72	0	0	72
30/12/96	1.8	0.43	0.36	16.0	18.4(17.6-19.9)	-	0	3*	412	415
30/12/96	0	0	0	0	22.0	40.0	413	0	2	415
14/1/97	1.98	1.09	0.61	16.0	25.5(22.9-28.9)	-	0	0	122	122
14/1/97	0	0	0	0	22.0	40.0	122	0	0	122
23/1/97	2.36	1.2	0.94	15.0	18.2(16.9-20.7)	-	0	4*	212	216
23/1/97	0	0	0	0	22.0	40.0	216	0	0	216
1/5/97	4.64	0.97	0.61	16.0	18.9(18.1-19.9)	-	0	1*	340	341
1/5/97	0	0	0	0	22.0	40.0	340	0	1	341
12/2/97	20	0.94	0.55	17.0	23.9(18.1-34.8)	84.4(53.7-93.5)	0	0	421	421
12/2/97	0	0	0	0	22.0	40.0	420	0	1	421
12/3/97	20	0.42	0.38	15.5	15.7(14.0-23.0)	85.2(62.7-90.3)	0	3*	77	80
12/3/97	0	0	0	0	22.0	40.0	80	0	0	80
17/3/97	30	0.64	0.43	16.0	16.3(11.8-24.1)	85.3(44.8-90.3)	0	1*	154	155
17/3/97	0	0	0	0	22.0	40.0	155	0	0	155
20/3/97	25	1.08	0.97	16.0	16.9(14.3-21.6)	88.5(71.8-95.2)	0	2*	250	255
20/3/97	0	0	0	0	22.0	40.0	253	0	2	255
24/3/97	23	0.78	0.57	17.0	17.4(15.3-24.1)	85.8(50.1-93.4)	0	0	120	120
24/3/97	0	0	0	0	22.0	40.0	120	0	0	120
9/4/97	30	1.32	0.48	16.25	21.1(18.8-25.5)	90.5(44.8-96.4)	0	0	110	110
9/4/97	0	0	0	0	22.0	40.0	110	0	0	110
14/4/97	25	1.01	0.76	17.0	18.9(18.1-19.5)	91.2(79.1-93.4)	0	3*	150	153
14/4/97	0	0	0	0	22.0	40.0	153	0	0	153
21/4/97	27	1.22	0.41	16.0	19.4(17.2-25.3)	87.3(60.3-92.8)	0	0	180	180
21/4/97	0	0	0	0	22.0	40.0	180	0	0	180

Date	Phosfume (kg)	Phosphine oncentration gm^{-3}		Exposure Time (h)	Temperature (°C)	R.H (%)	Two-spotted mite *Tetranychus urticae*			
		Initial	Final				Live	S. aff	Dead	Total
29/4/97	33	1.14	0.87	16.0	18.4(13.9-26.6)	91.7(62.3-96.4)	0	0	360	360
29/4/97	0	0	0	0	22.0	40.0	360	0	0	360
5/5/97	33	0.83	0.76	18.0	18.3(16.4-21.9)	83.4(66.1-94.6)	0	0	270	270
5/5/97	0	0	0	0	22.0	40.0	268	0	2	270
26/5/97	27	1.0	0.84	17.25	17.5(16.0-19.1)	90.6(85.3-97.0)	0	3*	160	163
26/5/97	0	0	0	0	22.0	40.0	163	0	0	163
3/6/97	26	1.04	0.48	16.0	18.3(16.7-19.9)	80.2(67.0-87.4)	0	0	320	320
3/6/97	0	0	0	0	22.0	40.0	318	0	2	320
15/7/97	20	0.86	0.48	16.5	17.7(14.9-20.5)	-	0	3*	40	43
15/7/97	0	0	0	0	22.0	40.0	43	0	0	43

*- all died within 24 h of fumigation.

Fumigation criteria

Exposure time: 16 h or more

Final phosphine concentration: 0.48 $g.m^{-3}$ or more

Temperature: more than 17.4°C or more

Table 4.56 Number of fumigations that satisfy the criteria

	Number of larvae severely affected	Number fumigated
Good		
14/1/97	-	122
12/2/97	-	421
24/3/97	-	120
21/4/97	-	180
29/4/97	-	360
5/5/97	-	270
3/6/97	-	320
1/5/97	1(0.28%)	341
14/4/97	3(1.96%)	153
26/5/97	3(1.84%)	163
15/7/97	3(7.0%)	43

Of the 11 fumigations conducted, seven fumigations gave a complete mortality of the exposed larvae (Table 4.56). However, in four fumigations a small number of larvae were severely affected and all these severely affected larvae had died within 24 h following fumigations.

A small number of individuals were severely affected in all the fumigations that did not meet the criteria (Table 4.57). The probable reason would be due to lower final phosphine concentration. However, all these individuals had died within 24 h following completion of fumigation.

Table 4.57 Fumigations that did not satisfy the criteria

	Number of larvae severely affected	Number fumigated
Marginal		
30/9/96	2(0.46%)	427
18/12/96	3(0.31%)	947
8/7/97	5(6.5%)	77
30/12/96	3(0.72%)	415
23/1/97	4(1.8%)	220
12/3/97	3(3.7%)	80
17/3/97	1(0.64%)	155
20/3/97	2(0.79%)	252

4.4.3.4 Adults

The response of adults of *T. urticae* to a range of phosphine concentrations, exposure times and different temperatures are presented in Table 4.58. In most fumigations a small number of exposed individuals were severely affected. All these severely affected individuals were dead within 24 h following fumigation.

Table 4.58 Toxicity of phosphine to adults of *T. urticae* fumigated with various concentrations, exposure times and temperatures in a 27 m^3 chamber at Emerald and Gembrooke.

Date	Phosfume® kg	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Two-spotted mite *Tetranychus urticae*			
		Initial	Final			Live	S.aff	Dead	Total
30/9/96	1.74	0.25	0.15	16.0	18.4(15.5-20.8)	0	2*	579	581
30/9/96	0	0	0	0	22.0	580	0	1	581
18/12/96	1.0	0.41	0.12	16.0	17.1	0	4	650	654
18/12/96	0	0	0	0	25.0	650	0	4	654
8/7/97	0.16	0.48	0.08	15.0	17.5(15.6-19.8)	0	5	94	99
8/7/97	0	0	0	0	25.0	99	0	0	99
30/12/96	1.8	0.43	0.36	16.0	18.4(17.6-19.9)	0	3*	796	799
30/12/96	0	0	0	0	22.0	795	0	4	799
14/1/97	1.98	1.09	0.61	16.0	25.5(22.9-28.9)	0	0	172	172
14/1/97	0	0	0	0	22.0	172	0	0	172
23/1/97	2.36	1.2	0.94	15.0	18.2(16.9-20.7)	0	2*	310	312
23/1/97	0	0	0	0	22.0	311	0	1	312
1/5/97	4.64	0.97	0.61	16.0	18.9(18.1-19.9)	0	1*	400	401
1/5/97	0	0	0	0	25.0	401	0	0	401

* - all died within 24 h after fumigation.

The results obtained from the 900 L chamber at Frankston are given in Table 4.60. Some fumigations gave a complete mortality of all the exposed individuals, also, in some fumigations a small number of individuals were severely affected. The time required for these severely affected individuals to die depends on the temperature to which they were exposed while fumigation. Those individuals exposed to comparatively higher temperatures (16.9 to 17.5°C) died earlier (24 h following fumigation) than those that were exposed to lower temperatures 15.7 to 16.9°C (48 to 62 h).

Table 4.59 Toxicity of phosphine to adults of *T. urticae* fumigated with various concentrations, exposure times and temperatures in a 900 L chamber.

Date	Phosfume® kg	Phosphine Concentration gm^{-3}		Exposure time (h)	Temperature (°C)	RH (%)	Two-spotted mite *Tetranychus urticae*			
		Initial	Final				Live	S. aff	Dead	Total
12/2/97	20	0.94	0.55	17.0	23.9(18.1-34.8)	84.4(53.7-93.5)	0	0	754	754
12/2/97	0	0	0	0	25.0	45.0	754	0	0	754
12/3/97	20	0.42	0.38	15.5	15.7(14.0-23.0)	85.2(62.7-90.3)	0	3#	45	48
12/3/97	0	0	0	0	25.0	45.0	47	0	1	48
17/3/97	30	0.64	0.43	16.0	16.3(11.8-24.1)	85.3(44.8-90.3)	0	2#	212	214
20/3/97	25	1.08	0.97	16.0	16.9(14.3-21.6)	88.5(71.8-95.2)	0	2#	180	182
20/3/97	0	0	0	0	25.0	45.0	182	0	0	182
24/3/97	23	0.78	0.57	17.0	17.4(15.3-24.1)	85.8(50.1-93.4)	0	0	160	160
24/3/97	0	0	0	0	25.0	45.0	160	0	0	160
9/4/97	30	1.32	0.48	16.25	21.1(18.8-25.5)	90.5(44.8-96.4)	0	0	140	140
9/4/97	0	0	0	0	25.0	45.0	138	0	2	140
14/4/97	25	1.01	0.76	17.0	18.9(18.1-19.5)	91.2(79.1-93.4)	0	0	60	60
14/4/97	0	0	0	0	25.0	45.0	60	0	0	60
21/4/97	27	1.22	0.41	16.0	19.4(17.2-25.3)	87.3(60.3-92.8)	0	0	200	200
21/4/97	0	0	0	0	25.0	45.0	200	0	0	200
29/4/97	33	1.14	0.87	16.0	18.4(13.9-26.6)	91.7(62.3-96.4)	0	0	450	450
29/4/97	0	0	0	0	25.0	45.0	450	0	0	450
5/5/97	33	0.83	0.76	18.0	18.3(16.4-21.9)	83.4(66.1-94.6)	0	0	210	210
5/5/97	0	0	0	0	25.0	45.0	210	0	0	210
26/5/97	27	1.0	0.84	17.25	17.5(16.0-19.1)	90.6(85.3-97.0)	0	2*	238	240
26/5/97	0	0	0	0	25.0	45.0	240	0	0	240
3/6/97	26	1.04	0.48	16.0	18.3(16.7-19.9)	80.2(67.0-87.4)	0	0	420	420
3/6/97	0	0	0	0	25.0	45.0	418	0	2	420
15/7/97	20	0.86	0.48	16.5	17.7(14.9-20.5)	-	0	1*	30	31
15/7/97	0	0	0	0	25.0	45.0	30	0	0	31

* - all dead within 24 h after fumigation.

- all dead 48 to 60 h after end of fumigation.

Fumigation criteria

Exposure time: 16 h or more

Temperature: 18.3°C or more

Phosphine concentration: 0.41 $g.m^{-3}$ or more

Of the nine fumigations that satisfy the criteria eight fumigations gave a complete mortality of all the exposed adults (Table 4.60). In one fumigation only one adult was severely affected due to unknown reasons.

Table 4.60 Number of fumigations that satisfy the criteria

	Number of larvae severely affected	Number fumigated
Good		
14/1/97	-	172
12/2/97	-	754
9/4/97	-	140
14/4/97	-	60
21/4/97	-	200
29/4/97	-	450
5/5/97	-	210
3/6/97	-	420
Marginal		
1/5/97	1(0.24%)	401

Ten fumigations out of eleven that did not satisfy the criteria did not give a complete mortality of all the exposed adults (Table 4.61). One to five individuals were severely affected in these fumigations. However, one fumigation that did not satisfy the criteria gave a complete mortality of all the exposed adults.

Table 4.61 The fumigations with phosphine that did not satisfy the criteria

	Number of larvae severely affected	Number fumigated
Good		
24/3/97	-	160
Marginal		
30/9/96	2(0.3%)	581
18/12/96	4(0.6%)	654
8/7/97	5(5.0%)	99
30/12/96	3(0.3%)	799
23/1/97	2(0.6%)	312
12/3/97	3(6.2%)	48
17/3/97	2(0.9%)	214
20/3/97	2(1.0%)	182
26/5/97	2(0.8%)	240
15/7/97	1(3.2%)	31

4.4.4 *The green peach aphid, Myzus persicae*

4.4.4.1 *Larvae*

Results of the phosphine fumigated larvae (at various concentrations, exposure times and temperatures) of *M. persicae* are presented in Table 4.62 and Table 4.63. A large number of larvae were severely affected when fumigated with a phosphine concentration of 1 $g.m^{-3}$, for 17.25 h at 17.5°C. However, number of individuals severely affected reduced considerably with phosphine concentrations of 0.94 to 1.04 $g.m^{-3}$, exposure periods of 15.5 to 17 h and temperature range of 17.7 to 23.9°C. Most of these severely affected larvae had died two to three hours after fumigation and only a small number was severely affected in the second assessment. Of these larvae, severely affected due to lower temperatures took longer time (48 to 60 h) for complete mortality than those exposed to comparatively higher temperatures (24 to 48 h).

Table 4.62 Toxicity of phosphine to larvae of *M. persicae* fumigated with various concentrations, exposure times and temperatures that were fumigated in a 900 L chamber at Frankston.

Date	hosfume® kg	Phosphine oncentration gm-3		Exposure time (h)	Temperature (°C)	RH (%)	Green peach aphid Myzus persicae			
		Initial	Final				Live	S. aff	Dead	Total
10/2/97	27	0.98	0.85	15.5	18.6(17.8-20.6)	-	0	3*	49	52
12/2/97	27	0.94	0.55	17.0	23.9(18.1-34.8)	84.4(53.7-93.5)	0	2*	16	18
12/2/97	0	0	0	0	19.0	45.0	18	0	0	18
26/5/97	27	1.0	0.84	17.25	17.5(16.0-19.1)	90.6(85.3-97.0)	0	70#	25	95
3/6/97	26	1.04	0.48	16.0	18.3(16.7-19.9)	80.2(67.0-87.4)	0	2*	20	22
3/6/97	0	0	0	0	19.0	45.0	31	0	1	32
15/7/97	20	0.86	0.48	16.5	17.7(14.9-20.5)	-	0	12#	5	17
15/7/97	0	0	0	0	19.0	45.0	7	0	0	7

* - all died 24 to 48 h after fumigation.

-90 per cent of individuals 24 h after end of fumigation and remaining died 48 to 60 h after end of fumigation.

Table 4.63 Toxicity of phosphine to larvae of *M. persicae* fumigated with various concentrations, exposure times and temperatures in a 27 m^3 chamber at Gembrook.

Date	Phosfume® kg	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Green peach aphid *Myzus persicae*			
		Initial	Final			Live	S.aff	Dead	Total
8/7/97	0.16	0.48	0.08	15.0	17.5(15.6-19.8)	0	18*	25	43
8/7/97	0	0	0	0	19.0	40	0	3	43

* - all died 24 to 48 h after fumigation.

4.4.4.2 Adults

Table 4.64 clearly indicates that most of the exposed adults were killed in all the fumigations and some were severely affected. However, in one fumigation (conducted on 26/5/97) a large number of individuals were severely affected, probably due to lower

Table 4.64 Toxicity of phosphine to adults of *M. persicae* fumigated with various concentrations, exposure times and temperatures in a 900 L chamber.

Date	Phosfume® kg	Phosphine Concentration gm-3		Exposure time (h)	Temperature (°C)	RH (%)	Green peach aphid *Myzus persicae*			
		Initial	Final				Live	S. aff	ead	Total
10/2/97	27	0.98	0.85	15.5	18.6 (17.8-20.6)	-	0	4*	42	46
10/2/97	0	0	0	0	19.0	55.0	46	0	0	46
12/2/97	27	0.94	0.55	17.0	23.9 (18.1-34.8)	4.4 (53.7-93.5)	0	7*	28	35
12/2/97	0	0	0	0	19.0	55.0	35	0	0	35
26/5/97	27	1.0	0.84	17.25	17.5(16.0-19.1)	90.6(85.3-97.0)	0	40#	10	50
26/5/97	0	0	0	0	19.0	55.0	50	0	0	50
3/6/97	26	1.04	0.48	16.0	18.3(16.7-19.9)	80.2(67.0-87.4)	0	9*	36	45
3/6/97	0	0	0	0	19.0	55.0	43	0	2	45
8/7/97	0.16	0.48	0.08	15.0	17.5(15.6-19.8)	-	0	18*	31	49
8/7/97	0	0	0	0	19.0	55.0	49	0	0	49
15/7/97	20	0.86	0.48	16.5	17.7(14.9-20.5)	-	0	1*	4	5
15/7/97	0	0	0	0	19.0	55.0	5	0	0	5

* - all died 24 to 48 h after fumigation.

- 27 died 24 hours, 10 died 48 h and 3 died 72 h after fumigation.

temperature. Most of the severely affected individuals generally took 4 to 24 h for a complete mortality: however, some took longer (48 to 72 h) to die.

4.4.5 The ornate aphid, Myzus ornatus

4.4.5.1 Larvae

Details of the fumigation trials conducted using different concentrations of phosphine with various exposure periods and temperatures are given in Table 4.65.

Table 4.65 Toxicity of phosphine to larvae of *M. ornatus* fumigated with various concentrations, exposure times and temperatures in a 900 L and 27 m^3 (Emerald) chamber.

Date	Phosfume® Kg	Phosphine oncentration gm^{-3}		Exposure time (h)	Temperature (°C)	Ornate aphid *Myzus ornatus*			
		Initial	Final			Live	S. aff	Dead	Total
0/12/96	1.8	0.43	0.36	16.0	18.4(17.6-19.9)	0	1*	49	50
0/12/96	0	0	0	0	19.0	50	0	0	50
14/1/97	1.98	1.09	0.61	16.0	25.5(22.9-28.9)	0	0	34	34
14/1/97	0	0	0	0	19.0	34	0	0	34
23/1/97	2.36	1.2	0.94	15.0	18.2(16.9-20.7)	0	2*	31	33
23/1/97	0	0	0	0	19.0	32	0	1	33
12/3/97	20	0.42	0.38	15.5	15.7 (14.0-23.0)	0	30#	6	36
17/3/97	30	0.64	0.43	16.0	16.3 (11.8-24.1)	0	12#	4	16
17/3/97	0	0	0	0	19.0	16	0	0	16
20/3/97	25	1.08	0.97	16.0	16.9 (14.3-21.6)	0	22#	18	40
20/3/97	0	0	0	0	19.0	39	0	1	40
24/3/97	23	0.78	0.57	17.0	17.4 (15.3-24.1)	0	4*	41	45
24/3/97	0	0	0	0	19.0	45	0	0	45
9/4/97	30	1.32	0.48	16.25	21.1(18.8-25.5)	0	0	10	10
9/4/97	0	0	0	0	19.0	10	0	0	10
5/5/97	33	0.83	0.76	18.0	18.3(16.4-21.9)	0	0	21	21
5/5/97	0	0	0	0	19.0	21	0	0	21

* - all died within 24 h after fumigation.

- 70 per cent died 24 h after fumigation and remaining died 48 to 72 h after fumigation.

In most of the trials a small number of larvae of *M. ornatus* was severely affected. This was observed only in the fumigations that were conducted at or below 18.4°C temperature. However, all these severely affected larvae were dead within 24 h following fumigation. Those individuals severely affected due to lower fumigation temperature (15.7°C) took longer (24 to 72 h) to die than those that were severely affected at 16.9°C (took 24 h to die).

Fumigation criteria

Exposure time: 16 h or more

Temperature: 18.3°C or more

Final phosphine concentration: 0.48 $g.m^{-3}$ or more

All the three fumigations that satisfy the criteria have given complete mortality of all the exposed individuals (Table 4.66).

Table 4.66 Fumigations with phosphine that satisfy the fumigation criteria

	Number of larvae severely affected	Number fumigated
Good		
14/1/97	-	34
9/4/97	-	10
5/5/97	-	21

Of the six fumigations that did not satisfy the criteria three were marginal, where small number of individuals were severely affected (Table 4.67). Three were poor in nature, a large number of individuals were severely affected.

Table 4.67 Fumigations with phosphine that did not satisfy the criteria

	Number of larvae severely affected	Number fumigated
Marginal		
30/12/96	1(2.0%)	50
23/1/97	2(6.0%)	33
24/3/97	4(9.0%)	45
Poor		
12/3/97	30(83.0%)	36
17/3/97	12(75.0%)	16
20/3/97	22(55.0%)	40

4.4.5.2 Adults

Table 4.68 shows the insecticidal efficacy of various concentrations of phosphine, exposure times and temperatures to the adults of *M. ornatus*. Complete mortality was achieved in some fumigations and number of individuals were severely affected in some fumigations. Number severely affected increased with lower fumigation temperatures. However, all the severely affected individuals had died 24 to 96 h after fumigation.

Table 4.68 Toxicity of phosphine to adults of *M. ornatus* fumigated with various concentrations, exposure times and temperatures in 900 L and 27 m^3 chamber at Emerald and Gembrooke.

Date	Phosfume® (kg)	Phosphine Concentration gm⁻		Exposure time (h)	Temperature (°C)	R.H (%)	Ornate aphid *Myzus ornatus*			
		Initial	Final				Live	S. aff	Dead	Total
8/12/96	1.0	0.41	0.12	16.0	17.1	-	0	49*	7	56
8/12/96	0	0	0	0	19.0	55.0	55	0	1	56
0/12/96	1.8	0.43	0.36	16.0	18.4(17.6-19.9)	-	0	0	37	37
0/12/96	0	0	0	0	19.0	55.0	37	0	0	37
14/1/97	1.98	1.09	0.61	16.0	25.5(22.9-28.9)	-	0	0	47	47
23/1/97	2.36	1.2	0.94	15.0	18.2(16.9-20.7)	-	0	0	23	23
23/1/97	0	0	0	0	19.0	55.0	23	0	0	23
12/3/97	20	0.42	0.38	15.5	15.7 (14.0-23.0)	85.2 (62.7-90.3)	0	7*	14	21
12/3/97	0	0	0	0	19.0	55.0	21	0	0	21
17/3/97	30	0.64	0.43	16.0	16.3 (11.8-24.1)	85.3 (44.8-90.3)	0	6*	3	9
17/3/97	0	0	0	0	19.0	55.0	9	0	0	9
20/3/97	25	1.08	0.97	16.0	16.9 (14.3-21.6)	88.5 (71.8-95.2)	0	2*	16	18
20/3/97	0	0	0	0	19.0	55.0	18	0	0	18
24/3/97	23	0.78	0.57	17.0	17.4 (15.3-24.1)	85.8 (50.1-93.4)	0	1*	29	30
24/3/97	0	0	0	0	19.0	55.0	30	0	0	30
9/4/97	30	1.32	0.48	16.25	21.1(18.8-25.5)	90.5(44.8-96.4)	0	0	14	14
9/4/97	0	0	0	0	19.0	55.0	14	0	0	14
5/5/97	33	0.83	0.76	18.0	18.3(16.4-21.9)	83.4(66.1-94.6)	0	0	15	15
5/5/97	0	0	0	0	19.0	55.0	15	0	0	15

* - all died 24 to 72 h after fumigation.

Fumigation criteria

Temperature: 18.2°C or more

Exposure time: 15 h or more

Final phosphine concentration: 0.43 $g.m^{-3}$ or more

All the five fumigations that satisfy the criteria gave complete mortality of all the exposed individuals (Table 4.69).

Table 4.69 The fumigations with phosphine that satisfy the criteria

	Number of larvae severely affected	Number fumigated
Good		
30/12/96	-	37
14/1/97	-	47
23/1/97	-	23
9/4/97	-	14
5/5/97	-	15

It is clear from this Table 4.70 that all the five fumigations that did not satisfy the criteria did not give a complete mortality of all the exposed individuals. Of these one is marginal and four were poor.

Table 4.70 The fumigations with phosphine that did not satisfy the criteria.

	Number of larvae severely affected	Number fumigated
Marginal		
24/3/97	1(3.3%)	30
Poor		
18/12/96	49(87.5%)	56
12/3/97	7(33.3%)	21
17/3/97	6(66.6%)	9
20/3/97	2(11.1%)	18

4.4.6 The cabbage aphid, Brevicoryne brassicae

4.4.6.1 Larvae

Response of the larvae of *B. brassicae* to various concentrations of phosphine, exposure times and temperatures are presented in Table 4.71. It is clear from these data that not all the fumigations gave a complete mortality of all the exposed individuals. In some fumigations a number of individuals were severely affected.

Table 4.71 Toxicity of phosphine to larvae of *B. brassicae* fumigated with various concentrations, exposure times and temperatures in a 900 L chamber.

Date	Phosfume® Kg	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	R.H (%)	Cabbage aphid *Brevicoryne brassicae*			
		Initial	Final				Live	S. aff	Dead	Total
17/3/97	30	0.64	0.43	16.0	16.3 (11.8-24.1)	85.3 (44.8-90.3)	0	42#	11	53
17/3/97	0	0	0	0	19.0	55.0	52	0	1	53
20/3/97	25	1.08	0.97	16.0	16.9 (14.3-21.6)	88.5 (71.8-95.2)	0	49#	5	54
20/3/97	0	0	0	0	19.0	55.0	54	0	0	54
24/3/97	23	0.78	0.57	17.0	17.4 (15.3-24.1)	85.8 (50.1-93.4)	0	29*	63	92
24/3/97	0	0	0	0	19.0	55.0	90	0	2	92
9/4/97	30	1.32	0.48	16.25	21.1(18.8-25.5)	90.5(44.8-96.4)	0	0	18	18
9/4/97	0	0	0	0	19.0	55.0	18	0	0	18
14/4/97	25	1.01	0.76	17.0	18.9(18.1-19.5)	91.2(79.1-93.4)	0	0	10	10
14/4/97	0	0	0	0	19.0	55.0	10	0	0	10
29/4/97	33	1.14	0.87	16.0	18.4(13.9-26.6)	91.7(62.3-96.4)	0	0	20	20
29/4/97	0	0	0	0	19.0	55.0	20	0	0	20
1/5/97	4.64	0.97	0.61	16.0	18.9(18.1-19.9)	-	0	0	69	69
1/5/97	0	0	0	0	19.00	55.0	67	0	2	69
5/5/97	33	0.83	0.76	18.0	18.3(16.4-21.9)	83.4(66.1-94.6)	0	0	45	45
5/5/97	0	0	0	0	19.0	55.0	43	0	2	45
26/5/97	27	1.0	0.84	17.25	17.5(16.0-19.1)	90.6(85.3-97.0)	0	12*	16	28
26/5/97	0	0	0	0	19.0	55.0	28	0	0	28
3/6/97	26	1.04	0.48	16.0	18.3(16.7-19.9)	80.2(67.0-87.4)	0	14*	20	34
3/6/97	0	0	0	0	19.0	55.0	34	0	0	34

* - all died within 24 h after end of fumigation.

- most of them (60 per cent) died 48 h after end of fumigation and remaining died within 48 to 72 h after fumigation.

The severely affected individuals died 24 to 72 h following completion of fumigation. Generally, those individuals that were fumigated with temperatures of 17.4°C or more died earlier (24 to 48 h) than the adults that were fumigated with temperatures of 16.3 or 16.9°C, where these severely affected adults took 48 to 72 h for complete mortality.

Fumigation criteria

Temperature: 18.3°C or more

Exposure time: 16 h or more

Final phosphine concentration: 0.48 $g.m^{-3}$ or more

Five fumigations that satisfy the criteria gave a complete mortality of all the exposed individuals (Table 4.72). However, in one fumigation large number of individuals were severely affected due to unknown reasons.

Table 4.72 The fumigations with phosphine that satisfy the criteria

	Number of larvae severely affected	Number fumigated
Good		
9/4/97	-	18
14/4/97	-	10
29/4/97	-	20
1/5/97	-	69
5/5/97	-	45
Poor		
3/6/97	14(41.0%)	34

All the four fumigations that did not satisfy the criteria resulted in large number of individuals severely affected (Table 4.73).

Table 4.73 The fumigations with phosphine that did not satisfy the criteria.

	Number of larvae severely affected	Number fumigated
Poor		
17/3/97	42(79.0%)	53
20/3/97	49(90.0%)	54
24/3/97	29(31.5%)	92
26/5/97	12(42.9%)	28

4.4.6.2 *Adults*

The toxicity of phosphine at various concentrations, exposure times and temperatures to adults of *B. brassicae* are presented in Tables 4.74. Some fumigations gave a complete mortality of all the exposed adults and in some fumigations a small number were severely affected. In some fumigations large number of individuals were severely

Table 4.74 Toxicity of phosphine to adults of *B. brassicae* moth fumigated with various concentrations, exposure times and temperatures in a 900 L and 27 m^3 (Gembrooke) chamber.

Date	Phosfume® (kg)	Phosphine oncentration gm-3		Exposure time (h)	Temperature (°C)	R.H (%)	Cabbage aphid *Brevicoryne brassicae*			
		Initial	Final				Live	S. aff	Dead	Total
1/5/97	4.64	0.97	0.61	16.0	18.9(18.1-19.9)	-	0	0	72	72
1/5/97	0	0	0	0	21.0	55.0	70	0	2	72
17/3/97	30	0.64	0.43	16.0	16.3 (11.8-24.1)	85.3 (44.8-90.3)	0	67#	14	81
17/3/97	0	0	0	0	21.0	55.0	81	0	0	81
20/3/97	25	1.08	0.97	16.0	16.9 (14.3-21.6)	88.5 (71.8-95.2)	0	60#	12	72
20/3/97	0	0	0	0	21.0	55.0	72	0	0	72
24/3/97	23	0.78	0.57	17.0	17.4 (15.3-24.1)	85.8 (50.1-93.4)	0	18*	54	72
24/3/97	0	0	0	0	19.0	55.0	72	0	0	72
9/4/97	30	1.32	0.48	16.25	21.1(18.8-25.5)	90.5(44.8-96.4)	0	0	20	20
9/4/97	0	0	0	0	19.0	55.0	20	0	0	20
14/4/97	25	1.01	0.76	17.0	18.9(18.1-19.5)	91.2(79.1-93.4)	0	0	15	15
14/4/97	0	0	0	0	19.0	55.0	15	0	0	15
29/4/97	33	1.14	0.87	16.0	18.4(13.9-26.6)	91.7(62.3-96.4)	0	0	30	30
29/4/97	0	0	0	0	19.0	55.0	30	0	0	30
5/5/97	33	0.83	0.76	18.0	18.3(16.4-21.9)	83.4(66.1-94.6)	0	0	60	60
5/5/97	0	0	0	0	19.0	55.0	60	0	0	60
26/5/97	27	1.0	0.84	17.25	17.5(16.0-19.1)	90.6(85.3-97.0)	0	17*	10	27
26/5/97	0	0	0	0	19.0	55.0	27	0	0	27
3/6/97	26	1.04	0.48	16.0	18.3(16.7-19.9)	80.2(67.0-87.4)	0	16*	12	28
3/6/97	0	0	0	0	19.0	55.0	27	0	1	28

* - all died within 24 to 48 h after fumigation.

- 60 per cent died 48 to 60 h after end of fumigation and remaining were dead 48 to 72 h after end of fumigation.

affected probably due to lower fumigation temperatures (18.3°C or less). The individuals that were severely affected due to lower than 17°C took longer to die (48 to 72 h) than those that were severely affected at or above 17°C (24 to 48 h).

Fumigation criteria

Exposure time: 16 h or more

Temperature: 18.3°C or more

Final phosphine concentration: 0.43 $g.m^{-3}$ or more

All the three fumigations (Table 4.75) that satisfy the criteria gave a complete control of all the exposed individuals.

Table 4.75 The fumigations with phosphine that satisfy the criteria

	Number of larvae severely affected	Number fumigated
Good		
14/1/97	-	34
9/4/97	-	10
5/5/97	-	21

Of the six fumigations that did not satisfy the criteria three were marginal and three were poor (Table 4.76).

Table 4.76 The fumigations with phosphine that did not satisfy the criteria

	Number of larvae severely affected	Number fumigated
Marginal		
30/12/96	1(2.0%)	50
23/1/97	2(6.0%)	33
24/3/97	1(3.3%)	30
Poor		
12/3/97	30(83.3%)	36
17/3/97	12(75.0%)	16
20/3/97	22(55.0%)	40

4.4.7 *The meat ant, Iridomyrmex purpureus*

4.4.7.1 *Adults*

Table 4.77 shows the results of phosphine fumigated adults of *I. purpureus* exposed to various concentrations, exposure times and temperatures in a 900 L chamber at Frankston. Except two fumigations all other fumigations has given a complete control of all the exposed adults.

Table 4.77 Toxicity of phosphine to adults of *I. purpureus* fumigated with various concentrations, exposure times and temperatures in a 900 L chamber.

Date	Phosfume® kg	Phosphine oncentration gm^{-3}		Exposure time (h)	Temperature (°C)	R.H (%)	Meat ant *Iridomyrmex purpureus*			
		Initial	Final				Live	S. aff	Dead	Total
17/3/97	30	0.64	0.43	16.0	16.3 (11.8-24.1)	85.3 (44.8-90.3)	18	2#	5	25
17/3/97	0	0	0	0	22.0	65.0	22	0	3	25
20/3/97	25	1.08	0.97	16.0	16.9 (14.3-21.6)	88.5 (71.8-95.2)	3	2*	76	81
20/3/97	0	0	0	0	22.0	65.0	79	0	2	81
24/3/97	23	0.78	0.57	17.0	17.4 (15.3-24.1)	85.8 (50.1-93.4)	0	0	30	30
24/3/97	0	0	0	0	22.0	65.0	26	0	4	30
9/4/97	30	1.32	0.48	16.25	21.1(18.8-25.5)	90.5(44.8-96.4)	0	0	15	15
9/4/97	0	0	0	0	22.0	65.0	14	0	1	15
21/4/97	27	1.22	0.41	16.0	19.4(17.2-25.3)	87.3(60.3-92.8)	0	0	46	46
21/4/97	0	0	0	0	22.0	65.0	44	0	2	46
29/4/97	33	1.14	0.87	16.0	18.4(13.9-26.6)	91.7(62.3-96.4)	0	0	30	30
29/4/97	0	0	0	0	22.0	65.0	30	0	0	30
26/5/97	27	1.0	0.84	17.25	17.5(16.0-19.1)	90.6(85.3-97.0)	0	0	40	40
26/5/97	0	0	0	0	22.0	65.0	40	0	0	40

* - all died within 48 h after fumigation.

\# - one died 48 h after fumigation and other died 96 h after fumigation.

In 17/3/97 trial a large number of adults survived, and some were severely affected, the probable reason for this would be either lower fumigation temperature or lower phosphine concentration or combination of both. The surviving individuals completed their life cycle in a way similar to the unfumigated one. It was observed that immediately

following fumigation and for up to 12 h 70 per cent of the fumigated adults had been narcotised (unable to move, showed paralysed symptoms and only spontaneous movement of legs). However, 24 h following fumigation, most of these adults recovered and responded in a similar way to unfumigated healthy individuals. On the other hand, the severely affected adults took 24 to 96 h for complete mortality.

Fumigation criteria

Exposure time: 16 h or more

Temperature: 17.4°C or more

Final phosphine concentration: 0.42 $g.m^{-3}$ or more

Table 4.78 clearly indicates that all the five fumigations that satisfy the criteria have given a complete mortality of all the exposed adults.

Table 4.78 The fumigations with phosphine that satisfy the criteria

	Number of larvae severely affected	Number fumigated
Good		
24/3/97	-	30
9/4/97	-	15
21/4/97	-	46
29/4/97	-	30
26/5/97	-	40

Two fumigations that did not satisfy the criteria did not give a complete control of all the exposed adults (Table 4.79).

Table 4.79 The fumigations with phosphine that did not satisfy the criteria

	Number of larvae severely affected	Number fumigated
Marginal		
20/3/97	5(6.1%)	81
Poor		
17/3/97	20(80.0%)	25

4.4.8 *The predatory mite, Phytoseiulus persimilis*

4.4.8.1 *Larvae*

The results obtained from the phosphine fumigations for the larvae of the predatory mite, fumigated with various concentrations, exposure times and temperatures are presented in Table 4.80.

Table 4.80 Toxicity of phosphine to larvae of *P. persimilis* fumigated with various concentrations, exposure times and temperatures in 900 L and 27 m^3 (Emerald) chamber.

Date	Phosfume® (g)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Predatory mite *Phytoseiulus persimilis*			
		Initial	Final			Live	S. aff	Dead	Total
10/2/97	20	0.98	0.85	15.5	18.6(17.8-20.6)	0	2*	41	43
10/2/97	0	0	0	0	22.0	42	0	1	43
12/2/97	20	0.94	0.55	17.0	23.9(18.1-34.8)	0	0	21	21
12/2/97	0	0	0	0	22.0	21	0	0	21
17/3/97	30	0.64	0.43	16.0	16.3 (11.8-24.1)	0	2*	63	65
17/3/97	0	0	0	0	22.0	63	0	2	65
20/3/97	25	1.08	0.97	16.0	16.9(14.3-21.6)	0	1*	45	46
20/3/97	0	0	0	0	22.0	46	0	0	46
24/3/97	23	0.78	0.57	17.0	17.4(15.3-24.1)	0	2*	39	41
24/3/97	0	0	0	0	22.0	41	0	0	41
9/4/97	30	1.32	0.48	16.25	21.1(18.8-25.5)	0	0	40	40
9/4/97	0	0	0	0	22.0	40	0	0	40
14/4/97	25	1.01	0.76	17.0	18.9(18.1-19.5)	0	0	49	49
14/4/97	0	0	0	0	22.0	49	0	0	49
21/4/97	27	1.22	0.41	16.0	19.4(17.2-25.3)	0	0	36	36
21/4/97	0	0	0	0	22.0	36	0	0	36
29/4/97	33	1.14	0.87	16.0	18.4(13.9-26.6)	0	0	30	30
29/4/97	0	0	0	0	22.0	30	0	0	30
1/5/97	4.64	0.97	0.61	16.0	18.9(18.1-19.9)	0	0	20	20
1/5/97	0	0	0	0	22.0	16	0	4	20
5/5/97	33	0.83	0.76	18.0	18.3(16.4-21.9)	0	0	25	25
5/5/97	0	0	0	0	22.0	25	0	0	25
26/5/97	27	1.0	0.84	17.25	17.5(16.0-19.1)	0	2*	120	122
26/5/97	0	0	0	0	22.0	118	0	4	122

Date	Phosfume® (g)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Predatory mite *Phytoseiulus persimilis*			
		Initial	Final			Live	S. aff	Dead	Total
3/6/97	26	1.04	0.48	16.0	18.3(16.7-19.9)	0	0	30	30
3/6/97	0	0	0	0	22.0	30	0	0	30
15/7/97	20	0.86	0.48	16.5	17.7(14.9-20.5)	0	3*	67	70
15/7/97	0	0	0	0	22.0	70	0	0	70

* - all died within 48 h after fumigation.

Most of the fumigations gave a complete control of all the exposed individuals. However, a small number of larvae were severely affected in some fumigations, especially fumigations conducted with temperatures of 18.6°C or less and/or the exposure times of 16 h or less. These severely affected larvae died within 24 h after fumigation.

Fumigation criteria

Temperature: 18.3°C or more

Exposure time: 16 h or more

Final phosphine concentration: 0.41 $g.m^{-3}$ or more

All the fumigations that satisfy criteria gave a complete mortality of all the exposed individuals (Table 4.81).

Table 4.81 The fumigations with phosphine that satisfy the criteria

	Number of larvae severely affected	Number fumigated
Good		
12/2/97	-	21
9/4/97	-	40
14/4/97	-	49
21/4/97	-	36
29/4/97	-	30
1/5/97	-	20
5/5/97	-	25
3/6/97	-	30

All the six fumigations that did not satisfy the criteria did not give a complete mortality of all the exposed individuals (Table 4.82). A small number of individuals were severely affected in all the fumigations.

Table 4.82 The fumigations with phosphine that did not satisfy the criteria

	Number of larvae severely affected	Number fumigated
Marginal		
10/2/97	2(4.5%)	43
17/3/97	2(3.0%)	65
20/3/97	1(2.2%)	46
24/3/97	2(4.9%)	41
26/5/97	2(1.6%)	122
15/7/97	3(4.3%)	70

4.4.8.2 Adults

The results of the phosphine fumigated predatory mite adults with various concentrations, exposure times and different temperatures are presented in Table 4.83.

Table 4.83 Toxicity of phosphine to adults of *P. persimilis* fumigated with various concentrations, exposure times and temperatures in a 900 L chamber.

Date	Phosfume® (kg)	Phosphine Concentration gm^{-3}		Exposure time (h)	Temperature (°C)	RH (%)	Predatory mite *Phytoseiulus persimilis*			
		Initial	Final				Live	S. aff	Dead	Total
10/2/97	20	0.98	0.85	15.5	18.6(17.8-20.6)	-	0	3*	54	57
10/2/97	0	0	0	0	22.0	65.0	57	0	0	57
12/2/97	20	0.94	0.55	17.0	23.9(18.1-34.8)	84.4(53.7-93.5)	0	0	29	29
12/2/97	0	0	0	0	22.0	65.0	29	0	0	29
17/3/97	30	0.64	0.43	16.0	16.3(11.8-24.1)	85.3(44.8-90.3)	0	3*	81	84
17/3/97	0	0	0	0	22.0	65.0	84	0	0	84
20/3/97	25	1.08	0.97	16.0	16.9(14.3-21.6)	88.5(71.8-95.2)	0	2*	63	65
20/3/97	0	0	0	0	22.0	65.0	65	0	0	65
24/3/97	23	0.78	0.57	17.0	17.4(15.3-24.1)	85.8(50.1-93.4)	0	4*	72	76

Date	Phosfume® (kg)	Phosphine Concentration gm^{-3}		Exposure time (h)	Temperature (°C)	RH (%)	Predatory mite *Phytoseiulus persimilis*			
		Initial	Final				Live	S. aff	Dead	Total
24/3/97	0	0	0	0	22.0	65.0	76	0	0	76
9/4/97	30	1.32	0.48	16.25	21.1(18.8-25.5)	90.5(44.8-96.4)	0	0	63	63
9/4/97	0	0	0	0	22.0	65.0	63	0	0	63
14/4/97	25	1.01	0.76	17.0	18.9(18.1-19.5)	91.2(79.1-93.4)	0	0	72	72
14/4/97	0	0	0	0	22.0	65.0	69	0	3	72
21/4/97	27	1.22	0.41	16.0	19.4(17.2-25.3)	87.3(60.3-92.8)	0	0	45	45
21/4/97	0	0	0	0	22.0	65.0	45	0	0	45
29/4/97	33	1.14	0.87	16.0	18.4(13.9-26.6)	91.7(62.3-96.4)	0	0	40	40
29/4/97	0	0	0	0	22.0	65.0	40	0	0	40
1/5/97	4.64	0.97	0.61	16.0	18.9(18.1-19.9)	-	0	0	45	45
1/5/97	0	0	0	0	22.0	65.0	45	0	0	45
5/5/97	33	0.83	0.76	18.0	18.3(16.4-21.9)	83.4(66.1-94.6)	0	0	30	30
5/5/97	0	0	0	0	22.0	65.0	30	0	0	30
26/5/97	27	1.0	0.84	17.25	17.5(16.0-19.1)	90.6(85.3-97.0)	0	2*	180	182
26/5/97	0	0	0	0	22.0	65.0	180	0	0	180
3/6/97	26	1.04	0.48	16.0	18.3(16.7-19.9)	80.2(67.0-87.4)	0	0	45	46
3/6/97	0	0	0	0	22.0	65.0	45	0	0	45
15/7/97	20	0.86	0.48	16.5	17.7(14.9-20.5)	-	0	3*	30	33
15/7/97	0	0	0	0	22.0	65.0	30		3	33

*- all died within 24 h after fumigation.

Complete mortality was achieved in most fumigations. A small number of individuals were severely affected in some fumigations.

Fumigation criteria

Temperature: 18.3°C or more

Exposure time: 16 h or more

Final phosphine concentration: 0.41 $g.m^{-3}$ or more

All the fumigations that satisfy criteria gave a complete mortality of all the exposed individuals (Table 4.84).

Table 4.84 The fumigations with phosphine that satisfy the criteria

	Number of larvae severely affected	Number fumigated
Good		
12/2/97	-	29
9/4/97	-	63
14/4/97	-	72
21/4/97	-	45
29/4/97	-	40
1/5/97	-	45
5/5/97	-	30
3/6/97	-	45

A small number of individuals were survived in all the fumigations that did not satisfy the criteria (Table 4.85).

Table 4.85 The fumigations with phosphine that did not satisfy the criteria

	Number of larvae severely affected	Number fumigated
Marginal		
10/2/97	3(5.2%)	57
17/3/97	3(3.6%)	84
20/3/97	2(3.0%)	65
24/3/97	4(5.2%)	76
26/5/97	2(1.0%)	182
15/7/97	3(9.0%)	33

4.4.9 The soft brown scale, Coccus hesperidum

4.4.9.1 Larvae

Response of the larvae of *C. hesperidum* to phosphine fumigations is given in Table 4.86. Fumigations were conducted with phosphine concentrations of 0.43 to 1.32 g.m^{-3}, exposure times of 15.5 to 18 h and temperatures of 17.5 to 25.5°C. All these fumigations gave complete mortality of all the exposed individuals.

Table 4.86 Toxicity of phosphine to larvae of *C. hesperidum* fumigated with various concentrations, exposure times and temperatures in a 27 m^3 chamber at Emerald.

Date	hosfume® (kg)	Phosphine Concentration gm^{-3}		Exposure time (h)	Temperature (°C)	R.H (%)	Soft brown scale *Coccus hesperidum*		
		Initial	Final				Live	Dead	Total
30/12/96	1.8	0.43	0.36	16.0	18.4(17.6-19.9)	-	0	47	47
30/12/96	0	0	0	0	19.0	55.0	45	2	47
14/1/97	1.98	1.09	0.61	16.0	25.5(22.9-28.9)	-	0	12	12
14/1/97	0	0	0	0	19.0	55.0	12	0	12
1/5/97	4.64	0.97	0.61	16.0	18.9(18.1-19.9)	-	0	27	27
1/5/97	0	0	0	0	19.0	55.0	27	0	27
10/2/97	20	0.98	0.85	15.5	18.6 (17.8-20.6)	0	0	127	127
10/12/97	0	0	0	0	19.0	55.0	121	6	127
9/4/97	30	1.32	0.48	16.25	21.1(18.8-25.5)	90.5(44.8-96.4)	0	60	60
9/4/97	0	0	0	0	19.0	55.0	60	0	60
14/4/97	25	1.01	0.76	17.0	18.9(18.1-19.5)	91.2(79.1-93.4)	0	60	60
14/4/97	0	0	0	0	19.0	55.0	58	2	60
21/4/97	27	1.22	0.41	16.0	19.4(17.2-25.3)	87.3(60.3-92.8)	0	40	40
21/4/97	0	0	0	0	19.0	55.0	40	0	40
29/4/97	33	1.14	0.87	16.0	18.4(13.9-26.6)	91.7(62.3-96.4)	0	63	63
29/4/97	0	0	0	0	19.0	55.0	59	4	63
5/5/97	33	0.83	0.76	18.0	18.3(16.4-21.9)	83.4(66.1-94.6)	0	25	25
5/5/97	0	0	0	0	19.0	55.0	24	1	25
26/5/97	27	1.0	0.84	17.25	17.5(16.0-19.1)	90.6(85.3-97.0)	0	20	20

4.4.9.2 Adults

Results of fumigating soft brown scale adults with various concentrations of phosphine, exposure times and different temperatures are presented in Tables 4.87. Apparently all the exposed individuals had been killed in all the fumigation trials. This suggests that the adults of *C. hesperidum* are highly susceptible to these fumigation conditions.

Table 4.87 Toxicity of phosphine to adults of *C. hesperidum* fumigated with various concentrations, exposure times and temperatures in a 900 L chamber and 27 m^3 chamber at Emerald.

Date	Phosfume® (kg)	Phosphine concentration (gm^{-3})		Exposure time (h)	Temperature (°C)	R.H (%)	Soft brown scale *Coccus hesperidum*		
		Initial	Final				Live	Dead	Total
30/12/96	1.8	0.43	0.36	16.0	18.4(17.6-19.9)	-	0	29	29
30/12/96	0	0	0	0	19.0	55.0	28	1	29
14/1/97	1.98	1.09	0.61	16.0	25.5(22.9-28.9)	-	0	19	19
14/1/97	0	0	0	0	19.0	55.0	19	0	19
1/5/97	4.64	0.97	0.61	16.0	18.9(18.1-19.9)	-	0	45	45
1/5/97	0	0	0	0	19.0	55.0	43	2	45
10/2/97	20	0.98	0.85	15.5	18.6 (17.8-20.6)	-	0	149	149
10/2/97	0	0	0	0	19.0	55.0	148	1	149
9/4/97	30	1.32	0.48	16.25	21.1(18.8-25.5)	90.5(44.8-96.4	0	40	40
9/4/97	0	0	0	0	19.0	55.0	40	0	40
14/4/97	25	1.01	0.76	17.0	18.9(18.1-19.5)	91.2(79.1-93.4	0	48	48
14/4/97	0	0	0	0	19.0	55.0	47	1	48
21/4/97	27	1.22	0.41	16.0	19.4(17.2-25.3)	87.3(60.3-92.8	0	55	55
21/4/97	0	0	0	0	19.0	55.0	55	0	55
29/4/97	33	1.14	0.87	16.0	18.4(13.9-26.6)	91.7(62.3-96.4	0	72	72
29/4/97	0	0	0	0	19.0	55.0	70	2	72
5/5/97	33	0.83	0.76	18.0	18.3(16.4-21.9)	83.4(66.1-94.6	0	30	30
5/5/97	0	0	0	0	19.0	55.0	30	0	30
26/5/97	27	1.0	0.84	17.25	17.5(16.0-19.1)	90.6(85.3-97.0	0	15	15
26/5/97	0	0	0	0	19.0	55.0	15	0	15

4.4.10 The European earwig, Forficula auricularia

4.4.10.1 Adults

A phosphine concentration of 0.41 $g.m^{-3}$ (final concentration of 0.12 $g.m^{-3}$) and an exposure period of 16 h at 17.1°C did not give a complete mortality of some adults of *F. auricularia* (Table 4.88). A small number of individuals had survived and completed their life-cycle as did the unfumigated healthy individuals. Also, a large number was severely affected in this trial; however, all these individuals had died 3 to

168 h following fumigation (Table 4.89). Most of the severely affected individuals were dead 3 to 72 h following fumigation and only a small number was still severely affected after 96 h.

Table 4.88 Toxicity of phosphine to the adults of *F. auricularia* fumigated with various concentrations, exposure times and temperatures in a 900 L chamber.

Date	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	European earwig *Forficula auricula*			
						Live	S.aff	Dead	Total
18/12/96	1.0	0.41	0.12	16.0	17.1	2	24	14	40
18/12/96	0	0	0	0	22.0	30	0	10	40

Table 4.89 Number of days required for the severely affected larvae to die.

Date	Phosphine concentration gm^{-3}	No. of larvae severely affected assessment after fumigation (h)						No. of days for complete mortality
		3	24	48	72	96	168	
18/12/96	0.41gm^{-3}	24	24	16	12	5	2	12

4.5 *Discussion*

4.5.1 *An intellectual framework*

As pointed out above, the experiments carried out at a commercial scale were very extensive. However, they were not as controlled as they would have been had they been carried out in the laboratory. Some intellectual framework in which to interpret the results was therefore sought. One approach was to examine the fumigation regime and classify them as being good, marginal or poor for each of the developmental stages studied. The second approach was to carry out a statistical analysis of the data and estimate which of the factors time, temperature or final concentration of phosphine have the most significant effect.

4.5.2 *Eggs*

It may be assumed prima facie that eggs may be one of the most difficult stages to kill because they are inactive. If this were the case any treatment that kills eggs would also kill other stages. Table 4.90 shows that the eggs of *E. postvittana* are indeed quite tolerant of phosphine, but only to roughly the same degree as the pre-pupal stage, and apparently less tolerant than the sixth instar. Significantly, eggs of *T. urticae* are apparently less tolerant to phosphine than the more active life stages.

A statistical analysis of the data indicates that the mortality of eggs of *E. postvittana* is most sensitive to the temperature of fumigation. The mortality of the eggs of *T. urticae* appear to be most sensitive of the concentration of phosphine.

The young eggs of *T. urticae* exhibited greater tolerance to phosphine fumigation than mature eggs. However, both the young and mature eggs of *E. postvittana* showed greater tolerance. The higher tolerance of eggs to phosphine fumigation in relation to active stages (larvae and adults) indicates that the relatively low uptake of fumigant by these stages is due to lower metabolic rates of developmental stages. Generally, the metabolism of insects varies with the stage of an insect stage, and the adults and larvae have higher metabolic rate compared with eggs or pupae.

In the fumigations trials it was observed that one to three days old eggs of *T. urticae* were comparatively tolerant to most of the phosphine fumigation than older eggs (four days and above), where some individuals survived and developed into normal larvae. Similarly, Bell (1976), averred that in general eggs of different stored product moths were highly tolerant to phosphine in their first day after oviposition which could be due to lower uptake of fumigants by these stages. Also, Vincent and Lindgren (1975) found that day old eggs of *T. variabile* were tolerant to phosphine, surviving phosphine dosage of 36 $g.h.m^{-3}$, but not 54 $g.h.m^{-3}$ over a three-day exposure at 21°C.

Table 4.90 Minimum requirements of various parameters for complete mortality of the exposed stages of different insect pests.

Insect species	Life stage	Exposure time (h)	Final phosphine concentration ($g.m^{-3}$)	Temperatur (°C)
S. ejectana	Larvae	15	0.25	20
	Pupae	17	0.55	23.9
E. postvittana	Eggs	17	0.55	23.9
	First to third instar lava	15	0.13	17.5
	Forth instar larvae	16	0.36	17.5
	Fifth instar larvae	16	0.48	21.1
	Sixth instar larvae	18	0.94	25.5
	Pre-pupae	17	0.55	23.9
	Pupae	15	0.08	17.4
T. urticae	Eggs	16	0.07	16.9
	Diapause larvae*			
	Larvae	16	0.48	17.4
	Adults	16	0.41	18.3
M. persicae	Larvae and adults**			
M. ornatus	Larvae	16	0.48	18.3
	Adults	15	0.43	18.2
B. brassicae	Larvae	16	0.48	18.3
	Adults	16	0.48	18.3
I. purpureus	Adults	16	0.42	17.4
P. presumes	Larvae and Adults	16	0.41	18.3
C. hesperidum	Larvae and Adults*			
F. auricularia	Adults***			

* - denotes all the exposed individuals were killed when assessed.

** - complete mortality was not achieved in the fumigation regime.

*** - data is not sufficient to analyse the results.

Similarly, Qureshi *et al.* (1965) found that one to three day old eggs of *T. mauritanicus* were highly tolerant to phosphine fumigation compared with four to seven-day old eggs. A concentration time product of 31.2 mg/L killed 23.1 per cent of the one-day

old eggs and the same concentration killed 100 per cent of the five to seven day old eggs. Phosphine reacts and inhibits a group of enzymes called cholinesterase (ChEs); these enzymes are important in toxicological studies. Comparatively the young eggs have lesser developed enzymes than the mature eggs. Al-Hakkah *et al.* (1989) established that developing embryos of a number of species exhibit low activity of this enzyme. As the eggs grow this enzyme develops progressively and increases its activity as it matures, hence young eggs are more resistant to phosphine fumigation than mature eggs.

The incubation studies show that the surviving eggs of *T. urticae,* subjected to phosphine fumigation, took longer to hatch than the unfumigated eggs. This indicates that phosphine could have retarded the development of young survived eggs. This supports the findings of Ho and Winks (1995) who found that the phosphine treated eggs of *L. bostrichophila* exhibited a delay in hatching. Also, Bond and Uptis (1973), reported that there was a delay in the development of *T. mauritanicus* eggs fumigated with phosphine. However, in these trials it was observed that there was no delay in hatching eggs of *E. postvittana* that were fumigated with various concentrations of phosphine.

The growth and reproductive capacity of those eggs of both species that survived were not affected by the phosphine fumigation; the larvae developed in a similar manner to the unfumigated eggs and completed their life-cycle.

4.5.3 Larvae

All the first and second instar larvae of *E. postvittana* and, second instar larvae of *S. ejectana* that were exposed to various concentrations of phosphine, temperatures and exposure times were killed. This was further confirmed in the second and third assessment made 24 and 48 h after the end of fumigation. It is apparent from these results that these stages did not have an efficient detoxifying mechanism similar to other stages. Moreover, the metabolism of these stages also differ from the mature

larvae, hence, these stages are highly vulnerable to phosphine fumigation. Severely affected larvae's mortality was high within 72 h following fumigation, and some larvae survived for 168 h. Not a single larva was severely affected beyond this time. This indicates that the toxic action of fumigants continues for several days after treatment.

The highest susceptibility of young larva of both *S. ejectana* and *E. postvittana* (first to third instar stages) to phosphine fumigation may also be due to different composition and different thickness of cuticular layer of the integument. Later instar larvae (late fourth, fifth and sixth instar) were slightly more tolerant to phosphine than the early instar larvae. Their physiology, living patterns and thickness of cuticular layer of the integument is entirely different from the younger stages. This is apparently a reflection of the increased protection of insects by the resistance mechanism known as protective stupefaction. In general, protective stupefaction reduces the uptake of toxic fumigant gases or detoxify the absorbed toxic gases to subacute levels leading to a delay in death, even though advanced symptoms of intoxication such as affected or severely affected were apparent.

Similarly, most of the larval and adult stages of *T. urticae* and *Myzus sp* were killed when assessed. A number of both larvae and adults were severely affected in some fumigations for up to 24 to 72 h. All the severely affected larvae of *T. urticae* died within 24 h after fumigation and adults required up to 60 h after fumigation for complete morality. All the severely affected larvae of *M. persicae* died within 60 h following fumigation and adults necessitated up to 72 h for complete mortality. On the other hand, both larvae and adults of *M. ornatus* and *B. brassicae* required up to 72 h for complete mortality.

4.5.4 Diapause stage

In general, diapause stages are induced by cold weather and/or over population in certain species of insects. This stage is characterised by zero or negligible growth and its metabolism remain low. As a result the phosphine uptake is expected to be low.

However, in all these fumigation trials all the diapause stages of *T. urticae* were killed in the first assessment itself. Similarly, all the diapause larvae of *S. ejectana* (exposed only on two trials due to their lack of availability) were killed at the first assessment. This may be either the exposure time and concentrations investigated were sufficient to make them susceptible.

4.5.5 Pupae

Most of the pupae of *S. ejectana* and *E. postvittana* exposed were killed, some survived and some severely affected. Only some pupae of *S. ejectana* were survived, some severely affected and others died. On the other hand, not a single pupa of *E. postvittana* survived, but a small number were severely affected and rest was dead.

Most of the young and some mature pupae of *S. ejectana* and *E. postvittana* responded with wriggling movements of abdominal segments while prodding during first 48 to 72 h after fumigation. However, these pupae did not show any response to external stimuli after this period. This indicates that the pupae were alive immediately after fumigation and died subsequently. Although a small number of pupae of *E. postvittana* were severely affected in two fumigations, the adults did not emerge fully from their pupal cases and subsequently died 24 to 48 h following emergence.

The survived adults of *S. ejectana* behaved similar to normal adults and the adults emerged from the severely affected pupae died within 24 to 36 h after emerging.

Phosphine may delay the growth and development of pupae. Bond and Uptis (1973) support this finding and they reported that the phosphine fumigated pupae of *T. mauritanicus* showed a delay in development. In these trials all the pupae that survived took longer (one to three days) to hatch than the unfumigated pupae. Most of the adults that emerged were of malformed. Only one adult was able to release from its pupal case and it died with in three days of emergence. Two other pupae were half emerged

from the pupae unable to release its wings from the pupal case and died within 24 h of emergence.

It is quite clear that as the pupae grew older, their sensitivity to phosphine increases (Bond 1980). In these trials, all the pupae that showed tolerance were from the age of one to three days. The reason for the susceptibility of old pupae would be their metabolism, which is higher resulting in more absorption of toxic fumigant gases.

4.5.6 Adults

The results from these trials indicate that adults of *T. urticae* and three species of aphids (*M. persicae, M. ornatus* and *B. brassicae*) were susceptible to phosphine fumigation. Although most of those exposed were killed in all the fumigations, a small number of individuals were severely affected at the end of fumigation. These were of mixed population of young and mature adults. However, all these were dead within 24 to 96 h following fumigation. This variation among individuals of same species of insects can be attributed to physiological or genetic variation that prevails in a population. Similarly, Qureshi *et al.* (1965) pointed out that one-day-old adults of *Sitophilus granarius* were more tolerant to phosphine than three-day-old adults. They found that susceptibility of adults gradually increases as their age and highest mortalities were recorded in older adults.

On the other hand in this fumigation all the exposed adults of *E. postvittana* were killed, presumably during fumigation.

4.5.7 Concluding Remarks

The aims of the experiments described in this chapter was to establish practical guidelines for fumigating Australian wildflowers on a commercial scale. Experiments

were carried out at three different geographical sites and it can be concluded that fumigations lasting about 16 h at a temperature of about 20°C and with a final phosphine concentration of about 0.5 $g.m^{-3}$ is sufficient to kill all of the life stages of the insects investigated. Of the active life stages that were not killed during the fumigation most of them died within 72 h. This is typically the time it takes to ship a consignment of flowers to their overseas distributor. Eggs that are not killed during fumigation can ultimately hatch, but their development time is prolonged as result of fumigation.

The results presented in this chapter indicate that phosphine with a final concentration of 0.94 $g.m^{-3}$, temperature of 25.5°C and exposure time of 18 h killed all the exposed insect pests and their developmental stages. Hence, it can be concluded that this fumigation regime is highly suitable for the postharvest disinfestation of wildflowers for commercial purposes. If the above mentioned fumigation conditions are not maintained (ie. less desirable fumigation conditions) a small number of individuals of various stages of different insect pests either survived (eggs of *E. postvittana*) or were severely affected (sixth instar larvae of *E. postvittana*). The presence of even single live insect pest is not tolerated by some countries, especially Japan. Hence, in this circumstance there are possibilities the entire consignment may be rejected because presence of insect pests.

Chapter - 5

Effects of Pestigas® and Phosfume® applied concurrently

Chapter 5

Effects of Pestigas® and Phosfume® applied concurrently

5.1 Scopes of the experiments

The studies on commercial scale fumigations reported in Chapter 4 has established that:

- although the majority of the active stages of insect pests exposed were killed, a small number was severely affected in most of the experiments; and

- a small number of developmental stages especially eggs of *T. urticae* and *E. postvittana,* and pupae of *S. ejectana* survived in some of the experiments.

Hence, there is a need to develop an appropriate strategy that will result in a completely disinfested product. To achieve this either exposure time or temperature or concentration has to be increased to a certain extent. However, increasing either any of these parameters is not feasible from the point of view of the vase life of flowers and or from the operational requirements of flower exporters. Long exposure times and high temperatures generally reduce the vase life of the flowers, reducing their value or making them unmarketable flowers. Moreover, long exposure times are inconvenient for flower exporters, as during certain seasons of the year (Christmas, St Valentine's day and Mothers' day) the demand for flowers is enormous. Hence, to meet the demand fumigations must be rapid. Also, maintaining higher temperatures for long times, particularly during the cooler months, increases the cost of energy requirements for heating the chamber.

It is well established that the lethality of fumigants can be increased by mixing them with other chemicals that would synergise or potentiate the action of the principal

fumigant. Thereby a complete kill of all the insect pests may possibly be achieved without increasing exposure time or temperature. Pyrethrum, which is a nerve poison, agitates or stirs the insect pests, resulting in their metabolic and respiration rates increasing. This leads that the insects to absorb more toxic gases.

These considerations prompted the candidate to study:

- the possibility of using Pestigas® (pyrethrum synergised with piperonyl butoxide) with Phosfume® and their insecticidal effects on all stages of a range of insect pests; and

- the phytotoxic effects of Pestigas® and Phosfume® combinations on four species of wildflowers and a species of cut flower. Details of this work are given in Chapter 7.

5.2 *Materials and methods*

5.2.1 *Pestigas®*

Pestigas® (4g/kg of pyrethrum synergised with 20 g/kg of piperonyl butoxide in carbon dioxide as a carrier gas), supplied in standard "G" type cylinder (17 kg tare weight and 6 kg net weight) by the British Oxygen Company Gases (BOCC), Preston, Victoria, was used for all the fumigation trials. The Phosfume® formulation used in these fumigations was similar to that described in the previous chapter (Chapter 4).

5.2.2 *27 m^3 modified shipping containers, located at Emerald and Gembrook*

Both the 27 m^3 chambers at Emerald and Gembrook had been installed with a solenoid valve to release Pestigas®, that is an aerosol. The fumigant was delivered by placing the cylinder on a 0 to 150 kg digital scale and the mass of fumigant delivered was

recorded. Ten minutes following the release of Pestigas®, Phosfume® was added to the chamber. The rationale of this strategy is that initial application of pyrethrum stirs or agitates the insects, and makes them likely to absorb more of the toxic phosphine gas, that was applied later.

5.2.3 *The 900 L chamber, Frankston*

As mentioned in the previous paragraph pyrethrums are administered as aerosols, which require special provisions to deliver them into the chamber. A stainless steel Jet Gun® supplied by British Oxygen Gases, Sydney, was used to transfer the Pestigas® from the cylinder into the chamber. This device has a capacity of 1,500 psi, that can deliver 5 g of Pestigas®/min or 19 L/min. Initially the Jet Gun® was filled with Pestigas® by attaching it to the Pestigas® cylinder. A calculated amount of Pestigas® was added into the chamber by fitting this gun on to the port of the chamber.

All other procedures (concentration measurement, purging the chamber at the end of fumigation and post-fumigation assessment of insects) followed were similar the phosphine fumigations that are described in detail in Chapter 4.

5.2.4 *Bioassay insects*

5.2.4.1 *Larvae of the leaf rolling moth, Strepsicrates ejectana*

Field collected larvae – Larvae of the leaf rolling moth (second to sixth instar stages) were collected randomly with their webbing shelters by cutting the stems (50 to 60 mm below the webbings from the plants) using secateurs. Collections were made every month from a commercial *T. calycina* plantation at Horsham, Victoria. The larvae were transferred to a laboratory at Knoxfield, Victoria in 2 to 4 L polyethylene containers (50

to 100 webbing shelters per container) covered with muslin cloth tops that were secured with rubber bands. A temperature of 20±1°C was maintained during this period. These larvae were fumigated with their webbing shelters within 24 to 30 h following collection. Remaining larvae were stored (for up to 20 to 25 days) for future fumigations. The webbing shelters were trimmed and transferred into rectangular, lidded polyethylene containers (10 to 12 larvae with webbings per container) with dimensions of 90 x 90 x 180 mm. Each container was supplied with 95 to 110 g of modified lightbrown apple moth medium. The reason for placing the larvae on the medium was that it maintains the larvae in a healthy condition without drying the shelters.

Laboratory reared larvae - Response of the laboratory reared larvae to various concentrations of phosphine, exposure times and temperatures were investigated by rearing field-collected various instar stages in modified lightbrown apple moth medium. Larvae of *S. ejectana* collected from field were removed from their webbing shelters and transferred into this medium and allowed to adjust to their environment. This medium lined (1.5 to 1.7 mm thickness) the bottom of the rectangular polyethylene containers and the surface was scored so that the larvae might take refuge in the grooves. The lids were each fitted with a tissue to absorb the condensed moisture, to preempt moulding or predatory mite attack. Moreover, this tissue also acted as an anchorage for the pupae. Just before pupation, the mature larvae move towards the lids and pupate on the tissue. The larvae were maintained at a controlled temperature of 22°C, and a relative humidity of 60 to 65 per cent. The conditions of the larvae were observed regularly; if moulding or predatory mite attacks were observed in the medium, the larvae were transferred into new containers filled with fresh medium. If the larvae were infested with mites or mould these were disposed of carefully by autoclaving them.

5.2.4.2 The sugar ant, *Camponotus consobrinus*

Adults of the common sugar ant *C. consobrinus* were collected from the storerooms or kitchen at the Institute for Horticultural Development, Knoxfield, Victoria. A lure using either 10 per cent honey solution or concentrated sugar solution soaked in absorbent cotton wool was placed on the bottom of 200 mL polyethylene cups. The traps were set 12 to 14 h before fumigation, during the night. When 50 to 60 ants had entered the cups they were covered with a muslin gauze cloth, secured with a rubber band and transferred to the fumigation site.

5.3 Results

5.3.1 The leaf rolling moth, *Strepsicrates ejectana*

5.3.1.1 Larvae

Field-collected larvae – Response of the larvae of *S. ejectana* to various concentrations of pyrethrum and phosphine, at different exposure times and temperatures are presented in Table 5.1. Although the phosphine concentrations were moderate to high (0.27 to 1.4 $g.m^{-3}$) more than 25 per cent of the larvae were severely affected at 4.5 to 5.5 h of exposure time and temperatures of 16.5 to 27.0°C. Mortality increased slightly when the exposure time increased to 6 h, with similar phosphine concentrations. The data from this table indicate that complete mortality of all the exposed larvae is possible by increasing the exposure time to 15 h or more, rather than increasing the concentration of phosphine. However, with fumigations of greater duration a small number of individuals were severely affected once the temperature or concentration of phosphine was not maintained adequately. Temperatures of 15.6°C or less and final phosphine concentrations of 0.15 $g.m^{-3}$ or less did not give a complete kill of all the exposed larvae.

Table 5.1 Insecticidal efficacy of pyrethrum-phosphine against third to sixth instar larvae of *S. ejectana* (field collected), exposed to various concentrations, exposure times and temperature in a 27 m^3 chamber at Emerald.

Date	Pyrethrum (gm^{-3})	Phosfume® (kg)	Phosphine Concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Leaf rolling moth *Strepsicrates ejectana*			
			Initial	Final			Live	S. aff	Dead	Total
2/2/95	4.4	-	0.94	0.82	4.5	28.0	0	16	35	51
2/2/95	0	0	0	0	0	22.0	50	0	1	51
8/2/95	4.4	-	1.25	1.18	4.5	22.5	0	14	27	41
8/2/95	0	0	0	0	0	22.0	41	0	0	41
2/3/95	4.4	-	1.21	1.17	4.5	27.0	0	20	24	44
2/3/95	0	0	0	0	0	22.0	44	0	0	44
30/3/95	4.4	-	1.3	1.26	5.5	18.5	0	0	6	6
30/3/95	0	0	0	0	0	22.0	6	0	0	6
28/6/95	4.4	-	1.33	1.1	6.0	18.7(17.5-19.9)	0	0	3	3
28/6/95	0	0	0	0	0	22.0	3	0	0	3
24/8/95	4.4	-	0.27	-	0.5	18.0	0	1	3	4
24/8/95	0	0	0	0	0	22.0	4	0	0	4
7/9/95	4.4	-	0.41	0.29	5.2	16.5	0	2	5	7
7/9/95	0	0	0	0	0	22.0	7	0	0	7
29/9/95	4.4	0.7	0.3	0.3	4.5	19.0	0	0	3	3
29/9/95	0	0	0	0	0	22.0	3	0	0	3
19/10/95	4.4	10	3.34	-	6.0	20.5(17.0-22.9)	0	0	36	36
19/10/95	0	0	0	0	0	22.0	36	0	0	36
26/10/95	4.4	1.960	1.0	0.9	6.0	20.3(19.5-21.4)	0	4	18	22
26/10/95	0	0	0	0	0	22.0	22	0	0	22
10/11/95	4.4	3.160	0.9	0.9	6.0	18.0	0	1	2	3
10/11/95	0	0	0	0	0	22.0	3	0	0	3
17/11/95	4.4	3.080	1.4	1.3	6.0	27.0	0	15	17	32
17/11/95	0	0	0	0	0	22.0	31	0	1	32
23/11/95	4.4	3.260	1.5	1.0	15.5	20.8(19.4-21.5)	0	0	21	21
23/11/95	0	0	0	0	0	22.0	20	0	1	21
30/11/95	4.4	3.120	1.4	1.0	`	20.5(19.6-22.8)	0	0	18	18
30/11/95	0	0	0	0	0	22.0	18	0	0	18
12/2/96	4.4	2	1.0	0.4	16.0	21.0(18.6-22.6)	0	0	30	30
12/2/96	0	0	0	0	0	22.0	28	0	2	30
19/2/96	4.6	2.02	1.0	0.6	16.0	21.0(16.3-23.1)	0	0	45	45
19/2/96	0	0	0	0	0	22.0	42	0	3	45
26/2/96	4.4	2.04	1.0	0.7	15.5	21.9(19.5-26.7)	0	0	103	103

Date	Pyrethrum (gm^{-3})	Phosfume® (kg)	Phosphine Concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Leaf rolling moth *Strepsicrates ejectana*			
			Initial	Final			Live	S. aff	Dead	Total
26/2/96	0	0	0	0	0	22.0	99	0	4	103
18/3/96	4.4	2.048	1.0	1.0	15.5	13.1(10.0-14.6)	0	1	24	25
18/3/96	0	0	0	0	0	22.0	25	0	0	25
25/3/96	4.4	2.02	1.0	0.7	16.0	21.7(18.1-27.2)	0	0	20	20
25/3/96	0	0	0	0	0	22.0	20	0	0	20
2/4/96	4.8	2.88	1.0	0.9	15.0	13.8(13.5-15.0)	0	3	12	15
2/4/96	0	0	0	0	0	22.0	15	0	0	15
29/5/96	4.4	2.52	1.0	0.9	18.0	18.2(15.4-19.9)	0	0	30	30
29/5/96	0	0	0	0	0	22.0	30	0	0	30
10/7/96	5.2	2.32	0.28	0.19	15.0	15.6(10.0-18.7)	0	0	59	59
10/7/96	0	0	0	0	0	22.0	58	0	1	59
18/7/96	4.4	1.12	0.28	0.18	15.25	17.4(10.1-20.3)	0	0	14	14
18/7/96	0	0	0	0	0	22.0	14	0	0	14
20/8/96	4.4	1.3	0.31	0.19	16.0	18.0	0	0	34	34
20/8/96	0	0	0	0	0	22.0	34	0	0	34
29/8/96	4.4	1.02	0.26	0.097	16.25	18.0(16.6-20.1)	0	1	132	133
29/8/96	0	0	0	0	0	22.0	129	0	4	133
4/9/96	4.6	0.92	0.27	0.11	16.25	18.1(15.2-20.8)	0	0	41	41
4/9/96	0	0	0	0	0	22.0	41	0	0	41
9/9/96	4.4	0.46	0.15	0.069	16.25	17.9(16.2-18.6)	0	2	22	24
9/9/96	0	0	0	0	0	22.0	24	0	0	24
23/9/96	4.4	0.6	0.22	0.07	16.0	18.9(16.9-20.8)	0	0	76	76
23/9/96	0	0	0	0	0	22.0	75	0	1	76
7/10/96	4.4	0.7	0.33	0.13	15.50	18.0(13.1-18.8)	0	2	23	25
7/10/96	0	0	0	0	0	22.0	25	0	0	25
16/10/96	4.4	0.7	0.15	0.12	16.0	17.1(12.5-19.2)	0	11	94	105
16/10/96	0	0	0	0	0	22.0	104	0	1	105
30/10/96	4.4	0.72	0.15	0.097	16.25	18.1(17.1-19.0)	0	30	58	88
30/10/96	0	0	0	0	0	22.0	86	0	2	88
12/11/96	4.4	1.12	0.27	0.15	16.0	18.1(17.5-18.9)	0	0	75	75
12/11/96	0	0	0	0	0	22.0	74	0	1	75
13/11/96	4.6	1.40	0.32	0.19	16.0	18.2(16.1-20.2)	0	0	96	96
13/11/96	0	0	0	0	0	22.0	95	0	1	96
17/2/97	4.8	0.7	0.44	0.32	15.75	26.5(22.1-33.4)	0	4	16	20
17/2/97	0	0	0	0	0	22.0	20	0	0	20
26/2/97	4.4	0.7	0.42	0.22	15.5	18.7(17.4-20.9)	0	3	11	14
26/2/97	0	0	0	0	0	22.0	14	0	0	14

Fumigation criteria

The procedure described in Chapter 4, Section 4.4.1.1 was followed in analysing the results. Insecticidal efficacy of pyrethrum-phosphine combinations was initially investigated by exposing various larval instars for 0.5 to 6 h with various concentrations and temperatures. Due to lack of availability of some instar stages at particular times, fumigations were conducted with one or more instars that were available. Twelve fumigations were conducted with the exposure times of 0.5 to 6 h. Only four gave a complete mortality of all the exposed larvae. The larvae exposed to fumigations conducted on 30/3/95, 28/6/95, 29/9/95 and 19/10/95 were third instar stages which can be killed easily, probably because of their higher metabolic rates. In addition to this, living pattern (webbing shelters are not compact, hence, toxic fumigants can easily reach the sides of the larvae) might make them vulnerable to phosphine fumigations. Hence, complete mortality of all the exposed larvae was achieved in these fumigations despite the short exposure time. Meticulous analysis of all the experimental results indicates that mortality of larvae depends on many factors including the instar stage and on a combination of fumigation factors (high temperature and/or sufficient phosphine concentration and/or longer exposure time). Hence, it is assumed that longer exposure times of 15 h or more are essential for quarantine treatment of flower consignments. Hence, in the light of the above facts, all the short exposure time experiments were not taken into consideration for analysis of results and only exposure times of 15 h or more were taken into consideration.

Criteria required for a satisfactory fumigation:

As in Chapter 4, criteria are proposed that will generally resulted in a satisfactory fumigation, ie. 100 per cent mortality. The basic idea is to define a benchmark set of conditions for mortality. In the case of larvae of *S. ejectana* the proposed criteria are:

Exposure time: 15 h or more

Final phosphine concentration: 0.15 $g.m^{-3}$ or more

Temperature: 15.6°C or more (Temperature-Time trajectories are presented in Appendix 5)

It can be seen from the Table 5.2 that 12 out of 14 fumigations that met the criteria were good (100 per cent mortality), and the remaining two were poor. In the case of poor fumigations, a small number of larvae was severely affected (three to four larvae) for reasons unknown. Only one larva was still severely affected for 96 to 168 h following fumigation, and all the other larvae had died within 72 h following fumigation.

Table 5.2 The fumigations with pyrethrum and phosphine that satisfy the criteria.

	Number of larvae severely affected	Number of larvae fumigated
Good		
23/11/95	-	21
30/11/95	-	18
12/2/96	-	30
19/2/96	-	45
26/2/96	-	103
25/3/96	-	20
29/5/96	-	30
10/7/96	-	59
18/7/96	-	14
20/8/96	-	34
12/11/96	-	75
13/11/96	-	96
Poor		
17/2/97	4(20.0%)	20
26/2/97	3(21.0%)	14

Table 5.3 indicates that out of nine fumigations that did not satisfy the fumigation criteria two were good, four were marginal and remaining three were poor. Complete mortality of all the exposed larvae were achieved during the 4/9/96 and 23/9/96 fumigations. The probable reason for this would be that in both these fumigations the temperature and exposure times were maintained above the required level which might have accelerated the mortality of the exposed larvae. In these fumigations only the final phosphine concentration was lower than the set criteria. In the marginal fumigation, all the larvae had died within 48 h following fumigation, except in one fumigation (9/9/96)

where a single larva was still severely affected 96 h following fumigation. However, in the poor fumigations the severely affected larvae took 24 to 168 h to die. In the poor fumigations, conducted on 2/4/96 and 30/10/96 all the severely affected larvae were dead 48 to 72 h following fumigation.

From these results it can be suggested that if an exporter requires nil or minimal chance of rejection of his container, he must satisfy all the criteria. Failure to meet the criteria results in a very high failure rate of the fumigations.

Table 5.3 The fumigations with pyrethrum-phosphine that did not meet the criteria

	Number of larvae severely affected	Number fumigated
Good		
4/9/96	-	41
23/9/96	-	76
Marginal		
18/3/96	1(4.0%)	25
29/8/96	1(0.75%)	133
9/9/96	2(8.3%)	24
7/10/96	2(8.0%)	25
Poor		
2/4/96	3(20.0%)	15
16/10/96	11(10.4%)	105
30/10/96	30(34.1%)	88

The number of days required for the severely affected larvae to die is given in Table 5.4. All these severely affected larvae were dead 24 to 168 h following fumigation. It is clear from this table that those larvae fumigated with short exposure periods of 0.5 to 6 h took longer to die (four to nine days) than those exposed for 15 h or more. In the case of long exposure fumigations all the severely affected larvae were dead within 24 to 96 days following fumigations. Furthermore, most of these severely affected larvae that were fumigated for 15 h or more, died 3 to 48 h following fumigation. Only one or two larvae were still severely affected between 24 to 96 h.

Table 5.4 Number of days required for complete mortality of severely affected larvae.

Date	Pyrethrum/ Phosphine	Exposure time (h)	Temperature (°C)	No. of larvae severely affected assessment time after fumigation (h)						o. of days for mortality	Instar Stage
				3	24	48	72	96	168		
2/2/95	4.4/0.94gm^{-3}	4.5	28.0	16	12	7	2	1	-	7	V and VI
8/2/95	4.4/1.25gm^{-3}	4.5	22.5	14	7	7	5	3	2	9	VI
2/3/95	2.2/1.21gm^{-3}	4.5	27.0	20	16	10	7	2	2	9	VI
24/8/95	4.4/0.27gm^{-3}	0.5	18.0	1	1	1	1	-	-	4	VI
7/9/95	4.4/0.41gm^{-3}	5.2	16.5	2	2	1	1	1	-	7	IV and V
26/10/95	4.4/1.0gm^{-3}	6.0	20.3(19.5-21.4)	4	4	4	2	1	1	8	V
10/11/95	4.4/0.9gm^{-3}	6.0	18.0	1	1	1	1	1	-	7	VI
17/11/95	4.4/1.4gm^{-3}	6.0	27.0	8	8	8	5	3	1	8	IV
18/3/96	4.6/1.0gm^{-3}	15.5	13.1(10.0-14.6)	1	1	-	-	-	-	2	V
2/4/96	4.8/1.0gm^{-3}	15.0	13.8(13.5-15.0)	3	3	1	-	-	-	4	VI
29/8/96	4.4/0.26gm^{-3}	16.25	18.0(16.6-20.1)	1	1	-	-	-	-	2	III
9/9/96	.4/0.15/0.069	16.25	17.9(16.2-18.6)	2	1	1	1			3	IV
7/10/96	4.4/0.33gm^{-3}	15.50	18.0(13.1-18.8)	2	-	-	-	-	-	1	V
16/10/96	4.4/0.15gm^{-3}	16.0	17.1(12.5-19.2)	11	-	-	-	-	-	1	IV
30/10/96	4.4/0.15gm^{-3}	16.25	18.1(17.1-19.0)	18	2	-	-	-	-	2	IV
17/2/97	.6/0.44/0.32	15.75	26.5(22.1-33.4)	4	4	2	1	1	-	4	VI
26/2/97	.4/0.42/0.22	15.5	18.7(17.4-20.9)	3	2	2	1	-	-	3	VI

Laboratory reared larvae - Mortality rates of larvae (first to sixth instar) of *S. ejectana* that were reared in modified lightbrown apple moth medium in the laboratory and fumigated with various concentrations of pyrethrum, phosphine, exposure times and temperatures are presented in Table 5.5. It is clear from this table that all the exposed larvae were killed in all the fumigations (including short exposure fumigations), indicating that these larvae were comparatively more susceptible than the larvae that were collected from the field. The possible reason for their susceptibility would be their different food or sheltering patterns.

Table 5.5 Insecticidal efficacy of pyrethrum-phosphine to the laboratory reared first to sixth instar larvae of *S. ejectana* with various concentrations, times and temperatures in a 27 m^3 chamber at Emerald.

Date	Pyrethrum gm^{-3}	hosfume® Kg	Phosphine Concentration gm^{-3}		Exposure time h	Temperature °C	Leaf rolling moth *Strepsicrates ejectana*		
			Initial	Final			Live	Dead	Total
2/2/95	120	-	0.94	0.82	4.5	28.0	0	24	24
2/2/95	0	0	0	0	0	22.0	21	3	24
28/6/95	120	-	1.33	1.1	6.0	18.7(17.5-19.9)	0	12	12
28/6/95	0	0	0	0	0	22.0	12	0	12
29/9/95	120	0.7	0.3	0.3	4.5	19.0	0	7	7
29/9/95	0	0	0	0	0	22.0	6	1	7
19/10/95	120	10	3.34	-	6.0	20.5(17.0-22.9)	0	16	16
19/10/95	0	0	0	0	0	22.0	14	2	16
26/10/95	120	1.960	1.0	0.9	6.0	20.3(19.5-21.4)	0	18	18
26/10/95	0	0	0	0	0	22.0	15	3	18
10/11/95	120	3.160	0.9	0.9	6.0	18.0	0	12	12
10/11/95	0	0	0	0	0	22.0	10	2	12
23/11/95	120	3.260	1.5	1.0	15.5	20.8(19.4-21.5)	0	21	21
23/11/95	0	0	0	0	0	22.0	20	1	21
30/11/95	120	3.120	1.4	1.0	15.0	20.5(19.6-22.8)	0	18	18
30/11/95	0	0	0	0	0	22.0	18	0	18
19/2/96	160	2.02	1.0	0.6	16.0	21.0(16.3-23.1)	0	15	15
19/2/96	0	0	0	0	0	22.0	15	0	15
26/2/96	120	2.04	1.0	0.7	15.5	21.9(19.5-26.7)	0	24	24
26/2/96	0	0	0	0	0	22.0	23	1	24
25/3/96	120	2.02	1.0	0.7	16.0	21.7(18.1-27.2)	0	14	14
25/3/96	0	0	0	0	0	22.0	14	0	14
18/7/96	120	1.12	0.28	0.18	15.25	17.4(10.1-20.3)	0	18	18
18/7/96	0	0	0	0	0	22.0	18	0	18
20/8/96	120	1.3	0.31	0.19	16.0	18.0	0	24	24
20/8/96	0	0	0	0	0	22.0	21	3	24
29/8/96	120	1.02	0.26	0.097	16.25	18.0(16.6-20.1)	0	32	32
29/8/96	0	0	0	0	0	22.0	32	0	32
9/9/96	120	0.46	0.15	0.069	16.25	17.9(16.2-18.6)	0	22	22
9/9/96	0	0	0	0	0	22.0	21	1	22
23/9/96	120	0.6	0.22	0.07	16.0	18.9(16.9-20.8)	0	25	25
23/9/96	0	0	0	0	0	22.0	25	2	27
7/10/96	120	0.7	0.33	0.13	15.50	18.0(13.1-18.8)	0	15	15

5.3.1.2 Pupae

Complete mortality of all the exposed pupae was obtained in both the short and long exposure time fumigations (Table 5.6). Fumigations were conducted with phosphine concentrations of 0.62 to 1.5 $g.m^{-3}$, exposure periods of 5.5 to 16 h and temperatures of 13.8 to 21.9°C. These pupae were from different ages (two to seven days old) and sexes.

Table 5.6 Insecticidal efficacy of pyrethrum-phosphine to pupae of *S. ejectana* exposed to different concentrations, exposure times and temperatures fumigated in a 27 m^3 chamber at Emerald.

Date	Pyrethrum gm^{-3}	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Leaf rolling moth *Strepsicrates ejectana*		
			Initial	Final			Live	Dead	Total
30/3/95	4.4	-	1.3	1.26	5.5	18.5	0	1	1
30/3/95	0	0	0	0	0	25.0	1	0	1
28/6/95	4.4	-	1.33	1.1	6.0	18.7(17.5-19.9)	0	1	1
28/6/95	0	0	0	0	0	25.0	1	0	1
26/10/95	4.4	1.960	1.0	0.9	6.0	20.3(19.5-21.4)	0	45	45
26/10/95	0	0	0	0	0	25.0	43	2	45
10/11/95	4.4	3.160	0.9	0.9	6.0	18.0	0	14	14
10/11/95	0	0	0	0	0	25.0	14	0	14
23/11/95	4.4	3.260	1.5	1.0	15.5	20.8(19.4-21.5)	0	16	16
23/11/95	0	0	0	0	0	25.0	16	0	16
30/11/95	4.4	3.120	1.4	1.0	15.0	20.5(19.6-22.8)	0	24	24
30/11/95	0	0	0	0	0	25.0	23	1	24
19/2/96	4.8	2.02	1.0	0.6	16.0	21.0(16.3-23.1)	0	12	12
19/2/96	0	0	0	0	0	25.0	12	0	12
26/2/96	4.4	2.04	1.0	0.7	15.5	21.9(19.5-26.7)	0	1	1
26/2/96	0	0	0	0	0	25.0	1	0	1
25/3/96	4.4	2.02	1.0	0.7	16.0	21.7(18.1-27.2)	0	3	3
25/3/96	0	0	0	0	0	25.0	3	0	3
2/4/96	4.8	2.88	1.0	0.9	15.0	13.8(13.5-15.0)	0	4	4
2/4/96	0	0	0	0	0	25.0	4	0	4
5/3/97	4.0	1.6	0.62	0.45	15.5	22.5(19.2-32.4)	0	3	3
5/3/97	0	0	0	0	0	22.0	3	0	3

5.3.2 *The lightbrown apple moth, Epiphyas postvittana*

5.3.2.1 *Eggs*

The results of the pyrethrum-phosphine fumigated eggs of *E. postvittana* are presented in Table 5.7. These eggs were exposed to various concentrations of phosphine, exposure times and temperatures. Complete mortality of the exposed eggs were achieved in most of the fumigations and only in one fumigation (25/6/97), a small number of two day old eggs survived. The probable reason for this would be either these eggs were exposed to lower phosphine concentrations (0.47 $g.m^{-3}$) or lower temperature (18°C) or a combination of both.

Table 5.7 Insecticidal efficacy of pyrethrum-phosphine to eggs of *E. postvittana* fumigated in a 900 L chamber.

Date	Pyrethrum gm^{-3}	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Lightbrown apple moth *Epiphyas postvttana*		
			Initial	Final			Live	Dead	Total
12/2/96	4.4	2.0	1.0	0.4	16.0	21.0 (18.9-22.6)	0	14	14
12/2/96	0	0	0	0	0	22.0	14	0	14
19/2/96	4.8	2.02	1.0	0.6	16.0	21.0 (16.3-23.1)	0	46	46
19/2/96	0	0	0	0	0	22.0	45	1	46
26/2/96	4.4	2.04	1.0	0.7	15.5	21.9 (19.5-26.7)	0	18	18
26/2/96	0	0	0	0	0	22.0	18	0	18
25/3/96	4.4	2.02	1.0	0.7	15.5	21.7 (18.1-27.2)	0	29	29
25/3/96	0	0	0	0	0	22.0	27	2	29
25/6/97	4.4	1.04	0.47	0.33	17.0	18.0 (16.0-20.2)	3*	597	600
25/6/97	0	0	0	0	0	22.0	590	10	600

*- Two days old eggs.

Fumigation criteria

Temperature: 21°C or more

Exposure time: 15.5 h or more

Final phosphine concentration: 0.4 $g.m^{-3}$ or more.

All the four fumigations that satisfy the criteria gave a complete mortality of the exposed eggs (Table 5.8).

Table5.8 The fumigations with pyrethrum-phosphine that satisfy the criteria.

	Number of eggs survived	Total number of eggs fumigated
Good		
12/2/96	-	14
19/2/96	-	46
26/2/96	-	18
25/3/96	-	29

Three eggs out of 600 survived and developed into normal larvae in the fumigation that did not satisfy the criteria.

Table5.9 The fumigations with pyrethrum-phosphine that did not satisfy the criteria.

	Number of eggs survived	Total number of eggs fumigated
Marginal		
25/6/97	3(0.5%)	600

5.3.2.2 Larvae

First, second and third instar larvae - The response of the first three larval instar stages of *E. postvittana* to the combined pyrethrum-phosphine fumigation with various concentrations and exposure times is presented in Table 5.10. The data clearly illustrate that these larval instar stages are highly susceptible to all the exposed concentrations, exposure times and temperatures.

Table 5.10 Toxicity of pyrethrum-phosphine to first to third instar larvae of *E. postvittana* exposed at various concentrations, exposure times and temperatures in a 27 m^3 chamber.

Date	Pyrethrum gm^{-3}	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Lightbrown apple mot *Epiphyas postvittana*		
			Initial	Final			Live	Dead	Total
12/2/96	4.4	2.0	1.0	0.4	16.0	21.0(18.9-22.6)	0	65	65
12/2/96	0	0	0	0	0	22.0	64	1	65
19/2/96	4.8	2.02	1.0	0.6	16.0	21.0(16.3-23.1)	0	50	50
19/2/96	0	0	0	0	0	22.0	50	0	50
26/2/96	4.4	2.04	1.0	0.7	15.5	21.9(19.5-26.7)	0	34	34
26/2/96	0	0	0	0	0	22.0	33	1	34
18/3/96	4.6	2.048	1.0	1.0	15.5	13.1(10.0-14.6)	0	160	160
18/3/96	0	0	0	0	0	22.0	151	9	160
25/3/96	4.4	2.02	1.0	0.7	15.5	21.7(18.1-27.2)	0	48	48
25/3/96	0	0	0	0	0	22.0	46	2	48
2/4/96	4.6	2.88	1.0	0.9	15.0	13.8 (13.5-15)	0	69	69
2/4/96	0	0	0	0	0	22.0	65	4	69
29/5/96	4.6	2.52	1.0	0.9	18.0	18.2(15.4-19.9)	0	64	64
29/5/96	0	0	0	0	0	22.0	64	0	64
10/7/96	5.2	2.32	0.3	0.19	15.0	15.6(10.0-18.7)	0	120	120
10/7/96	0	0	0	0	0	22.0	117	3	120
18/7/96	4.4	1.12	0.3	0.18	15.25	17.4(10.1-20.3)	0	48	48
18/7/96	0	0	0	0	0	22.0	46	2	48
20/8/96	4.4	1.3	0.26	0.097	16.0	18.0	0	47	47
20/8/96	0	0	0	0	0	22.0	47	0	47
29/8/96	4.4	1.02	0.27	0.11	16.25	18.0(16.6-20.1)	0	34	34
29/8/96	0	0	0	0	0	22.0	34	0	34
4/9/96	4.6	0.92	0.15	0.069	16.25	18.1(15.2-20.8)	0	120	120
4/9/96	0	0	0	0	0	22.0	120	0	120
9/9/96	4.4	0.46	0.23	0.18	16.25	17.9(16.2-18.6)	0	47	47
9/9/96	0	0	0	0	0	22.0	47	0	47
18/9/96	4.4	0.7	0.22	0.07	16.0	19.2(17.8-20.6)	0	127	127
18/9/96	0	0	0	0	0	22.0	122	5	127
23/9/96	4.4	0.6	0.25	0.15	16.0	18.9(16.9-20.8)	0	90	90
23/9/96	0	0	0	0	0	22.0	93	7	90
7/10/96	4.4	0.7	0.33	0.13	15.5	18.0(13.1-18.8)	0	36	36
7/10/96	0	0	0	0	0	22.0	33	3	36
16/10/96	4.4	0.7	0.15	0.12	16.0	17.1(12.5-19.2)	0	47	47

Date	Pyrethrum gm^{-3}	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	ightbrown apple mot *Epiphyas postvittana*		
			Initial	Final			Live	Dead	Total
16/10/96	0	0	0	0	0	22.0	42	5	47
30/10/96	4.4	0.72	0.15	0.097	16.25	18.4(17.1-19.0)	0	74	74
30/10/96	0	0	0	0	0	22.0	74	0	74
12/11/96	4.4	1.12	0.27	0.15	16.0	18.1(17.5-18.9)	0	45	45
12/11/96	0	0	0	0	0	22.0	44	1	45
13/11/96	4.6	1.40	0.32	0.19	16.0	18.2(16.1-20.2)	0	45	45
13/11/96	0	0	0	0	0	22.0	45	0	45
23/1/97	4.8	0.7	0.44	0.32	15.75	26.5(22.1-33.4)	0	29	29
23/1/97	0	0	0	0	0	22.0	25	4	29
17/2/97	4.4	0.7	0.42	0.22	15.5	18.7(17.4-20.9)	0	25	25
17/2/97	0	0	0	0	0	22.0	25	0	25
1/5/97	4.6	0.92	0.34	0.23	16.5	17.8(13.2-18.8)	0	24	24
1/5/97	0	0	0	0	0	22.0	24	0	24

All the first, second and third instar larvae of the lightbrown apple moth that were exposed to pyrethrum-phosphine combination were killed following fumigation. These are the minimum fumigation conditions that killed these stages:

Temperature:13.1°C or more

Exposure time: 15 h or more

Final phosphine concentration: 0.15 $g.m^{-3}$ or more.

Fourth instar larvae - The fourth instar larvae are comparatively tolerant to pyrethrum-phosphine fumigations compared with early instar larvae (Table 5.11). Complete mortality of all the exposed larvae was achieved in all the fumigations, except in eight fumigations where a small number of larvae were severely affected.

Table 5.11 Insecticidal efficacy of pyrethrum-phosphine to fourth instar larvae of *E. postvittana* exposed to various concentrations, exposure times and temperatures in a 27 m^3 chamber.

Date	Pyrethrum gm^{-3}	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Lightbrown apple moth *Epiphyas postvittana*			
			Initial	Final			Live	S.aff	Dead	Total
12/2/96	120	2.0	1.0	0.4	16.0	21.0(18.9-22.6)	0	0	21	21
12/2/96	0	0	0	0	0	22.0	21	0	0	21
19/2/96	160	2.02	1.0	0.6	16.0	21.0(16.3-23.1)	0	0	30	30
19/2/96	0	0	0	0	0	22.0	30	0	0	30
26/2/96	120	2.04	1.0	0.7	15.5	21.9(19.5-26.7)	0	0	24	24
26/2/96	0	0	0	0	0	22.0	24	0	0	24
18/3/96	140	2.048	1.0	1.0	15.5	13.1(10.0-14.6)	0	4	19	23
18/3/96	0	0	0	0	0	22.0	23	0	0	23
25/3/96	120	2.02	1.0	0.7	15.5	21.7(18.1-27.2)	0	0	24	24
25/3/96	0	0	0	0	0	22.0	23	0	1	24
2/4/96	160	2.88	1.0	0.9	15.0	13.8 (13.5-15)	0	6	54	60
2/4/96	0	0	0	0	0	22.0	59	0	1	60
29/5/96	140	2.52	1.0	0.9	18.0	18.2(15.4-19.9)	0	0	31	31
29/5/96	0	0	0	0	0	22.0	30	0	1	31
10/7/96	200	2.32	0.3	0.19	15.0	15.6(10.0-18.7)	0	2	26	28
10/7/96	0	0	0	0	0	22.0	27	0	1	28
18/7/96	120	1.12	0.3	0.18	15.25	17.4(10.1-20.3)	0	3	45	48
18/7/96	0	0	0	0	0	22.0	47	0	1	48
20/8/96	120	1.3	0.26	0.097	16.0	18.0	0	0	31	31
20/8/96	0	0	0	0	0	22.0	31	0	0	31
29/8/96	120	1.02	0.27	0.11	16.25	18.0(16.6-20.1)	0	0	14	14
29/8/96	0	0	0	0	0	22.0	14	0	0	14
4/9/96	140	0.92	0.15	0.069	16.25	18.1(15.2-20.8)	0	3	34	37
4/9/96	0	0	0	0	0	22.0	36	0	1	37
9/9/96	120	0.46	0.23	0.18	16.25	17.9(16.2-18.6)	0	0	27	27
9/9/96	0	0	0	0	0	22.0	27	0	0	27
18/9/96	120	0.7	0.22	0.07	16.0	19.2(17.8-20.6)	0	0	34	34
18/9/96	0	0	0	0	0	22.0	33	0	1	34
23/9/96	120	0.6	0.25	0.15	16.0	18.9(16.9-20.8)	0	0	84	84
23/9/96	0	0	0	0	0	22.0	82	0	2	84
7/10/96	120	0.7	0.33	0.13	15.5	18.0(13.1-18.8)	0	0	24	24
7/10/96	0	0	0	0	0	22.0	24	0	0	24
16/10/96	120	0.7	0.15	0.12	16.0	17.1(12.5-19.2)	0	1	42	43
16/10/96	0	0	0	0	0	22.0	43	0	0	43

Date	Pyrethrum gm^{-3}	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Lightbrown apple moth *Epiphyas postvittana*			
			Initial	Final			Live	S.aff	Dead	Total
30/10/96	120	0.72	0.15	0.097	16.25	18.4(17.1-19.0)	0	4	14	18
30/10/96	0	0	0	0	0	22.0	18	0	4	18
12/11/96	120	1.12	0.27	0.15	16.0	18.1(17.5-18.9)	0	1	24	25
12/11/96	0	0	0	0	0	22.0	21	0	1	25
13/11/96	140	1.40	0.32	0.19	16.0	18.2(16.1-20.2)	0	0	15	15
13/11/96	0	0	0	0	0	22.0	15	0	0	15
23/1/97	160	0.7	0.44	0.32	15.75	26.5(22.1-33.4)	0	0	26	26
23/1/97	0	0	0	0	0	22.0	25	0	1	26
17/2/97	120	0.7	0.42	0.22	15.5	18.7(17.4-20.9)	0	0	24	24
17/2/97	0	0	0	0	0	22.0	21	0	3	24
1/5/97	140	0.92	0.34	0.23	16.5	17.8(13.2-18.8)	0	0	63	63

Fumigation criteria

Temperature: 17.8°C or more

Exposure time: 15.5 h or more

Phosphine concentration: complete mortality can be achieved with the initial phosphine concentration of 0.15 $g.m^{-3}$, provided the final phosphine concentration is 0.12 $g.m^{-3}$ or more. Complete mortality was not achieved with this initial phosphine concentration with the final phosphine concentration of 0.097 $g.m^{-3}$ (fumigated on 30/10/96). Moreover, an initial phosphine concentration of 0.26 $g.m^{-3}$ and a final concentration of 0.069 $g.m^{-3}$ (fumigated on 20/8/96) gave a complete mortality of all the exposed larvae.

Table 5.12 clearly indicates that of the 16 fumigations that satisfy the criteria only one fumigation did not give a complete mortality of all the exposed larvae. In this fumigation only a single larva was severely affected and this larva died within 48 h following fumigation.

Table 5.12 The fumigations with pyrethrum-phosphine that satisfy the criteria.

	Number of larvae severely affected	Number fumigated
Good		
12/2/96	-	21
19/2/96	-	30
26/2/96	-	24
25/3/96	-	24
29/5/96	-	31
20/8/96	-	31
29/8/96	-	14
9/9/96	-	27
18/9/96	-	34
23/9/96	-	84
7/10/96	-	24
13/11/96	-	15
23/1/97	-	26
17/2/97	-	24
1/5/97	-	63
Marginal		
12/11/96	1(4.0%)	25

Of the seven fumigations that did not satisfy the fumigation criteria, four were marginal and three were poor (Table 5.13). In the marginal fumigations one to three larvae were severely affected and in the poor fumigations four to six larvae were severely affected.

Table 5.13 The fumigations with pyrethrum-phosphine that did not satisfy the criteria.

	Number of larvae severely affected	Number fumigated
Marginal		
10/7/96	2(7.1%)	28
18/7/96	3(6.25%)	48
4/9/96	3(8.1%)	37
16/10/96	1(2.3%)	43
Poor		
18/3/96	4(17.4%)	23
2/4/96	6(10.0%)	60
30/10/96	4(22.2%)	18

The number of days required for the severely affected larvae for complete mortality varies greatly and most of the larvae died within 3 to 48 h after fumigation (Table 5.14). Only a small number (one to three) remained severely affected for 72 to 168 h. Those larvae that were fumigated at lower temperatures (18/3/96 and 2/4/96) necessitated 168 h to obtain complete mortality, and all other larvae fumigated with comparatively high temperature died within 78 h following fumigation.

Table 5.14 Number of days required for the severely affected fourth instar larvae to die.

Date	Phosphine concentration gm^{-3}		No. of larvae severely affected assessment after fumigation (h)						No. of days for complete mortality
			3	24	48	72	96	168	
18/3/96	1.0	1.0	4	4	4	2	2	1	9
2/4/96	1.0	0.9	6	6	6	3	2	2	11
10/7/96	0.3	0.19	2	1	1	0	0	0	2
18/7/96	0.3	0.18	3	2	2	2	0	0	3
4/9/96	0.15	0.069	3	3	1	0	0	0	2
16/10/96	0.15	0.12	1	1	1	1	0	0	3
30/10/96	0.15	0.097	4	3	2	1	0	0	3
12/11/96	0.27	0.15	1	1	1	0	0	0	2

Fifth instar larvae - Results of the pyrethrum-phosphine fumigated fifth instar larvae are given in Table 5.15. Various concentrations of phosphine, exposure times and temperatures were investigated. Data from this table indicate that not all the fumigations have given complete mortality of all the exposed larvae, a small number was severely affected in some fumigations.

Table 5.15 Insecticidal efficacy of pyrethrum-phosphine to fifth instar larvae of *E. postvittana* Exposed to various concentrations, exposure times and temperatures in a 27 m^3 chamber.

Date	Pyrethrum gm^{-3}	Phosfume® (kg)	Phosphine oncentration gm^{-3}		Exposure time (h)	Temperature (°C)	Lightbrown apple moth *Epiphyas postvittana*			
			Initial	Final			Live	S.aff	Dead	Total
12/2/96	120	2.0	1.0	0.4	16.0	21.0(18.9-22.6)	0	0	37	37
12/2/96	0	0	0	0	0	22.0	37	0	0	37
19/2/96	160	2.02	1.0	0.6	16.0	21.0(16.3-23.1)	0	0	56	56
19/2/96	0	0	0	0	0	22.0	55	0	1	56
26/2/96	120	2.04	1.0	0.7	15.5	21.9(19.5-26.7)	0	0	28	28
26/2/96	0	0	0	0	0	22.0	28	0	0	28
18/3/96	140	2.048	1.0	1.0	15.5	13.1(10.0-14.6)	0	18	60	78
18/3/96	0	0	0	0	0	22.0	78	0	0	78
25/3/96	120	2.02	1.0	0.7	15.5	21.7(18.1-27.2)	0	0	18	18
25/3/96	0	0	0	0	0	22.0	18	0	0	18
2/4/96	160	2.88	1.0	0.9	15.0	13.8 (13.5-15)	0	21	49	70
2/4/96	0	0	0	0	0	22.0	70	0	0	70
29/5/96	140	2.52	1.0	0.9	18.0	18.2(15.4-19.9)	0	0	46	46
29/5/96	0	0	0	0	0	22.0	45	0	1	46
10/7/96	200	2.32	0.3	0.19	15.0	15.6(10.0-18.7)	0	4	16	20
10/7/96	0	0	0	0	0	22.0	19	0	1	20
18/7/96	120	1.12	0.3	0.18	15.25	17.4(10.1-20.3)	0	7	48	55
18/7/96	0	0	0	0	0	22.0	54	0	1	55
29/8/96	120	1.02	0.27	0.11	16.25	18.0(16.6-20.1)	0	0	65	65
29/8/96	0	0	0	0	0	22.0	63	0	2	65
4/9/96	140	0.92	0.15	0.069	16.25	18.1(15.2-20.8)	0	4	25	29
4/9/96	0	0	0	0	0	22.0	27	0	2	29
9/9/96	120	0.46	0.23	0.18	16.25	17.9(16.2-18.6)	0	0	31	31
9/9/96	0	0	0	0	0	22.0	30	0	1	31
18/9/96	120	0.7	0.22	0.07	16.0	19.2(17.8-20.6)	0	0	72	72
18/9/96	0	0	0	0	0	22.0	70	0	2	72
23/9/96	120	0.6	0.25	0.15	16.0	18.9(16.9-20.8)	0	0	14	14
23/9/96	0	0	0	0	0	22.0	12	0	2	14
7/10/96	120	0.7	0.33	0.13	15.5	18.0(13.1-18.8)	0	0	20	20
7/10/96	0	0	0	0	0	22.0	20	0	0	20
6/10/96	120	0.7	0.15	0.12	16.0	17.1(12.5-19.2)	0	6	16	22
6/10/96	0	0	0	0	0	22.0	21	0	1	22
0/10/96	120	0.72	0.15	0.097	16.25	18.4(17.1-19.0)	0	0	18	18
0/10/96	0	0	0	0	0	22.0	16	0	2	18

Date	Pyrethrum gm^{-3}	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Lightbrown apple moth *Epiphyas postvittana*			
			Initial	Final			Live	S.aff	Dead	Total
2/11/96	120	1.12	0.27	0.15	16.0	18.1(17.5-18.9)	0	0	21	21
2/11/96	0	0	0	0	0	22.0	21	0	0	21
3/11/96	140	1.40	0.32	0.19	16.0	18.2(16.1-20.2)	0	0	16	16
3/11/96	0	0	0	0	0	22.0	16	0	0	16
23/1/97	160	0.7	0.44	0.32	15.75	26.5(22.1-33.4)	0	0	27	27
23/1/97	0	0	0	0	0	22.0	27	0	0	27
17/2/97	120	0.7	0.42	0.22	15.5	18.7(17.4-20.9)	0	0	42	42
17/2/97	0	0	0	0	0	22.0	40	0	2	42
1/5/97	140	0.92	0.34	0.23	16.5	17.8(13.2-18.8)	0	2	24	26
1/5/97	0	0	0	0	0	22.0	24	0	2	26

Fumigation criteria

Temperature: 17.9°C or more

Exposure time: 15.5 h or more

Phosphine concentration: Generally, an initial phosphine concentration of 0.15 $g.m^{-3}$ can give a complete mortality of all the exposed larvae provided if the final phosphine concentration is maintained at 0.097 $g.m^{-3}$ or more.

It can be seen from this table that all the fumigations that satisfied the criteria gave a complete mortality of all the exposed larvae (Table 5.16).

Table 5.16 The fumigations with pyrethrum-phosphine that satisfy the criteria

	Number of larvae severely affected	Number fumigated
Good		
12/2/96	-	37
19/2/96	-	56
26/2/96	-	28
25/3/96	-	18
29/5/96	-	46
29/8/96	-	65
9/9/96	-	31

	Number of larvae severely affected	Number fumigated
Good		
18/9/96	-	72
23/9/96	-	14
7/10/96	-	20
30/10/96	-	18
12/11/96	-	21
13/11/96	-	16
23/1/97	-	27
17/2/97	-	42

This table indicates that all the fumigations that did not satisfy the criteria failed to give complete mortality of all the exposed larvae (Table 5.17). Of the seven fumigations one was marginal and six were poor. A large number of individuals were severely affected in some fumigations, particularly in the 18/3/96 and 2/4/96 fumigations where the temperature was 13.1 and 13.8°C respectively.

Table 5.17 The fumigations with pyrethrum-phosphine that did not satisfy the criteria.

	Number of larvae severely affected	Number fumigated
Marginal		
1/5/97	2(7.7%)	26
Poor		
18/3/96	18(23.0%)	78
2/4/96	21(30.0%)	70
10/7/96	4(20.0%)	20
18/7/96	7(12.7%)	55
4/9/96	4(14.0%)	29
16/10/96	6(27.0%)	22

The times required for these severely affected larvae to die are given in Table 5.18. Most of these larvae died within 3 to 48 h following fumigation and only one or two larvae died within 96 to 168 h following fumigation. Those severely affected larvae

that necessitated longer hours (168 h) to die were fumigated at lower temperatures (13.1 and 13.8°C).

Table 5.18 Number of days required for complete mortality of the severely affected fifth instar larvae.

Date	Phosphine concentration gm^{-3}		No. of larvae severely affected assessment after fumigation (h)						No. of days for complete mortality
			3	24	48	72	96	168	
18/3/96	1.0	1.0	18	7	4	2	2	1	10
2/4/96	1.0	0.9	21	12	5	3	2	2	12
10/7/96	0.3	0.19	4	1	1	1	1	0	4
18/7/96	0.3	0.18	7	2	2	2	1	0	4
4/9/96	0.15	0.069	1	1	1	0	0	0	2
16/10/96	0.15	0.12	6	6	2	1	0	0	3
1/5/97	0.34	0.23	2	2	1	1	0	0	3
14/5/97	0.34	0.23	6	6	2	1	0	0	3

Results of fumigating sixth instar larvae of *E. postvittana* with various concentrations of pyrethrum and phosphine are given in Table 5.19. Some fumigations did not give a complete mortality of all the exposed larvae. The reason could be that either the temperature and/or exposure time maintained was sub-lethal for the exposed larvae. In one fumigation 21.4 per cent of the larvae survived and developed into normal adults that were exposed to 0.5 h (fumigated on 24/8/95. There was no difference in egg laying capacity of the adults that emerged from the fumigated survived larvae and the control larvae.

Table 5.19 Insecticidal efficacy of pyrethrum-phosphine to sixth instar larvae of *E. postvittana* exposed to various concentrations, exposures and temperatures in a 27 m^3 chamber at Emerald and Gembrooke.

Date	Pyrethrum gm^{-3}	Phosfume® Kg	Phosphine concentration gm^{-3}		xposure time (h)	Temperature (°C)	Lightbrown apple moth *Epiphyas postvittana*			
			Initial	Final			Live	S.aff	Dead	Total
24/8/95	120	0.5	0.27	0.15	0.5	18.0	9	11	22	42
24/8/95	0	0	0	0	0	22.0	33	0	0	33
12/2/96	120	2.0	1.0	0.4	16.0	21.0(18.9-22.6)	0	0	21	21
12/2/96	0	0	0	0	0	22.0	21	0	0	21
19/2/96	160	2.02	1.0	0.6	16.0	21.0(16.3-23.1)	0	0	42	42
19/2/96	0	0	0	0	0	22.0	42	0	0	42
26/2/96	120	2.04	1.0	0.7	15.5	21.9(19.5-26.7)	0	0	24	24
26/2/96	0	0	0	0	0	22.0	24	0	0	24
18/3/96	140	2.048	1.0	1.0	15.5	13.1(10.0-14.6)	0	21	48	69
18/3/96	0	0	0	0	0	22.0	68	0	1	69
25/3/96	120	2.02	1.0	0.7	15.5	21.7(18.1-27.2)	0	0	43	43
25/3/96	0	0	0	0	0	22.0	43	0	0	43
2/4/96	160	2.88	1.0	0.9	15.0	13.8 (13.5-15.0)	0	14	39	53
2/4/96	0	0	0	0	0	22.0	52	0	1	53
29/5/96	140	2.52	1.0	0.9	18.0	18.2(15.4-19.9)	0	0	29	29
29/5/96	0	0	0	0	0	22.0	29	0	0	29
10/7/96	200	2.32	0.3	0.19	15.0	15.6(10.0-18.7)	0	12	31	43
10/7/96	0	0	0	0	0	22.0	42	0	1	43
18/7/96	120	1.12	0.3	0.18	15.25	17.4(10.1-20.3)	0	9	48	57
18/7/96	0	0	0	0	0	22.0	57	0	0	57
29/8/96	120	1.02	0.27	0.11	16.25	18.0(16.6-20.1)	0	2	34	36
29/8/96	0	0	0	0	0	22.0	33	0	3	36
4/9/96	140	0.92	0.15	0.069	16.25	18.1(15.2-20.8)	0	3	25	28
4/9/96	0	0	0	0	0	22.0	27	0	1	28
9/9/96	120	0.46	0.23	0.18	16.25	17.9(16.2-18.6)	0	0	31	31
9/9/96	0	0	0	0	0	22.0	31	0	0	31
18/9/96	120	0.7	0.22	0.07	16.0	19.2(17.8-20.6)	0	4	94	98
18/9/96	0	0	0	0	0	22.0	90	0	4	94
23/9/96	120	0.6	0.25	0.15	16.0	18.9(16.9-20.8)	0	0	45	45
23/9/96	0	0	0	0	0	22.0	45	0	0	45
7/10/96	120	0.7	0.33	0.13	15.5	18.0(13.1-18.8)	0	1	57	58
7/10/96	0	0	0	0	0	22.0	58	0	0	58
6/10/96	120	0.7	0.15	0.12	16.0	17.1(12.5-19.2)	0	4	63	67

Date	Pyrethrum gm^{-3}	Phosfume® Kg	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Lightbrown apple moth *Epiphyas postvittana*			
			Initial	Final			Live	S.aff	Dead	Total
6/10/96	0	0	0	0	0	22.0	65	0	2	67
0/10/96	120	0.72	0.15	0.097	16.25	18.4(17.1-19.0)	0	4	17	21
0/10/96	0	0	0	0	0	22.0	16	0	1	17
2/11/96	120	1.12	0.27	0.15	16.0	18.1(17.5-18.9)	0	0	31	31
2/11/96	0	0	0	0	0	22.0	30	0	1	31
3/11/96	140	1.40	0.32	0.19	16.0	18.2(16.1-20.2)	0	0	64	64
3/11/96	0	0	0	0	0	22.0	63	0	1	64
4/12/96	160	1.0	0.48	0.1	16.0	18.0(15.7-19.9)	0	1	59	60
4/12/96	0	0	0	0	0	22.0	56	0	4	60
23/1/97	160	0.7	0.44	0.32	15.75	26.5(22.1-33.4)	0	0	29	29
23/1/97	0	0	0	0	0	22.0	27	0	2	29
17/2/97	120	0.7	0.42	0.22	15.5	18.7(17.4-20.9)	0	2	24	26
17/2/97	0	0	0	0	0	22.0	25	0	1	26
1/5/97	140	0.92	0.34	0.23	16.5	17.8(13.2-18.8)	0	0	23	23
1/5/97	0	0	0	0	0	22.0	22	0	1	23
5/3/97	120	0.04	0.62	0.45	15.5	22.5 (19.2-32.4)	0	3	142	145
5/3/97	0	0	0	0	0	22.0	144	0	1	145
25/6/97	120	11	0.47	0.33	17.0	18.0(16.0-20.2)	0	4	86	110
25/6/97	0	0	0	0	0	22.0	104	0	6	110

Fumigation criteria

Temperature: 18.1°C or more

Exposure time: 15.5 h or more

Phosphine concentration: 0.15 $g.m^{-3}$ or more.

Table 5.20 indicates that nine fumigations out of eleven have given a complete mortality of all the exposed larvae. Only in two fumigations a small number of larvae were severely affected due to reasons unknown.

Table 5.20 The fumigations with pyrethrum-phosphine that satisfy the criteria.

	Number of larvae severely affected	Number fumigated
Good		
12/2/96	-	21
19/2/96	-	42
26/2/96	-	24
25/3/96	-	43
29/5/96	-	29
23/9/96	-	45
12/11/96	-	31
13/11/96	-	64
23/1/97	-	29
Marginal		
17/2/97	2(7.7%)	26
5/3/97	3(2.1%)	145

This table (Table 5.21) clearly indicates that of the 15 fumigations that did not satisfy the criteria two fumigations have given a complete mortality of all the exposed sixth instar larvae. Six were marginal and seven were poor.

Table 5.21 The fumigations with pyrethrum-phosphine that did not satisfy the criteria.

	Number of larvae severely affected	Number fumigated
Good		
9/9/96	-	31
1/5/97	-	23
Marginal		
29/8/96	2(5.5%)	36
18/9/96	4(4.1%)	98
7/10/96	1(1.7%)	58
16/10/96	4(6.0%)	67
4/12/96	1(1.6%)	60
25/6/97	4(4.0%)	100
Poor		
18/3/96	21(30.0%)	69
2/4/96	14(26.4%)	53
10/7/96	12(27.9%)	43
18/7/96	9(16.0%)	57

	Number of larvae severely affected	Number fumigated
Poor		
4/9/96	3(10.8%)	28
30/10/96	4(19.0%)	21
24/8/95	20*(48.0%)	42

* - Nine individuals survived and developed into normal pupae.

Table 5.22 indicates that the severely affected larvae fumigated with short exposure time fumigations required more than168 h to die. In most of the long exposure time fumigations all the severely affected larvae died within 72 h following fumigation and some remained severely affected for 168 h.

Table 5.22 Number of days required for the severely affected larvae for complete mortality.

Date	Phosphine concentration gm^{-3}		No. of larvae severely affected assessment after fumigation (h)						No. of days for complete mortality
	Initial	Final	3	24	48	72	96	168	
24/8/95	0.27	0.15	11	11	7	5	5	5	15
18/3/96	1.0	1.0	21	5	4	2	2	1	10
2/4/96	1.0	0.9	14	7	6	3	2	2	11
10/7/96	0.3	0.19	2	11	9	3	1	1	7
18/7/96	0.3	0.18	9	9	7	5	2	1	7
29/8/96	0.27	0.11	2	1	1	1	-	-	3
4/9/96	0.15	0.069	3	3	1	1	-	-	3
18/9/96	0.22	0.07	4	2	1	1	-	-	3
7/10/96	0.33	0.13	1	1	1	1	-	-	3
16/10/96	0.15	0.12	4	4	2	1	-	-	3
30/10/96	0.15	0.097	4	4	1	1	1	-	4
4/12/96	0.48	0.1	1	1	1	1	-	-	3
17/2/97	0.42	0.22	2	2	1	1	1	-	4
5/3/97	0.62	0.45	3	2	2	-	-	-	2
25/6/97	0.47	0.33	4	2	1	-	-	-	2

5.3.2.3 Pupae

Data in Table 5.23 show that all the exposed pupae were killed in all the fumigation trials that were exposed to different concentrations of pyrethrum, phosphine, exposure times and temperatures.

Table 5.23 Insecticidal efficacy of pyrethrum-phosphine to pupae of *E. postvittana* exposed to different concentrations of phosphine, times and temperatures in a 27 m^3 chamber at Emerald.

Date	Pyrethrum gm^{-3}	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure Time (h)	Temperature (°C)	Lightbrown apple moth *Epiphyas postvittana*		
			Initial	Final			Live	Dead	Total
24/8/95	120	0.5	0.27	0.15	0.5	18.0	0	14	14
24/8/95	0	0	0	0	0	22.0	12	2	14
12/2/96	120	2.0	1.0	0.4	16.0	21.0(18.9-22.6)	0	21	21
12/2/96	0	0	0	0	0	22.0	21	0	21
19/2/96	160	2.02	1.0	0.6	16.0	21.0(16.3-23.1)	0	25	25
19/2/96	0	0	0	0	0	22.0	24	1	25
26/2/96	120	2.04	1.0	0.7	15.5	21.9(19.5-26.7)	0	27	27
26/2/96	0	0	0	0	0	22.0	25	2	27
2/4/96	160	2.88	1.0	0.9	15.0	13.8 (13.5-15)	0	6	6
2/4/96	0	0	0	0	0	25.0	6	0	6
29/5/96	140	2.52	1.0	0.9	18.0	18.2(15.4-19.9)	0	60	60
10/7/96	200	2.32	0.3	0.19	15.0	15.6(10.0-18.7)	0	16	16
10/7/96	0	0	0	0	0	25.0	16	0	16
18/7/96	120	1.12	0.3	0.18	15.25	17.4(10.1-20.3)	0	6	6
18/7/96	0	0	0	0	0	25.0	6	0	6
20/8/96	120	1.3	0.26	0.097	16.0	18.0	0	5	5
20/8/96	0	0	0	0	0	25.0	5	0	5
29/8/96	120	1.02	0.27	0.11	16.25	18.0(16.6-20.1)	0	12	12
29/8/96	0	0	0	0	0	25.0	12	0	12
6/10/96	120	0.7	0.15	0.12	16.0	17.1(12.5-19.2)	0	12	12
6/10/96	0	0	0	0	0	25.0	12	0	12
1/5/97	140	0.92	0.34	0.23	16.5	17.8(13.2-18.8)	0	25	25
1/5/97	0	0	0	0	0	25.0	24	1	25
5/3/97	120	0.04	0.62	0.45	15.5	22.5(19.2-32.4)	0	16	16
5/3/97	0	0	0	0	0	25.0	14	2	16
25/6/97	4.4	11	0.47	0.33	17.0	18.0(16.0-20.2)	0	50	50

The results indicate that the fumigations conducted under these conditions are highly lethal to the exposed pupae. Results indicate that even 0.5 h of exposure time was highly lethal to pupae. In all the trials almost equal numbers of one to three days and four to seven days old pupae of both male and female pupae were exposed. A small number of pupae from the control experiments died either naturally or as a result of being handled.

Fumigation criteria

Temperature: 13.8°C or more

Exposure time: 0.5 h or more

Phosphine concentration: 0.097 $g.m^{-3}$ or more

It is clear from the Table 5.24 that complete mortality of all the exposed pupae was achieved in all the fumigation trials.

Table 5.24 The fumigations with pyrethrum-phosphine that satisfy the criteria.

	Number of pupae severely affected	Number fumigated
Good		
24/8/95	-	14
12/2/96	-	21
19/2/96	-	25
26/2/96	-	27
2/4/96	-	6
29/5/96	-	60
10/7/96	-	16
18/7/96	-	6
20/8/96	-	5
29/8/96	-	12
16/10/96	-	12
1/5/97	-	25
5/3/97	-	16
25/6/97	-	50

5.3.3 *The two-spotted mite, Tetranychus urticae*

5.3.3.1 *Eggs*

Data from the fumigation of eggs of *T. urticae* with pyrethrum and phosphine show that these stages are comparatively tolerant to phosphine fumigation (Table 5.25 and 5.26).

Table 5.25 Insecticidal efficacy of pyrethrum-phosphine to eggs of *T. urticae* exposed to different concentrations of phosphine, times and temperatures in a 27 m^3 chamber at Emerald.

Date	Pyrethrum gm^{-3}	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Two-spotted mite *Tetranychus urticae*		
			Initial	Final			Live	Dead	Total
17/11/95	10 s	3.080	1.4	1.3	6.0	27.0	18	7	25
17/11/95	0	0	0	0	0	22.0	25	0	25
23/11/95	10 s	3.260	1.5	1.0	15.5	20.8(19.4-21.5)	0	45	45
23/11/95	0	0	0	0	0	22.0	43	2	45
30/11/95	10 s	3.120	1.4	1.0	15.0	20.5(19.6-22.8)	0	50	50
30/11/95	0	0	0	0	0	22.0	47	3	50
12/2/96	120	2.0	1.0	0.4	16.0	21.0(18.9-22.6)	0	1944	1944
12/2/96	0	0	0	0	0	22.0	1903	41	1944
19/2/96	160	2.02	1.0	0.6	16.0	21.0(16.3-23.1)	0	486	486
19/2/96	0	0	0	0	0	22.0	471	15	486
26/2/96	120	2.04	1.0	0.7	15.5	21.9(19.5-26.7)	0	945	945
26/2/96	0	0	0	0	0	22.0	938	7	945
18/3/96	140	2.048	1.0	1.0	15.5	13.1(10.0-14.6)	5	738	743
18/3/96	0	0	0	0	0	22.0	739	4	743
25/3/96	120	2.02	1.0	0.7	16.0	21.7(18.1-27.2)	0	947	947
25/3/96	0	0	0	0	0	22.0	941	6	947
2/4/96	160	2.88	1.0	0.9	15.0	13.9(13.5-15.0)	36	1463	1499
2/4/96	0	0	0	0	0	22.0	1490	9	1499
29/5/96	140	2.52	1.0	0.9	18.0	18.2(15.4-19.9)	0	311	311
29/5/96	0	0	0	0	0	22.0	308	3	311
10/7/96	200	2.32	0.28	0.19	15.0	15.6(10.0-18.7)	21	1047	1068
10/7/96	0	0	0	0	0	22.0	1002	45	1047
18/7/96	120	1.12	0.28	0.18	15.25	17.4(10.1-20.3)	14	750	764
18/7/96	0	0	0	0	0	22.0	760	4	764
20/8/96	120	1.3	0.31	0.19	16.0	18.0	0	1050	1050

Date	Pyrethrum gm^{-3}	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Two-spotted mite *Tetranychus urticae*		
			Initial	Final			Live	Dead	Total
20/8/96	0	0	0	0	0	22.0	1042	8	1050
29/8/96	120	1.02	0.26	0.097	16.15	18.0(16.6-20.1)	0	1188	1188
29/8/96	0	0	0	0	0	22.0	1178	10	1188
4/9/96	140	0.92	0.27	0.11	16.15	18.1(15.2-20.8)	0	949	949
4/9/96	0	0	0	0	0	22.0	940	9	949
9/9/96	120	0.46	0.15	0.069	16.15	17.9(16.2-18.6)	0	839	839
9/9/96	0	0	0	0	0	22.0	828	11	839
18/9/96	120	0.7	0.23	0.18	16.0	19.2(17.8-20.6)	0	1356	1356
18/9/96	0	0	0	0	0	22.0	1342	14	1356
23/9/96	120	0.6	0.22	0.07	16.0	18.9(16.9-20.8)	0	375	375
23/9/96	0	0	0	0	0	22.0	371	4	375
7/10/96	120	0.7	0.33	0.13	15.50	18.0(13.1-18.8)	0	1257	1257
7/10/96	0	0	0	0	0	22.0	1250	7	1257
16/10/96	120	0.7	0.15	0.12	16.0	17.1(12.5-19.2)	7	1530	1537
16/10/96	0	0	0	0	0	22.0	1520	17	1537
30/10/96	120	0.72	0.15	0.097	16.25	18.1(17.1-19.0)	11	1010	1021
30/10/96	0	0	0	0	0	22.0	1018	3	1021
12/11/96	120	1.12	0.27	0.15	16.0	18.1(17.5-18.9)	0	1750	1750
12/11/96	0	0	0	0	0	22.0	1745	5	1750
13/11/96	140	1.40	0.32	0.19	16.0	18.2 16.1-20.2)	0	546	546
13/11/96	0	0	0	0	0	22.0	538	8	546
17/2/97	160	0.7	0.44	0.32	15.75	26.5(22.1-33.4)	0	120	120
17/2/97	0	0	0	0	0	22.0	116	4	120
26/2/97	120	0.7	0.42	0.22	15.5	18.7(17.4-20.9)	3	630	633
26/2/97	0	0	0	0	0	22.0	628	3	631
14/5/97	140	0.92	0.34	0.23	16.5	17.8(13.2-18.8)	30	250	250
14/5/97	0	0	0	0	0	22.0	248	2	250
8/7/97	4.4	0.16	0.48	0.08	15.0	17.5(15.6-19.8)	630	770	1400
8/7/97	0	0	0	0	0	22.0	1380	20	1400

It is clear from this table that short exposure fumigations of 6 h are not lethal to either young or mature eggs. Seventy-two per cent of the fumigated young and mature eggs survived, and developed into normal adults. The eggs that survived developed in a similar manner to the eggs in the control; this indicates that exposing them for short periods did not delay the development of eggs. On the other hand, most of the long exposure fumigations of 15 h or more killed all the exposed eggs, provided temperature

and phosphine concentration are maintained above lethal limit (described below-fumigation criteria).

Table 5.26 Insecticidal efficacy of pyrethrum-phosphine to eggs of *T. urticae* exposed to different concentrations, exposure times and temperatures in a 27 m^3 chamber at Gembrook.

Date	Pyrethrum gm^{-3}	Phosfume kg	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	RH (%)	Two-spotted mite *Tetranychus urticae*		
			Initial	Final				Live	Dead	Total
4/12/96	160	1.0	0.48	0.1	16.0	18.0(15.7-19.9)	-	29	1572	1601
4/12/96	0	0	0	0	0	22.0	45.0	1590	11	1601
5/3/97	4	0.04	0.62	0.45	15.5	22.5 (19.2-32.4)	89.8 (55.5-95.0)	0	120	120
5/3/97	0	0	0	0	0	22.0	45.0	110	10	120
25/6/97	4.4	11	0.47	0.33	17.0	18.0(16.0-20.2)	79.5(62.2-85.3)	5	745	750

Fumigation criteria

Exposure time: 15 h or more

Final phosphine concentration: 0.69 $g.m^{-3}$ or more

Temperature: 17.9°C or more

Out of 22 fumigations, 18 fumigations gave complete mortality of all the exposed eggs (Table 5.27). However, a small number of eggs survived and developed into normal larvae in four fumigations. This is probably due to either lower initial temperatures or lower final phosphine concentrations during fumigation.

Table 5.27 Number of fumigations with pyrethrum-phosphine satisfy the criteria.

	Number of eggs survived	Total number of eggs fumigated
Good		
23/11/95	-	45
30/11/95	-	50
12/2/96	-	1944
19/2/96	-	486
26/2/96	-	945
25/3/96	-	947
29/5/96	-	311
20/8/96	-	1050

	Number of eggs survived	Total number of eggs fumigated
Good		
29/8/96	-	1188
4/9/96	-	949
9/9/96	-	839
18/9/96	-	1356
23/9/96	-	375
7/10/96	-	1257
12/11/96	-	1750
13/11/96	-	546
17/2/97	-	120
5/3/97	-	120
Marginal		
4/12/96	29(1.8%)	1601
25/6/97	5(0.6%)	750
30/10/96	11(1.1%)	1021
26/2/97	3(0.5%)	633

All the eight fumigations that did not meet the fumigation criteria did not give complete mortality of all the exposed eggs (Table 5.28). The reason for the failure would be probably due to the lower initial temperature and/or lower final phosphine concentration.

Table 5.28 Fumigations with pyrethrum-phosphine that did not satisfy the criteria.

	Number of eggs survived	Number fumigated
Marginal		
18/3/96	5 (0.6%)	743
2/4/96	36 (2.4%)	1499
10/7/96	21(2.0%)	1068
18/7/96	14(1.8%)	764
16/10/96	7(0.45%)	1537
Poor		
8/7/97	630(45.0%)	1400
14/5/97	30(10.7%)	280
17/11/95	18(72.0%)	25

The time required for the fumigated surviving eggs to emerge is presented in the Table 5.29. The surviving eggs required longer times to hatch than the eggs that were not fumigated.

Table 5.29 Number of *T. urticae* eggs survived and time required to hatch.

Date	Phosphine oncentration gm⁻		Exposure time h	Number of eggs emerged (days after fumigation)									No. Hatched	Per cent Hatched
	Initial	Final		4	5	6	7	8	9	10	11	12		
17/11/95	1.4	1.3	6.0	16*	2	0	0	0	0	0	0	0	18	72.0
18/3/96	1.0	1.0	15.5	0	1	0	3	1	0	0	0	0	5	0.67
2/4/96	1.0	0.9	15.0	0	4	12	16	3	1	0	0	0	36	2.4
16/10/96	0.15	0.12	16.0	0	3	3	2	1	1	0	0	0	10	0.7
30/10/96	0.15	0.097	16.25	1	0	5	4	1	0	0	0	0	11	1.1
26/2/97	0.42	0.22	15.5	0	0	0	3	0	0	0	0	0	3	0.5
14/5/97	0.34	0.23	16.5	2	3	0	10	5	0	3	6	1	30	12
8/7/97	0.16	0.48	15.0	0	100	180	250	20	18	34	21	7	630	45

* - 40 per cent of these eggs hatched 3 days following fumigation.

On the other hand, all the surviving eggs that were exposed to short exposure of 6 h hatched four to five days following fumigation. Eggs that were exposed to longer exposure time of 15.5 to 16.5 h began to hatch four days after fumigation and peak numbers hatched five to eight days following fumigation and emergence continued for up to 12 days. The proportion of eggs that survived increased as the exposure time and/or concentration and/or temperature were maintained below lethal levels.

Incubation studies show that as the egg matures, tolerance to phosphine fumigations also reduces significantly and it ultimately becomes nil tolerance (Table 5.30). The data in this table clearly illustrate that of all the ages of eggs that survived one-day old represents 60 per cent followed by two-day old eggs (20 per cent survival). However, only a few of the four-day old eggs survived. The eggs of five-day old and above were highly susceptible to the exposed phosphine fumigation conditions and complete mortality was achieved in all the fumigations. The surviving eggs completed their life-cycles in a manner similar to unfumigated eggs. On the other hand, a uniform

emergence was observed in the control eggs and most of these eggs emerged two to five days following the set up of the experiment.

Table 5.30 Percentage of different age eggs survived that were fumigated with different concentrations of pyrethrum, phosphine, exposure times and temperatures.

Age of eggs (days)	Percentage survived, %
1	60
2	20
3	15
4	5
5	0
6 and above	0

5.3.3.2 Larvae

A short exposure time (6 h) with high concentrations of phosphine (1.4 $g.m^{-3}$ or more) was found to be highly toxic to the larvae exposed, and all the exposed individuals were killed (Table 5.31). However, a small number of larvae were severely affected with a phosphine concentration of 1 $g.m^{-3}$ at the same exposure time. All except one larva were dead within 48 h following fumigation, and the single severely affected larva took between 60 and 72 h to die. On the other hand, all the larvae that were exposed to 15 h or more with a phosphine concentration of 0.15 to 1 $g.m^{-3}$ at 13.1 to 26.5°C died when assessed. Generally, a phosphine concentration of 0.15 $g.m^{-3}$, and an exposure time of 16 h killed all the exposed larvae. The data from this table indicate that larvae of *T. urticae* are more susceptible to long exposure fumigations than short exposure fumigation.

Table 5.31 Mortality data for the larvae of *T. urticae* fumigated with various concentrations of pyrethrum, phosphine, exposure time and temperatures in the 27 m^3 chambers at Emerald and Gembrook and the 900 L chamber at Frankston.

Date	Pestigas® (g)	Phosfume ® kg	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Two-spotted mite *Tetranychus urticae*		
			Initial	Final			Live	Dead	Total
19/10/95	10sec	10	3.34	-	6.0	20.5(17.0-22.9)	0	36	36
19/10/95	0	0	0	0	0	22.0	34	2	36
26/10/95	120	1.96	1.0	0.9	6.0	20.3(19.5-21.4)	0	27	27
26/10/95	0	0	0	0	0	22.0	24	3	27
17/11/95	10 s	3.080	1.4	1.3	6.0	27.0	0	45	45
17/11/95	0	0	0	0	0	22.0	40	4	45
23/11/95	10 s	3.260	1.5	1.0	15.5	20.8(19.4-21.5)	0	50	50
23/11/95	0	0	0	0	0	22.0	48	2	50
30/11/95	10 s	3.120	1.4	1.0	15.0	20.5(19.6-22.8)	0	900	900
30/11/95	0	0	0	0	0	22.0	888	12	900
12/2/96	120	2.0	1.0	0.4	16.0	21.0(18.9-22.6)	0	507	507
12/2/96	0	0	0	0	0	22.0	506	6	507
19/2/96	160	2.02	1.0	0.6	16.0	21.0(16.3-23.1)	0	238	238
19/2/96	0	0	0	0	0	22.0	229	9	238
26/2/96	120	2.04	1.0	0.7	15.5	21.9(19.5-26.7)	0	219	219
26/2/96	0	0	0	0	0	22.0	214	5	219
18/3/96	140	2.048	1.0	1.0	15.5	13.1(10.0-14.6)	0	426	426
18/3/96	0	0	0	0	0	22.0	418	8	426
25/3/96	120	2.02	1.0	0.7	16.0	21.7(18.1-27.2)	0	272	272
25/3/96	0	0	0	0	0	22.0	260	12	272
2/4/96	160	2.88	1.0	0.9	15.0	13.8(13.5-15.0)	0	438	438
2/4/96	0	0	0	0	0	22.0	431	7	438
29/5/96	140	2.52	1.0	0.9	18.0	18.2(15.4-19.9)	0	184	184
29/5/97	0	0	0	0	0	22.0	180	4	184
10/7/96	200	2.32	0.28	0.19	15.0	15.6(10.0-18.7)	0	276	276
10/7/96	0	0	0	0	0	22.0	269	7	276
18/7/96	120	1.12	0.28	0.18	15.25	17.4(10.1-20.3)	0	836	836
18/7/96	0	0	0	0	0	22.0	822	14	836
20/8/96	120	1.3	0.31	0.19	16.0	18.0	0	537	537
20/8/96	0	0	0	0	0	22.0	530	7	537
29/8/96	120	1.02	0.26	0.097	16.15	18.0(16.6-20.1)	0	436	436
29/8/96	0	0	0	0	0	22.0	424	12	436

Date	Pestigas® (g)	Phosfume® kg	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Two-spotted mite *Tetranychus urticae*		
			Initial	Final			Live	Dead	Total
4/9/96	140	0.92	0.27	0.11	16.15	18.1(15.2-20.8)	0	363	363
4/9/96	0	0	0	0	0	22.0	358	5	363
9/9/96	120	0.46	0.15	0.069	16.15	17.9(16.2-18.6)	0	618	618
9/9/96	0	0	0	0	0	22.0	607	11	618
18/9/96	120	0.7	0.23	0.18	16.0	19.2(17.8-20.6)	0	475	475
18/9/96	0	0	0	0	0	22.0	470	5	475
23/9/96	120	0.6	0.22	0.07	16.0	18.9(16.9-20.8)	0	179	179
23/9/96	0	0	0	0	0	22.0	174	5	179
7/10/96	120	0.7	0.33	0.13	15.50	18.0(13.1-18.8)	0	844	844
7/10/96	0	0	0	0	0	22.0	841	3	844
16/10/96	120	0.7	0.15	0.12	16.0	17.1(12.5-19.2)	0	959	959
16/10/96	0	0	0	0	0	22.0	954	5	959
30/10/96	120	0.72	0.15	0.097	16.25	18.1(17.1-19.0)	0	479	479
30/10/96	0	0	0	0	0	22.0	470	9	479
12/11/96	120	1.12	0.27	0.15	16.0	18.1(17.5-18.9)	0	719	719
12/11/96	0	0	0	0	0	22.0	702	17	719
13/11/96	140	1.40	0.32	0.19	16.0	18.2(16.1-20.2)	0	246	246
13/11/96	0	0	0	0	0	22.0	240	6	246
4/12/96	160	1.0	0.48	0.1	16.0	18.0(15.7-19.9)	0	640	640
4/12/96	0	0	0	0	0	22.0	636	4	640
17/2/97	160	0.7	0.44	0.32	15.75	26.5(22.1-33.4)	0	354	354
17/2/97	0	0	0	0	0	22.0	349	5	354
26/2/97	120	0.7	0.42	0.22	15.5	18.7(17.4-20.9)	0	270	270
26/2/97	0	0	0	0	0	22.0	268	2	270
14/5/97	140	0.92	0.34	0.23	16.5	17.8(13.2-18.8)	0	250	250
14/5/97	0	0	0	0	0	22.0	245	5	250
8/7/97	120	0.16	0.48	0.08	15.0	17.5(15.6-19.8)	0	72	72
8/7/97	0	0	0	0	0	22.0	69	3	72
5/3/97	4	0.04	0.62	0.45	15.5	22.5 (19.2-32.4)	0	212	212
5/3/97	0	0	0	0	0	22.0	210	2	212
25/6/97	4.4	11	0.47	0.33	17.0	18.0(16.0-20.2)	0	120	120
25/6/97	0	0	0	0	0	22.0	118	2	120

5.3.3.3 Diapause larvae

All the diapause larvae died during long exposure fumigations with the two concentrations of pyrethrum, phosphine, exposure times and temperatures investigated (Table 5.32). These results suggest that diapause larvae are susceptible to the below mentioned pyrethrum-phosphine combined treatment. On the other hand, there were no mortalities from the control batch.

Table 5.32 Insecticidal efficacy of pyrethrum-phosphine to diapause larvae of *T. urticae* exposed to two concentrations, exposure times and temperatures in a 27 m^3 chamber at Gembrook.

Date	Pestigas® gm^{-3}	Phosfume® kg	Phosphine oncentration gm^{-3}		Exposure time h	Temperature °C	RH (%)	Two-spotted mite *Tetranychus urticae*		
			Initial	Final				Live	Dead	Total
5/3/97	4	0.04	0.62	0.45	15.5	22.5(19.2-32.4)	89.8(55.5-95.0)	0	72	72
5/3/97	0	0	0	0	0	22.0	45 .0	72	0	72
5/6/97	4.4	11	0.47	0.33	17.0	18.0(16.0-20.2)	79.5(62.2-85.3)	0	40	40
6/5/97	0	0	0	0	0	22.0	45.0	43	0	43

5.3.3.4 Adults

The responses of adult *T. urticae* to being fumigated with pyrethrum-phosphine are presented in Table 5.33. A small number of individuals were severely affected in some fumigations. However, all the severely affected individuals were dead 24 to 72 h after the end of the fumigation. The severely affected adults that were exposed to short exposure fumigations generally took longer (48 to 72 h) to die, and those adults that were fumigated for long periods of time died earlier (24 h). A small number of control adults had died either due to handling or as a result of natural mortality.

Table 5.33 Mortality data for the adults of *T. urticae* fumigated with various concentrations of pyrethrum, phosphine, exposure times and temperatures in a 27 m^3 chamber at Emerald, Gembrooke and 900 L chamber at Frankston.

Date	Pestigas® (g)	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Two-spotted mite *Tetranychus urticae*			
			Initial	Final			Live	S.aff	Dead	Total
19/10/95	10sec	10	3.34	-	6.0	20.5(17.0-22.9)	0	0	63	63
19/10/95	0	0	0	0	0	22.0	59	0	4	63
26/10/95	120	1.960	1.0	0.9	6.0	20.3(19.5-21.4)	0	1	54	55
26/10/95	0	0	0	0	0	22.0	52	0	3	55
17/11/95	10 s	3.080	1.4	1.3	6.0	27.0	0	0	35	35
17/11/95	0	0	0	0	0	22.0	32	0	3	35
23/11/95	10 s	3.260	1.5	1.0	15.5	20.8(19.4-21.5)	0	0	400	400
23/11/95	0	0	0	0	0	22.0	396	0	4	400
30/11/95	10 s	3.120	1.4	1.0	15.0	20.5(19.6-22.8)	0	0	599	599
30/11/95	0	0	0	0	0	22.0	585	0	14	599
12/2/96	120	2.0	1.0	0.4	16.0	21.0(18.9-22.6)	0	0	720	720
12/2/96	0	0	0	0	0	22.0	713	0	7	720
19/2/96	160	2.02	1.0	0.6	16.0	21.0(16.3-23.1)	0	0	402	402
19/2/96	0	0	0	0	0	22.0	400	0	2	402
26/2/96	120	2.04	1.0	0.7	15.5	21.9(19.5-26.7)	0	0	337	337
26/2/96	0	0	0	0	0	22.0	331	0	6	337
18/3/96	140	2.048	1.0	1.0	15.5	13.1(10.0-14.6)	0	0	572	572
18/3/96	0	0	0	0	0	22.0	567	0	5	572
25/3/96	120	2.02	1.0	0.7	16.0	21.7(18.1-27.2)	0	0	388	388
25/3/96	0	0	0	0	0	22.0	381	0	7	388
2/4/96	160	2.88	1.0	0.9	15.0	13.8(13.5-15.0)	0	0	623	623
2/4/96	0	0	0	0	0	22.0	611	0	12	623
29/5/96	140	2.52	1.0	0.9	18.0	18.2(15.4-19.9)	0	0	286	286
29/5/96	0	0	0	0	0	22.0	281	0	5	286
10/7/96	200	2.32	0.28	0.19	15.0	15.6(10.0-18.7)	0	0	993	993
10/7/96	0	0	0	0	0	22.0	979	0	10	989
18/7/96	120	1.12	0.28	0.18	15.25	17.4(10.1-20.3)	0	0	455	455
18/7/96	0	0	0	0	0	22.0	450	0	5	455
20/8/96	120	1.3	0.31	0.19	16.0	18.0	0	0	584	584
20/8/96	0	0	0	0	0	22.0	584	0	0	584
29/8/96	120	1.02	0.26	0.097	16.15	18.0(16.6-20.1)	0	7	546	553
29/8/96	0	0	0	0	0	22.0	546	0	0	546
4/9/96	140	0.92	0.27	0.11	16.15	18.1(15.2-20.8)	0	0	718	718

Date	Pestigas® (g)	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Two-spotted mite *Tetranychus urticae*			
			Initial	Final			Live	S.aff	Dead	Total
4/9/96	0	0	0	0	0	22.0	718	0	0	718
9/9/96	120	0.46	0.15	0.069	16.15	17.9(16.2-18.6)	0	4	732	736
9/9/96	0	0	0	0	0	22.0	734	0	2	736
18/9/96	120	0.7	0.23	0.18	16.0	19.2(17.8-20.6)	0	0	659	659
18/9/96	0	0	0	0	0	22.0	662	0	1	663
23/9/96	120	0.6	0.22	0.07	16.0	18.9(16.9-20.8)	0	3	219	222
23/9/96	0	0	0	0	0	22.0	219	0	0	219
7/10/96	120	0.7	0.33	0.13	15.50	18.0(13.1-18.8)	0	0	723	723
7/10/96	0	0	0	0	0	22.0	723	0	0	723
16/10/96	120	0.7	0.15	0.12	16.0	17.1(12.5-19.2)	0	0	1135	1135
16/10/96	0	0	0	0	0	22.0	1124	0	11	1135
30/10/96	120	0.72	0.15	0.097	16.25	18.1(17.1-19.0)	0	6	675	681
30/10/96	0	0	0	0	0	22.0	681	0	0	681
12/11/96	120	1.12	0.27	0.15	16.0	18.1(17.5-18.9)	0	0	1226	1226
12/11/96	0	0	0	0	0	22.0	1220	0	13	1233
13/11/96	140	1.40	0.32	0.19	16.0	18.2(16.1-20.2)	0	0	325	325
13/11/96	0	0	0	0	0	22.0	321	0	4	325
14/12/96	160	1.0	0.48	0.1	16.0	18.0(15.7-19.9)	0	0	697	697
14/12/96	0	0	0	0	0	22.0	695	0	2	697
17/2/97	160	0.7	0.44	0.32	15.75	26.5(22.1-33.4)	0	0	474	474
17/12/96	0	0	0	0	0	22.0	474	0	0	474
26/2/97	120	0.7	0.42	0.22	15.5	18.7(17.4-20.9)	0	3	545	548
26/2/97	0	0	0	0	0	22.0	580	0	4	584
14/5/97	140	0.92	0.34	0.23	16.5	17.8(13.2-18.8)	0	0	180	180
14/5/97	0	0	0	0	0	22.0	180	0	0	180
8/7/97	120	0.16	0.48	0.08	15.0	17.5(15.6-19.8)	0	2	94	96
8/7/97	0	0	0	0	0	22.0	94	0	0	94
5/3/97	4	0.04	0.62	0.45	15.5	22.5 (19.2-32.4)	0	0	117	117
5/3/97	0	0	0	0	0	22.0	117	0	0	117
25/6/97	4.4	11	0.47	0.33	17.0	18.0(16.0-20.2)	0	0	180	180
25/6/97	0	0	0	0	0	22.0	181	0	1	182

Fumigation criteria

Temperature: 13.1°C or more.

Exposure time: 15 h or more.

Final phosphine concentration: 0.1 $g.m^{-3}$ or more

All the 23 fumigations that satisfy the criteria gave a complete mortality of all the exposed adults (Table 5.34).

Table 5.34 The fumigations with pyrethrum-phosphine that satisfy the criteria.

	Number of adults severely affected	Number fumigated
Good		
23/11/95	-	400
30/11/95	-	599
12/2/96	-	720
19/2/96	-	402
26/2/96	-	337
18/3/96	-	572
25/3/96	-	388
2/4/96	-	623
29/5/96	-	286
10/7/96	-	993
18/7/96	-	455
20/8/96	-	584
4/9/96	-	718
18/9/96	-	659
7/10/96	-	723
16/10/96	-	1135
12/11/96	-	1226
13/11/96	-	325
14/12/96	-	697
17/2/97	-	474
14/5/97	-	180
5/3/97	-	117
25/6/97	-	180

All the six fumigations that did not satisfy the criteria did not give a complete mortality of all the exposed adults (Table 5.35). In these trials, a small number of individuals were severely affected.

Table 5.35 The fumigations with pyrethrum-phosphine that did not satisfy the criteria.

	Number of larvae severely affected	Number fumigated
Marginal		
29/8/96	7(1.2%)	553
9/9/96	4(0.54%)	736
23/9/96	3(1.35%)	222
30/10/96	6(0.9%)	681
26/2/97	3(0.5%)	548
8/7/97	2(2.1%)	96

5.3.4 The green peach aphid, Myzus persicae

5.3.4.1 Larvae and adults

Both the larvae and adults of *M. persicae* (reared in capsicum seedlings) were highly susceptible to the combined application of pyrethrum-phosphine at both short and long exposure periods. In these fumigations 100 per cent mortality was achieved (Table 5.36). Fumigation trials were conducted with phosphine concentrations of 0.41 to 3.34 $g.m^{-3}$, exposure times of 4.5 to 15.5 h and temperatures of 16.5 to 20.5°C.

Table 5.36 Insecticidal efficacy of pyrethrum-phosphine to larvae and adults of *M. persicae* exposed to various concentrations, exposure times and temperatures in a 27 m^3 chamber.

Date	Pestigas® (g)	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Green peach aphid *Myzus persicae*		
			Initial	Final			Live	Dead	Total
7/9/95	10sec	7min	0.41	0.29	5.2	16.5	0	2,400	2,400
7/9/95	0	0	0	0	0	19.0	2,200	200	2,400
29/9/95	10sec	5min	0.3	0.3	4.5	19.0	0	2,400	2,400
29/9/95	0	0	0	0	0	19.0	2250	150	2,400
5/10/95	10sec	5.4	0.62	0.55	6.0	18.0	0	8,000	8,000
5/10/95	0	0	0	0	0	19.0	7,500	500	8,000
19/10/95	10sec	10	3.34	-	6.0	20.5(17.0-22.9)	0	7,500	7,500
19/10/95	0	0	0	0	0	19.0	7,400	100	7,500
10/11/95	10sec	3.16	0.9	0.9	6.2	18.0	0	8,000	8,000
10/11/95	0	0	0	0	0	19.0	7,750	250	8,000
17/11/95	10sec	3.08	1.4	1.3	6.0	27.0	0	6,400	6,400
17/11/95	0	0	0	0	0	19.0	6,300	100	6,400
23/11/95	10sec	3.26	1.5	1.0	15.5	22.0	0	468*	468
23/11/95	0	0	0	0	0	19.0	461	7	468
30/11/95	10sec	3.12	1.4	1.0	15.0	20.5(19.6-22.8)	0	929*	929
30/11/95	0	0	0	0	0	19.0	927	2	929

* - including 75 and 120 alate aphids.

5.3.5 *The cabbage aphid, Brevicoryne brassicae*

5.3.5.1 *Larvae*

The results of the pyrethrum-phosphine fumigated larvae of the cabbage aphid are given in Table 5.37.

Table 5.37 Insecticidal efficacy of pyrethrum-phosphine to larvae of *B. brassicae*, exposed to various concentrations, exposure times and temperatures in a 27 m^3 chamber at Emerald.

Date	Pestigas® (g)	Phosfume® (kg)	Phosphine Concentration gm^{-3}		Exposure Time (h)	Temperature (°C)	Cabbage aphid *Brevicoryne brassicae*			
			Initial	Final			Live	S.aff	Dead	Total
14/5/97	140	0.92	0.34	0.23	16.5	17.8(13.2-18.8)	0	24	45	69
14/5/97	0	0	0	0	0	19.0	65	0	4	69
5/3/97	4	0.04	0.62	0.45	15.5	22.5 (19.2-32.4)	0	42	39	81
5/3/97	0	0	0	0	0	19.0	78	0	3	81
25/6/97	4.4	11	0.47	0.33	17.0	18.0(16.0-20.2)	0	5	41	46
25/6/97	0	0	0	0	0	19.0	41	0	5	46

In all the trials a number of larvae was severely affected and the proportion of larvae severely affected reduced as the exposure time or temperature increased. However, all these severely affected individuals had died within 24 to 48 h after fumigation. Of all the fumigations, comparatively higher mortality of the larvae was achieved with a pyrethrum concentration of 4.4 $g.m^{-3}$, phosphine concentration of 0.47 $g.m^{-3}$ (final concentration of 0.33 $g.m^{-3}$) and an exposure of 17 h at 18.0°C (range of 16.0°C to 20.2°C).

5.3.5.2 *Adults*

The responses of adult cabbage aphids to different concentrations of pyrethrum and phosphine at various exposure times and temperatures are similar to those of larvae (Table 5.38). Higher mortalities were obtained in the fumigations that were exposed to 16.5 h or more. More than 65 per cent of the exposed adults were severely affected

after fumigations lasting 15.5 h. This suggests that exposure time is more important than temperature or concentration of phosphine for a complete mortality of the adults of *B. brassicae*. However, there was no difference in mortality of all the severely affected individuals, and all the adults were dead 24 to 48 h following fumigation. Comparatively higher mortality of exposed adults were obtained with a pyrethrum concentration of 4.4gm^{-3} and phosphine concentration of 0.47 g.m^{-3} (final concentration of 0.33 g.m^{-3}) exposed to 17 h at 18°C (range of 16 to 20.2°C).

Table 5.38 Insecticidal efficacy of pyrethrum-phosphine to adults of *B. brassicae*, exposed to various concentrations, exposure times and temperature in a 27 m^3 chamber at Emerald.

Date	yrethru gm^{-3}	Phosfume® (kg)	Phosphine Concentration gm^{-3}		Exposure Time (h)	Temperature (°C)	Cabbage aphid *Brevicoryne brassicae*			
			Initial	Final			Live	S. aff	Dead	Total
14/5/97	4.4	0.92	0.34	0.23	16.5	17.8(13.2-18.8)	0	14	40	54
14/5/97	0	0	0	0	0	19.0	49	0	5	54
5/3/97	4.0	0.04	0.62	0.45	15.5	22.5 (19.2-32.4)	0	216	117	333
5/3/97	0	0	0	0	0	19.0	327	0	6	333
25/6/97	4.4	11	0.47	0.33	17.0	18.0(16.0-20.2)	0	3	25	28
25/6/97	0	0	0	0	0	19.0	25	0	3	28

5.3.6 The ornate aphid, Myzus ornatus

5.3.6.1 Larvae

Results of the pyrethrum-phosphine fumigated larvae of *M. ornatus* are given in Table 5.39. In all the fumigations (long exposure) about 50 per cent or less of the population was severely affected. All these severely affected had died 48 h following fumigation. The majority of the severely affected individuals died within 24 h following fumigation and only five or six individuals were still severely affected for 48 h.

Table 5.39 Insecticidal efficacy of pyrethrum-phosphine to larvae of *M. ornatus* aphid, exposed to various concentrations, exposure times and temperature in a 27 m^3 chamber at Emerald.

Date	Pyrethrum gm^{-3}	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Ornate aphid *Myzus ornatus*			
			Initial	Final			Live	S.aff	Dead	Total
4/9/96	140	0.92	0.27	0.12	16.25	18.1(15.2-20.8)	0	79	121	200
4/9/96	0	0	0	0	0	19.0	199	0	1	200
9/9/96	120	0.46	0.23	0.18	16.15	17.9(16.2-18.6)	0	41	99	140
9/9/96	0	0	0	0	0	19.0	138	0	2	140
7/10/96	120	0.7	0.33	0.13	15.5	18.0(13.1-18.8)	0	36	45	81
7/10/96	0	0	0	0	0	19.0	81	0	0	81
6/10/96	120	0.7	0.15	0.12	16.0	17.1(12.5-19.2)	0	21	40	61
6/10/96	0	0	0	0	0	19.0	61	0	0	61
0/10/96	120	0.72	0.15	0.097	16.25	18.1(17.1-19.0)	0	12	18	30
0/10/96	0	0	0	0	0	19.0	30	0	0	30
2/11/96	120	1.12	0.27	0.15	16.0	18.1(17.5-18.9)	0	9	12	21
2/11/96	0	0	0	0	0	19.0	21	0	0	21
1/11/96	120	1.94	0.07	0.04	4.5	13.3(10.7-15.3)	1	2	6	9
1/11/96	0	0	0	0	0	19.0	9	0	0	9
4/12/96	160	1.0	0.48	0.1	16.0	18.0(15.7-19.9)	0	4	12	16
4/12/96	0	0	0	0	0	19.0	16	0	0	16
14/5/97	140	0.92	0.34	0.23	16.5	17.8(13.2-18.8)	0	0	100	100
14/5/97	0	0	0	0	0	19.0	98	0	2	100

5.3.6.2 Adults

Results obtained on adults of *M. ornatus* subjected to fumigations with a mixture of pyrethrum-phosphine indicate that these stages also responded in a way similar to larval stages (Table 5.40). Large numbers of adults were severely affected at the end of fumigation; however, the majority of these were dead within 24 h following fumigations. Only one to seven individuals were still severely affected after another 48 h.

Table 5.40 Insecticidal efficacy of pyrethrum-phosphine to adults of *M. ornatus*, exposed to various concentrations, exposure times and temperature in a 27 m^3 chamber at Emerald.

Date	Pyrethru gm^{-3}	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Ornate aphid *Myzus ornatus*			
			Initial	Final			Live	S.aff	Dead	Total
11/11/96	120	1.94	0.07	0.04	4.5	13.3(10.7-15.3)	0	12	7	19
11/11/96	0	0	0	0	0	18.0	19	0	0	19
4/12/96	160	1.0	0.48	0.1	16.0	18.0(15.7-19.9)	0	3	43	46
4/12/96	0	0	0	0	0	18.0	46	0	0	46
4/9/96	140	0.92	0.27	0.12	16.25	18.1(15.2-20.8)	0	215	348	563
4/9/97	0	0	0	0	0	19.0	549	0	14	563
9/9/96	120	0.46	0.23	0.18	16.15	17.9(16.2-18.6)	0	96	120	216
9/9/96	0	0	0	0	0	19.0	204	0	12	216
7/10/96	120	0.7	0.33	0.13	15.5	18.0(13.1-18.8)	0	154	158	312
7/10/97	0	0	0	0	0	19.0	309	0	3	312
16/10/96	120	0.7	0.15	0.12	16.0	17.1(12.5-19.2)	0	170	120	290
16/10/97	0	0	0	0	0	19.0	284	0	6	290
30/10/96	120	0.72	0.15	0.097	16.25	18.1(17.1-19.0)	0	15	24	39
30/10/96	0	0	0	0	0	19.0	35	0	4	39
12/11/96	120	1.12	0.27	0.15	16.0	18.1(17.5-18.9)	0	2	29	31
12/11/96	0	0	0	0	0	19.0	28	0	3	31
13/11/96	140	1.40	0.32	0.19	16.0	18.2(16.1-20.2)	0	9	25	34
13/11/96	0	0	0	0	0	19.0	34	0	0	34
14/5/97	140	0.92	0.34	0.23	16.5	17.8(13.2-18.8)	0	12	31	43
14/5/97	0	0	0	0	0	19.0	39	0	4	43

5.3.7 The Meat ant, Iridomyrmex purpureus

5.3.7.1 Adults

The data in Table 5.41 indicate that all the exposed adults of *I. purpureus* were killed in all the fumigations. Fumigations were conducted with phosphine concentrations of 0.15 to 0.44 $g.m^{-3}$, exposure times of 15.75 to 16.25 h and temperatures of 17.1 to 26.5°C. A small number of individuals from the control experiments was dead, presumably due to

handling. The results indicate that adults of *I. purpureus* are highly susceptible to the below mentioned pyrethrum and phosphine fumigations.

Table 5.41 Insecticidal efficacy of pyrethrum-phosphine to adults of *I. purpureus* exposed to various concentrations, exposure times and temperature in a 27 m^3 chamber at Emerald.

	Pyrethrum gm^{-3}	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Meat Ants *Iridomyrmex purpureus*		
			Initial	Final			Live	Dead	Total
20/8/96	120	1.3	0.26	0.097	16.0	18.0	0	73	73
20/8/96	0	0	0	0	0	19.0	72	1	73
29/8/96	120	1.02	0.27	0.11	16.25	18.0(16.6-20.1)	0	57	57
29/8/96	0	0	0	0	0	19.0	57	0	57
4/9/96	140	0.92	0.15	0.069	16.25	18.1(15.2-20.8)	0	150	150
4/9/96	0	0	0	0	0	19.0	148	2	150
9/9/96	120	0.46	0.23	0.18	16.25	17.9(16.2-18.6)	0	180	180
9/9/96	0	0	0	0	0	19.0	178	2	180
18/9/96	120	0.7	0.22	0.07	16.0	19.2(17.8-20.6)	0	175	175
18/9/96	0	0	0	0	0	19.0	174	1	175
16/10/96	120	0.7	0.15	0.12	16.0	17.1(12.5-19.2)	0	125	125
16/10/96	0	0	0	0	0	19.0	125	0	125
30/10/96	120	0.72	0.15	0.097	16.25	18.1(17.1-19.0)	0	130	130
30/10/96	0	0	0	0	0	19.0	128	2	130
12/11/96	120	1.12	0.27	0.15	16.0	18.1(17.5-18.9)	0	235	235
12/11/96	0	0	0	0	0	19.0	230	5	235
13/11/96	140	1.40	0.32	0.19	16.0	18.2(16.1-20.2)	0	121	121
13/11/96	0	0	0	0	0	19.0	120	0	121
17/2/97	160	0.7	0.44	0.32	15.75	26.5(22.1-33.4)	0	45	45
17/2/97	0	0	0	0	0	19.0	45	0	45
14/5/97	140	0.92	0.34	0.23	16.5	17.8(13.2-18.8)	0	24	24

5.3.8 *The sugar ant, Camponotus consobrinus*

5.3.8.1 *Adults*

Complete mortality of all the exposed adults of *C. consobrinus* was obtained in all the fumigation experiments (Table 5.42). Fumigations were conducted with phosphine concentrations of 0.3 to 1.0 $g.m^{-3}$, exposure times of 15 to 18 h and temperatures of 13.1 to 21.7°C.

Table 5.42 Insecticidal efficacy of pyrethrum-phosphine to adults of *C. consobrinus,* exposed to various concentrations, exposure times and temperature in a 27 m^3 chamber at Emerald.

Date	Pyrethru (g)	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Common sugar ants *Camponotus consobrinus*		
			Initial	Final			Live	Dead	Total
/10/96	120	0.7	0.33	0.13	15.5	18.0(13.1-18.8)	0	92	92
/10/96	0	0	0	0	0	22.0	90	2	92
8/3/96	140	2.048	1.0	1.0	15.5	13.1(10.0-14.6)	0	78	78
8/3/96	0	0	0	0	0	22.0	76	2	78
5/3/96	120	2.02	1.0	0.7	15.5	21.7(18.1-27.2)	0	15	15
5/3/96	0	0	0	0	0	22.0	15	0	15
2/4/96	160	2.88	1.0	0.9	15.0	13.8 (13.5-15)	0	67	67
2/4/96	0	0	0	0	0	22.0	64	3	67
9/5/96	140	2.52	1.0	0.9	18.0	18.2(15.4-19.9)	0	28	28
9/5/96	0	0	0	0	0	22.0	28	0	28
8/7/96	120	1.12	0.3	0.18	15.25	17.4(10.1-20.3)	0	67	67
8/7/96	0	0	0	0	0	22.0	67	0	67

5.3.9 *The tubular black thrips, Haplothrips victoriensis*

5.3.9.1 *Adults*

Complete mortality of the exposed adults of *H. victoriensis* was achieved with all the concentrations, temperatures and exposure time investigated (Table 5.43). The results suggest that a pyrethrum concentration of 4.0 to 4.2 $g.m^{-3}$ and a phosphine concentration

of 1 $g.m^{-3}$ with an exposure time of 15.5 h at two temperatures (13.1 and 21.9°C) are highly lethal to all the exposed adults.

Table 5.43 Insecticidal efficacy of pyrethrum-phosphine to the adults of *H. victoriensis*, exposed to various concentrations, exposure times and temperature in a 27 m^3 chamber at Emerald.

Date	Pyrethrum gm^{-3}	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Tabular black thrips *H. victoriensis*		
			Initial	Final			Live	Dead	Total
26/2/96	120	2.04	1.0	0.7	15.5	21.9(19.5-26.7)	0	47	47
26/2/96	0	0	0	0	0	22.0	45	2	47
18/3/96	140	2.048	1.0	1.0	15.5	13.1(10.0-14.6)	0	92	92
18/3/96	0	0	0	0	0	22.0	91	1	92

5.3.10 The predatory mite, Phytoseiulus persimilis

5.3.10.1 Larvae and adults

Results of the pyrethrum-phosphine fumigated larvae and adults of *P. persimilis* are given in Table 5.44. The data from this table clearly illustrate that complete mortality of all the exposed individuals is possible with phosphine concentrations of 0.34 to 0.42gm^{-3}, exposure times of 15.5 to 16.5 h at temperatures of 17.8 to 18.7°C.

Table 5.44 Insecticidal efficacy of pyrethrum-phosphine to larvae of *P. persimilis*, exposed to various concentrations, exposure times and temperature in a 27 m^3 chamber at Emerald.

Date	Pyrethrum g	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Predatory mite *Phytoseiulus persimilis*		
			Initial	Final			Live	Dead	Total
26/2/97	120	0.7	0.42	0.22	15.5	18.7(17.4-20.9)	0	49	49
26/2/97	0	0	0	0	0	22.0	47	2	49
14/5/97	140	0.92	0.34	0.23	16.5	17.8(13.2-18.8)	0	30	30
14/5/97	0	0	0	0	0	22.0	30	0	30

5.3.11 Other species - the rose aphid, *Macrosiphum rosae (Linnaeus)*, the common centipede, *Scolopendra morsitans, Linnaeus*, the European earwig, *Forficula auricularia*, pupae of Geometrid, Shield bug *Poecilotoma perconfusca* and the jumping spider.

Tables 5.45 to 5.53 clearly illustrates complete mortality of all the exposed individuals have been achieved in all the fumigation trials. It can be assumed that all these insect species and their exposed stages were highly susceptible to the exposed pyrethrum and phosphine fumigations.

Table 5.45 Insecticidal efficacy of pyrethrum-phosphine to the adults of *M. rosae*, exposed to various concentrations, exposure times and temperature in a 27 m^3 chamber at Emerald.

Date	yrethru (g)	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Rose aphid *Macrosiphum rosae*		
			Initial	Final			Live	Dead	Total
2/11/95	120	0.54	-	-	0.5	23.3 (22.8-23.8)	0	49	49
2/11/95	0	0	0	0	0	19.0	45	4	49
5/10/95	120	5.42	0.62	0.55	6.0	18.0	0	64	64
5/10/95	0	0	0	0	0	19.0	63	1	64

Table 5.46 Insecticidal efficacy of pyrethrum-phosphine to the adults of *F. auricularia*, exposed to various concentrations, exposure times and temperature in a 27 m^3 chamber at Emerald.

Date	yrethru (g)	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure Time (h)	Temperature (°C)	European earwig *Forficula auricula*		
			Initial	Final			Live	Dead	Total
9/9/95	120	1.5	0.3	0.3	4.5	19.0	0	8	8
9/9/95	0	0	0	0	0	22.0	8	0	8
0/11/95	120	3.160	0.9	0.9	6.0	18.0	0	12	12
0/11/95	0	0	0	0	0	22.0	12	0	12
2/4/96	160	2.88	1.0	0.9	15:0	13.8 (13.5-15)	0	1	1
2/4/96	0	0	0	0	0	22.0	1	0	1
9/5/96	140	2.52	1.0	0.9	18:0	18.2 (15.4-20)	0	2	2
9/5/96	0	0	0	0	0	22.0	2	0	2

Table 5.47 Insecticidal efficacy of pyrethrum-phosphine to pupae of Geometrid, exposed to various concentrations, exposure times and temperature in a 27 m^3 chamber at Emerald.

Date	Pyrethrum (g)	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Geometrid		
			Initial	Final			Live	Dead	Total
4/9/96	140	0.92	0.15	0.069	16.15	18.1(15.2-20.8)	0	6	6
4/9/96	0	0	0	0	0	22.0	6	0	6
8/9/96	120	0.7	0.22	0.07	16.0	19.2(17.8-20.6)	0	4	4
8/9/96	0	0	0	0	0	22.0	4	0	4
/10/96	120	0.7	0.33	0.13	15.5	18.0(13.1-18.8)	0	2	2

Table 5.48 Insecticidal efficacy of pyrethrum-phosphine to larvae of Geometrid, exposed to various concentrations, exposure times and temperature in a 27 m^3 chamber at Emerald.

Date	Pyrethrum (g)	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature °C	Geometrid		
			Initial	Final			Live	Dead	Total
4/9/96	140	0.92	0.15	0.069	16.15	18.1 (15.2-20.8)	0	12	12
4/9/96	0	0	0	0	0	22.0	10	0	12
18/9/96	120	0.7	0.22	0.07	16.0	19.2 (17.8-20.6)	0	7	7
18/9/96	0	0	0	0	0	22.0	7	0	7
7/10/96	120	0.7	0.33	0.13	15.5	18.0(13.1-18.8)	0	4	4
7/10/96	0	0	0	0	0	22.0	4	0	4

Table 5.49 Insecticidal efficacy of pyrethrum-phosphine to adults of *S. morsitans,* Linnaeus, exposed to various concentrations, exposure times and temperature in a 27 m^3 chamber at Emerald.

Date	yrethru (g)	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposur time(h)	Temperature (°C)	Common centipede *S. morsitans*		
			Initial	Final			Live	Dead	Total
9/9/95	120		0.3	0.3	4.5	19.0	0	5	5
9/9/95	0	0	0	0	0	21.0	5	0	5

Table 5.50 Insecticidal efficacy of pyrethrum-phosphine to immature and adult stages of *P. perconfusca,* exposed to various concentrations, exposure times and temperature in a 27 m^3 chamber at Gembrook.

Date	Pyrethrum gm^{-3}	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Shield bug *Poecilotoma perconfusca*		
			Initial	Final			Live	Dead	Total
16/10/96	120	0.7	0.15	0.12	16.0	17.1(12.5-19.2)	0	16	16
16/10/96	0	0	0	0	0	21.0	16	0	16
13/11/96	140	1.40	0.32	0.19	16.0	18.2(16.1-20.2)	0	24	24
13/11/96	0	0	0	0	0	21.0	24	0	24

Table 5.51 Insecticidal efficacy of pyrethrum-phosphine to adults of the *P. perconfusca*, exposed to various concentrations, exposure times and temperature in a 27 m^3 chamber at Emerald.

Date	Pyrethru Gm^{-3}	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Shield bug *Poecilotoma perconfusca*			
			Initial	Final			Live	S. aff	Dead	Total
11/11/96	120	1.94	0.07	0.04	4.5	3.3(10.7-15.3)	0	10	24	34
11/11/96	0	0	0	0	0	21.0	34	0	0	34

Table 5.52 Insecticidal efficacy of pyrethrum-phosphine to immature stages of *P. perconfusca*, exposed to various concentrations, exposure times and temperature in a 27 m^3 chamber at Emerald.

Date	yrethru (g)	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Shield bug *Poecilotoma perconfusca*		
			Initial	Final			Live	Dead	Total
16/10/96	120	0.7	0.15	0.12	16.0	17.1(12.5-19.2)	0	29	29
16/10/96	0	0	0	0	0	19.0	29	0	29
13/11/96	140	1.4	0.32	0.19	16.0	18.2(16.1-20.2)	0	67	67

Table 5.53 Insecticidal efficacy of pyrethrum-phosphine to the adults of jumping spiders, exposed to various concentrations, exposure times and temperatures in a 27 m^3 chamber at Emerald.

Date	Pyrethrum gm^{-3}	Phosfume® (kg)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Jumping spiders		
			Initial	Final			Live	Dead	Total
4/9/96	140	0.92	0.15	0.069	16.15	18.1(15.2-20.8)	0	6	6
4/9/96	0	0	0	0	0	21.0	6	0	6
18/9/96	120	0.7	0.22	0.07	16.0	19.2(17.8-20.6)	0	4	4
18/9/96	0	0	0	0	0	21.0	4	0	4
7/10/96	120	0.7	0.33	0.13	15.5	18.0(13.1-18.8)	0	21	21
7/10/96	0	0	0	0	0	21.0	21	0	21
16/10/96	120	0.7	0.15	0.12	16.0	17.1(12.5-19.2)	0	12	12
16/10/96	0	0	0	0	0	21.0	12	0	12
13/11/96	140	1.40	0.32	0.19	16.0	18.2(16.1-20.2)	0	14	14
13/11/96	0	0	0	0	0	21.0	14	0	14

5.4 *Discussion*

It was shown in Chapter 4 that phosphine in reasonably efficacious as a fumigant of wildflowers. However, in some treatments the survival of some stages of the insects may have compromised the quarantine requirements of importing countries. It is hypothesised that phosphine synergised with pyrethrum may be more effective, and experiments have been conducted to test this hypothesis.

As in the phosphine alone experiments, the trials were carried out on a commercial scale. Furthermore, the intrinsic nature of field experiments results in their not being replicable. However, to be able to compare the phosphine only and phosphine-pyrethrum experiments the device of specifying good, marginal and poor fumigation conditions were implemented. Although, quite crude, it has been found to be a useful comparator. The results for the pyrethrum-phospine experiments that are analogous to those presented in Table 4.90, for the phosphine only case are presented in Table 5.54.

Table 5.54 Minimum requirements of various parameters for a complete mortality of exposed insect pests.

Insect	Stage	Temperature (°C)	Exposure (hours)	Final phosphine concentration (gm^{-3})
S. ejectana	3rd to 6th instar larvae (collected from the field)	15.6	15	0.15
	First to sixth instar larvae (reared in the laboratory)	*		
	Pupae	*		
E. postvittana	Eggs	21	15.5	0.4
	First to third instar larvae	13.1	15	0.15
	Fourth instar larvae	17.8	15.5	0.12
	Fifth instar larvae	17.9	15.5	0.097
	Sixth instar larvae	18.1	15.5	0.15
	Pupae	13.8	0.5	0.97
T. urticae	Eggs	17.9	15	0.69
	Larvae	*		
	Diapause larvae	*		
	Adults	13.1	15	0.1
M. persicae	Larvae and adults	*		
B. brassicae	Larvae and adults	**		
M. ornatus	Larvae and adults	**		
I. purpureus	Adults	*		
C. consobrins	Adults	*		
. persimilus	Larvae and adults	**		

*- Complete mortality of all the exposed individuals.

**- Complete mortality was not achieved in all the exposed fumigations.

In the case of *E. postvittana* the results indicate that eggs of the former species are perhaps slightly more susceptible to phosphine synergised with pyrethrum. The evidence for this is that 'good' fumigations were achieved at a lower temperature, shorter time and lower final phosphine concentrations than in the phosphine alone case. In the case of the eggs of *T. urticae* there is no evidence that pyrethrum enhances the efficacy of phosphine.

It is possible to generalise the results and conclude that phosophine synergised with pyrethrum is more effective against larvae, pupae and adults than phosphine alone. In the case of *S. ejectana* the third to sixth stage instar larvae were all killed at a lower temperature and with a lower final concentration of synergised phosphine than with non-synergised phospine. Larvae of *E. postvittana* were all killed at lower temperatures when they were fumigated with a mixture of pyrethrum and phosphine.

In the experiments carried out on larvae and adults of *B. brassicae* and *M. ornatus*, the conditions of the pyrethrum-phosphine mixture were such that all of the treatments were sub-lethal. As it was not possible to declare an effective fumigation regime. The pyrethrum-phosphine mixture proved to be highly effective against both the adults and larval stages of *M. persicae* and *P. persimilis*. The mixture was also lethal to adults of *I. purpureus* and *C. consobrinus*.

These experiments investigated the potential of using pyrethrum-phosphine for short (4.5 to 6 h) and long (15 h or more) exposure time for the postharvest disinfestation of wildflowers as an alternative to methyl bromide. A range of concentrations and temperatures was investigated. Very often, the required concentrations of phosphine, exposure times and temperatures for disinfestation of wild flowers depend on insect species, and their developmental stages. Also, results from these trials indicate that response of some individuals of the same population of some insect species to pyrethrum-phosphine fumigation differed slightly. Some individuals of the following insect species and their stages were severely affected for a certain period of time following fumigation:

- third to sixth instar larvae of the field collected *S. ejectana;*

- eggs (a small number survived in one fumigation), and fourth, fifth and sixth instar larvae of the *E. postvittana*;
- eggs and adults of *T. urticae*; and
- larvae and adults of *B. brassicae and M. ornatus*.

The number of individuals severely affected varied among species and stages of insects exposed. Results indicate that number of individuals severely affected were higher for the larvae of *S. ejectana* and *E. postvittana* than all other insects and their stages exposed (mentioned above). Also, results indicate that comparatively higher numbers of individuals were severely affected in short exposure time fumigations than long exposure time fumigations.

The following insect species and stages showed a uniform mortality to the exposed pyrethrum-phosphine fumigation:

- laboratory reared larvae and pupae of *S. ejectana*;
- first, second and third instar larvae, and pupae of *E. postvittana*;
- larvae and diapause larvae of *T. urticae*;
- larvae and adults of *M. persicae* and *P. persimilis*;
- adults of *H. victoriensis, I. purpureus C. consobrinus, M. rosae, S. morsitans, F. auricularia, Geometrid, P. perconfusca* and the jumping spider.

The entire laboratory reared larvae and field collected pupae of the *S. ejectana* were killed that were exposed to short exposure time fumigations. The susceptibility of this laboratory reared larvae can be related to its artificial rearing medium and/or its living pattern, where absence of compact shelter webbings might have facilitated the easy access of toxic phosphine to the target sites. Similarly, all the exposed larvae and adults of *M. persicae* were killed. Also, it was observed that all the larvae of the *T. urticae* and adults of *S. morsitans, M. rosae* and *F. auricularia* were killed. Hence, it can be assumed from these results that these stages are highly susceptible to pyrethrum-phosphine fumigation. The reason is probably a result of their different metabolisms, physiology or living patterns.

Results indicate that pyrethrum-phosphine fumigation is highly toxic, provided all other criteria (temperature, exposure time and phosphine concentration) are met, to a range of insect pests and their stages that commonly infest wildflowers. However, in some fumigations, although all the fumigation criteria are met, a small number of individuals of some insect pests were severely affected due to unknown reasons. From the results, it can be concluded that fumigation failure may occur when one or more of the variable (temperature and/or exposure time and/or phosphine concentration) is not maintained adequately.

5.4.1 Short exposure time fumigations

Short exposure periods (4 to 6.5 h) with a range of phosphine concentrations did not give a complete kill of even the active stages (larvae) of *S. ejectana* and *E. postvittana*. Few larvae of *S. ejectana* (fourth to sixth instar stages) and *E. postvittana* (only sixth instar stage was exposed due to unavailability) and a small number of the adults of *T. urticae* were severely affected regardless of the concentration of phosphine or temperature that they were exposed.

The severely affected sixth instar larvae exposed to 6 h or less took longer to die (15 days) and all other severely affected instar stages died within 5 days of fumigation. Higher proportions (mostly fourth and fifth instar stages) died within 48 h after fumigation. Moreover, the colour changes of the dead larvae were gradual. The dead larvae retained their colour for the 24 to 48 h but after this period the thoracic region became brown and this progressed towards abdomen. The larvae became blackened five to seven days following fumigation.

In view of these results it can be concluded that short exposures (4.5 to 6 h) are not highly effective against some insect pests of wildflowers and they are unlikely to satisfy the quarantine requirements of importing countries. In view of these results it can be assumed that, at higher concentrations of phosphine (in these trials 1.0 to 3.4 $g.m^{-3}$) the insects exposed were detoxifying a substantial amount of toxic gas absorbed or they were narcotised at these concentrations. Narcotisation protect the larvae from the lethal

effects of phosphine. Hence, it can be concluded that, longer exposure times with comparatively lower concentrations of phosphine are necessary for the insects to absorb lethal amount of phosphine without detoxifying it.

5.4.2 Long exposure time fumigations

Long exposure times of 14 h or more are highly toxic to most of the insect pests and their stages exposed than that of short exposure fumigations. In most of the long exposure time fumigations a uniform mortality of the most of the exposed individuals were achieved. In the long exposure time fumigations, however, a small number of individuals of various species (larvae of *S. ejectana*, *E. postvittana*, *B. brassicae, M. ornatus* and adults of *M. ornatus* and *T. urticae*) were severely affected. Also, small number of eggs of *E. postvittana* and *T. urticae* had survived in some fumigations. The probable reason for this would be lower temperature and/or lower phosphine concentrations during fumigations. In the case of *S. ejectana* and *E. postvittana* larvae the severely affected were of third to sixth instar stages, and the number of individuals severely affected increased with the age of the instar stages.

In case of eggs, only young eggs (one to three days old) of *E. postvittana* and *T. urticae* survived in some fumigations, and all the mature eggs killed in all the fumigations. Similarly, Lindgren and Vincent (1966) found that a-day-old eggs of *T. confusum* were comparatively tolerant to phosphine than two to six-day old eggs. The same observations were made by Querashi *et al.* (1965) on eggs of *T. mauritanicus* fumigated with phosphine. Bell (1976) also found that the initial egg developmental stages were highly tolerant to phosphine fumigation, but subsequently the resistance reduces as they mature. The surviving eggs emerged later (2 to 3 days) than the control eggs, and this suggests that there were marked differences in the developmental periods for the fumigated and control eggs. However, the surviving individuals developed in the same way as the controls.

It can be concluded from these results that phosphine and pyrethrum might have acted independently and had different modes of toxic action, i.e. independent joint action. Notably, phosphine is a respiratory poison that reacts with the respiratory chain, and pyrethrum is a nerve poison that agitates the insects. The initial stimulatory effects of pyrethrum on crawling insects might have prompt them to absorb larger amounts of toxic phosphine because of their increased activity. It is clear that when two fumigant chemicals are applied jointly, and they exert their toxicity on different sites of insects and produce different toxic symptoms, the joint action is more significant than if they acted independently. In this case, two independent poisons with single dominant effect on two sites of an insect may accelerate the mortality. Because of the site of action of these fumigants were different (pyrethrum-a nerve poison and phosphine-a respiratory poison), these are complementary and the combined effect was significant.

These fumigation trials clearly show that phosphine concentration of 0.69 $g.m^{-3}$, temperature of 21°C and exposure time of 15.5 h killed all the exposed insect pests of wildflowers. This fumigation regime is highly suitable for commercial postharvest disinfestation of wildflowers.

Chapter - 6

Phytotoxicity

Chapter 6

Phytotoxicity

6.1 Scopes of the experiments

The quality and value of flowers are determined by their fresh appearance which is related to their longevity. As a result it is utmost importance for the exporters to reduce any practice after harvest that would shorten vase life. Sometimes, wildflowers are infested with a range of insect pests, and because of the quarantine restrictions imposed by the importing countries, fumigation of wildflowers destined for export is imperative. Generally, most fumigant chemicals are not only toxic to insect pests, but also to foliage and flowers. Hence, it is likely that vase life of the fumigated flowers and foliage may be reduced considerably. Other than this, exposing the flowers or foliage to temperatures of 18°C or more for long exposure times (14 h or more) during fumigation is also likely to reduce the vase life considerably. The end of vase life is marked by wilting and discolouration of flowers, and the browning or blackening of the foliage.

Preliminary laboratory experiments carried out by the candidate established that standard phosphine formulations (1 per cent phosphine in nitrogen as a carrier gas) did not reduce the vase life of the exposed wildflowers. These were exposed to a phosphine concentration of 0.69 to 1.1 $g.m^{-3}$ for 4 to 5 h at 18°C. However, these exposure times and/or concentrations did not result in complete mortality of the two insect pests exposed. Hence, a more comprehensive set of experiments was designed to determine:

- the toxicity of various concentrations of phosphine (applied as Phosfume® that contains 2 per cent phosphine in carbon dioxide as a carrier gas) to a range of insect pests and their developmental stages,

- the effects of various concentrations of combined application of Pestigas®-Phosfume® on the vase life of the exposed flowers and lethality to a range of insect pests and

- the feasibility of using long exposure time fumigation (14 h or more) and a range of temperatures (13 to 27°C) on the vase life of three botanically diverse wildflower species, as well as toxicity to the exposed insect pests.

This chapter reports the results of the phytotoxicity studies.

6.2 *Materials and methods*

A range of export quality flowers and foliage bunches (Table 6.1) that were placed in buckets filled with 4 to 5 L of water was obtained from flower outlets and commercial flower exporters in Victoria, 24 to 48 h following harvest. Flowers were selected randomly from the bunches and the bases of the stems (10 to 15 mm) were cut under water using secateurs. Selected flowers were transferred into 20 L plastic buckets (five to nine stems per bucket) that were filled with 7 to 9 L of tap water (Figure 6.1).

Table 6.1 Flowers and foliage that were fumigated with phosphine and pyrethrum-phosphine combination.

Species	Common name	Family
Anigozanthos pulcherrimus	Kangaroo Paw	Haemodoraceae
Banksia coccinea	Scarlet Banksia	Proteaceae
Eucalyptus (foliage)	Eucalyptus	Myrtaceae
Leucadendron floridum	Floridum	Proteaceae
Protea cynaroides	King Protea	Proteaceae
Thryptomene calycina	Grampian thryptomene	Myrteaceae
	Carnation	

The control and the flowers that were to be fumigated were labelled and placed in separate buckets. Flowers for fumigation were placed on a trolley, transferred into the fumigation chamber and placed in a range of locations. The control flowers were placed adjacent to the fumigation chamber and maintained at a similar temperature to the fumigation chamber. Fumigant delivery into the chamber and temperature maintenance is discussed in detail in Chapter 4, Section 4.2.3.2. A phosphine concentration of 0.15 to 1.2 $g.m^{-3}$ and exposure periods of 14 to 18 h in a temperature range of 13 to 25.5°C were investigated. The reason for choosing this temperature range was dictated by the need to obtain complete mortality of different insect species and their developmental stages.

Once the fumigation was completed, the fumigant residues were aired off from the flowers using a vacuum cleaner for 30 to 45 min. These flowers were transported to the Institute for Horticultural Development, Knoxfield where they transferred into different 1 to 1.5 L glass vases (three to five stems per vase) filled with 500 to 700 mL of tap water. Similarly, the control flowers were transferred into similar glass jars and labelled. These were maintained at a typical room temperature of 20±2°C, using an electric fan heater. The temperature in this room was monitored by placing temperature sensors near to the flowers (Hobos). The rationale for choosing this temperature is once the flowers are fumigated these were generally displayed in retail outlets or in the consumers' houses at this temperature. A photoperiod of 18 h of light and 6 h of dark was maintained using two 15W fluorescent lamps that were controlled by a timer. The lights were located 2,500 mm above the flowers. The condition of the control and fumigated flowers was observed daily for wilting, drooping, drying, browning, or blackening and these were compared with control flowers for up to seven days. The water was replaced every two days to reduce the likelihood of micro-organisms developing.

The responses of various species of flowers and foliage to different concentrations of phosphine, exposure times and temperatures differ considerably, hence, for each flower and foliage different criteria were used to determine the vase life. Generally, the flowers will reach consumers one to three days following fumigation, hence, the vase life was

assessed for up to seven days following fumigation. The fumigated and control flowers were rated zero to eight based on appearance, and these were compared with the control flowers.

Table 6.2 Phytotoxicity index, for assessing the toxicity of fumigants.

Rating	Flowers	Foliage
0	No damage to petals or bracts	No damage to leaves or stem
1	Slight discolouration or marginal browning of 10 per cent or less of flowers/bracts	Marginal necrosis of 10 per cent or less of leaves
2	Some discolouration, but marketable	Marginal necrosis of 10 to 25 per cent of leaves
3	Discolouration of 25 per cent or more of flowers	Marginal necrosis of 25 to 50 per cent of leaves
4	Increased discolouration	Increased necrosis of 50 per cent or more of leaves
5	Entire flower browned and wilting, unmarketable	Browning of whole leave, not suitable for vase life
6	Flower closed	Showering of leaves
7	Showering of flowers	Foliage destroyed
8	Flowers destroyed	

6.3 Results

Immediately after fumigation and for up to 24 h none of the fumigated flowers responded differently from those that were not fumigated.

6.3.1 The kangaroo paw, *Anigozanthus pulcherrimus*

6.3.1.1 Exposure to Phosfume®

Results of the phosphine-fumigated flowers and foliage of *A. pulcherrimus* are presented in Table 6.3. Phosphine with concentrations of 0.64 to 0.98 $g.m^{-3}$ with exposure times of 16 to 18 h did not affect the vase life of the flowers or young leaves exposed. However, the mature leaves were comparatively sensitive to a phosphine concentration of 0.94 $g.m^{-3}$ or

above. The mature leaves that were fumigated at this concentration showed slight marginal browning (25 per cent) four to five days following fumigation.

Table 6.3 The toxicity of phosphine to *A. pulcherrimus* exposed to various concentrations, exposures and temperatures.

Date	Phosphine Concentration gm^{-3}		Exposure time (h)	Temperature (°C)	RH (%)	Phytotoxicity		Vase life (days)	
	Initial	Final				Flowers	Foliage	Flowers	Foliage
10/2/97	0.98	0.85	15.5	18.6(17.8-20.6)	-	0	1	9	8
10/2/97	0	0	0	22.0	65.0	0	0	9	8
12/2/97	0.94	0.55	17.0	23.9(18.1-34.8)	84.4(53.7-93.5)	1	1	8	8
12/2/97	0	0	0	22.0	65.0	0	0	9	8
17/3/97	0.64	0.43	16.0	16.3(11.8-24.1)	85.3(44.8-90.3)	0	0	9	8
17/3/97	0	0	0	22.0	65.0	0	0	9	9
20/3/97	1.08	0.97	16.0	16.9(14.3-21.6)	88.5(71.8-95.2)	0	1	9	8
20/3/97	0	0	0	17.0	65.0	0	0	9	8
24/3/97	0.78	0.57	17.0	17.4(15.3-24.1)	85.8(50.1-93.4)	0	0	9	8
24/3/97	0	0	0	18.0	65.0	0	0	9	8
9/4/97	1.32	0.48	16.25	21.1(18.8-25.5)	90.5(44.8-96.4)	1	1	8	7
9/4/97	0	0	0	22.0	65.0	0	0	9	8
14/4/97	1.01	0.76	17.0	18.9(18.1-19.5)	91.2(79.1-93.4)	1	1	9	8
14/4/97	0	0	0	19.0	65.0	0	0	9	8
21/4/97	1.22	0.41	16.0	19.4(17.2-25.3)	87.3(60.3-92.8)	1	1	8	7
21/4/97	0	0	0	19.0	65.0	0	0	9	8
29/4/97	1.14	0.87	16.0	18.4(13.9-26.6)	91.7(62.3-96.4)	1	1	9	7
29/4/97	0	0	0	18.0	65.0	0	0	9	8
5/5/97	0.83	0.76	18.0	18.3(16.4-21.9)	83.4(66.1-94.6)	0	0	9	8
5/5/97	0	0	0	18.0	65.0	0	0	9	8
26/5/97	1.0	0.84	17.25	17.5(16.0-19.1)	90.6(85.3-97.0)	1	1	9	8
26/5/97	0	0	0	18.0	65.0	0	0	9	8
3/6/97	1.04	0.48	16.0	18.3(16.7-19.9)	80.2(67.0-87.4)	1	1	9	8
3/6/97	0	0	0	18.0	65.0	0	0	9	8
15/7/97	0.86	0.48	16.5	17.7(14.9-20.5)	-	0	0	9	8
15/7/97	0	0	0	18.0	65.0	0	0	9	8

Similarly, phosphine concentrations of 1 $g.m^{-3}$ or more were slightly toxic to all the flowers and foliage of *A. pulcherrimus* exposed. However, 50 per cent of mature foliage showed marginal browning three to four days following fumigation exposed to phosphine concentrations of 1.01 to 1.2 $g.m^{-3}$. This was followed by browning of whole leaves (50 per cent) 60 to 72 h following the appearance of initial symptoms. Similar symptoms appeared in young leaves five to six days following fumigation. Also, 25 per cent of the mature flowers showed slight discolouration of the petals six to seven days following fumigation at this phosphine concentration. Higher temperatures of 20°C or more reduced the vase life slightly. The symptoms were similar to those mentioned above, but these appeared on mature foliage (25 per cent) three to five days following fumigation. However, both young leaves and all flowers were not affected.

6.3.1.2 Exposure to Pestigas®-Phosfume® combination

There was no considerable difference between fumigated flowers and control flowers that were fumigated with a pyrethrum concentration of 4.4 to 4.8 $g.m^{-3}$, phosphine concentration of 0.28 to 0.31 $g.m^{-3}$ exposed for 15 to 16 h at 15.6 to 18.0°C. However, both the flowers and leaves showed browning at higher phosphine concentrations (1 $g.m^{-3}$ or above). The affected flowers and foliage responded as mentioned in Section 6.3.1.1. The results show that addition of pyrethrum did not change the response of the vase life of flowers and foliage of *A. pulcherrimus* to phosphine.

Table 6.4 The toxicity of pyrethrum-phosphine to *A. pulcherrimus* exposed to various concentrations, exposures and temperatures.

Date	Pyrethrum gm^{-3}	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Phytotoxicity		Vase life (days)	
		Initial	Final			Flowers	Foliage	Flowers	Foliage
23/11/95	120	1.5	1.0	15.5	20.8 (19.4-21.5)	1	1	8	7
23/11/95	0	0	0	0	22.0	0	0	9	8
30/11/95	120	1.4	1.0	15.0	20.5 (19.6-22.8)	1	1	8	7
30/11/95	0	0	0	0	22.0	0	0	9	8
12/2/96	120	1.0	0.4	16.0	21.0 (18.6-22.6)	1	1	9	8
12/2/96	0	0	0	0	22.0	0	0	9	8
19/2/96	160	1.0	0.6	16.0	21.0(16.3-23.1)	1	1	9	8
19/2/96	0	0	0	0	22.0	0	0	9	8
26/2/96	120	1.0	0.7	15.5	21.9 (19.5-26.7)	1	1	9	8
26/2/96	0	0	0	0	22.0	0	0	9	8
18/3/96	140	1.0	1.0	15.5	13.1 (10.0-14.6)	1	1	9	8
18/3/96	0	0	0	0	22.0	0	0	9	8
25/3/96	120	1.0	0.7	16.0	21.7 (18.1-27.2)	1	1	9	8
25/3/96	0	0	0	0	22.0	0	0	9	8
2/4/96	160	1.0	0.9	15.0	13.8 (13.5-15.0)	1	1	9	8
2/4/96	0	0	0	0	22.0	0	0	9	8
29/5/96	140	1.0	0.9	18.0	18.2 (15.4-19.9)	1	1	9	8
29/5/96	0	0	0	0	22.0	0	0	9	8
10/7/96	200	0.28	0.19	15.0	15.6 (10.0-18.7)	0	0	9	8
10/7/96	0	0	0	0	22.0	0	0	9	8
18/7/96	120	0.28	0.18	15.25	17.4 (10.1-20.3)	0	0	9	8
18/7/96	0	0	0	0	22.0	0	0	9	8
20/8/96	120	0.31	0.19	16.0	18.0	0	0	9	8
20/8/96	0	0	0	0	22.0	0	0	9	8

6.3.2 *The scarlet banksia, Banksia coccinea*

6.3.2.1 *Exposure to Phosfume®*

Both the flowers and foliage of *B. coccinea* that were fumigated with phosphine with the concentration range of 0.25 to 1.2 $g.m^{-3}$ for 15 to 16 h did not exhibit symptoms of phytotoxicity (Table 6.5) when the temperature range was 18.2 to 18.4°C. On the other hand, at higher temperatures (25.5°C) a small number (15 per cent) of flowers showed slight browning of their tips five to seven days following fumigation. However, the mature leaves responded differently to the flowers. Twenty-five per cent of these leaves showed slight marginal yellowing, which was followed by drying symptoms (12 to 18 h following appearance of initial symptoms). A slight upward curling of mature leaves was also observed seven to eight days following fumigation. The young leaves remained in good condition for up to seven days after fumigation under these conditions. On the other hand, the control flowers remained in good conditions for seven days and more.

Table 6.5 The toxicity of phosphine to *B. coccinea* exposed to various concentrations, exposures and temperatures.

Date	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Phytotoxicity		Vase life (days)	
	Initial	Final			Flowers	Foliage	Flowers	Foliage
30/9/96	0.25	0.15	16.0	18.4(15.5-20.8)	0	0	9	8
30/9/96	0	0	0	19.0	0	0	9	8
30/12/96	0.43	0.36	16.0	18.4(17.6-19.9)	0	0	9	8
30/12/96	0	0	0	19.0	0	0	9	8
14/1/97	1.09	0.61	16.0	25.5(22.9-28.9)	1	1	9	8
14/1/97	0	0	0	25.0	0	0	9	8
23/1/97	1.2	0.94	15.0	18.2(16.9-20.7)	0	0	9	8
23/1/97	0	0	0	19.0	0	0	9	8

These results suggest that fumigating *B. coccinea* with phosphine (concentration of 0.25 to 1.2 $g.m^{-3}$ and an exposure period of 15 to 16 h) is not toxic if the temperature is less than about 20°C. But exposure to higher temperatures causes slight damage to both flowers and foliage

6.3.2.2 Exposure to Pestigas®-Phosfume® combination

Similarly, a pyrethrum concentration of 4.4 to 5 $g.m^{-3}$ in phosphine did not reduce the vase life of both young and mature flowers and foliage of *B. coccinea* (Table 6.6). All the flowers that were fumigated with this combination responded similarly as described above. However, if the temperature maintained during fumigation was above 26.5°C the vase life of the flowers and foliage was reduced slightly.

Table 6.6 The toxicity of pyrethrum and phosphine to *B. coccinea* exposed to various concentrations, exposures and temperatures.

Date	Pyrethrum gm^{-3}	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Phytotoxicity		Vase life (days)	
		Initial	Final			Flowers	Foliage	Flowers	Foliage
12/2/96	120	1.0	0.4	16.0	21.0 (18.9-22.6)	0	1	12	11
12/2/96	0	0	0	0	21.0	0	0	12	12
19/2/96	160	1.0	0.6	16.0	21.0 (16.3-23.1)	0	1	12	12
19/2/96	0	0	0	0	22.0	0	0	12	12
26/2/96	120	1.0	0.7	15.5	21.9 (19.5-26.7)	0	1	12	12
26/2/96	0	0	0	0	22.0	0	0	12	12
18/3/96	140	1.0	1.0	15.5	13.1 (10.0-14.6)	0	0	12	12
18/3/96	0	0	0	0	14.0	0	0	12	12
2/4/96	160	1.0	0.9	15.0	13.8 (13.5-15)	0	0	12	12
2/4/96	0	0	0	0	14.0	0	0	12	12
29/5/96	140	1.0	0.9	18.0	18.2 (15.4-19.9)	0	0	12	12
29/5/96	0	0	0	0	18.0	0	0	12	12
10/7/96	200	0.3	0.19	15.0	15.6 (10.0-18.7)	0	0	12	12
10/7/96	0	0	0	0	16.0	0	0	12	12
18/7/96	120	0.3	0.18	15.25	17.4 (10.1-20.3)	0	0	12	12
18/7/96	0	0	0	0	18.0	0	0	12	12
20/8/96	120	0.26	0.097	16.0	18.0	0	0	12	11
20/8/96	0	0	0	0	18.0	0	0	12	12
29/8/96	120	0.27	0.11	16.25	18.0 (16.6-20.1)	0	0	12	12
29/8/96	0	0	0	0	18.0	0	0	12	12
16/10/96	120	0.15	0.12	16.0	17.1(12.5-19.2)	0	0	12	11
16/10/96	0	0	0	0	18.0	0	0	12	11
30/10/96	120	0.15	0.097	16.25	18.4 (17.1-19.0)	0	0	12	12
30/10/96	0	0	0	0	19.0	0	0	12	11
12/11/96	120	0.27	0.15	16.0	18.1 (17.5-18.9)	0	0	12	12
12/11/96	0	0	0	0	19.0	0	0	12	12
13/11/96	140	0.32	0.19	16.0	18.2 (16.1-20.2)	0	0	12	12
13/11/96	0	0	0	0	19.0	0	0	12	12
23/1/97	160	0.44	0.32	15.75	26.5(22.1-33.4)	1	1	12	11
23/1/97	0	0	0	0	25.0	0	0	12	12
17/2/97	120	0.42	0.22	15.5	18.7(17.4-20.9)	0	0	12	12
17/2/97	0	0	0	0	19.0	0	0	12	11

6.3.3 *The king protea, Protea cynaroides*

6.3.3.1 *Exposure to Phosfume®*

No visible damage to flowers or foliage was observed to King proteas fumigated with a range of phosphine concentrations (0.25 to 1.09 $g.m^{-3}$), exposure times (15 to 16 h) and temperatures (18.2 to 25.5°C) (Table 6.7). However, slight marginal blackening of 25

Table 6.7 The toxicity of phosphine to *P. cynaroides* exposed to a range of concentrations, exposures and temperatures.

Date	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Phytotoxicity		Vase life (days)	
	Initial	Final			Flowers	Foliage	Flowers	Foliage
30/9/96	0.25	0.15	16.0	18.4(15.5-20.8)	0	0	12	12
30/9/96	0	0	0	18.0	0	0	12	12
30/12/96	0.43	0.36	16.0	18.4(17.6-19.9)	0	0	12	11
30/12/96	0	0	0	18.0	0	0	12	12
14/1/97	1.09	0.61	16.0	25.5(22.9-28.9)	1	1	12	12
14/1/97	0	0	0	25.0	0	0	12	12
23/1/97	1.2	0.94	15.0	18.2(16.9-20.7)	0	1	12	11
23/1/97	0	0	0	19.0	0	0	12	11

per cent of young and mature leaves was observed four to five days following fumigation with a phosphine concentration of 1.2 $g.m^{-3}$. The blackening spread towards the centre of the leaves, rapidly turning the whole leaf black within three days following initial symptoms. Although the symptoms were observed generally on both young and mature leaves, mature leaves became black at a faster rate than the young leaves. Similarly, the bracts of flowers (10 per cent) exhibited browning symptoms with this concentration four to five days following fumigation. This browning was followed by inward curling of 50 per cent of the bracts. However, the buds were not affected by the higher phosphine

concentration or temperatures, and the fumigated buds opened in the same manner as the control buds.

6.3.3.2 Exposure to Pestigas®-Phosfume® combination

The results of the pyrethrum and phosphine fumigated King proteas are presented in Table 6.8. All the flowers that were fumigated with a pyrethrum concentration of 4.4 to 5 $g.m^{-3}$ and a phosphine concentration of 0.15 to 1 $g.m^{-3}$, for 15 to 18 h of exposure with a temperature range of 13.1 to 21.9°C did not show any reduction in vase life. However, marginal browning of leaves was observed four to five days following fumigation with a phosphine concentration of 1.2 $g.m^{-3}$. Seventy-five per cent of the affected foliage showed accelerated browning one to two days following the appearance of initial symptoms, and the whole leaf turned to black five to seven days following fumigation. All the flowers and young leaves could not be differentiated from those of the unfumigated batch.

Table 6.8 The toxicity of phosphine to *P. cynaroides* exposed to a range of concentrations, exposures and temperatures.

Date	Pyrethrum gm^{-3}	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Phytotoxicity		Vase life (days)	
		Initial	Final			Flowers	Foliage	Flowers	Foliage
12/2/96	120	1.0	0.4	16.0	21.0(18.9-22.6)	0	1	12	12
12/2/96	0	0	0	0	22.0	0	0	12	12
19/2/96	160	1.0	0.6	16.0	21.0(16.3-23.1)	0	1	12	11
19/2/96	0	0	0	0	22.0	0	0	12	12
26/2/96	120	1.0	0.7	15.5	21.9(19.5-26.7)	0	1	12	12
26/2/96	0	0	0	0	22.0	0	0	12	12
18/3/96	140	1.0	1.0	15.5	13.1(10.0-14.6)	0	1	12	12
18/3/96	0	0	0	0	14.0	0	0	12	12
2/4/96	160	1.0	0.9	15.0	13.8(13.5-15)	0	1	12	11
2/4/96	0	0	0	0	14.0	0	0	12	11
29/5/96	140	1.0	0.9	18.0	18.2(15.4-19.9)	0	1	12	11
29/5/96	0	0	0	0	18.0	0	0	12	11
10/7/96	200	0.3	0.19	15.0	15.6(10.0-18.7)	0	0	12	12
10/7/96	0	0	0	0	16.0	0	0	12	12
18/7/96	120	0.3	0.18	15.25	17.4(10.1-20.3)	0	0	12	12
18/7/96	0	0	0	0	18.0	0	0	12	12
20/8/96	120	0.26	0.097	16.0	18.0	0	0	12	11
20/8/96	0	0	0	0	18.0	0	0	12	11
29/8/96	120	0.27	0.11	16.25	18.0(16.6-20.1)	0	0	12	12
29/8/96	0	0	0	0	18.0	0	0	12	12
16/10/96	120	0.15	0.12	16.0	17.1(12.5-19.2)	0	0	12	11
16/10/96	0	0	0	0	18.0	0	0	12	12
12/11/96	120	0.27	0.15	16.0	18.1(17.5-18.9)	0	0	12	12
12/11/96	0	0	0	0	18.0	0	0	12	12
13/11/96	140	0.32	0.19	16.0	18.2(16.1-20.2)	0	0	12	12
13/11/96	0	0	0	0	18.0	0	0	12	11
23/1/97	160	1.2	0.94	15.0	18.2(16.9-20.7)	0	1	12	12
23/1/97	0	0	0	0	27.0	0	0	12	12
17/2/97	120	0.42	0.22	15.5	18.7(17.4-20.9)	0	0	12	12
17/2/97	0	0	0	0	19.0	0	0	12	11

6.3.4 *The Grampian thryptomene, Thryptomene calycina*

6.3.4.1 *Exposure to Phosfume®*

No visible damage to either the flowers or foliage of *T. calycina* was observed when fumigated with phosphine concentrations of 0.25 to 1.09 $g.m^{-3}$ and exposure periods of 15 to 16 h at temperatures of 18.2 to 18.4°C (Table 6.9). Higher concentrations of phosphine (1.2 $g.m^{-3}$) reduced the vase life of foliage and flowers slightly. Fifty per cent of the lower (mature) leaves showed slight browning three to four days following fumigation and this followed by desiccation and shedding within six days following fumigation. Fifty per cent of the young (top) leaves showed slight desiccation four to five days following fumigation, however, no colour change or dropping was observed.

Table 6.9 The toxicity of phosphine to *T. calycina* exposed to a range of concentrations, exposures and temperatures.

Date	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Phytotoxicity		Vase life (days)	
	Initial	Final			Flowers	Foliage	Flowers	Foliage
30/9/96	0.25	0.15	16.0	18.4(15.5-20.8)	0	0	9	9
30/9/96	0	0	0	18.0	0	0	9	9
30/12/96	0.43	0.36	16.0	18.4(17.6-19.9)	0	0	9	8
30/12/96	0	0	0	18.0	0	0	9	9
14/1/97	1.09	0.61	16.0	25.5(22.9-28.9)	0	0	9	9
14/1/97	0	0	0	25.0	0	0	9	8
23/1/97	1.2	0.94	15.0	18.2(16.9-20.7)	1	1	9	9
23/1/97	0	0	0	18.0	0	0	9	9

Similarly, 75 per cent of the old flowers and 25 per cent of the young flowers showed yellowing of their petals seven days following fumigation and this followed by desiccation. Twenty five percent of these old flowers were shed seven days following fumigation. Five per cent of the young flowers and 15 per cent of the mature leaves dropped from both the

control and fumigated flowers 6 to 7 days following fumigation. However, no considerable damage to buds was observed at both high and low concentrations of phosphine. Sixty to sixty five per cent of the buds opened three to five days following fumigation, and for the controls this was 75 per cent.

6.3.4.2 Exposure to Pestigas®-Phosfume® combination

Initial application of pyrethrum did not affect the vase life of the fumigated flowers of *T. calycina* (Table 6.10). The flowers fumigated responded similarly to the phosphine fumigated flowers and are discussed in the previous paragraph. These results confirm that phosphine with concentrations of 0.15 to 1.09 $g.m^{-3}$, for 15 to 18 h of exposure at 13.1 to 18.4°C are not toxic to *T. calycina* flowers.

Table 6.10 The toxicity of pyrethrum-phosphine to *T. calycina* exposed to various concentrations, exposures and temperatures.

Date	Pyrethrum gm^{-3}	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Phytotoxicity		Vase life (days)	
		Initial	Final			Flowers	Foliage	Flowers	Foliage
12/2/96	120	1.0	0.4	16.0	21.0 (18.9-22.6)	0	1	9	8
12/2/96	0	0	0	0	22.0	0	0	9	9
19/2/96	160	1.0	0.6	16.0	21.0 (16.3-23.1)	0	1	9	9
19/2/96	0	0	0	0	22.0	0	0	9	9
26/2/96	120	1.0	0.7	15.5	21.9 (19.5-26.7)	0	1	9	9
26/2/96	0	0	0	0	22.0	0	0	9	9
18/3/96	140	1.0	1.0	15.5	13.1 (10.0-14.6)	0	1	9	9
18/3/96	0	0	0	0	14.0	0	0	9	9
2/4/96	160	1.0	0.9	15.0	13.8 (13.5-15)	0	1	9	9
2/4/96	0	0	0	0	14.0	0	0	9	9
29/5/96	140	1.0	0.9	18.0	18.2 (15.4-19.9)	0	1	9	9
29/5/96	0	0	0	0	18.0	0	0	9	9
10/7/96	200	0.3	0.19	15.0	15.6 (10.0-18.7)	0	0	9	9
10/7/96	0	0	0	0	16.0	0	0	9	9
18/7/96	120	0.3	0.18	15.25	17.4 (10.1-20.3)	0	0	9	9
18/7/96	0	0	0	0	18.0	0	0	9	9
20/8/96	120	0.26	0.097	16.0	18.0	0	0	9	9
20/8/96	0	0	0	0	18.0	0	0	9	9
29/8/96	120	0.27	0.11	16.25	18.0 (16.6-20.1)	0	0	9	9
29/8/96	0	0	0	0	18.0	0	0	9	9
16/10/96	120	0.15	0.12	16.0	17.1(12.5-19.2)	0	0	9	9
16/10/96	0	0	0	0	18.0	0	0	9	9

6.3.5 Carnations

6.3.5.1 Exposure to Phosfume®

Phosphine with a concentration range of 0.25 to 1.2 $g.m^{-3}$ for 15 to 16 h of exposure at 18.2 to 25.5°C temperature range reduced the vase life of both flowers and foliage considerably (Table 6.11). The symptoms of damage to leaves were observed 24 h following fumigation, and flowers were seem to be damaged 48 h following fumigation. The initial symptoms were flabby leaves, followed by wilting in 6 to 12 h. Those fumigated with higher concentration of phosphine (1 $g.m^{-3}$ and above) showed blackening of leaves three days following fumigation. Similarly, flowers also showed wilting and desiccation four to six days after fumigation. Hence, it is concluded that carnations were highly susceptible to phosphine fumigations and further fumigations using pyrethrum and phosphine were not conducted. On the other hand, all the control flowers were in good condition for up to seven days.

Table 6.11 The toxicity of phosphine to *Carnations* exposed to a range of concentrations, exposures and temperatures.

Date	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Phytotoxicity		Vase life (days)	
	Initial	Final			Flowers	Foliage	Flowers	Foliage
30/9/96	0.25	0.15	16.0	18.4 (15.5-20.8)	1	2	2	2
30/9/96	0	0	0	22.0	0	1	7	7
30/12/96	0.43	0.36	16.0	18.4 (17.6-19.9)	3	3	2	2
30/12/96	0	0	0	22.0	0	1	7	7
14/1/97	1.09	0.61	16.0	25.5 (22.9-28.9)	4	4	2	1
14/1/97	0	0	0	22.0	0	0	7	7
23/1/97	1.2	0.94	15.0	18.2(16.9-20.7)	4	4	2	1
23/1/97	0	0	0	22.0	0	0	7	7

6.4 Discussion

One of the important criteria that must be taken into consideration in postharvest disinfestation of flowers using fumigants is not only that the selected fumigant must disinfest the flower consignment in an environmentally friendly way, but the vase life of the flowers must be maintained. Flowers are basically senescent tissues, hence, they will die after a certain period of time. Stress factors, mainly exposing them to fumigant chemicals and/or high temperatures would accelerate the senescent process. For most of these species the estimated average number of days flowers were marketable was particularly correlated with the concentration of phosphine and temperature to which the flowers were exposed. The results show that most of the wildflowers can be safely fumigated using either pyrethrum-phosphine or phosphine alone in a concentration range of 0.15 to 1 $g.m^{-3}$. Moreover, phosphine is an advantageous fumigant for the postharvest disinfestation of wildflowers, mainly because of low or nil residue levels remaining after fumigation.

As mentioned in the literature review, temperature is one of the important factors that determine the success of a fumigation process. On the other hand, higher temperatures are inimical to a long vase life of the flowers. Irving and Honnor (1994) indicated that higher temperatures of 20°C or more is the most detrimental factor reducing the vase life of carnations. This was substantiated by the findings of Weller *et al.* (1995) who found that phosphine concentration of 0.1 mm^{-3} for 5 h or 0.25 $g.m^{-3}$ for 2 h reduced the vase life of exposed carnations significantly. However, they found that phosphine fumigation did not reduce the vase life of the following flowers: *Banksia coccinea*, Billy Buttons, *Pycnosorus globosa,* Kangaroo paw, *Anigozanthus spp, Protea cynaroides*, Protea- Pink Ice (Protea hybrid) Riceflower, *Ozothamnus disomifolius* and Waxflower, *Chamelaucium uncinatum.* All these flowers were fumigated with a phosphine concentration of 0.1 mm^{-3} for 5 h or 0.25 $g.m^{-3}$ for 2 h. However, they reported that of all the fumigants investigated (methyl bromide, ethyl formate, hydrogen cyanide, phosphine and carbonyl sulphide) phosphine seems to be the least toxic fumigant to all the cut and wildflowers exposed. Also, Hawkes

(1973) found that phosphine with a Concentration Time Product of 36 mg.h/L (24 and 48 h exposure time) at a temperature of 20°C damaged the exposed chrysanthemum cuttings (cv. Freedom and Pollyanne), however, no damage was observed at 15°C. In all these experiments conducted as part of this research a temperature of 18°C or more was maintained during exposure. All the carnation flowers and foliage exposed to this temperature range showed reduced vase life. However, the vase life of all the wildflowers exposed was not significantly affected up to a temperature of 21.9°C.

Foliage is comparatively more susceptible to higher temperature than flowers. This was evidenced by the fact that slight damage to most of the flowers was observed once the fumigation temperature was above 25°C. However, slight damage to foliage of most of the wildflower species was observed at or above 18°C that will not reduce the vase-life considerably. These results show that wildflowers can be safely fumigated with a temperature range of 13 to 25.5°C. The reason for the wildflowers to withstand comparatively higher temperature would be due to the fact that most of these wildflowers are adapted to the hot climates of Australia, hence, they can withstand this temperature range.

The results suggest that when pyrethrum (concentration of 4.4 to 5 $g.m^{-3}$) is applied in combination with phosphine it is not toxic to any of the wildflowers and foliage exposed. Similarly, phosphine concentrations of 0.15 to 1.09 $g.m^{-3}$ were not toxic to all the exposed flowers except carnations. However, a phosphine concentration of 1.09 $g.m^{-3}$ and above was toxic to most of the foliage exposed. Similarly, these fumigation conditions killed all the exposed active stages of insect species.

From these results it is clear that the exposure times investigated (14 to 18 h) did not reduce the vase life of all the wildflowers exposed. The results obtained by Karuanratane *et al.* (1997) support the above findings.

Chapter - 7

The control of temperature - some practical considerations

Chapter 7

The control of temperature – some practical considerations.

7.1 Scopes of the experiments

Temperature is a key variable that governs the lethality of phosphine against both developing and active stages of insects. The reason includes the fact that the diffusive and penetrative capacities of fumigants are generally enhanced by higher temperatures, and lower temperatures tend to induce stratification. Furthermore, the sorption of fumigant molecules by the commodities and structure of the chamber is significantly higher at lower temperatures. As a result of uneven distribution of fumigant molecules or stratification and consequent fumigation failures are quite possible. Moreover, purging the chamber takes a longer time at lower temperatures due to lower rates of diffusion and higher sorption of fumigant molecules by the commodities.

Perhaps most importantly, temperature generally influences metabolism of insects and an increased in metabolism consequently increases the rate of respiration. In turn, higher respiration increases the rate of absorption of toxic gases, and this is especially important in the case of phosphine as it is a respiratory poison. These phenomena are positively related to temperature. In contrast, most cut and wildflowers prefer lower temperatures, and higher temperatures generally reduce the vase life as discussed in detail in Chapter 6.

The temperature requirements for a higher metabolism, and resulting higher respiration vary substantially among individuals of a population, insect species, strains and developmental stages. However, a temperature range of 18 to 20°C seems to present a window of opportunity for the timely fumigation of insects without damaging the flowers.

As described in Chapters 4 and 5 large scale experiments have been carried out to investigate the lethality of phosphine to insect pests under a range of conditions. In this chapter we shall pay particular attention to the effects of temperature on the insects.

In keeping with the spirit of the research, the observations will serve pragmatic and practical purposes. Specifically we shall study:

- the lethality of various concentrations of phosphine and exposure times to a range of temperatures (13 to 28°C) and to various insect pests, including their developmental stages;

- the time required to heat the chamber to the desired level under various climatic conditions (Winter, Spring, Autumn and Summer), using two different heaters - a 1kW industrial heater, and a 1 to 3kW industrial heater; and

- the temperatures at strategic locations in the chamber, and the ability to maintain the desired temperature during exposure time.

As a result of carrying out these experiments, the research outcomes provide sound practical advice on the implementation of phosphine fumigation of wildflowers.

7.2 *Materials and methods*

The ambient temperatures during the seasons of the year vary significantly in the eastern states of Australia, and this is more pronounced in Victorian and South Australian regions. Also, most of the flower plantations and fumigation facilities are located in inland areas of this region, where the winter over-night temperatures can be sub zero. This was substantiated by the data obtained from an export oriented *T. calycina* plantations at Horsham, Victoria, where the over-night temperatures commonly plunge to –2.7 to –4.1°C

during June and July (Chapter 3, Section 3.3). Moreover, during most part of the year except summer and some periods of autumn and spring the daily temperatures were also substantially lower, and they were not suitable for fumigation (12 to 15°C). Hence, the only solution to this impediment is to artificially heat the chamber during fumigation. The heating capacity of the heater is important as it must provide sufficient heat to increase the temperature to the desired level, and maintain the temperature at this level there. Furthermore, chambers are filled with flowers and foliage in buckets and the buckets are filled with water that had been stored in cool temperature of 4°C. Consequently, the chamber also cools down to similar temperatures.

The selected heater must withstand these conditions and maintain the temperature at the desired level during the exposure time. On the other hand, the chances of over heating the chamber (that may destroy all the flowers and foliage) are high if it is not fitted with a thermostat and sensor. It is always important to maintain the temperature within the range of ±0.5 to 1°C of the recommended level. Higher or lower than these temperatures reduce the vase life of the flowers and increase the chances of survival of the exposed insect pests respectively. Also, because of its highly flammable nature, temperatures higher than those recommended may increase the chances of fire hazards.

The temperature measurements were carried out at Emerald using a 27 m^3 rectangular modified steel shipping container, and at Frankston in a 900 L steel cylindrical chamber. The temperature recordings were obtained at Emerald while the chamber was filled with two metal trolleys, the dimension and other details are discussed in detail in Chapter 4, Section 4.2.4. These trolleys were loaded with three to four 20L plastic buckets filled with 7 to 9 L of tap water. The flowers and foliage (five to seven bunches of various species) were placed in these buckets. The Frankston trials were conducted while the chamber was filled with plastic buckets with flowers and water, similar to the Emerald fumigations. The experiments were carried out three to five times a month (weekly intervals), starting from 16:00 or 17:00 and completed by the following morning 8 or 9 h. These chambers were used either for commercial or experimental fumigations. Hence, in some instances the

The airflow rate measured at the exit of the air delivery duct was typically 0.25 m^3/s. Installing an industrial heater in the air supply duct did little to impede gas circulation in the chamber. Adequate controlled heating of a fumigation chamber is essential as the minimum temperature required for effective fumigation is 16 to 20°C and the temperature should not rise much above 20°C otherwise the vase life of the flowers may be adversely affected. When domestic heater was used in the experimental fumigation chamber it failed to provide adequate heating when ambient temperatures were 2°C or less.

The temperatures in the chamber during fumigation period was obtained at 15 or 30 min intervals by installing 3 "T" type thermocouples at strategic locations. The thermocouples' positive lead was made of copper and negative lead was made of 55 per cent copper, the remaining being nickel. These sensors can record a temperature range of -200 to 350°C. The thermocouple sensors inside the chamber were sealed with silicon rubber (Silastic®) and covered with small plastic tubes. This is to avoid direct contact of sensors with phosphine because of its corrosive properties and the corroded sensors generally give false readings. These thermocouples were connected to a Datataker 505®, supplied by Data Electronics (Aust) Pty Ltd, Melbourne, externally through an aperture in the wall of the chamber. This aperture was sealed with silicon rubber (Silastic®).

The locations of thermocouples were as follows:

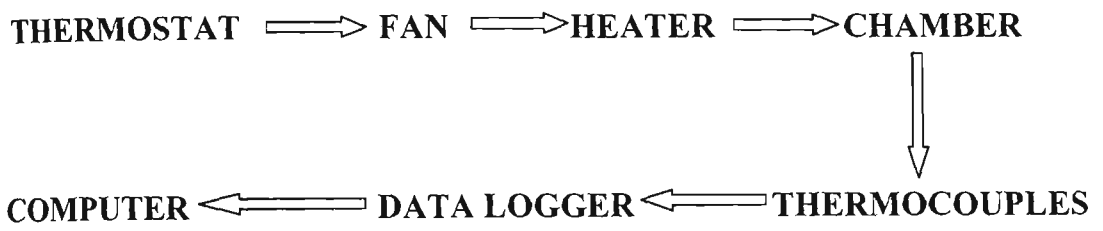
Thermocouple 1 Ambient, just outside the chamber, 300 to 400 mm from the ground, and tied on a 2m pole (sensors hanging freely in the air).

Thermocouple 2 5,800 mm from the heater, 190 mm from the floor (attached near to the corner, front wall of the chamber).

Thermocouple 3 2,200 mm from the heater, 900 mm from the floor (centre, middle tier of the chamber).

Thermocouple 4 2,000 mm from the heater, 180 mm from the floor (middle bottom tier of the chamber).

Schematic representation of the heating system



7.4 Temperature measurements at Frankston

Due to its smaller volume, a 1kW industrial heater was used in all the fumigation trials carried out at Frankston. The heater was mounted on the floor of the chamber (550 mm from the front door, 30mm below the sliding basket), and the sensor was attached semi permanently just below the sliding basket. Hence, this can be moved to different locations of the chamber. The heater was connected to a junction box (capacity of 250V) to supply power, that was fitted next to the heater. This heater was fitted with internal and external switches. The temperature was recorded in every 15 min intervals by placing 1 or 2 temperature recorders (Hobo Data Takers® XT, supplied by Onset Computer Corporation, U.S.A) on the sliding basket among the bioassay insects. These loggers can record a temperature range of -37 to 46°C. A software package called Log Book 2 was used for unloading the stored data from these loggers. A fan was fitted next to the junction box to assist the circulation of the fumigant gases. Hence, a uniform distribution of temperature in the chamber was attained.

7.5 Results

7.5.1 Time required to heat the 27 m^3 chamber using a 1kW heater

The results of the temperature recordings obtained during various seasons of the year are presented in Table 7.1. Time required to reach a temperature range of 17°C or more in the chamber varies greatly and this depends on the ambient temperature at the time of fumigation.

During winter months (especially mid-winter) the 1kW heater necessitates longer times (60 to 255 min) to heat the chamber to the desired level. This may lead to fumigation failures, as the eggs of *T. urticae* require higher temperatures during fumigation for at least 12 h of the total exposure period. If the temperature is less than the recommended one for 240 min or more there are possibilities that not only the developmental stages would survive, but also a small number of mature larvae of the leaf rolling moth can be severely affected.

On the other hand, during late autumn the time required to heat the chamber is comparatively shorter (40 to 60 min), when the ambient temperature is somewhat low. However, this low temperature fumigation did not affect the mortality rate of several insect pests. Except during mid and late summer all other periods require a period of heating the chamber. Generally, the summer ambient temperature reaches 38°C or more, however, the chamber temperature generally remained less than this temperature (26°C). This would probably due to the reflective properties of the coatings or due to the location of the chamber, where it is located in the shade of buildings, and the thermal inertia of the structure.

On the other hand, the temperature was maintained at the desired level during exposure time by this 1kW heater (Figure 7.1). However, on two occasions (early and mid autumn) the temperature in the chamber did not exceed 15°C. In these circumstances some larvae of *S. ejectana* were severely affected and some eggs of *T. urticae* survived despite the

concentration of phosphine and exposure time being lethal to all the other insect pests exposed (Table 7.4 and 7.5). The presence of even a single live or severely affected insect pest among the consignment leads to a complete rejection of the consignment at the ports of importing countries. Hence, in view of these results these fumigations were not successful.

Table 7.1 Time required to heat the 27 m^3 chamber located at Emerald, during different seasons of the year (1995-96), using a 1kW heater.

Season	Ambient temperature (°C)	Temperature in the chamber (°C)	Time required to reach 17°C (in minutes)		
			Average	Standard deviation	Range
Winter June	10.1 (6.4 - 15.2)	18.7 (15.0 - 19.2)	120.0	71.18	60 - 200
July	6.1 (3.1-9.5)	17.9 (14.0 - 18.5)	168.75	91.13	90 - 255
August	7.2 (4.5 - 8.2)	18.0 (14.1 - 20.1)	159.75	74.78	70 - 240
Spring September	8.1 (7.2 - 11.3)	18.4 (15.5 - 20.8)	125.0	26.45	60 - 150
October	15.5 (10.9 - 18.5)	18.0 (15.1 - 18.8)	22.5	6.4	15 - 30
November	16.5 (11.5 - 20.8)	18.1 (15.0 - 18.9)	7.5	6.45	0 - 15
Summer December	18.2 (12.6 - 25.3)	18.4 (15.6 - 19.9)	4.5	3.87	0 - 9
January	21.6 (14.1 - 27.0)	25.5 (22.9 - 28.9)	0	-	0
February	20.4 (7.2 - 28.9)	21.0 (18.3 - 23.1)	0	-	0
Autumn March	18.4 (6.5 - 26.2)	21.7 (14.7 - 27.2)	4.25	0.95	3 - 5
April	14.4 (5.4 - 18.4)	17.4 (14.4 - 24.1)	26.0	11.43	14 - 40
May	12.1 (5.2 - 16.4)	18.7 (12.6 - 19.9)	52.5	9.57	40 - 60

The temperatures at the strategic locations of the chamber are uniform (Figure 7.1). This is likely to be a result of the excellent mixing of the heated air. Generally, the thermocouple (Thermocouple 1) located next to the door recorded 0.5 to 1°C higher than all other thermocouples, and this could be due to its location (attached to the wall) near to a poorly insulated wall. Comparatively lower temperatures were recorded in the centre, 190 mm above from floor of the chamber (Thermocouple 3). However, this temperature was above the recommended level.

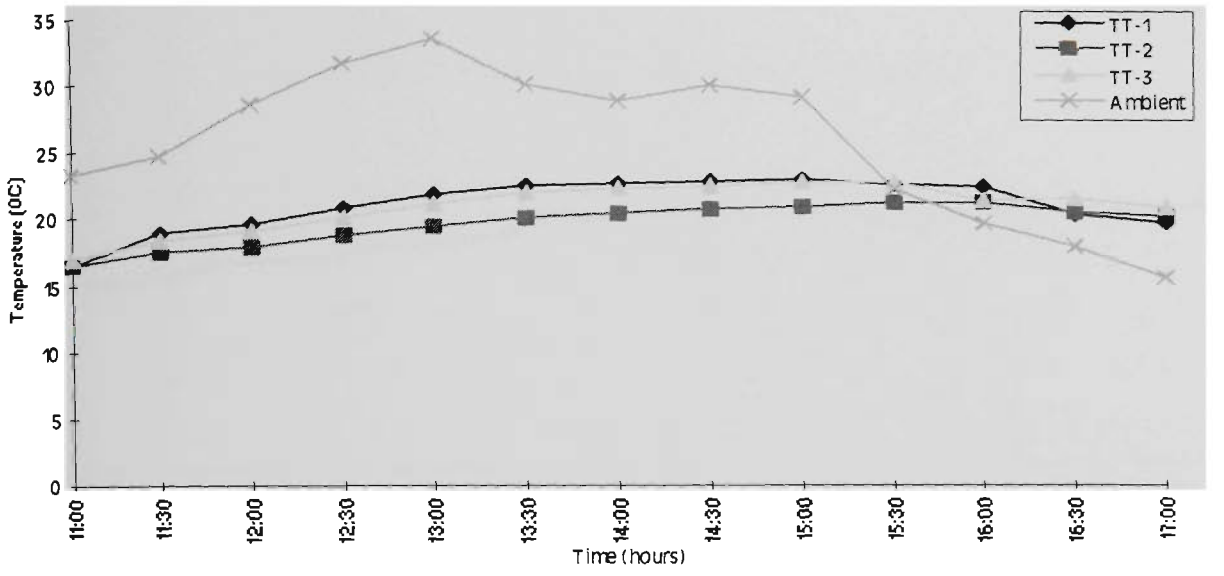


Figure 7.1 Temperature distribution in the chamber (27 m^3) at different locations using a 1kW heater during summer (19/10/1995).

Figure 7.2 clearly indicates that during winter times the 1kW heater failed to raise the temperature above 15°C. This temperature is sublethal to certain insect pests and fumigation failure is highly possible.

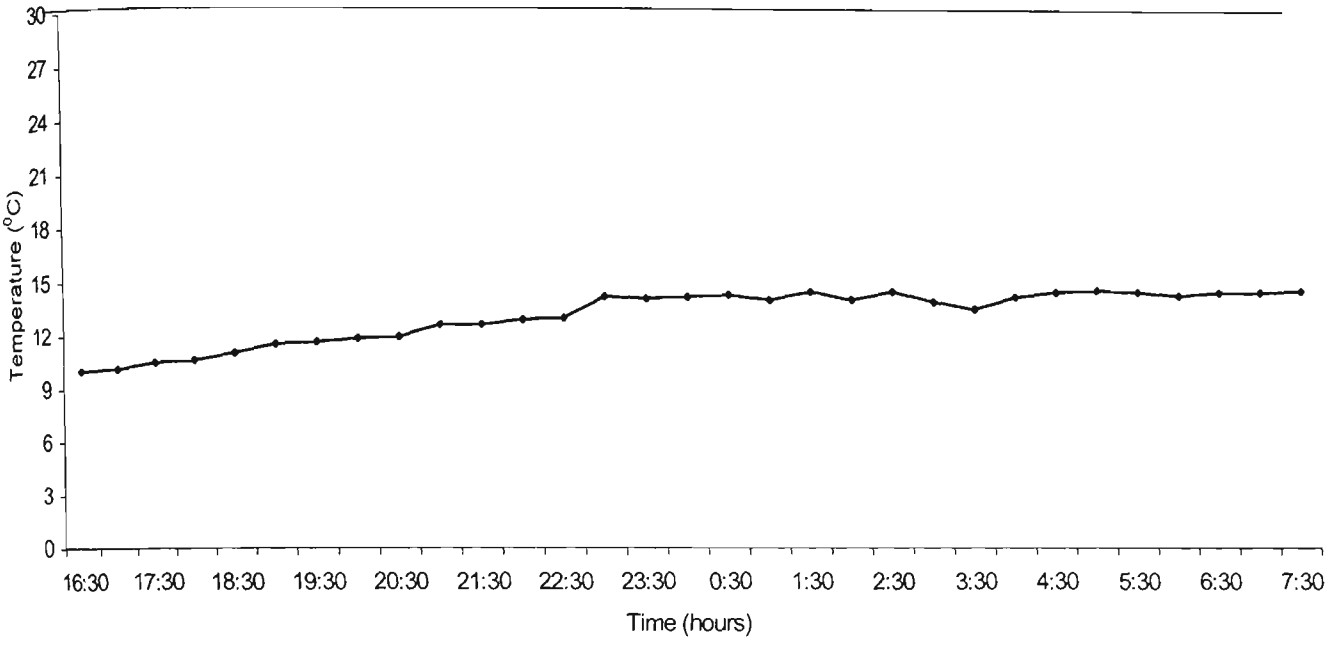


Figure 7.2.Temperatures resulting from a 1kW heater in a 27 m^3 chamber (location among the flowers) on 18/3/96.

It is clear from Figure 7.3, the 1kW heater failed to maintained the temperature at the desired level in the chamber during fumigation. The temperature during first five hours of fumigation was above 18°C, however, the temperature fell slowly and reached to a minimum of 10°C.

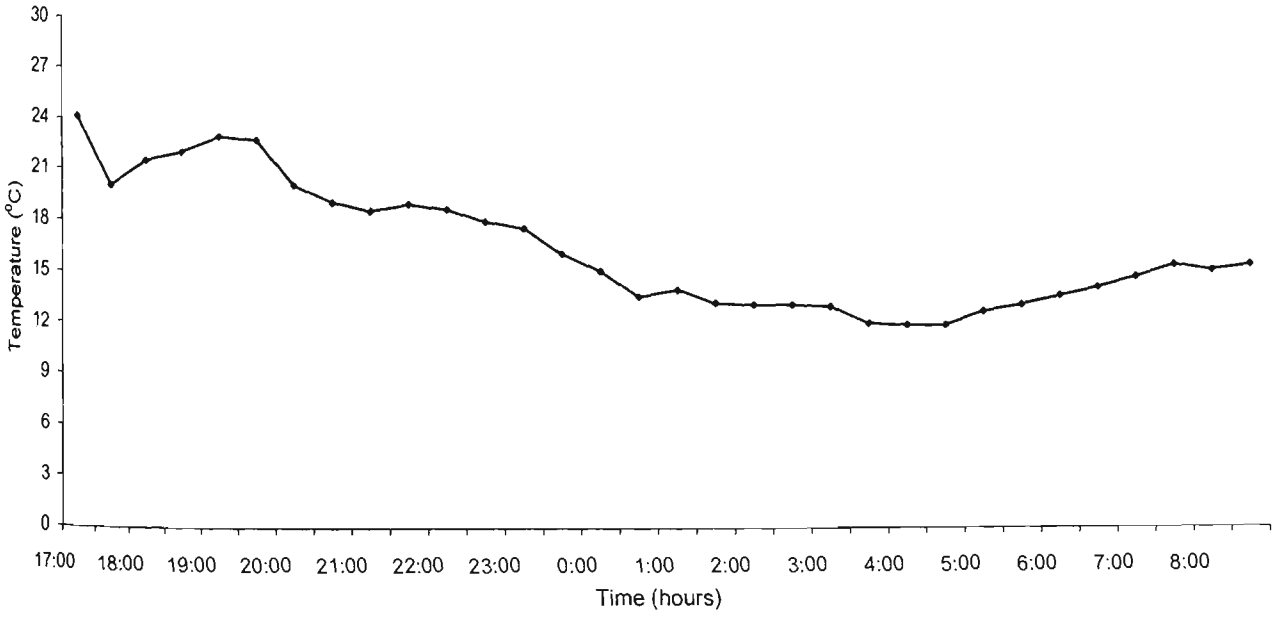


Figure 7.3 Temperatures resulting from a 1kW heater in a 27 m^3 chamber (location among the flowers) on 17/3/97.

7.5.2 Time required to heat the 27 m^3 chamber using a 3kW heater

Compared to the 1kW domestic heater, the 3kW industrial heater warms the chamber rapidly, except in mid winter where 20 to 30 min are sufficient to reach the desired level (Table 7.2). Moreover, most of the time, the temperature was maintained very close to the desired level (18°C or more) during exposure, without over heating the chamber. However, in January and February the temperature in the chamber reached more than 25°C. As mentioned in the previous section (Section 7.6.1), due to its white coating and location the influence of high temperature on the chamber would be minimal. Figure 7.4 indicates that the time required to reach 18°C or more is 4 h and the temperature was maintained at this level during fumigation.

Table 7.2 Time required to heat the 27 m^3 chamber located at Emerald during different seasons of the year, using a 3kW industrial heater.

Season	Ambient temperature (°C)	Temperature in the chamber (°C)	Time required to reach 17°C (in minutes)		
			Average	Standard deviation	Range
Winter June	12.1 (5.4 - 16.2)	18.0 (10.6 - 20.1)	14.5	3.31	10 - 18
July	7.5 (4.6 - 11.7)	18.0 (10.5 - 20.1)	23.0	3.55	20 - 30
August	8.1 (4.9 - 12.0)	18.1 (9.6 - 20.8)	11.25	7.5	5 - 25
Spring September	12.1 (4.1 - 19.7)	18.4 (11.5 - 20.8)	7.5	5.25	3 - 15
October	14.4 (6.9 - 18.5)	18.0 (13.1 - 18.8)	5.25	2.87	2 - 9
November	15.9 (12.1 - 20.5)	18.2 (12.1 - 20.2)	2.25	2.62	0 - 5
Summer December	20.5 (14.5 - 24.4)	18.4 (15.6 - 19.9)	0.5	1.0	0 - 2
January	21.3 (12.1 - 28.1)	25.5 (22.9 - 28.9)	0	-	0
February	22.8 (11.5 - 27.9)	26.5 (22.1 - 33.4)	0	-	0
Autumn March	19.8 (9.9 - 24.0)	21.5 (18.1 - 19.9)	0	-	0
April	16.5 (7.2 - 19.8)	18.9 (12.1 - 19.9)	2.5	2.88	0 - 5
May	10.5 (4.5 - 12.6)	17.8 (13.2 - 18.8)	11.5	3.78	6 – 14

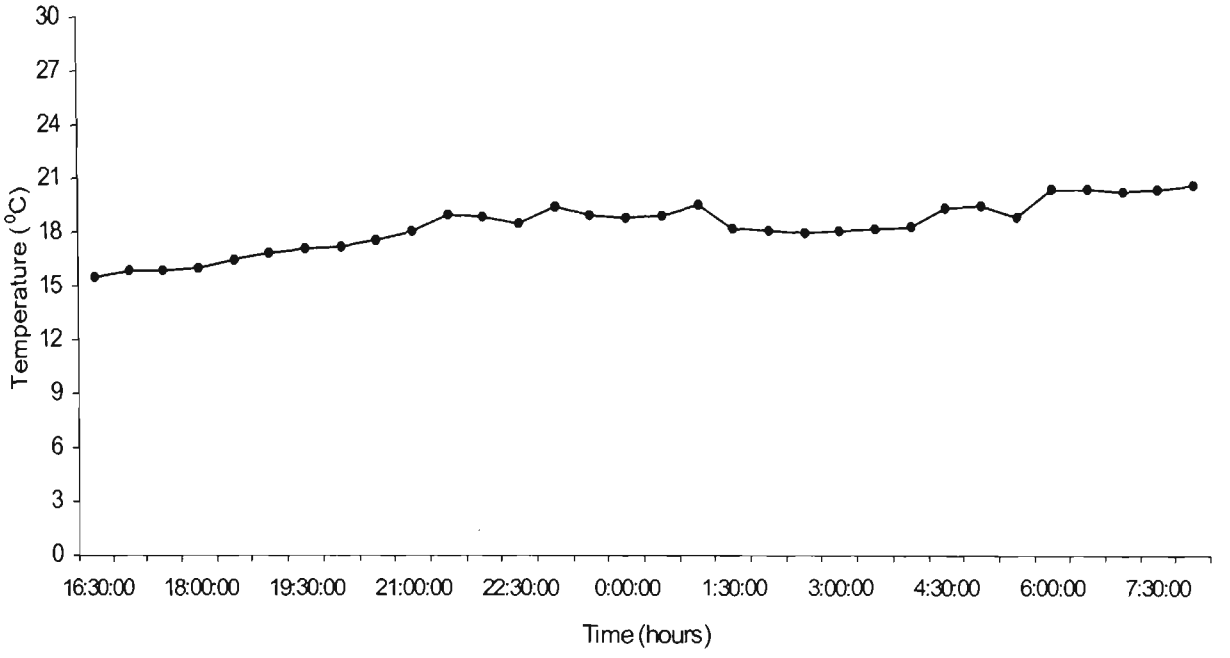


Figure 7.4 Temperature distribution (thermocouple located among the flowers) in the chamber ($27m^3$) during exposure, using a 3kW industrial heater on 30/9/96.

7.5.3 *Time required to heat the 900 L chamber using 1kW heater*

The temperature rose rapidly (0 to 14 min) to 17°C or above and heating was rapid during all seasons of the year, in spite of lower ambient temperatures (Table 7.3). This is due to the small volume of the chamber and its low thermal capacitance. The average time taken to heat the chamber to the required level was 10.7 min (range of 2 to 14 min) during mid winter, when the ambient temperature fell below 14.7°C (range of 10 to 18°C) (Table 7.3). At all other periods of the year, the heating time required was insignificant. Similar to other heaters, during summer months the initial heating period was not necessary, as the chamber was already heated above the required level. However, the overnight ambient temperature during most part of the year fell to less than 10°C, but the chamber temperature was maintained at the desired level during exposure time. Hence, this results suggest that a heater is essential all seasons of the year.

Table 7.3 Heating time of 1kW heater in a 900 L steel chamber, during various periods of the year (1996-97).

Season	Ambient temperature (°C)	Temperature in the chamber (°C)	Time required to reach 17°C or more (minutes)		
			Average	Standard deviation	Range
Winter June	11.2 (3.3 - 12.3)	18.3(16.7-19.9)	9.14	1.27	2 - 12
July	9.3 (2.1 - 11.7)	17.7(14.9-20.5)	10.7	1.8	9 - 14
August	12.6 (4.2 - 14.4)	18.5 (16.7-20.59)	6.14	1.57	4 - 9
Summer February	24.6 (18.1 - 27.9)	23.9 (18.1 - 34.8)	0	-	0
Autumn March	20.7 (12.6 - 24.0)	22.5 (19.2-32.4)	0	-	0
April	17.1 (9.0 - 20.7)	21.1(18.8-25.5)	1.71	1.11	0 - 3
May	11.7 (5.7 - 14.0)	21.1(18.8-25.5)	4.4	1.27	2 - 6

The Figure 7.5 indicates that 1kW heater is sufficient to heat the chamber and maintain the temperature at the desired level during fumigation.

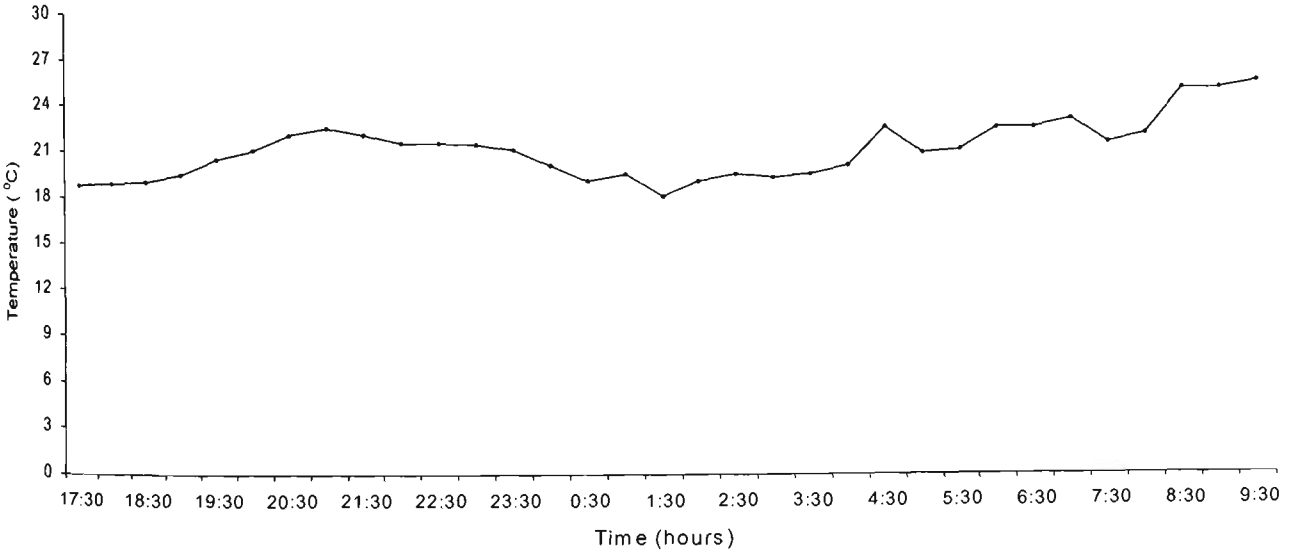


Figure 7.5 Temperature distribution in the chamber (900 L) during exposure, using a 1kW industrial heater on 9/4/97.

7.5.4 *Effects of temperature on the mortality of bioassay insects*

7.5.4.1 *Larvae of the leaf rolling moth Strepsicrates ejectana*

Response of the larvae of leaf rolling moths to various temperatures (13.1 to 17.4°C), fumigated with various concentrations of pyrethrum (4.4 to 4.6 $g.m^{-3}$) and phosphine (0.28 to 1 $g.m^{-3}$), and various exposure times (15 to 15.5 h) are presented in Table 7.4. The data from this table clearly illustrates the lethal effects of high temperature on insects. A temperature of 15.5°C or below was not lethal to all of the larvae exposed, regardless of concentrations of fumigants and exposure time.

Table 7.4 Lethality of pyrethrum-phosphine to larvae and pupae of *S. ejectana* at different temperatures.

Date	Pestigas® (g)	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Leaf rolling moth *Strepsicrates ejectana*				
		Initial	Final			Larvae			Pupae	
						S. aff	Dead	Total	Dead	Total
18/3/96	140	1.0	1.0	15.5	13.1 (10.0-14.6)	1*	24	25	0	0
2/4/96	160	1.0	0.9	15.0	13.8 (10.5-15.0)	3*	12	15	4	4
18/7/96	120	0.28	0.18	15.25	17.4 (10.1-20.3)	0	14	14	14	14
10/7/96	200	0.28	0.19	15.0	15.6 (10.0-18.7)	0	59	59	0	0

* Sixth instar larvae

7.5.4.2 *Eggs of the two-spotted mite Tetranychus urticae*

The toxicity of phosphine to eggs, larvae, and adults of *T. urticae* at various temperatures, and exposure times are presented in Table 7.5. Survival of a small number of young eggs (one to three days old) at lower temperatures give firm evidence that phosphine is lethal only if the temperatures are high enough. These results suggest that a temperature of 15.6°C killed all the exposed eggs. The higher temperature presumably would have increased the diffusion of toxic gases into the eggs through the chorion, consequently all

the exposed eggs died. In other two fumigation where the eggs were exposed to comparatively higher phosphine concentrations (1 $g.m^{-3}$) and slightly longer exposure time (15.5 h, fumigated on 18/3/96) and the same exposure time (15 h, fumigated on 2/4/96) failed to kill all the exposed eggs. It is clear from this table, in both these fumigations, only the temperature was maintained below 15.6°C, and it can be concluded that the temperatures of 13.8°C or less are not toxic to the exposed eggs.

Table 7.5 Lethality of pyrethrum-phosphine to eggs of *T. urticae* at different temperatures.

Date	Pyrethrum gm^{-3}	Phosphine concentration gm^{-3}		Exposure time (h)	Temperature (°C)	Twospotted mite *Tetranychus urticae*						
		Initial	Final			Eggs			Larvae		Adults	
						Live	Dead	Total	Dead	Total	Dead	Total
18/3/96	140	1.0	1.0	15.5	13.1(10.0-14.6)	5*	1138	1143	426	426	572	572
2/4/96	160	1.0	0.9	15.0	13.8(13.5-15.0)	36*	1463	1499	438	438	623	623
10/7/96	200	0.28	0.19	15.0	15.6(12.0-18.7)	-	1047	1047	276	276	993	993

* - Young eggs (one to three days old).

Furthermore, all the hatched eggs developed in to normal larvae, and development proceeds similar to the unfumigated individuals. However, the eggs took seven to nine days to hatch more than the unfumigated ones, presumably phosphine may have delayed or arrested the development for a certain period. On the other hand, all the unfumigated 1 to 2 day old eggs hatched within two to five days under similar conditions (25°C and 40 to 45 per cent relative humidity). Moreover, the fecundity of the surviving individuals was not affected and this was described in detail in Chapter 5. Although, the temperature of the 2/4/97 trial was comparatively higher than the 18/3/96 trial, a large number of eggs survived in the later trials. This could be due to the effects of comparatively long exposure periods maintained at the 18/3/96 fumigation.

7.6 Discussion

7.6.1 Heating efficiency of 1kW and 3kW heaters

The distribution, penetration, and diffusion of fumigant chemicals are influenced by temperature. Generally, higher temperatures increase the penetrative capacity, and molecular diffusion coefficients of fumigants; properties which are essential for a successful fumigation process. Higher temperatures favour uniform distribution of fumigants without stratification. On the other hand, lower temperatures favour sorption, which reduces the availability of toxic gases to the insects. The larvae of leaf rolling moth construct webbing shelters from the second instar stage, using 3 to 90 individual leaves. These leaves are joined together by silken threats secreted by the larvae. Hence, these webbing shelters are compact and fumigants require higher penetrative capacity to reach and act the sites of larvae.

The heater was equipped with an on/off controller, hence, the desired temperature was maintained without over or under heating the chamber (Table 7.1). Moreover, the temperature distribution studies show that uniform distribution of temperature in all the locations of the chamber was achieved. This is a result of turbulent flow of the recirculating air and fumigant mixture, which increases the effective thermal diffusivity by several orders of magnitude. Hence, a suitable recirculation fan is essential to distribute the temperature and fumigants in the chamber. Moreover, the turbulence plays an important role in distributing the temperature in eddies to the corners of the chamber. Over-heating in certain parts of the chamber and wide swings in temperature distribution would result if there is no proper distribution. Although the summer chamber temperature was higher than the recommended temperature, the temperature in the chamber during fumigation was lower than the ambient temperature, possibly as a result of transpiration which imposes a latent heat load on the chamber. Also, this chamber was placed under a shed that prevents the sun's rays impinging directly on the chamber. Higher temperatures

of 21°C or above are not conducive for a long vase life of all the foliage and flowers (wild flowers) exposed and this has been discussed in detail in Chapter 6.

Of the two heaters used, the 3kW industrial heater delivered the required temperature more rapidly than the 1kW industrial heater. Also, the 3kW heater maintained the temperature of the chamber at the desired level during all seasons of the year. The 1kW heater failed to maintain the temperature during certain periods of the year and in this circumstance a small number of insect pests were survived or severely affected. The 1kW industrial heater used in the 900 L chamber is sufficient to raise and maintain the required temperature during all seasons of the year.

7.6.2 Effects of temperature on toxicity of phosphine to bioassay insects

The results show that at sub optimum temperatures (13 to 14°C) young eggs of *T. urticae* are difficult to kill, even at longer exposure times, and higher concentrations of phosphine that were lethal to other insect pests, and their developmental stages. The relative tolerance of eggs of various insect pests to phosphine fumigation is discussed in detail in Chapter 2, Section 2.8. In these trials all the mature (four to six days old) eggs died and a small number of young eggs had survived. This supports the findings of Lindgren and Vincent (1966) and Howe (1973). Howe (1973) pointed out the relative susceptibility of mature eggs to typical phosphine fumigations compared with young eggs coincide with the formation of more or less completely developed larvae within the chorion. Hence, the metabolism of the mature egg is similar to that of larvae, hence the absorption and subsequent toxicity are comparatively higher than on young eggs. Moreover, the mid egg development period in which blastoderm is forming is also vulnerable to moderate levels of phosphine concentration.

On the other hand, complete mortality of most of the active stages indicate that the fumigation was successful. However, once a consignment of flower reaches importing

countries (which requires two to three days or longer) there are chances that eggs may hatch and the consignment will be rejected due to the presence of live insects. Moreover, control failures due to lower temperature have been reported by several authors. They include studies on acarid mites (*Tyrophagus putrescentiae* and *Caloglyphus berlesei* (Mich) by Jalil *et al.* (1970), on *T. confusum* studied by Kenaga (1961) and on *T. confusum*, *S. oryzae* and *S. granarius* by Lindgren and Vincent (1966).

Except for the larvae of the leaf rolling moth, active stages of all other insect species are highly susceptible in the temperature range considered. A small number of these larvae (fifth and sixth instar stages) were severely affected and this suggests that the temperature that is lethal varies substantially among different species of insets and their developmental stages.

It is quite clear from the results that there are considerable variations in metabolism of insects at different temperatures and based on temperatures, the rate of development also vary i.e. rapid, slow or nil. Generally, lower temperatures reduce the metabolism and retard the development of active as well as developmental stages. Similarly, the enzyme reactions of insects depend on the temperature: at lower temperatures these reactions are considerably slowed (Fields 1992). As pointed out in the literature review, the site of action of phosphine is initially on cytochrome oxidase (Kashi 1981) and then on the nervous system. Hence, for phosphine to be toxic to insects these enzymes must be active. Furthermore, normally the insects are active at higher temperatures and while they are active, the spiracles are open fully thus allowing more diffusion of respiratory gases. However, while in a resting position or at lower temperatures the tracheal valves are almost closed, therefore, allowing less toxic gases into the system. From these trials it was observed that higher temperatures are not only lethal to active stages but also to inactive stages such as eggs and pupae. Moreover, lower phosphine concentrations are sufficient for a complete kill of a range of insect pests and its developmental stages at higher temperatures. This would be due to the fact that at higher temperatures the penetration and diffusive capacity of fumigants are generally more.

In brief, the rate of volatility and absorption of a fumigant by an insect is influenced by temperature. From these results it can be concluded that the detoxifying process, that protect the insects partially or wholly from the toxic effects of phosphine decline rapidly as the temperature increases. At lower temperatures the uptake of toxic fumigants will be relatively low requires either longer exposure time or higher phosphine concentration. However, this will not always give a complete control of certain species of insects and its developmental stages.

Chapter - 8

Distribution of phosphine in a 27 m^3 chamber at Emerald

Chapter 8

Distribution of phosphine in a 27 m^3 fumigation chamber at Emerald

8.1 Scope of the experiments

One of the critical factors that determines the success of a fumigation process is the requirement that sufficient concentrations of phosphine are maintained in the chamber during exposure. The volume of gas in the chamber is considered to be a non-perfectly mixed air space. Hence, there are possibilities that under or over exposure of toxic gases in certain parts of the chamber due to poor mixing of the fumigant. This necessitates monitoring the distribution of phosphine at various locations of the chamber, at various time intervals.

Exposures to sublethal or high concentrations of phosphine may either lead to the survival of insect pests that may elicit resistance to phosphine or narcotise the insects. In the past two decades a number of fumigants have been phased out either due to their ineffectiveness against insects, or their harmful effects on human beings and/or to the environment. Recently methyl bromide has been added in this list due to its ozone depleting potential. Research evidence shows that phosphine is one of the efficient alternatives to methyl bromide, provided if it is used judiciously. It is unlikely that any alternative fumigant or fumigant mixture to phosphine will be found in the near future, hence, the need to preserve this fumigant becomes imperative. However, already there are reports that some insect species are developing resistance towards phosphine (Price and Mills 1988) either due to high or low phosphine concentrations. The amount of toxic gas required in a particular atmosphere to kill every individual of an insect population is important in determining dosage required for individual species and developmental stages.

Hence, utmost care must be taken to maintain the required concentrations of phosphine during exposure.

The majority of the flower exporters use either weighing scales or a timer to deliver fumigants into the chamber; they seldom measure the concentration of phosphine in the chamber during fumigation. When a predetermined mass of Phosfume® or timed dose is delivered either using a timer or scale based on the volume of the chamber, there are possibilities that either the expected concentration of phosphine will not be achieved. Higher concentrations of phosphine not only affect the mortality of the exposed insects but also reduce the vase life of the exposed flowers. Similarly, lower than recommended concentrations may not kill all the exposed individual of an insect population.

From a practical point of view, growers and distributors of wildfowers need to be able to manage fumigations simply, but effectively. For this reason experiments to investigate the following were designed to:

- Distribution of phosphine at strategic locations of the chamber (27 m^3) at various intervals using phosphine detection tubes (Dräger® 0 to 4 or 50 to 1000ppm);

- Determine the concentration of phosphine achieved in the chamber by using different methods of delivery (using a timer and a weighing scale);

- Compare the amount of active ingredient (phosphine) remaining in the chamber at the end of exposure during both short and long exposure fumigations; and

- The gas holding capacity of the chamber.

8.2 *Materials and methods*

Phosphine concentration in the chamber was measured by installing six semi rigid nylon gas lines that has an internal diameter of 2 mm. The gas lines were installed in the following order:

Gas line 1: 3800 mm from the fumigant delivery point, 100 mm from the floor and on the side wall of the chamber (near to the door) where the delivery nozzle was fitted.

Gas line 2: 1500 to 2000 mm from the delivery point, 900 mm from the floor among the flowers and near to the side wall that is fitted with the delivery nozzle.

Gas line 3: Similar to gas line 2, but just outside the flowers.

Gas line 4:2000 to 2200 mm from the delivery point among the flowers and 180 mm from the floor.

Gas line 5: Similar to gas line 4, but just outside the flowers.

These lines (that were between 6000 and 7200 mm long) were connected to a multiport valve fabricated from stainless steel. The multiport valve (30 valves) was located external to the fumigation chamber, and the gas lines were led through an aperture in the rear wall of the chamber. This valve enabled the gas lines to be sampled sequentially by opening and closing the appropriate taps fitted to a manifold. Each tap was fitted with a filter that prevents the entry of dirt or foreign material that may block the sampling ports. Six of these ports were connected with the gas lines and all other lines were closed. On top of the equipment there were two 5 mm internal diameter air inlet ports to purge this equipment once the gas sampling was completed.

A 10 mm internal diameter outlet was fitted to the base of the equipment that acts as an exhaust port. A semiflexible polyethylene tube (length of 200 mm with an internal diameter of 7 mm) was fitted to the exhaust port of the multiport valve. This tube was connected to the inlet of a compressor pump (240V, 50Hz) supplied by Dynavac Engineering, Melbourne, Australia. The exhaust port of the pump was connected to a similar size polyethylene tube (internal diameter of 10 mm) and placed 5000 mm away from the gas sampling point and 1500 mm from the floor.

Phosphine concentration measurement: Concentration of phosphine in the chamber was obtained by purging the gas lines. Purging was carried out by pumping gas from the gas line for two or three minutes which had been found adequate to calibrated the gas lines and multiport equipment.

Phosphine concentrations were obtained 10 min (generally it requires 5 to 7 min to distribute uniformly in the chamber following administration) after the release of phosphine. For short exposure periods (4.5 to 6 h), concentrations were measured in every 30 to 45 min following the initial measurement until the end of the exposure period. For overnight fumigations (14 h or more), phosphine concentrations were obtained 10 min following the release of Phosfume® and just prior (1 to 2 min) to the end of exposure period. This procedure was followed due to the constraints in the after hours access to the fumigation site.

Distribution of phosphine was assisted by installing a heavy-duty industrial fan on the rear wall of the chamber in an enclosure, and this is discussed in detail in Chapter 4, Section 4.2.3.

The mass of Phosfume® required to give a desired concentration of phosphine in the chamber was calculated as follows;

Calculation; $\mathbf{W = Y / Z \times X}$

where; **W** = concentration of phosphine required (in parts per million).

Y = proportion of active ingredient (phosphine) in the Phosfume® cylinder.

Z = volume of chamber (m^3).

X = mass of Phosfume® required (kg).

Hence, $\mathbf{X = W \times Z / Y}$

The quantity of phosphine added into the chamber is based on the predetermined weight or a timer to give the desired concentration of phosphine. However, it is not always possible to obtain the required concentration of phosphine due to a number of reasons, such as the amount of gas in the cylinderor ambient temperature). Hence, as part of this work a correlation was established based on the weight of gas in the cylinder, ambient temperature, mass of Phosfume® released into the chamber and the resulting concentration of phosphine in the chamber. The initial weight of the cylinder was measured by placing the cylinder on a digital scale (capacity of 150 kg) and the mass of Phosfume® delivered (by switching on the timer for a predetermined time of 1 to 10 sec of multiple bursts). The amount of Phosfume® delivered was calculated by deducting the final weight from the initial weight of the cylinder. Ten minutes following this, the phosphine concentration in the chamber was determined using phosphine detector tubes (Dräger®, supplied by Dräger Australia, Melbourne) as this would give a fairly accurate estimate of the concentration of phosphine achieved in the chamber. If the required concentration of phosphine was not achieved, an additional mass of Phosfume® was delivered into the chamber and the concentration was measured 5 to 7 min later.

To be effective, fumigation must be conducted in a gas-tight chamber, otherwise the toxic gases may escape into atmosphere resulting in fumigation failure. Hence, pressure decay tests were conducted periodically to test the gas tightness of the chamber. Once the door and vent of the chamber were closed the gas lines were connected to the multiport valve.

The exhaust port of the multiport equipment was connected to a manometer supplied by the Dwyer Control Gages, USA. This manometer measures pressures up to 254 mm of water guageThe indicator was red gauge oil with a specific gravity of 0.826).

The chamber was pressurised by using a domestic vacuum cleaner. Once the required pressure was reached (indicated by the rise in oil level from the manometer, generally within two to three minutes) the vacuum cleaner was switched off. The pressure drop and the time taken for the drop were recorded using the manometer and a stopwatch. If the manometer readings indicated that the gas loss was rapid, the chamber was sprayed with concentrated soap water. This was carried out while the chamber was in the pressurised state, and the formation of gas bubbles of soap water from the chamber clearly indicated the points of the leaks. The identified leaks were sealed with Silastic® (silicon rubber) and allowed to cure for 10 min in hot weather (18°C or above) or 5 to 7 h in cool weather (15°C or less).

8.3 Results

Concentrations of phosphine (parts per million) achieved in different locations of the chamber during several fumigations are presented in Table 8.1. Data from this table clearly indicate that the concentration of phosphine achieved in the chamber varies considerably, depending on mass of Phosfume® in the cylinder. The amount of phosphine achieved is positively correlated with the weight of the cylinder. Generally, if the cylinder is 54 kg or above it releases comparatively more active ingredient (phosphine), hence comparatively shorter releasing time is sufficient. Moreover, the ambient temperature also determines the amount of active ingredient delivered into the chamber ie. higher the temperature, the higher the quantity of phosphine released into the chamber. This is presumably the result of higher pressures associated with higher temperatures. Hence, it is clear that the amount of phosphine released by predetermined time did not give the expected concentration of phosphine.

Table 8.1 Concentration of phosphine (ppm) achieved in the 27 m^3 chamber at Emerald by using predetermined time and mass of Phosfume®.

Date	Ambient temperature (°C)	Weight of cylinder (kg)	Time (sec) estimated*	Time added (sec)	Mass of Phosfume added (kg)	Concentration of phosphine (ppm) in the chamber
12/2/96	16.5	63.06	95	97	2.94	690
19/2/96	22.5	60.8	95	112	2.18	700
26/2/96	24.5	58.96	90	108	2.04	610
18/3/96	14.9	56.76	95	90	2.048	690
25/3/96	16.7	54.58	95	115	2.02	650
2/4/96	15.0	52.92	95	390	2.88	700
29/8/96	13.5	67.12	30	90	0.92	190
4/9/96	12.7	66.28	30	80	0.92	200
9/9/96	11.0	65.28	25	60	0.56	110
18/9/96	16.0	64.66	30	50	0.7	170
23/9/96	14.0	64.02	30	40	0.6	160
30/9/96	12.0	63.42	25	70	0.8	110
7/10/96	16.0	61.6	25	70	0.7	240
16/10/96	16.0	61.02	25	50	0.7	110
30/10/96	16.0	60.28	25	60	0.72	110
12/11/96	15.5	59.5	25	35	0.82	120
13/11/96	20.5	57.58	30	35	0.9	160
4/12/96	21.6	49.14	60	1190	1.0	360
30/12/96	18.0	56.62	25	60	0.76	120
14/1/97	30.0	54.6	60	120	1.02	320
23/1/97	24.0	52.76	15	250	1.34	150
17/2/97	30.0	50.32	30	160	0.7	320
26/2/97	19.0	49.8	20	150	0.58	210
1/5/97	14.0	48.58	20	200	1.6	190
14/5/97	12.0	74.68	15	70	0.66	150

*- These are the estimates made by a commercial grower, and they would be used in practice.

Statistical analysis:

Various factors (weight of the cylinder, time added and mass added) influencing the amount of phosphine gas attained in the chamber was analysed using Genstat 5. Relationship among different variables is given in the correlation matrix. The most important factor that influencing the amount of phosphine gas delivered in the chamber is given in the regression coefficients.

Correlation matrix

ambtemp	1.000			
wtcyl	-0.559	1.000		
timeadd	0.225	-0.483	1.000	
massadd	0.085	-0.224	0.130	1.000

	ambtemp	wtcyl	timeadd	massadd

Estimates of regression coefficients

	estimate	s.e.	t(23)
Constant	2.68	1.35	1.99
wtcyl	-0.0251	0.0227	-1.10

The statistical analysis indicates that the amount of active ingredient achieved in the chamber was positively correlated with the weight of the cylinder. The regression coefficient indicates that $e^{-0.0251} = 0.97$, ie for every kg decrease in weight of phosphine; odds of concentration of phosphine attained in the chamber increased by a factor of 0.97.

Tables 8.2 to 8.7 show the concentrations of phosphine recorded at various intervals and locations on various days from the chamber during short exposure fumigations. Generally, higher concentrations of phosphine were recorded in the initial sampling (10 min following release) and the concentration reduced gradually as time progressed. However, the desired concentration of phosphine was maintained during exposure. Phosphine in general is not sorbed by the commodities or the chamber: hence, the reason for the small reduction would be either leakage or breakdown due to higher temperature. The results indicate that there was continuous loss of phosphine from the chamber and the rate of loss depends on the size and number of leaks.

Occasionally, Gas line - 1 (near to the door), 2 (inside the flowers on the top tier of the trolley) and 4 (among the flowers lower tier of the trolley) of the chamber recorded lower initial concentrations than the middle or final readings (Table 8.3 and 8.4). One possible reason for this could be that Phosfume® was stored in sub zero temperatures, hence, immediately after release and to a certain time phosphine might have sunk to the bottom of the chamber (stratification). Once these gas was heated to the chamber temperature, it began to diffuse through the chamber, hence, higher than initial recordings are likely. From these tables it is apparent that there was no considerable differences of phosphine concentrations that were recorded among different locations of the chamber.

Generally, the lowest concentrations of phosphine were recorded from Gas line 4, which was located near to the floor. Also, there are possibilities for comparatively lower phosphine concentrations in the corners or wall of the chamber due to leakage in which the air dilutes the phosphine, or phosphine does not reach these sites due to the circulation pattern or eddies.

Table 8.2 Concentration of phosphine (in parts per million) recorded at different locations of the chamber at different times fumigated on 2/2/95.

Time	Temperature (°C)	Concentration (ppm)	Average (ppm)
Gas line - 1			
11.30	28.0	680	
13.35	27.0	620	640.0
14.50	25.0	610	
Gas line - 2			
11.35	28.0	720	
13.40	27.5	650	660.0
14.55	25.0	600	
Gas line - 3			
11.40	28.0	600	
13.45	27.0	600	610.0
14.59	25.0	610	
Gas line - 4			
11.50	28.0	590	
13.50	27.0	590	570.0
15.03	25.5	530	
Gas line - 5			
12.00	28.0	700	
13.55	27.0	590	630.0
15.06	25.5	600	

Table 8.3 Concentration of phosphine (in parts per million) recorded at different locations of the chamber at different times fumigated on 26/10/95.

Time	Temperature (°C)	Concentration (ppm)	Average conc.(ppm)
Gas line - 1			
11.50	20.5	650	
13.10	21.5	600	600.0
15.30	22.5	550	
Gas line - 2			
11.56	21.5	700	
13.15	22.5	780	770.0
15.36	23.5	850	
Gas line - 3			
12.06	21.5	700	
13.25	22.5	650	650.0
15.46	23.5	600	
Gas line - 4			
12.16	21.5	650	
13.30	22.5	600	620.0
15.50	23.5	600	
Gas line -5			
12.26	21.5	720	
13.45	22.5	700	690.0
15.56	23.5	650	

Table 8.4 Concentration of phosphine (in parts per million) recorded at different locations of the chamber at different times fumigated on 30/3/95.

Time	Temperature (°C)	Concentration (ppm)	Average conc.(ppm)
Gas line - 1			
10.55	21.0	850	
13.55	25.0	830	860.0
14.56	26.5	900	
Gas line - 2			
10.58	21.0	900	
14.00	25.0	860	860.0
15.05	26.5	800	
Gas line - 3			
11.04	21.0	900	
14.10	25.5	880	890.0
15.11	26.5	870	
Gas line - 4			
11.10	21.0	800	
14.15	25.5	900	840.0
15.17	26.5	820	
Gas line -5			
11.18	21.0	900	
14.20	26.0	890	870.0
15.22	26.5	820	

Table 8.5 Concentration of phosphine (in parts per million) recorded at different locations of the chamber at different times fumigated on 26/10/95.

Time	Temperature (°C)	Concentration (ppm)	Average (ppm)
Gas line -1			
9.50	24.0	550	
10.55	25.0	520	530.0
11.00	21.5	500	
Gas line - 2			
9.55	28.0	720	
11.10	27.5	700	790.0
12.15	24.5	600	
Gas line - 3			
10.00	28.0	650	
11.25	25.0	600	620.0
12.29	24.5	600	
Gas line - 4			
10.10	28.0	700	
11.30	27.0	650	650.0
12.33	24.5	600	
Gas line - 5			
10.30	28.0	700	
11.55	25.0	690	660.0
12.46	24.5	600	

Table 8.6 Concentration of phosphine (in parts per million) recorded at different locations of the chamber at different times fumigated on 17/11/95.

Time	Temperature (°C)	Concentration (ppm)	Average (ppm)
Gas line - 1			
9.30	18.0	1020	
10.35	17.0	1000	990.0
12.50	15.5	950	
Gas line - 2			
9.35	18.0	1050	
10.40	17.5	1000	990.0
12.55	14.5	900	
Gas line - 3			
9.40	18.0	900	
10.45	15.0	850	850.0
12.59	14.5	790	
Gas line - 4			
9.50	18.0	590	
10.50	17.0	590	570.0
13.03	14.5	530	
Gas line - 5			
12.00	28.0	700	
13.55	25.0	590	630.0
15.06	24.5	600	

Table 8.7 Concentration of phosphine (in parts per million) recorded at different locations of the chamber at different times fumigated on 10/11/95.

Time	Temperature (°C)	Concentration (ppm)	Average (ppm)
Gas line - 1			
10.10	14.5	810	
14.48	20.0	850	830.0
Gas line - 2			
10.16	18.5	880	
15.00	19.5	880	880.0
Gas line - 3			
10.32	18.5	1000	
15.08	19.5	950	980.0
Gas line - 4			
10.40	18.5	960	
15.14	19.5	990	980.0
Gas line - 5			
10.43	18.5	1050	
15.20	19.5	930	990.0

These tables above clearly demonstrate that under practical fumigation conditions loss of toxic gas is likely and this depends on a number of factors (temperature, leakage, wind velocity and concentration of phosphine in the chamber). Therefore, it is necessary to obtain gas samples at least both just after release of phosphine and just before the end of fumigation. It is always important to maintain the recommended phosphine concentrations during the exposure periods. This will avoid any survivors that may lead to the development of resistant to phosphine fumigation.

The initial and final phosphine concentrations obtained from the short and long exposure fumigations in the 27 m^3 chamber at Emerald are presented in Table 8.8. Differences between initial and final concentrations are negligible in most of the short exposure fumigations. However, on two occasions the differences are substantial and this could be

Table 8.8 Initial and final concentration of phosphine concentrations obtained during various temperatures and exposure times from the 27 m^3 chamber at Emerald.

Date	Exposure time (h)	Temperature (°C)	Initial concentration (g.m^{-3})	Final concentration (g.m^{-3})
2/2/95	4.5	28.0	0.94	0.82
8/2/95	4.5	22.5	1.25	1.18
2/3/95	4.5	27.0	1.21	1.17
30/3/95	5.5	18.5	1.3	1.26
28/6/95	6.0	18.7 (17.5-19.9)	1.33	1.1
7/9/95	5.2	16.5	0.41	0.29
29/9/95	4.5	19.0	0.3	0.3
26/10/95	6.0	20.3 (19.5-21.4)	1.0	0.9
23/11/95	15.5	20.8 (19.4-21.5)	1.5	1.0
30/11/95	15.0	20.5 (19.6-22.8)	1.4	1.0
12/2/96	16.0	21.0 (18.6-22.6)	1.0	0.4
19/2/96	16.0	21.0(16.3-23.1)	1.0	0.6
26/2/96	15.5	21.9 (19.5-26.7)	1.0	0.7
18/3/96	15.5	13.1 (10.0-14.6)	1.0	1.0
25/3/96	16.0	21.7 (18.1-27.2)	1.0	0.7
2/4/96	15.0	13.8 (13.5-15.0)	1.0	0.9
29/5/96	18.0	18.2 (15.4-19.9)	1.0	0.9
10/7/96	15.0	15.6 (10.0-18.7)	0.28	0.19
18/7/96	15:25	17.4 (10.1-20.3)	0.28	0.18
29/8/96	16.25	18.0 (16.6-20.1)	0.26	0.097
4/9/96	16.25	18.1 (15.2-20.8)	0.27	0.11
9/9/96	16.25	17.9 (16.2-18.6)	0.15	0.069
18/9/96	15.50	19.2 (17.8-20.6)	0.23	0.18
23/9/96	16.0	18.9 (16.9-20.8)	0.22	0.07
30/9/96	16.0	18.4 (15.5-20.8)	0.25	0.15
7/10/96	15.50	18.0(13.1-18.8)	0.33	0.13
16/10/96	16.0	17.1(12.5-19.2)	0.15	0.12
30/10/96	16.25	18.1 (17.1-19.0)	0.15	0.097
12/11/96	16.0	18.1 (17.5-18.9)	0.27	0.15
13/11/96	16.0	18.2 (16.1-20.2)	0.32	0.19
30/12/96	16.0	18.4 (17.6-19.9)	0.43	0.36
14/1/97	16.0	25.5 (22.9-28.9)	1.09	0.61
23/1/97	15.0	18.2 (16.9-20.7)	1.2	0.94

due to the leakage from the chamber. On the other hand, considerable differences between initial and final concentrations had been recorded in the long exposure fumigations. This may be due to a number of factors higher temperatures, leakages and natural breakdown of phosphine with time.

Most importantly, the loss of fumigants may be accelerated by temperature differences between the chamber and ambient. The chamber temperature was generally maintained at 17°C or above and the overnight ambient temperature was generally less than 5°C during most parts of the year (winter and spring). Hence, this accelerates the loss of temperature along with the toxic gases into atmosphere through leaks. This was less pronounced in short fumigations because they are conducted during the day when the temperature differences were not substantial. Moreover, the exposure time itself was short, hence, the quantity of toxic gas lost would be less.

Statistical analysis was carried out using Genstat 5 to find out the factors influencing the amount of phosphine gas remained in the chamber. It is assumed that temperature and the exposure are the important parameters determining the amount of phosphine gas remained in the chamber.

Estimates of regression coefficients

	estimate	s.e.	t(36)
Constant	-0.0320	0.0671	-0.48
expo	-0.01005	0.00484	-2.08

The regression coefficient indicates that $e^{-0.01005} = 0.99$, ie for every hour increase in exposure time; the odds of the final concentration remaining in the chamber decreases by a factor of 0.99.

Concentrations of phosphine obtained during long exposure fumigations from the 900 L chambers at Frankston are presented in Table 8.9. Data from this table clearly indicates that differences between initial and final concentrations of phosphine in some fumigations varied considerably. This could be probably due to the losses associated with leakage.

Table 8.9 Initial and final concentration of phosphine obtained during various temperatures and exposure times from the 900 L chamber at Frankston.

Date	Exposure time (h)	Temperature (°C)	R.H (%)	Initial concentration ($g.m^{-3}$)	Final concentration ($g.m^{-3}$)
10/2/97	15.5	18.6(17.8-20.6)	-	0.98	0.85
12/2/97	17.0	23.9(18.1-34.8)	84.4(53.7-93.5)	0.94	0.55
5/3/97	15.5	22.5(19.2-32.4)	89.8(55.5-95.0)	0.62	0.45
12/3/97	15.5	15.7(14.0-23.0)	85.2(62.7-90.3)	0.42	0.38
17/3/97	16.0	16.3(11.8-24.1)	85.3(44.8-90.3)	0.64	0.43
20/3/97	16.0	16.9(14.3-21.6)	88.5(71.8-95.2)	1.08	0.97
24/3/97	17.0	17.4(15.3-24.1)	85.8(50.1-93.4)	0.78	0.57
9/4/97	16.25	21.1(18.8-25.5)	90.5(44.8-96.4)	1.32	0.48
14/4/97	17.0	18.9(18.1-19.5)	91.2(79.1-93.4)	1.01	0.76
21/4/97	16.0	19.4(17.2-25.3)	87.3(60.3-92.8)	1.22	0.41
29/4/97	16.0	18.4(13.9-26.6)	91.7(62.3-96.4)	1.14	0.87
5/5/97	18.0	18.3(16.4-21.9)	83.4(66.1-94.6)	0.83	0.76
26/5/97	17.25	17.5(16.0-19.1)	90.6(85.3-97.0)	1.0	0.84
3/6/97	16.0	18.3(16.7-19.9)	80.2(67.0-87.4)	1.04	0.48
25/6/97	17.0	18.0(16.0-20.2)	79.5(62.2-85.3)	0.47	0.33
15/7/97	16.5	17.7(14.9-20.5)	-	0.86	0.48
21/7/97	16.75	17.8(16.2-19.4)	-	0.42	0.14
6/8/97	16.5	18.4(16.2-20.1)	-	0.44	0.25
13/8/97	16.25	18.5(16.7-20.5)	65.0(50.0-72.0)	1.0	0.69
19/8/97	16.5	18.3(16.5-20.4)	-	1.0	0.57

The results of the pressure decay tests carried out at Emerald are given in Table 8.10. Data from this table indicate that there is a slight leakage from the chamber most of the time. However, severe leakage was detected in one occasion (25/10/95). These data show that the chamber was fairly gas tight, however, due to the smaller molecular weight of

phosphine, the loss of phosphine was considerable. Leakage was mainly located from the joints of the chamber; presumably caused by the pressure created while delivery of Phosfume®. These leaks were sealed with Silastic®, once the leaks were sealed the losses were reduced considerably, and there was no substantial difference between initial and final phosphine concentrations. Generally, a reduction of water level from 25.4 to 12.7 inches over a period of 100 sec or more is the indication that the chamber is gas tight. If the water level recedes in 60 sec or above is considered to be fairly gas tight and some sealing is necessary. However, the chamber is considered to be leaky and unsuitable for fumigations once the water level recedes rapidly. For example, on 25/10/95 the pressure in the chamber fell to 12.7 inches from 25.4 inches of water in 39 sec, indicating the chamber was very leaky, hence sealing is necessary.

Table 8.10 Gas holding capacity of the 27 m^3 chamber at Emerald.

Date	Time (sec)	Water level (mm)	
		From	To
29/5/95	68.0	20.32	10.16
14/5/95	75.0	5.08	2.54
5/10/95	69.0	20.32	10.16
25/10/95 (before sealing)	39.0	25.4	12.7
25/10/95 (after sealing)	63.0	25.4	12.7
30/11/95	45.72	25.4	12.7
14/1/97	117.0	25.4	12.7

8.4 Discussion

Monitoring concentrations of fumigant during the exposure period enables the likelihood of the effectiveness of treatments to be assessed and remedial action to be taken as necessary. Various defects in fumigant retention, distribution and application may lead to

inefficient treatment. The success of fumigation treatments on a commercial scale can be judged by the following criteria:

- The treated flowers must be found free of live insects.

- The quantity of fumigant used must be kept as low as possible, compatible with its efficacy.

For a successful fumigation not only are sufficient exposure times, temperatures and concentrations of phosphine essential, but also, uniform distribution of the fumigants are equally important. In most of the trials, the concentration of phosphine achieved in all locations of the chamber were above the required level and this indicates that phosphine distributes uniformly without stratification. However, a slight variation of phosphine concentration among different locations was also observed, but at a level that would presumably not influence the mortality of the exposed insect pests. Moreover, the molecular weight of phosphine is low ie. 34.04 and this facilitates in higher diffusion ability of phosphine than any other fumigants. The results suggests that phosphine can be distributed uniformly with minimum assistance from a fan in an enclosure without stratification.

Although, it was thought earlier that due to its explosive properties, phosphine cannot be recirculated (Monro, 1961a), it appears that phosphine can be recirculated safely by using low power recirculation fan, having fan tip speeds of less than 40 m per sec (Green *et al.* 1984). In all these trials an industrial fan was used successfully.

The distribution of fumigant in the chamber was fast and mostly uniform, hence, the ability of an insect to find a fumigant-free space is remote. In some fumigations, only a slight variation of phosphine concentration among different parts of the chamber was recorded and this did not influence the mortality of the insect pests exposed.Diffusion due to higher temperatures facilitates the distribution of fumigant gases evenly. Otherwise, stratification

of phosphine in the corners and bottom of the chamber are quite possible. Likewise, distribution of phosphine in the chamber depends on airflow ducts installed in the chamber, that determines how the gas is distributed in the chamber. The air movement in the chamber was closely related to increased effectiveness of fumigants by equally distributing the fumigants and temperature in the chamber.

Fumigant loss from an enclosure mainly depends on pressure in the enclosure, and the size and flow characteristic of the leaks. In addition to this, gas composition of the chamber (molecular diffusion) also determines the gas loss over a period of time. This suggests that losses are mainly by diffusion and permeation. It is clear from these data that if the chamber was gas tight the loss of phosphine would be nil. However, the losses were substantial from the leaks and this may lead to fumigation failures.

In general, the losses of phosphine from the chamber through leaks are significant due to its composition, and higher temperature than that of atmosphere. The literature suggests a number of reasons for the gas loss and uneven distribution of fumigants in the chamber. Cotton *et al.* (1936) found that, wind causes poor distribution and retention of fumigant gases in an enclosure. Moreover, in a well-sealed enclosure, thermal expansion and contraction considerably reduce the retention of the toxic gases and this can be overcome by insulating the system. This chamber was insulated except on the door, hence, during cool weather, loss of heat from this location is highly likely. Radiation and over-heating of chamber during hot weather can be reduced by using heat reflecting white coating.

Fumigant distribution in chambers can be studied by using phosphine detection tubes (Dräger®), and this is very effective for both identifying and measuring concentration of gases at both high and low levels. This was substantiated by the results obtained from bioassay insects, whenever the concentration of phosphine is less than the recommended (obtained from Dräger® tubes) there was correspondingly a number of individuals either survived or were affected.

It is clear from the concentration studies that most of the phosphine was retained in the chamber during exposure period if the chamber was gas tight. The temperature also plays an important role in diffusion, and diffusion is more rapid at higher temperatures than at lower temperature. Moreover, the convectional currents activated by the temperature differences in the flower bunches with bucket of water and wall of the chamber could also be responsible for the upward, downward and side movements of phosphine in the chamber.

The pressure decay tests indicate that occasional pressure tests are necessary, because increased pressure during fumigant delivery blows the seals of the chamber, hence, resealing becomes imperative. Generally, a pressure drop of 0.4 to 0.8 range at 60 sec or more holds phosphine sufficiently during exposure. However, once the pressure drop was more ie. 1 to 0.5 at 39 sec the chamber is not at all gas tight and would loose substantial amount of phosphine to the atmosphere during exposure, and fumigation failure is highly likely. Resistance to phosphine by certain insect pests that causes serious impediments to fumigation have been attributed to repeated fumigations of commodities which were not properly sealed i.e. exposed to sublethal concentrations of phosphine .

Concentration of phosphine achieved in the chamber varies greatly and this is positively correlated with the weight of the cylinder. A possible reason could be that the amount of active ingredient (phosphine) at these weights of cylinder would be less and higher proportion were of carrier gas (carbon dioxide). The cylinder delivers higher quantities of active ingredient only when it is more than half full. These fumigation trials show that the present timer system which was adjusted to deliver a desired quantity of fumigant is unreliable and most of the time it under delivers and some times the leakage from the chamber also accelerates the loss of toxic gases.

Chapter - 9

Conclusions

Chapter 9

Conclusions

The aim of this research is to identify an efficient alternate fumigant and/or fumigant mixtures for the posthavest disinfestation of Australian wildflowers destined for export markets. Methyl bromide has been used effectively to disinfest a range of wildflowers, which is highly toxic to insect pests and do not cause any significant reduction of vase-life of exposed flowers. Hence, a number of criteria were taken into consideration in this research. They were:

- selected fumigant has to be highly toxic to insect pest's of wildflowers;
- selected fumigant should be nil or less phytotoxic;
- investigate economically important insect pests of wildflowers and their feeding behaviour.

Initially, laboratory experiments were carried out (described in the Appendix 1) using carbon disulphide and phosphine. At this stage, the leaf rolling moth and tubular black thrips were used as bioassays. The results show that although carbon disulphide was highly toxic to all the exposed insect pests, it is also highly phtotoxic in nature. The exposed foliage of *T. calycina* began to blacken and fell off of the stem within hours following fumigation. The flowers also showed similar symptoms 24 h following fumigation. Hence, it was hypothesised that carbon disulphide is highly insecticidal and highly phytotoxic in nature and further experiments with carbon disulphide were not conducted. Phosphine, on the other hand, did not kill the exposed insect pests with an exposure time of 4 h, however increasing the exposure time to 5 h accelerated mortality of insects considerably. Also, fumigated flowers and foliage did not show any

phytotoxic symptoms at these fumigation regimes. Hence, it was concluded to investigate insecticidal efficacy and phytotoxicity of phosphine at large scale fumigation trials using a range of exposure times, temperatures and concentrations that can be economically viable and technologically feasible for the commercial flower exporters.

Chapter 3 describe the life-cycle and biology of one of the unstudied native Australian insect pest of Grampian thryptomene called the leaf rolling moth *S. ejectana*. The studies concluded that adults lay eggs twice a year: one in summer and the other in the winter. Hence, there are two generations per year are highly possible. Generally, egg laying starts five to seven days following emergence of adults. This insect undergoes egg, larva (six instar stages), pupa and adults. Under normal conditions it requires 63 to 91 days to complete its life-cycle at the temperature range of 19 to 22^{0}C. However, if the weather conditions are not feasible (winter) the life-cycle may be prolonged. The larval stage requires 35 to 52 days to become pupa. It was also noted during winter moths the larva undergoes a quiescent stage.

Toxicity of phosphine at various concentrations, exposure times and temperatures are presented in Chapter 4. Trials were conducted extensively, simulated to large-scale commercial levels. However, it was observed due to its large-scale nature it was difficult to control certain parameters (especially temperature and concentration of phosphine). Hence, a frame-work was developed to analyse the results. Results of various fumigation regimes for individual insects and their stages were classified as Good (those fumigation regimes that give 100 per cent mortality), Marginal (those fumigation regimes that give 90 to 99 per cent mortality) and Poor (89 per cent and less mortality). Following analysis of results, it was established that most of the fumigations are effective against most of the exposed insect pests and their stages. Fumigation regime with a final phosphine concentration of 0.5 g.m^{-3}, temperature of 20^{0}C and exposure time of 16 h or more is highly effective against all the exposed insect pests and their stages, where a complete mortality of all the exposed insect pests were obtained. If one or more of the above mentioned parameters are not maintained adequately a small number of insects were either survived or severely affected. Especially, the eggs of *T. urticae* and eggs and sixth instar larvae of *E. postvittana* are

comparatively tolerant than that of other insects. In most of the fumigations a number of different insects and their stages were severely affected. Most of these severely affected individuals died within 24 h after fumigation and only a small number were severely affected for up to seven days. Under the International Plant Protection Convention, importing country has the rights to either reject and/or refumigate a consignment of flowers if life insects are found, which is costly to flower exporters. Hence, certain modifications were made in the next set of fumigations and they are discussed in detail in the next paragraph.

The synergistic effects of pyrethrum added to phosphine fumigations are described in Chapter 5. It was hypothesised by adding a nerve poison (pyrethrum) five to ten minutes before adding phosphine would reduce the temperature, exposure time and concentration of phosphine requirements for a complete kill of exposed insect pests. The rational behind this theory is: pyrethrum attacks the nerve system of insects which obviously make the insects to stir. This will automatically make the insects to respire more, hence, insect will absorb more toxic phosphine gas and succumb to death. In these experiments effects of short (4.5 to 6 h) and long (14 h or more) exposure times on mortality on insects were investigated. The results indicate that short exposure times are not effective, a small number of eggs of *T. urticae* survived and some *S. ejectana* larvae and *T. urticae* adults were severely affected. However, this exposure time fumigations killed all the exposed *T. urticae* larvae. It was observed increasing the exposure time to 6 h increased the mortality rate slightly. The long exposure time fumigations are highly effective and only in some fumigations a small number of individuals were severely affected. This is particularly in fumigations where some of the fumigation parameters (temperature or phosphine concentration) were not maintained sufficiently. Tables in Chapter 4 (Table 4.90) and Chapter 5 (Table 5.54) clearly illustrate the advantages of pyrethrum-phosphine combination over phosphine alone fumigations. The temperature, exposure time and phosphine concentration requirement for a complete mortality of exposed insect pests were of lower for the pyrethrum-phosphine fumigations than that of phosphine alone fumigations.

Results presented in Chapter 4 and 5 clearly illustrate that both Phosfume® and Pestigas®+Phosfume® are highly suitable for successful commercial postharvest disinfestation of wildflowers. The fumigation regime required for commercial successful postharvest disinfestation is presented in Table 9.1.

Table 9.1 Fumigation regime for the commercial successful postharvest disinfestation of wildflowers.

Fumigant	Concentration ($g.m^{-3}$)	Exposure time (h)	Temperature (°C)
Phosfume®	0.94	18	25.5
Pestigas®+Phosfume®	0.15	15.5	18.1

Another important phenomenon observed in these fumigation trials is that laboratory reared *S. ejectana* larvae (reared on modified lightbrown apple moth medium LBAM) are highly susceptible to all the fumigation regimes. Complete mortality of all the exposed larvae was achieved in all the fumigation trials, in contrast to the field collected larvae, which were comparatively tolerant to most of the fumigation regimes.

Phytotoxic studies conducted in these trials were reported in Chapter 6. Pyrethrum-phosphine or phosphine alone fumigations are not highly toxic to the exposed wildflowers. The vase-life was not affected for seven days, which is the time period which is sufficient to reach the customers. Generally, the foliages are comparatively susceptible to these fumigations than that of flowers.

Chapter 7 compares the heating efficiency and the capacity to maintain the required temperature of two different (1kW and 3kW industrial) heaters in a 27 m^3 modified chamber during all seasons of the year. The time required to heat the chamber differs greatly during different periods of the year. The 1kW industrial heater required long time (60 to 255 min) to heat the chamber during winter time, however, the 3kW industrial heater took only minutes to heat the chamber. This may leads to fumigation failures. However, during autumn season 1kW heater 40 to 60 min to heat the chamber.

The 3kW heater is very efficient in heating the chamber and maintained the chamber at the required temperature level – without over heating or under heating of chamber.

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Appendix - 1

A Preliminary Screening of Candidate Fumigants

Appendix 1

A Preliminary Screening of Candidate Fumigants

A.1.1 Scope of the experiments

The insect species that have resulted in rejections at Japanese ports were ascertained from exporters and quarantine authorities and attempts were made to transport these insects to Melbourne and establish colonies. Initially, it was determined that the larvae *S. ejectana* and *H. victoriensis* were causes of rejections at the ports of Japan. Further investigations revealed that *Thryptomene* in the Horsham area of Victoria is highly infested with larvae of *S. ejectana*. In the initial stages of the work reported in this thesis, very little was known about the response of Australian wildflowers to candidate fumigants that might supplant methyl bromide.

Williams (1996) had established that ethyl formate and metham sodium were unlikely to be suitable for fumigating wildflowers because of their phytotoxicity. However, carbon disulphide and phosphine appeared to be potentially useful. Williams' (1996) bio-assays had been carried out using *O. surinamensis* and the larvae of *S. ejectana* as test species. Typical concentrations and exposure times studied by Williams (1996) were 650 mg/L and 1.5 h for carbon disulphide, and 1mg/L for 4 h for phosphine. Carbon disulphide at this concentration range did not give a complete kill of all the *O. surinamensis*. On the other hand, phosphine concentrations investigated proved to be somewhat marginal to obtain both insect mortality and for negligible phytotoxicity, but they provided a first order estimate of the conditions that may have proved effective against *S. ejectana.*

The laboratory experiments described in this appendix provide somewhat more data obtained by the candidate on the response of active stages of *S. ejectana* and *H. victoriensis* to carbon disulphide and phosphine, and on the phytotoxicity of these fumigants on *T. calycina*, *P. cynaroides*, *B. coccinia, L. coniferum* and carnations. The

principal motivation of this element of the research is to provide preliminary, or range-finding data, that would act as a guide to the carrying out of more comprehensive experiments to be carried out on a commercial scale.

A.1.2 Materials and methods

A.1.2.1 Selection of fumigants

Alternatives to methyl bromide were reviewed initially by means of a literature search and by personal communications with Dr P. Williams and Australian wildflower export industry. It was considered for these experiments that fumigants which are already available, and that have potential insecticidal action but require further investigation would be studied. Hence, carbon disulphide and phosphine were chosen for the initial laboratory experiments. Although carbon disulphide is not in as widespread use as phosphine, it has many desirable properties, ie. short exposure times are sufficient for complete kills, and it is highly toxic to a range of insect species. At present phosphine is being used extensively for fumigation of a range of commodities, especially grains, and to a certain extent of dried fruits (sultanas and dates), nuts (almonds), and some perishables (citrus and grapefruit).

The literature suggests that although phosphine requires a longer exposure time, it is highly toxic to a range of insect pests and their developmental stages. Also, the long term environmental hazards posed by phosphine are very small or zero as it is converted into hypophosphites or hydrophosphites. Moreover, if proper aeration and safety measures are taken, the health hazards to humans may be contained to acceptably safe levels. Research indicates there is a possibility that insects can develop resistance to phosphine fumigation if insufficient exposure times and/or concentrations are maintained. Hence, these parameters were taken into consideration when designing the fumigation experiments. All the live, affected, and severely affected individuals that were remaining after the phosphine fumigations were maintained carefully, and their behaviour and fecundity were studied separately. Once the studies were completed, the

survivors were disposed of carefully by autoclaving them to minimise any possibilities of developing resistant strains.

A.1.2.2 Fumigation chambers

A.1.2.2.1 Carbon disulphide

The fumigation trials with carbon disulphide were carried out in a pressure tested, wooden lidded 4.2 L wide mouthed (12 mm) glass cylinder (450 mm high and 12 mm wide). This cylinder was provided with 10 gas sampling ports (3.0mm internal diameter) spaced at 40 mm intervals along its length. These ports were fitted with rubber septums (5 mm diameter) for gas delivery and sampling. An absorbent cotton wool pad was attached on the centre of the lid (60 mm diameter and 15 mm thickness) into which the carbon disulphide was injected, and from where it volatilised into the chamber.

A.1.2.2.2 Phosphine

Glass jars that were pressure tested and with capacities of 4.5 L (280 mm high and 135 mm diameter with a 85 mm opening), and 9.7 L (385 mm high, 180 mm diameter with a 130 mm opening) and fitted with metal lids were used for phosphine fumigations. At the centre of the lids, rubber septums (5 mm diameter) were fitted through a drill hole to facilitate fumigant delivery, and sampling. These chambers were made air-tight by lining them with silicon rubber (Silastic®) on the edges of the septum and lid.

A.1.2.3 Collection of insects

Bunches of *T. calycina* infested with the larvae of leaf rolling moths *S. ejectana* (third to sixth instar stages) and the adults of tubular black thrips *H. victoriensis* were

collected each month from a large scale export-oriented commercial plantation (Austflora Pacific Pty Ltd) in the Grampians in Western Victoria. These were transported to the State Chemistry Laboratory, Melbourne, within 10 to 12 h of collection. The plantations were regularly sprayed with insecticides; hence, a separate block (1 hac) was maintained for experimental purposes in an isolated location in the plantation to protect it from any chemical treatment. The bunches of *T calycina* were selected randomly from this plot, and were cut from bases of the plants (60 to 90mm from the ground) using secateurs. These bunches, infested with insects, were kept in 20 L plastic buckets (10 to 12 bunches per bucket) filled with 7 to 9 L of tap water and maintained at a temperature of 20±2°C.

An iron-framed cage (dimensions of 450 x 600 x 400 mm) fitted on its sides and top with muslin cloth, was used for housing infested bunches of *T. calycina.* A photoperiod of 12 h light and 12 h darkness was maintained using a 15W fluorescent light and this was fitted 500 mm from the canopy outside the cage. To keep the *Thryptomene* foliage in fresh condition, and to avoid wilting, 2 to 3 mL of glycerol with a purity of 99% (supplied by the Sigma Chemicals, USA) was added to the buckets. This kept the flowers and foliage in good condition for 10 to 12 days. Although the foliage began to dry after this period, the larvae of *S. ejectana* were maintained in a healthy condition for up to 20 to 25 days. However, the thrips were not active under these conditions. Hence, these insects were inoculated into fresh foliage at intervals of 15 to 20 days.

A.1.2.4 Bio-assay and control insects

Insects were chosen randomly from these bunches, and divided into two equal lots; one was used for fumigation and the other portion was maintained separately as control insects. Similar environmental conditions were maintained for these batches, and the states of these insects were assessed and compared with fumigated insects regularly. The control insects were maintained until they complete their life cycle, and some of

this were used for fumigation purpose rest were disposed carefully by autoclaving them.

A.1.2.5 Phytotoxicity studies

The toxicities of phosphine and carbon disulphide to flowers and foliage of *T. calycina*, *P. cynaroides*, *B. coccinia, L. coniferum* and carnations (Dianthus) were investigated. Carnations are generally highly susceptible to high temperatures and the presence of fumigants, and these provided a useful benchmark against which other species could be compared. The flowers used in the experiments were obtained from flower sellers in Melbourne and this had been generally harvested 1 to 2 days before fumigation, and/or from Horsham (harvested a day before fumigation). These flowers were kept in 20 L plastic buckets filled with 7 to 9 L of tap water and maintained in a temperature range of 19±1°C. A photoperiod of 12 h of light and 12 h of darkness was maintained using two 15 W fluorescent lamps.

Five to ten minutes before fumigation, flowers and foliage were selected randomly for fumigation and a similar number was maintained as a control. If the bunch had any wilted, bruised, or browned flowers or leaves they were rejected. The selected flowers were cut 15 to 20 mm from the base of their stems under water using secateurs. The reason for cutting stems under water is that it prevents the entry of air into the water transporting route (phloem) as the presence of any air bubbles may block the water uptake. Vase life assessments were conducted by comparing fumigated and unfumigated flowers that were maintained under identical conditions. An initial assessment was made 10 to 30 min following the end of fumigation and a second assessment was made 24 h after fumigation. The assessments continued for up to seven days, observing the flowers and foliage at 24 h intervals. In order to make the observations as objective as possible the following indications were considered to signal the end of vase life:

- *Thryptomene calycina* - browning and/or drying and/or dropping of 25 per cent or more of the foliage and flowers.

- *Banksia coccinea* - these were fumigated with five to seven leaves and a single flower. Blackening of the edges of two or more leaves was considered to indicate the end of vase life.

- *Protea cyanorides* - these were fumigated with seven to nine leaves and a single flower. Blackening of two or more leaves and browning of the edges of bracts (two or more bracts) was considered to mark the end of vase life.

- *Leucadendron coniferum* - these were fumigated with 15 to 20 leaves and a flower. Blackening of edges of four or more leaves or flowers (two or more bracts) was considered to signify the end of vase life.

- Carnation - wilting or marginal browning of 25 per cent or more of leaves or flowers was considered to indicate the end of vase life.

Table A.1 Phytotoxicity index, for assessing the toxicity of fumigants.

Rating	Flowers	Foliage
0	No damage to petals or bracts	No damage to leaves or stem
1	Slight discolouration or marginal browning of 10 per cent or less of flowers/bracts	Marginal necrosis of 10 per cent or less of leaves
2	Some discolouration, but marketable	Marginal necrosis of 10 to 25 per cent of leaves
3	Discolouration of 25 per cent or more of flowers	Marginal necrosis of 25 to 50 per cent of leaves
4	Increased discolouration	Increased necrosis of 50 per cent or more of leaves
5	Entire flower browned and wilting, unmarketable	Browning of whole leave, not suitable for vase life
6	Flower closed*	Showering of leaves*
7	Showering of flowers*	Foliage destroyed

A.1.2.6 Fumigation procedure

All the laboratory fumigations were carried out in a standard air-tight ventilated fume hood fitted with a door. The flower stems of different species (two or three species and each species was represented by five to seven stems per fumigation) were tied together with jute fibre or rubber bands. These were transferred into a 50 mL glass bottle that was filled with 30 to 40 mL of tap water. A semi flexible nylon tube was attached to the side of this bottle using masking tape and the flower bunch was tied to this tube. This tube also facilitated handling whilst transferring the flower bunches into the fumigation chamber. Thrips were placed among the foliage of flower bunches, and the leaf rolling moth larvae were placed among the flower bunches, and on the bottom of the chamber with their webbing shelters. This gave an indication on the distribution of fumigant gases at different locations of the chamber.

A.1.2.6.1 Carbon disulphide

Carbon disulphide was supplied as a liquid fumigant by Sigma Chemicals, Melbourne, in a 3 L glass jar. A known amount (100 to 120 mL) of carbon disulphide was poured directly into a 250 mL glass beaker under the fume hood while the extraction fan was operating. The required quantity of carbon disulphide was withdrawn into a glass syringe (volume of 30 mL), and injected into the centre of the cotton wool from which it vaporised into the jar. The fumigation chamber was made air-tight by tying aluminium foil around the lid of the chamber. Initially, the toxicity of carbon disulphide was investigated after a long exposure time (2.5 h), and a lower concentration (650 mg/L). Later, a short exposure time (2 h), and a comparatively higher concentration (742 mg/L) was investigated. The duration of the exposure was calculated from the time the carbon disulphide was added in to the chamber.

A.1.2.6.2 Phosphine

Laboratory experiments were carried out using standard phosphine (1 per cent phosphine in nitrogen as a carrier gas) supplied in high pressure standard cylinder (J type, 17 kg net weight and 34 kg tare weight). Because of its lower molecular weight, phosphine diffuses rapidly in all directions. Hence, in order to reduce the probability of being exposed to the toxic fumes, the fume hood door was lowered to the base of its floor, allowing only a 120 to 150 mm gap for handling the equipment and gas. The fan was switched on during the transfer of fumigant gases and during exposure, as well as whilst airing off at the end of the treatment. Phosphine was initially transferred from the cylinder through a regulator into a glass gas bottle (300 mL) or aluminium gas bag (capacity of 5 to 7 L) filled to 75 per cent of its volume. The gas bottle and gas bag were fitted with a rubber septum (5 mm diameter) for the delivery and sampling of the fumigant. A large number (volume of 2 to 25 mL) of gas tight syringes was employed, for transferring the phosphine gas.

Initially, a negative pressure was created in the fumigation jar by withdrawing a known amount of air from the chamber. The required amount of fumigant was withdrawn into the calibrated gas-tight syringe through the septum of the gas bag or gas bottle, and this was transferred into the fumigation jar through the septum. Before administering phosphine, the jar was made air-tight by coating a thick layer of silicon rubber (Silastic®) around the edge of lid, and this was allowed to cure for 20 to 25 min. The fumigations were deemed to have begun immediately after phosphine was delivered in to the chamber. The exposure times studied were 4, 4.5 and 5 h, with a phosphine concentration range of 0.67, 0.97 and 1.1 $g.m^{-3}$ respectively.

A.1.2.6.3 Temperature control

A domestic fan heater (Ringgrip®) that can be adjusted to fan only (delivers unheated air), medium 1,200 W (delivers warm air) and high 2,400 W (delivers hot air) was used

for maintaining a temperature of 19±1°C within the fume hood. This heater was placed in front of the fume hood (2000 to 2500 mm away from the door) resting on the floor. The temperature in the chamber was monitored by means of a thermometer that was placed inside the fume hood. The temperature was maintained at the desired level during fumigation: if the temperature was higher or lower than that desired, the heating level was adjusted, or the distance between heater and the fume hood was increased or decreased. This gave the desired temperature in the chamber during the treatments.

A.1.2.7 Post-fumigation procedure

A.1.2.7.1 Flowers

Once the exposure to the fumigants was completed, the extraction fan of the fume hood was switched on and the lid of the fumigation jar was opened for 30 min to purge the chamber during the airing off process. The fume hood was completely sealed in order to prevent the diffusion of fumigant gases into the surrounding atmosphere. After this period uncontaminated air was applied to the chamber through a polyethylene pipe from the fume hood for 15 to 20 min to flush out any remaining fumigant residues.

Once, the airing off was completed, the fumigated and unfumigated flowers were transferred separately into various 1 to 1.5 L glass beakers filled with deionised or tap water (750 to 1000 mL), labelled and maintained at room temperature (20±1°C). A photoperiod of 10 h light, and 14 h darkness was maintained by means of two 15W fluorescent lights. The conditions of the flowers and foliage were observed daily except Saturdays and Sundays for symptoms of phytotoxicity ie desiccation, browning or blackening, drying, showering of flowers and/or foliage, drooping. These were compared with unfumigated flowers and foliage. The water was replenished every two days, and the flowers were transferred into new beakers with fresh water every five days to reduce the likelihood of microbial infections.

A.1.2.7.2 Insects

Once the purging was completed the fumigated leaf rolling moth larvae were transferred into rectangular plastic trays (dimension of 180 x 180 x 360 mm). The webbing shelters were opened using forceps and/or needles; the larvae were transferred (based on instar stages) into different lidded plastic Pectic dishes (10 to 12 mm diameter) and labelled. The base of the chamber and the foliage of fumigated plants were searched for larvae that had emerged from their shelters during fumigation. These were labelled and transferred separately into different plastic Pectic dishes. The separated insects were assessed within 15 to 20 min of removal from their shelters. The condition of these larvae was observed under a microscope with a light source consisting of a 15W halogen lamp. The criteria used for determining the states of the fumigated insects are discussed in detail in Chapter 5, Section 5.2.6. Once assessed, these larvae were maintained in lidded polyethylene containers (10 to 12 larvae per container) with dimensions of 180 × 90 × 75 mm.

The affected or severely affected larvae were not able to construct their own webbing shelters, hence, they were placed among existing webbing shelters to give them some protection against drying. The larvae that appeared to be dead in the initial assessment (two to three hours after fumigation) were assessed twice more (4 and 24 h following first assessment) when they were observed for any gross reaction to the fumigation (time taken for colour change, oozing of fluids from their bodies) that definitively indicated their mortality. Once these studies were completed, the larvae were disposed of carefully by autoclaving them. The affected and severely affected larvae were assessed with minimum disturbance to them by placing them under a light source and prodded with a fine hair camel brush every 24 h. All the fumigated and control insects were maintained at a temperature of 20±1°C.

The thrips were transferred into lidded 10 to 12 mm diameter lidded plastic Petrie dishes, using a fine hair camel brush. These adults were classified as live, affected, severely affected or dead based on their response to the external stimuli. All the fumigated individuals were transferred separately into lidded Petrie dishes to which

small amounts of fumigated *T. calycina* foliage had been added, and maintained in a temperature range of 20±1°C. The conditions of these severely affected individuals were assessed once every three days until they died; a procedure that minimised disturbing them during observation. Conditions and reactions to the fumigant by the dead individuals had assessed for 72 h at 24 h intervals.

A.1.2.8 Results

A.1.2.8.1 Carbon disulphide

Larvae of the leaf rolling moth, Strepsicrates ejectana

The toxicity of carbon disulphide to larvae of *S. ejectana* at various exposure times and concentrations is presented in Table A.2. Complete mortality of all the exposed larvae (third to sixth instar stages) was obtained at the two different concentrations of carbon disulphide (650 and 742 mg/L) and exposure times (2 to 2.5 h) investigated. This implies that carbon disulphide is highly toxic to the larvae of leaf rolling moths. Moreover, all the larvae remained in the webbing shelters during exposure; not a single larva was found on the bottom of the chamber or hanging among the foliage. This suggests that carbon disulphide penetrated sufficiently into the webbing shelters, and acted instantaneously on the target sites of the insects that are sensitive to the fumigant. Similarly, all the insects that remained at the bottom of the chamber, and among the foliage were killed which indicates that carbon disulphide distributed to all locations of the chamber sufficiently.

A constant temperature of 18.0±0.5°C was maintained during exposure. There was no over-heating or under-heating of chamber, or any part of the fume-hood during exposure.

Table A.2 Insecticidal efficacy of carbon disulphide to third to sixth instar larvae of *S. ejectana* at two concentrations and exposure times at 18°C.

Date	Concentration (mg/L)	Exposure time (h)	*S. ejectana*		
			Live	Dead	Total
27/9/94	650	2.5	0	34	34
27/9/94	0	0	30	2	32
28/9/94	650	2.5	0	46	46
28/9/94	0	0	46	0	46
29/9/94	742	2.0	0	24	24
29/9/94	0	0	23	1	24
3/10/94	742	2.0	0	27	27
3/10/94	0	0	27	0	27

Tubular black thrips, Haplothrips victoriensis

Table shows the results obtained from the fumigation experiments at two concentrations of carbon disulphide (650 and 742 mg/L), and two exposure times (2 to 2.5 h) on tubular black thrips. Both these concentrations and exposure times investigated are highly toxic to the adult thrips. A concentration of carbon disulphide of 650 mgL^{-1} and an exposure time of 2.5 h killed all these exposed adults. Moreover, it was observed that all the exposed adults were found on the bottom of the chamber at the end of fumigation.

Table A.3 Insecticidal efficacy of carbon disulphide to adult tubular black thrips *H. victoriensis* with two concentrations, exposure times, at 18°C.

Date	Concentration (mg/L)	Exposure time (h)	*H. victoriensis*		
			Live	Dead	Total
27/9/94	650	2.5	0	16	16
27/9/94	0	0	12	4	16
28/9/94	650	2.5	0	24	24
28/9/94	0	0	15	9	24
29/9/94	742	2.0	0	34	34
29/9/94	0	0	27	7	34
3/10/94	742	2.0	0	18	18
3/10/94	0	0	14	4	18

Control insects

In all the experiments a small number of *S. ejectana* larvae had died before reaching the pupal stage either due to handling or due to natural mortality. All other larvae were healthy and completed their life cycle. However, survival rates among the unfumigated thrips were comparatively lower than larvae of the leaf rolling moths. Mortality was mainly due to lack of fresh feed and some had died due to natural mortality or while handling.

Phytotoxicity studies

Results of the carbon disulphide fumigated flowers and foliage of four species of wildflowers and a cut flower are presented in Table A.4. Immediately following fumigation, the *T. calycina* foliage and flowers that were fumigated with two concentrations and exposure times investigated appeared to be in good condition. However, those fumigated with a carbon disulphide concentration of 650 mg/L and an exposure period of 2.5 h showed accelerated browning (25 per cent of the mature leaves) on the edges 12 h after the exposure. This browning progressed towards the centre and eventually the entire leaves turned to brown and then blackened. Similarly, young leaves also showed accelerated browning 24 to 48 h following fumigation. This was followed by defoliation within three to four days of fumigation. Moreover, the old flowers (near to the middle, and base of the stem) showed signs of yellowing, and browning 24 h after exposure.

One day after this, symptoms of shrivelling and drying were observed. This was followed by unacceptable dropping of 75 per cent of the flowers within four to five days of fumigation. Young flowers and buds were not affected during the first three days. However, shrinking, and bristling of young flowers was observed four days after fumigation, and this led to the dropping of 50 per cent of these flowers. Moreover, buds of the fumigated flowers did not open; whereas 75 per cent of the buds opened on the unfumigated stems three to six days following the start of the experiments.

Similarly, fumigating at higher concentrations (742 mg/L) with 2 h of exposure time reduced the vase life of both flowers and foliage in a manner similar to that mentioned in the previous paragraph. But the browning and dropping of both flowers and foliage were rapid and complete defoliation was observed within 48 h after fumigation. However, immediately after fumigation and for up to 12 h all the flowers and foliage remained similar to control flowers.

Immediately following fumigation all the exposed *B. coccinea* flowers and foliage remained similar in appearance to the unfumigated ones. However, 75 per cent of the mature leaves showed marginal browning 12 h and 18 h following fumigation when they had been fumigated with a carbon disulphide concentration of 650 mg/L and 742 mg/L respectively. The young leaves were unaffected for 48 to 54 h following fumigation, but 25 per cent of these leaves showed accelerated browning at both these concentrations after this period. The browning spreads towards the centre of the leaves 12 to 24 h following the appearance initial symptoms. On the other hand all the flowers were in good condition for up to six days, but a slight browning of the tips was observed seven to nine days following fumigation.

All the *L. coniferum* flowers and foliage that were fumigated with two concentrations of carbon disulphide and exposure times remained similar to unfumigated flowers for six hours. That foliage fumigated with higher concentration of carbon disulphide (742 mg/L) showed marginal blackening of the margins of both young and mature leaves within 12 to 24 h after fumigation. However, at 650 mg/L most of the mature foliage (75 per cent) showed marginal browning 30 h following fumigation and younger leaves showed similar browning 48 h after fumigation. At both concentrations of carbon disulphide, petals (50 per cent) showed marginal wilting 48 to 72 h following fumigation. This browning spread to whole leaves within 24 h of initial symptoms. Fifty per cent of the affected petals dropped off from the stem 12 to 18 h after the appearance of initial symptoms.

Observations made 10 to 15 min after fumigation on the *P. cynaroides* showed no symptoms of phytotoxicity, and they appeared similar to the control bunches.

However, 24 h after fumigation 50 per cent of the mature leaves (near to the base of the stem) showed a blackening of the edges. This blackening spread towards the centre of leaves at both the concentrations of carbon disulphide investigated. The entire leaf turned to black within 48 h of the initial symptoms. However, the same age leaves of unfumigated *Proteas* remained in good condition for 10 to 11 days. On the other hand, the young leaves (the first four leaves from the top) were in good condition for the first four days after fumigation that were exposed to 650 mg/L of carbon disulphide for 2.5 h. However, these also showed signs of browning five days after fumigation.

Table A.4 Toxicity of carbon disulphide to various species of flowers and foliage fumigated with two concentrations and exposure times.

Species	Concentration (mg/L)	Exposure time (h)	Phytotoxicity		Vase life days	
			Flowers	Foliage	Flowers	Foliage
Thryptomene calycina	650	2.5	7	8	1	1
Thryptomene calycina	0	0	0	0	9	8
Thryptomene calycina	742	2.0	8	8	1	1
Thryptomene calycina	0	0	0	0	9	8
Banksia coccinea	650	2.5	6	7	3	2
Banksia coccinea	0	0	0	0	12	10
Banksia coccinea	742	2.0	6	6	2	2
Banksia coccinea	0	0	0	0	12	10
Protea cynaroides	650	2.5	6	6	3	3
Protea cynaroides	0	0	0	0	11	10
Protea cynaroides	742	2.0	6	6	3	3
Protea cynaroides	0	0	0	0	11	11
Leucadendron coniferum	650	2.0	7	8	2	2
Leucadendron coniferum	0	0	0	0	8	9
Leucadendron coniferum	742	2.5	8	8	2	2
Leucadendron coniferum	0	0	0	0	8	8
Carnation (*Dianthus*)	650	2.5	7	7	1	1
Carnation (*Dianthus*)	0	0	0	0	7	7
Carnation (*Dianthus*)	742	2.0	8	8	0	0
Carnation (*Dianthus*)	0	0	0	0	7	7

On the other hand, browning was rather rapid for the young leaves that were fumigated with a carbon disulphide concentration of 742 mg/L with an exposure period of 2 h. The flowers also showed browning of bracts (50 per cent) 4 to 5 days after treatment after exposure to both concentrations and exposure times.

One to three hours following fumigation both the mature and young leaves of Carnation that were fumigated with a carbon disulphide concentration of 650 mg/L showed wilting symptoms. The flowers and young leaves showed wilting 6 to 12 h after fumigation. Wilting proceeded to discolouration of flowers and blackening of foliage within 48 to 60 h following fumigation. Similarly, those flowers and foliage that were exposed to higher concentration of carbon disulphide (742 mg/L) showed wilting symptoms immediately after fumigation. This wilting proceeds to discolouration of both flowers and foliage within 12 h following fumigation.

On the other hand, all the unfumigated flowers and foliage remained in good condition for between 7 and 12 days.

A.1.2.8.2 Phosphine

Larvae of the leaf rolling moth, Strepsicrates ejectana

Tables A.5 to A.8 shows the result of the phosphine fumigations carried out with various exposure times (4 to 5 h) and concentrations (0.69 to 1.1 $g.m^{-3}$) to third to sixth instar larvae of *S. ejectana*. Of these larval stages, a similar pattern of response had been recorded for the third and fourth instar larvae, likewise, fifth and sixth instar larvae responded similarly. Although 4 h of exposure time did not give complete mortality of most of the larvae fumigated, increasing the exposure time to 5 h gave a comparatively higher mortality. Complete mortality of all the third and fourth instar larvae was achieved with a phosphine concentration of 1.1 $g.m^{-3}$ exposed for 5 h (Tables A.5 and A.6).

Mortality rates were less for the fifth and sixth instar larvae at a lower concentration of phosphine (0.69 $g.m^{-3}$) and a shorter exposure time (4 h), however, the mortality rate became increasingly higher with increasing exposure time and concentration of phosphine. Comparatively, a small number of fifth and sixth instar larvae of *S. ejectana* was severely affected immediately after exposure to a higher concentration (1.1 $g.m^{-3}$) and longer exposure time (5 h), and all the others were dead.

Table A.5. Insecticidal efficacy of phosphine to third instar larvae of *S. ejectana*

Date	Concentration ($g.m^{-3}$)	Exposure time (h)	*S. ejectana*			
			Live	S. aff	Dead	Total
4/10/94	0.69	4.0	0	3	16	19
4/10/94	0	0	17	0	2	19
5/10/94	0.69	4.0	0	4	11	15
5/10/94	0	0	15	0	0	15
6/10/94	0.97	4.0	0	1	18	19
6/10/94	0	0	18	0	1	19
7/10/94	0.97	4.0	0	1	15	16
7/10/94	0	0	16	0	0	16
10/10/94	0.97	4.5	0	1	28	29
10/10/94	0	0	29	0	0	29
11/10/94	0.97	4.5	0	2	34	36
11/10/94	0	0	32	0	4	36
12/10/94	0.97	4.5	0	0	18	18
12/10/94	0	0	15	0	3	18
13/10/94	1.1	5.0	0	0	18	18
13/10/94	0	0	17	0	1	18
14/10/94	1.1	5.0	0	0	15	15
14/10/94	0	0	14	0	1	15
18/10/94	1.1	5.0	0	0	30	30
18/10/94	0	0	30	0	0	30

The behaviour of these severely affected larvae was monitored regularly by observing their feeding habits, faeces and movement of appendages and mouth parts. The feeding pattern was studied by observing signs of feeding in the medium (nibbling), and the presence of faeces, that were removed daily. The absence of faeces beyond three days of fumigation indicated that the larvae probably did not feed for one to two days

following fumigation. Moreover, there was no sign of feeding on the medium, on the other hand, the unfumigated larvae showed feeding signs (nibbling on top of the medium and presence of faeces). Also, the severely affected larvae were immobile and showed only slight movements of their mouth parts or appendages when they were prodded under a light source.

In all these trials, a constant temperature of 18±0.5°C was maintained in all parts of chamber during exposure to the fumigant.

Table A.6 Insecticidal efficacy of phosphine to fourth instar larvae of *S. ejectana.*

Date	Concentration ($g.m^{-3}$)	Exposure time (h)	*S. ejectana*			
			Live	S. aff	Dead	Total
4/10/94	0.69	4.0	0	2	12	14
4/10/94	0	0	12	0	2	14
5/10/94	0.69	4.0	0	3	12	15
5/10/94	0	0	12	0	3	15
6/10/94	0.97	4.0	0	1	20	21
6/10/94	0	0	20	0	1	21
7/10/94	0.97	4.0	0	0	16	16
7/10/94	0	0	18	0	0	18
10/10/94	0.97	4.5	0	1	29	30
10/10/94	0	0	27	0	3	30
11/10/94	0.97	4.5	0	0	30	30
11/10/94	0	0	28	0	2	30
12/10/94	0.97	4.5	0	1	18	19
12/10/94	0	0	16	0	3	19
13/10/94	1.1	5.0	0	0	18	18
13/10/94	0	0	16	0	2	18
14/10/94	1.1	5.0	0	0	16	16
14/10/94	0	0	18	0	2	20
18/10/94	1.1	5.0	0	0	24	24
18/10/94	0	0	30	0	0	30

Table A.7 Insecticidal efficacy of phosphine to fifth instar larvae of *S. ejectana.*

Date	Concentration ($g.m^{-3}$)	Exposure time (h)	*S. ejectana*			
			Live	S. aff	Dead	Total
4/10/94	0.69	4.0	0	12	11	33
4/10/94	0	0	31	0	2	33
5/10/94	0.69	4.0	0	10	6	16
5/10/94	0	0	15	0	1	16
6/10/94	0.97	4.0	0	9	12	21
6/10/94	0	0	20	0	1	21
7/10/94	0.97	4.0	0	10	15	25
7/10/94	0	0	30	0	0	30
10/10/94	0.97	4.5	0	12	28	40
10/10/94	0	0	36	0	0	36
11/10/94	0.97	4.5	0	18	34	52
11/10/94	0	0	46	0	4	50
12/10/94	0.97	4.5	0	15	18	33
12/10/94	0	0	27	0	3	30
13/10/94	1.1	5.0	0	1	18	19
13/10/94	0	0	20	0	1	21
14/10/94	1.1	5.0	0	3	15	18
14/10/94	0	0	18	0	2	20
18/10/94	1.1	5.0	0	6	30	36
18/10/94	0	0	30	0	0	30

Table A.8 Insecticidal efficacy of phosphine to sixth instar larvae of *S. ejectana.*

Date	oncentration ($g.m^{-3}$)	Exposure time (h)	*S. ejectana*			
			Live	S. aff	Dead	Total
4/10/94	0.69	4.0	0	14	16	30
4/10/94	0	0	28	0	2	30
5/10/94	0.69	4.0	0	10	5	15
5/10/94	0	0	12	0	3	15
6/10/94	0.97	4.0	0	9	14	23
6/10/94	0	0	22	0	1	23
7/10/94	0.97	4.0	0	14	20	34
7/10/94	0	0	34	0	2	36
10/10/94	0.97	4.5	0	12	16	28
10/10/94	0	0	28	0	0	28
11/10/94	0.97	4.5	0	15	21	36
11/10/94	0	0	32	0	4	36
12/10/94	0.97	4.5	0	10	15	25
12/10/94	0	0	22	0	3	25
13/10/94	1.1	5.0	0	2	35	37
13/10/94	0	0	20	0	1	21
14/10/94	1.1	5.0	0	1	31	32
14/10/94	0	0	28	0	2	30
18/10/94	1.1	5.0	0	4	36	40
18/10/94	0	0	38	0	2	40

These statistical analyses indicate that the sixth instar larvae is the most tolerant to phosphine. This result is consistent with those obtained in the commercial scale tests reported in Chapter 4.

The numbers of third and fourth instar larvae that were found outside their webbing shelters at the end of fumigation are presented in Table A.9 and A.10. These results clearly illustrate that most of the third and fourth instar larvae emerged from their shelters during exposure. These were found mostly on the bottom of the chamber and some were found hanging on the foliage or from branches of the flowers. All these larvae were dead immediately after fumigation. A small number of larvae that remained inside their webbing shelters were severely affected at phosphine

Table A.9 Numbers of third instar *S. ejectana* larvae found outside and inside of their webbing shelters at the end of exposure time.

Date	Concentration ($g.m^{-3}$)	Exposure time (h)	Inside			Outside		
			S.aff	Dead	Total	S.aff	Dead	Total
4/10/94	0.69	4.0	3	1	4	0	15	15
5/10/94	0.69	4.0	4	2	6	0	9	9
6/10/94	0.97	4.0	1	3	4	0	15	15
7/10/94	0.97	4.0	1	4	5	0	11	11
10/10/94	0.97	4.5	1	3	4	0	25	25
11/10/94	0.97	4.5	2	4	6	0	30	30
12/10/94	0.97	4.5	0	3	3	0	15	15
13/10/94	1.1	5.0	0	6	6	0	12	12
14/10/94	1.1	5.0	0	5	5	0	10	10
18/10/94	1.1	5.0	0	7	7	0	23	23

Table A.10 Number of fourth instar *S. ejectana* larvae found outside and inside of their webbing shelters at the end of exposure time.

Date	Concentration ($g.m^{-3}$)	Exposure time (h)	Inside			Outside		
			S.aff	Dead	Total	S.aff	Dead	Total
4/10/94	0.69	4.0	2	4	6	0	8	8
5/10/94	0.69	4.0	2	3	5	0	10	10
6/10/94	0.97	4.0	1	4	5	0	16	16
7/10/94	0.97	4.0	0	4	4	0	12	12
10/10/94	0.97	4.5	1	6	7	0	23	23
11/10/94	0.97	4.5	0	2	2	0	28	28
12/10/94	0.97	4.5	1	3	4	0	15	15
13/10/94	1.1	5.0	0	6	6	0	12	12
14/10/94	1.1	5.0	0	2	2	0	14	14
18/10/94	1.1	5.0	0	4	4	0	20	20

concentrations of 0.69 to 0.97 $g.m^{-3}$ and exposures of 4 to 4.5 h. However, complete mortality of all these third and fourth instar larvae was achieved with increasing phosphine concentration to 1.1 $g.m^{-3}$ and an exposure time to 5 h.

The data presented in these tables indicate that phosphine presumably caused some larvae to abandon their shelters during fumigation. These are more vulnerable to death than those that remained inside their shelters. However, a comparatively higher phosphine concentration (1.1 $g.m^{-3}$) and exposure time (5 h) killed all the larvae irrespective of their position (inside or outside of their shelters). This suggests that both the exposure time and concentration were sufficient for the phosphine to diffuse into the shelters and act on the vulnerable sites of the insects. The results of the fumigation of fifth to sixth instar larvae with phosphine are presented in Table A.11 and A.12. These data indicate that comparatively large numbers of larvae remained inside their webbing shelters during the exposure period. The webbing shelters of these stages were compact and made of 60 to 90 individual leaves; hence, it would be somewhat difficult for the phosphine to penetrate and reach the sites of the larvae. As a result a large number of fifth and sixth instar larvae were severely affected in all of these fumigations. However, increasing the phosphine concentration to 1.1 $g.m^{-3}$ and exposure time to 5 h increased the mortality rate of these instar stages. Mortality rate among these larvae were less than those of larvae that were found outside their shelters.

Table A.11 Number of fifth instar *S. ejectana* larvae found outside and inside of their webbing shelters at the end of exposure time.

Date	Concentration ($g.m^{-3}$)	Exposure time (h)	Inside			Outside		
			S.aff	Dead	Total	S.aff	Dead	Total
4/10/94	0.69	4.0	10	15	25	2	6	8
5/10/94	0.69	4.0	7	4	11	3	2	5
6/10/94	0.97	4.0	6	8	14	3	4	7
7/10/94	0.97	4.0	6	12	18	4	3	7
10/10/94	0.97	4.5	7	21	28	5	7	12
11/10/94	0.97	4.5	12	20	32	6	14	20
12/10/94	0.97	4.5	11	12	23	4	6	10
13/10/94	1.1	5.0	1	11	12	0	7	7
14/10/94	1.1	5.0	2	12	14	1	3	4
18/10/94	1.1	5.0	4	21	25	2	9	11

Table A.12 Number of sixth instar *S. ejectana* larvae found outside and inside of their webbing shelters at the end of exposure time.

Date	Concentration ($g.m^{-3}$)	Exposure time (h)	Inside			Outside		
			S.aff	Dead	Total	S.aff	Dead	Total
4/10/94	0.69	4.0	9	10	19	5	6	11
5/10/94	0.69	4.0	7	3	10	3	2	5
6/10/94	0.97	4.0	7	9	16	2	5	7
7/10/94	0.97	4.0	11	15	26	3	5	8
0/10/94	0.97	4.5	7	12	19	5	4	9
1/10/94	0.97	4.5	11	14	25	4	7	11
2/10/94	0.97	4.5	7	12	19	3	3	6
3/10/94	1.1	5.0	2	21	23	0	14	14
4/10/94	1.1	5.0	1	23	24	0	8	8
8/10/94	1.1	5.0	4	21	25	0	15	15

The number of days required for the severely affected larvae (third to sixth instars) of *S. ejectana* to die when they were exposed to 3 concentrations of phosphine are presented on Table A.13, A.14, A.15 and A.16. These larvae took two to nine days to die, depending on the larval stage, exposure time and the concentration of phosphine to which they were exposed. Generally, the older (fifth to sixth instar stages) larvae took seven to nine days for complete mortality. All the severely affected fourth instar died within three to four days after fumigation (Table A.13) and two to three days are

Table A.13 Number of days required for the severely affected third instar larvae to die.

Date	Phosphine ($g.m^{-3}$)	No. of larvae severely affected assessment after fumigation (h)						No. of days for complete mortality
		3	24	48	72	96	168	
4/10/94	0.69	3	1	1	0	0	0	2
5/10/94	0.69	4	2	2	2	0	0	3
6/10/94	0.97	1	1	1	1	0	0	3
7/10/94	0.97	1	1	1	0	0	0	2
10/10/94	0.97	1	1	1	1	0	0	3
11/10/94	0.97	2	2	2	0	0	0	2

sufficient for a complete mortality of all the severely affected third instar larvae. Moreover, these third instar larvae that were exposed to a longer exposure time (5 h) and higher concentration (1.1 $g.m^{-3}$) died two to three days earlier than the larvae that were fumigated for a shorter exposure time (4 h) and comparatively lower phosphine concentrations (0.69 and 0.97 $g.m^{-3}$).

Table A.14 Number of days required for the severely affected fourth instar larvae to die.

Date	Phosphine ($g.m^{-3}$)	No. of larvae severely affected assessment after fumigation (h)						No. of days for complete mortality
		3	24	48	72	96	168	
4/10/94	0.69	2	2	2	2	1	1	6
5/10/94	0.69	3	3	3	2	2	2	6
6/10/94	0.97	1	1	1	1	0	0	3
10/10/94	0.97	1	1	1	1	1	0	4
12/10/94	0.97	1	1	1	1	1	0	4

Table A.15 Number of days required for the severely affected fifth instar larvae to die.

Date	Phosphine gm^{-3}	No. of larvae severely affected assessment after fumigation (h)						No. of days for complete mortality
		3	24	48	72	96	168	
4/10/94	0.69	12	12	7	7	2	1	9
5/10/94	0.69	10	10	9	5	3	3	9
6/10/94	0.97	9	9	9	4	4	2	7
7/10/94	0.97	10	10	10	7	7	3	8
10/10/94	0.97	12	12	7	7	6	3	8
12/10/94	0.97	15	15	9	5	3	3	9
13/10/94	1.1	1	1	1	1	1	1	7
14/10/94	1.1	3	3	3	2	2	2	7
18/10/94	1.1	6	6	6	4	4	3	7

Table A.16 Number of days required for the severely affected sixth instar larvae to die.

Date	Phosphine gm^{-3}	No. of larvae severely affected assessment after fumigation (h)						No. of days for complete mortality
		3	24	48	72	96	168	
4/10/94	0.69	14	14	9	9	5	4	9
5/10/94	0.69	10	10	7	7	5	5	9
6/10/94	0.97	9	9	5	5	5	4	8
7/10/94	0.97	14	14	7	6	3	3	9
10/10/94	0.97	12	10	9	7	7	4	8
11/10/94	0.97	15	12	9	9	5	3	9
12/10/94	0.97	10	9	9	9	6	5	8
13/10/94	1.1	2	2	2	2	2	2	7
14/10/94	1.1	1	1	1	1	1	1	7
18/10/94	1.1	4	4	2	2	2	2	7

Tubular black thrips, ***Haplothrips victoriensis***

Complete mortality of all the adult *H. victoriensis* exposed was achieved with phosphine concentrations (0.69 $g.m^{-3}$, 0.97 $g.m^{-3}$ and 1.1 $g.m^{-3}$) and an exposure times of 4, 4.5 and 5 h respectively at 18°C (Table A.17). Furthermore, almost half the exposed adults were severely affected once the temperature of the fumigation fell to 12°C. These severely affected adults showed independent movement of their mouth parts and other appendages under a light source without prodding them. However, all these severely affected adults had died within three to four days after fumigation. This results suggest that adults of *H. victoriensis* are susceptible to phosphine fumigation at a concentration range of 0.69 to 1.1gm^{-3} and an exposure range of 4 to 5 h at 18°C.

Table A.17 Insecticidal efficacy of phosphine to adults of *H. victoriensis*.

Date	Concentration ($g.m^{-3}$)	Exposure time (h)	*H. victoriensis*			
			Live	S. aff	Dead	Total
4/10/94	0.69	4.0	0	0	15	15
4/10/94	0	0	14	0	6	20
5/10/94	0.69	4.0	0	0	14	14
5/10/94	0	0	15	0	3	18
6/10/94	0.97	4.0	0	0	18	18
6/10/94	0	0	17	0	4	21
7/10/94	0.97	4.0	0	0	20	20
7/10/94	0	0	18	0	3	21
10/10/94	0.97	4.5	0	0	23	23
10/10/94	0	0	21	0	3	24
11/10/94	0.97	4.5	0	0	15	15
11/10/94	0	0	15	0	4	19
12/10/94	0.97	4.5	0	0	23	23
12/10/94	0	0	21	0	3	24
13/10/94	1.1	5.0	0	0	14	14
13/10/94	0	0	14	0	2	16
17/10/94	1.1	5.0*	0	11	10	21
17/10/94	0	0	20	0	4	24
18/10/94	1.1	5.0	0	0	16	16
18/10/94	0	0	14	0	2	16

* - Temperature was below 18°C (12°C).

Phytotoxicity

All the exposed *T. calycina, B. coccinea,* and *P. cynaroides* flowers and foliage appeared to be unaffected immediately after fumigation, and seven days following fumigation (Table A.18). These were fumigated at various concentrations (0.69 to 1.1 $g.m^{-3}$), and exposure times at a constant temperature of 18^0C. Hence, these fumigation conditions have no deleterious effects on the vase life of these exposed wildflowers. However, a slight discolouration of mature foliage (10 per cent) of *T. calycina* was observed seven days following fumigations with a phosphine concentration of 1.1 $g.m^{-3}$ and an exposure time of 5 h.

The flowers, and foliage of *L. coniferum* remained in good condition immediately after fumigation at all the concentrations of phosphine and exposure times investigated. However, a slight browning appeared on the edges of mature leaves five days following fumigations at phosphine concentrations of 1.1 $g.m^{-3}$, and an exposure time of 5 h at 18°C. Similar browning of the edges was observed on the unfumigated flowers nine days after the setting up of experiment. This browning progressed towards centre of leaves; as a result, the whole leaf became brown. This leads to complete blackening of leaves within six to seven days following fumigation. Moreover, most of the flowers (75 per cent) and young leaves were in good condition, and they remained similar in appearance to the unfumigated ones. However, a small number (two to three) of bracts and young leaves (25 per cent) showed slight browning at their margins six days following fumigation at this concentration (1.1 $g.m^{-3}$) and exposure time (5 h). On the other hand, all the flowers and foliage that were exposed to phosphine concentrations of 0.69 and 0.97 $g.m^{-3}$ for 4 and 4.5 h were not affected for 8 days (foliage) and 9 (flowers) days.

Carnations, on the other hand, showed slight wilting (drooping) of mature leaves (75 per cent) 24 h after fumigation. However, the flowers and young leaves remained in good condition (similar to the control flowers) for the first 48 h following fumigation. These were fumigated with a phosphine concentration of 0.69 to 0.97 $g.m^{-3}$ for 4 and 4.5 h of exposure time respectively. However, flowers and young leaves showed wilting symptoms within 60 h of fumigation, and this wilting leads to curling and browning of entire petals. Similarly, both the young and mature leaves showed accelerated browning and drying five days after fumigation. Increasing the phosphine concentration to 1.1 $g.m^{-3}$ and exposure time to 5 h shortened the vase life to one to two days. Both the flowers and foliage showed wilting symptoms 12 h following fumigation and this proceeded to curling and discolouration of mature leaves.

Table A.18 Toxicity of phosphine to various flower species fumigated with different concentrations and exposure times.

Species	Concentration (mg/L)	Exposure time (h)	Phytotoxicity		Vase life (days)	
			Flowers	Foliage	Flowers	Foliage
Thryptomene calycina	0.69	4.0	0	0	9	7
Thryptomene calycina	0	0	0	0	9	7
Thryptomene calycina	0.97	4.0	0	0	9	7
Thryptomene calycina	0	0	0	0	9	7
Thryptomene calycina	0.97	4.5	0	0	9	7
Thryptomene calycina	0	0	0	0	9	7
Thryptomene calycina	1.1	5.0	0	0	8	7
Thryptomene calycina	0	0	1	2	9	6
Banksia coccinea	0.69	4.0	0	0	9	8
Banksia coccinea	0	0	0	0	9	8
Banksia coccinea	0.97	4.0	0	0	9	8
Banksia coccinea	0	0	0	0	9	8
Banksia coccinea	0.97	4.5	0	0	9	8
Banksia coccinea	0	0	0	0	9	8
Banksia coccinea	1.1	5.0	0	0	9	8
Banksia coccinea	0	0	0	0	9	8
Protea cynaroides	0.69	4.0	0	0	9	8
Protea cynaroides	0	0	0	0	9	8
Protea cynaroides	0.97	4.0	0	0	9	8
Protea cynaroides	0	0	0	0	9	8
Protea cynaroides	0.97	4.5	0	0	9	8
Protea cynaroides	0	0	0	0	9	8
Protea cynaroides	1.1	5.0	0	0	9	8
Protea cynaroides	0	0	0	0	9	8
Leucadendron coniferum	0.69	4.0	0	0	9	9
Leucadendron coniferum	0	0	0	0	9	8
Leucadendron coniferum	0.97	4.0	0	0	9	8
Leucadendron coniferum	0	0	0	0	9	8
Leucadendron coniferum	0.97	4.5	0	0	9	8
Leucadendron coniferum	0	0	0	0	9	8
Leucadendron coniferum	1.1	5.0	1	1	6	5
Leucadendron coniferum	0	0	0	0	9	8

Species	Concentration (mg/L)	Exposure time (h)	Phytotoxicity		Vase life (days)	
			Flowers	Foliage	Flowers	Foliage
Carnation (*Dianthus*)	0.69	4.0	3	4	3	2
Carnation (*Dianthus*)	0	0	0	0	6	6
Carnation (*Dianthus*)	0.97	4.0	3	4	3	2
Carnation (*Dianthus*)	0	0	0	0	5	6
Carnation (*Dianthus*)	0.97	4.5	3	4	3	2
Carnation (*Dianthus*)	0	0	0	0	6	5
Carnation (*Dianthus*)	1.1	5.0	5	6	1	1
Carnation (*Dianthus*)	0	0	0	0	6	5

These results indicate that wildflowers can be fumigated with phosphine (concentration of 0.69 to 1.1 $g.m^{-3}$ with an exposure period of 4 to 5 h) for the postharvest disinfestation without substantially reducing the vase life of the flowers. Similarly, unfumigated flowers remained in good condition for up to seven to nine days. It is well established that most cut flowers are highly sensitive to temperature and the concentration of fumigants, hence, it is important to reduce either one or both of these variables. However, both these variables are important for a successful fumigation process and failure to maintain either of these parameters may lead to survival of some exposed insect pests. It is therefore important to establish a window of efficacy which results in the mortality of pest biota, but which maintain vase life.

In these preliminary trials the insecticidal efficacy of phosphine was investigated only on two insect species, and phytotoxicty studies were conducted on four export oriented species of wildflowers and one species of cutflower. The susceptibility or tolerance of different insect species and their developmental stages to various fumigants differ substantially, hence, it can be assumed that this concentration of phosphine, and exposure time at a particular temperature that gave a complete kill of these insect species does not necessarily give the same results on other insect species and their developmental stages. Therefore it is imperative to investigate the insecticidal efficacy of phosphine to various species of insects that infest the wildflowers. Based on these results, phosphine was selected for large scale fumigation trials and the feasibility of using various dose rates and exposure time had investigated.

A.1.2.9 Discussion

Initial laboratory experiments are essential to determine the feasibility of using candidate fumigants on large scale, and this gives an opportunity for reducing the time, and cost involved in conducting large scale trials. Furthermore, it is plausible that in laboratory experiments fumigants are readily available to the insects due to the small volume of the chamber, whereas in large scale fumigations deeper penetration and perhaps higher dosages are necessary. Hence, certain modifications are imperative to use and interpret the results from large scale experiments. It is anticipated that higher concentrations of phosphine, exposure times or temperature are necessary for complete mortality of all the insect pests exposed on a commercial scale.

A.1.2.9.1 Insecticidal efficacy of carbon disulphide and phosphine

In making comparison between these two fumigants, it is obvious that, although, carbon disulphide killed all the exposed insect pests, the concentrations and exposure times investigated were highly toxic to the wildflowers. On the other hand, fumigations using phosphine did not give a complete kill of all the *S. ejectana* larvae exposed for 4 or 4.5 h and a phosphine concentration of 0.69 and 0.97 $g.m^{-3}$ respectively.

The mortality of all the exposed insects increases considerably as the exposure time increased to five hours from four hours. Also, increasing the phosphine concentration to 1.1 $g.m^{-3}$ from either 0.69 or 0.97 $g.m^{-3}$, subsequently increased the mortality rate. A few exposed third and fourth instar larvae were severely affected at phosphine concentrations of 0.67 and 0.97 $g.m^{-3}$ and 4 to 4.5 h of exposure respectively. However, increasing the exposure time to 5 h and the phosphine concentration to 1.1 $g.m^{-3}$ killed all these instar stages. On the other hand, a comparatively large number of fifth and sixth instar larvae was severely affected, and the rest died with phosphine concentrations of 0.67 or 0.97 $g.m^{-3}$ at 4 and 4.5 h of exposure time respectively.

Hence, if phosphine is to be used commercially it is likely that a combination of higher concentrations, exposure times and temperatures may be necessary.

The data from the experiments indicate that adults of *H. victoriensis* are of susceptible to all the concentrations of phosphine (0.69 to 1.1 $g.m^{-3}$) and exposure times (4.5 to 5 h) investigated. However, 54 per cent of the adults were severely affected once the fumigation temperature was not maintained at 18°C.

All *S. ejectana* larvae that were exposed to carbon disulphide remained in their shelters during exposure, which indicates that the fumigants penetrate in sufficient concentrations to be lethal to the larvae. It can be assumed that once it reaches the site of the insects, carbon disulphide might have killed the larvae very quickly. On the other hand, a number of larvae that were fumigated with phosphine were found hanging on foliage or on the bottom of the chamber. The number of larvae that emerged from their shelters varied considerably, and this depended on concentration of phosphine and larval instar stage. Generally, a large number third and fourth instar larvae emerged from their shelters when exposed to a lower phosphine concentration than higher concentration. But in the case of mature larvae (fifth and sixth instar) there was no considerable difference between the number of larvae emerging from their shelters.

Of all the instar stages exposed, a larger number of third and fourth instar larvae emerged from their shelters than mature stages. Generally, the shelters were made of 25 to 95 individual leaves. Early instar stages (third and fourth) use 25 to 40 leaves and are not as compact as mature larval shelters. Hence, phosphine can penetrate into their shelters quite easily and rapidly and act at the sites of the larvae. The results suggest that due to its slow action the insects were stirred by the phosphine hence they abandoned their shelters. Mature larvae use 50 to 90 leaves for their webbing shelters, and these act as a barrier for the phosphine to penetrate into the shelters.

Mortality of the larvae that were found outside their shelters was higher than that those found inside the shelters. Once they emerged from their shelters they were exposed

directly to toxic phosphine gas, hence they were more prone to death than those that remained inside their shelters.

Reactions to the two fumigants by the larvae following their death were entirely different. The phosphine fumigated larvae (dead) changed in colour from creamy yellow to brown within 48 to 60 h after dying, generally starting from the posterior and spreading towards the head. This proceeds to the complete blackening of the whole larvae within 68 h. However, the dead larvae that had been fumigated with carbon disulphide usually changed their colour to black starting from the abdomen and proceeding towards the head this occurred within 12 to 24 h after fumigation, and complete blackening occurs 48 to 60 h after death. Generally, the colour change was slower for 5th and 6th instar larvae than early stages for the phosphine fumigated larvae, but this reaction was similar for all stages of larvae that were fumigated with carbon disulphide. Generally, the colour change was followed by oozing of body fluid from the abdomen.

A.1.2.9.2 ***Phytotoxicity***

Vase life of the flowers is important, as it determines their marketability. Both concentrations of carbon disulphide (concentration of 650 and 742 mg/L) accelerated the blackening of the leaves of *Banksia spp*, *Protea spp* within 24 h of fumigation. Moreover, the foliage and florets of *T. calycina* had dropped off three to four days of treatment. On the other hand, all the fumigated (at a concentration of 657 $mg.L^{-1}$ and an exposure time of 2 h) carnations showed symptoms of wilting immediately after fumigation, and drying followed in 48 h. This indicates that carbon disulphide is highly toxic to the flowers investigated. In view of these results, and those reported by Williams (1996) further experiments using carbon disulphide were not carried out.

On the other hand, the concentration of phosphine and exposure times investigated were toxic to both exposed insect pests without affecting the vase life of most of the flowers. Slight damage to foliage and flowers of *L. coniferum* had been observed once

the concentrations of phosphine were high (1.1 $g.m^{-3}$) with a comparatively long exposure time (5 h); however, the damage was negligible. The results show that carnations were highly susceptible to phosphine fumigation, all the flowers and foliage were damaged to an unacceptable level within one to three days following completion of fumigation. In view of these results phosphine, was selected for large scale fumigation trials for the postharvest disinfestation of wildflowers.

A.1.2.9.3 The prospects for large scale fumigation trials

All these fumigation trials with phosphine were conducted using standard phosphine (one per cent phosphine in nitrogen as a carrier gas). Insects are generally narcotised in high concentration nitrogen atmospheres, and this enables them to withstand anoxic conditions for some time without any harmful effects (Bond *et al* 1967 b, Bond *et al* 1969 and Kashi 1981). Once this narcotised condition is created, the insects are protected from the toxic action of phosphine. This supports the findings of Kashi (1981) who found that in nitrogen and phosphine atmospheres (phosphine concentration of 10 mgL^{-1} and exposure time of 12 to 24 h) the mortality rate of five species of stored products insects (*T. castaneum, T. confusum, R. dominica, S. oryzae,* and *S. granarius*) were very low (1.2 to 3.2 per cent). He concluded that these insects were protected from the toxic action of phosphine due to the presence of nitrogen. Similarly, in these trials a number of larvae was severely affected, and all others were killed from the same population. This could be either due to the genetic or physiological variability among the individuals of the same population, or due to the narcotising effects of nitrogen, mixed with phosphine.

One of the recent research findings on fumigation techniques has resulted in new ways of applying phosphine. Mixing phosphine with carbon dioxide as a carrier gas may augment the insecticidal properties of phosphine. Carbon dioxide has been used as a fumigant for many decades (Saayakan, 1938, Desmarchelier and Wohlgemuth 1984 and Price 1984). It can be assumed that the phosphine-carbon dioxide combination would assist in both the biological actions, and the distribution of fumigants within the

treatment enclosure. This is substantiated by the findings of Banks (1984), who found that a phosphine-carbon dioxide combination in enclosed space acts more quickly than pure phosphine in air.

Similarly, findings of Bond and Buckland (1978) indicate that toxicity of various fumigants increase with the addition of carbon dioxide. They found that toxicity of phosphine to adults of *T. castaneum* and *S. granarius* increased more than 2 and 3 fold respectively when phosphine was combined with 30 per cent carbon dioxide at 25^0C. They speculated that the mechanism involved in the action of carbon dioxide could be that it promoted the spiracular opening of the insects that in turn increases the uptake of phosphine. Moreover, the earlier findings of Saaykan (1938) supports this view. In his experiments using carbon dioxide he found that less than a concentration of five per cent of carbon dioxide generally increases the respiration rate of *Tribolium spp.* However, recent research findings show that the efficacy of carbon dioxide decreases or ceases when the level exceeds an upper limit. Quan (1990), observed that the potential action of carbon dioxide for the adults of *T. castaneum* was only at 4 to 6 per cent levels combined with 0.006 mg/L of phosphine. Moreover, Rajendran (1990) claimed that a carbon dioxide level of more than 10 per cent in combination with 0.01 mg/L of phosphine failed to increase the mortality of *T. castaneum* adults.

In addition to this, carbon dioxide and phosphine fumigation in general reduces the time required for degassing from the system, as a consequence of reduced phosphine concentration and presence of the carbon dioxide. Hence, the large scale experiments were planned to conduct using Phosfume® (2 per cent phosphine in carbon dioxide as a carrier gas). The exposure time required and associated biological responses are often limiting factors in the phosphine fumigations especially for perishable commodities fumigation. However, it may be that exposure time can be shortened to a certain extent if carbon dioxide is mixed with phosphine.

Appendix - 2

Insects growing medium

Appendix 2

ARTIFICIAL DIET FOR INSECTS

A.2.1 Modified lightbrown apple moth medium.

Haricot beans	**534 g (Cooked)**	**Ascorbic acid**	**8 g**
Yeast	**80 g**	**Paraben**	**5 g**
Agar	**20 g**	**Sorbic acid**	**2.5 g**
Hot water	**800 ml**	**Phosphoric & propionic acid**	**5 ml**

Method.

- Cook haricot beans in microwave (approximately 40 to 50 min) until tender.
- Add agar to hot water stir and heat on high in microwave until almost boiling approximately 2 min 30 sec) stirring every minute.
- Blend beans, agar mix and yeast in electric mixture.
- When the temperature has fallen to approximately 60^0C add acids and mix well.
- Pour hot into "Chinese take away" containers (61 x 40 x 25 mm) *ca.* 93 to 97 g/container.
- When the media cools down, divide it into 20 to 25 squares using small knife.
- Transfer 3 to 4 masses of mature eggs (dark brown in colour)/container and cover the lid with tissue to avoid condensation and moulding of medium.

- Once the larvae turn to 2nd instar larvae (10 to 15 days at 22^0C and 60 to 65 per cent r.h.) transfer excess larvae into other containers. Maintain 15 to 20 larvae/container.

- Once pupated, transfer 6 pupae/350ml plastic cups.

- Place 6 male and 6 female adults/350 ml plastic cups. Put 8 to 10 holes on the lids using needles.

- Feed the adult moths using 10% honey solution, which contains antioxident (ascorbic acid) and antifungal agent (sorbic acid and paraben) to prevent mould growth, use a cotton wool pad placed in a small plastic lid or cups.

- To store eggs for future breeding, store only green eggs self seal plastic bags at 1^0C, and use within 2 months.

A.2.2 Honey solution.

Honey	**180 ml**	**Sorbic acid**	**1.8 g (0.1%)**
Hot water	**1800 ml**	**Paraben**	**1.8 g**
Ascorbic acid	**10.8 g (0.6%)**	**Ethanol 70%**	**10 ml**

- Dissolve sorbic acid and paraben in ethanol (their water solubility is low)

- Pour honey jar into graduated 1000 ml beaker. Pour enough hot water to dissolve the honey. Pour this into 3000 ml conical flasks and add the rest of the hot water. Mix until all honey is dissolved.

- When cool add the ascorbic acid and the solution of sorbic acid and paraben in alcohol.

Appendix - 3

Statistical Analysis

Appendix 3

Statistical Analysis.

A.3.1 Statistical Analysis of Results of fumigation trials (for Appendix 1, and Chapters 4, 5 and 7)

The relationship between the proportion of dead individuals (eggs, larvae, pupae and adults) and the following variables were examined: date, mass of Phosfume® added (kg), initial phosphine concentration ($g.m^{-3}$), final phosphine concentration ($g.m^{-3}$), exposure time (h), temperature (°C) and relative humidity (per cent). To do this, Pearson correlation coefficients were first calculated. Then a model with a binomial distribution and a logit link function was used to express the proportion dead in terms of the explanatory variables. A stepwise procedure was used, in which only variables making a significant contribution to the model were included. Models were fitted using Genstat 5, a commercial statistical package.

A.3.2 Detailed description of the statistical analysis for the third instar larvae of the leaf rolling moth, *S. ejectana*.

Responses of a number of insect species to a range of fumigation strategies are given in the main body of the thesis. This section gives detail description of the statistical analysis of the result for larvae of the leaf rolling moth that were exposed to various concentrations and exposure times. For all other insect pests and their stages we highlight the key features (correlation matrix and regression analysis) and significance of the analyses.

1: Units: indicates number of input added, in this case number of fumigations.

4: Read (Channel 2): calculates maximum, mean and minimum of the added data

5: Calculate: This step calculates per cent of dead individuals by dividing the number of dead individuals by the total.

The correlation matrix and regression analysis is particularly important. The correlation is a measure of closeness of the relationship between two variables. In this analysis, correlation among various parameters (phosphine concentration, exposure time-hours and the resulting per cent dead of the exposed insects) were investigated. The positive and highest value is deemed to have greater influence over the mortality than other parameters.

Estimate of regression coefficient: Regression is a technique for fitting a straight line that relates one quantitative variable to another. This deals primarily with the means of one variable and how their location changes with another variable. Hence, a correlation coefficient indicates something about a joint relationship between variables (degree of closeness of linear relationship between two variables y and x), a regression coefficient indicates that if the value of independent variable is altered then the value of the dependent variable is altered by a certain amount on the average. From this analysis that the regression coefficient indicates that $e^{6.59}=727$, i.e. for every degree Celsius increase of fumigant temperature it is anticipated that mortality of the larvae of the leaf rolling moth increased by a factor of 727.

The 't' statistics allow one to test whether the selected parameter differs significantly from zero, keeping other parameters fixed. If the estimate of variance is supplied, then the 't-statistics' actually have a standard normal distribution, indicated by the column heading 't()'. In general the degrees of freedom for tests of significance are put inside t(). The t(*) indicates infinity. This generally happens if the dispersion from the data, and hence, it assumes that it is one (which corresponds exactly to a binomial distribution). So it uses normal distribution value i.e. 1.96 for multiplying the standard

error. A normal distribution is just a t distribution with infinite degrees of freedom. Standard error of the observation is estimated simply by the square root of the residual mean square.

The residual mean deviances indicate which of the parameters are important and they are listed in descending order. In this particular analysis phosphine concentration was found to be the most important parameter that determines the mortality of the exposed larvae.

The summary of the analyses dealing with variance, which subdivides the total sum of squares, corrected for the mean, between that explained by the regression and that which is not explained. This table has a standard form with columns for the degrees of freedom (d.f), variance, mean deviance and deviance ratio.

Table A.3.1 Insecticidal efficacy of phosphine to third instar larvae of *S. ejectana*

Date	**Concentration (gm^{-3})**	**Exposure time (h)**	***S. ejectana***			
			Live	S. aff	Dead	Total
4/10/94	0.69	4.0	0	3	16	19
5/10/94	0.69	4.0	0	4	11	15
6/10/94	0.97	4.0	0	1	18	19
7/10/94	0.97	4.0	0	1	15	16
10/10/94	0.97	4.5	0	1	28	29
11/10/94	0.97	4.5	0	2	34	36
12/10/94	0.97	4.5	0	0	18	18
13/10/94	1.1	5.0	0	0	18	18
14/10/94	1.1	5.0	0	0	15	15
18/10/94	1.1	5.0	0	0	30	30

```
1 units [10]
2 factor [levels = 1] date
3 open '141.dat' ; channel = 2
4  read [channel = 2] date, phcon, exptme, saff, dead, total
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Phocon	0.6900	0.9530	1.1000	10	0
Exptme	4.000	4.450	5.000	10	0
Saff	0.000	1.200	4.000	10	0
Dead	11.00	20.30	34.00	10	0
Total	15.00	21.50	36.00	10	0

Identifier	Values	Missing	Levels
date	10	0	1

5 calc pdead = dead/total

6 correlate [print = corr] phcon, exptme, pdead

*** Correlation matrix ***

	Phosphine concentration	Exposure time	Per cent dead
Phosphine concentration	1.000		
Exposure time	0.785	1.000	
Per cent dead	0.922	0.699	1.000

7 tabulate [class = date; print = mean] pdead

Mean

date

1 0.9370

8

9 model [dist = binomial] dead; nbinomial = total

10 terms date, phcon, exptme

10 step date, phcon, exptme

*** Step 1: Residual mean deviances ***

0.5593 Adding phcon

0.8346 Adding exptme

2.1265 No change

Chosen action: Adding phcon

***** Regression Analysis *****

Response variate: dead

Binomial totals: total

Distribution: Binomial

Link function: Logit

Fitted terms: Constant, phcon

*** Summary of analysis ***

	df	Deviance	Mean Deviance	Deviance Ratio
Regression	1	14.664	14.6643	14.66
Residual	8	4.479	0.5593	
Total	9	19.139	2.1265	

Change -1 -14.664 14.6643 14.66

* MESSAGE: ratios are based on dispersion parameter with value 1

* MESSAGE: The following units have high leverage:

1 0.55

2 0.43

*** Estimates of regression coefficients ***

	Estimate	s.e	t(*)
Constant	-3.90	1.70	-2.30
Phosphine concentration	7.52	2.03	3.71

* MESSAGE: s.e.s are based on dispersion parameter with value 1

12 stop

End of job. Maximum of 3501 data units used at line 11 (85743 left)

The regression coefficient indicates that $e^{7.52}$ = 1844, i.e. for every gm^{-3} increase in phosphine concentration; it is most likely that the third instar larvae of leaf rolling moth mortality increase by a factor of 1844.

A.3.3 Key features of the statistical analysis of results presented in Appendix 1

A.3.3.1 Fourth instar larvae of *S. ejectana* (Appendix 1, Table A.6, Page 385)

Correlation matrix

	Phosphine concentration	Exposure time	Per cent dead
Phosphine concentration	1.000		
Exposure time	0.785	1.000	
Per cent dead	0.928	0.623	1.000

Estimates of regression coefficients

	Estimate	**Standard error**	**t(*)**
Constant	-4.23	2.08	-2.03
Phosphine concentration	8.34	2.53	3.29

The regression coefficient indicates that $e^{8.34}$ = 4188, i.e. for every $g.m^{-3}$ increase in phosphine concentration; odds of the fourth instar larvae of leaf rolling moth mortality increased by a factor of 4188.

A.3.3.2 Fifth instar larvae of *S. ejectana* (Appendix 1, Table A.7, Page 386)

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-4.564	0.966	-4.72
Phosphine concentration	5.41	1.01	5.35

The regression coefficient indicates that $e^{5.41} = 223$, i.e. for every $g.m^{-3}$ increase in phosphine concentration; it is most likely that the fifth instar larvae of leaf rolling moth mortality will increase by a factor of 223.

A.3.3.4 Sixth instar larvae of *S. ejectana* (Appendix 1, Table A.8, Page 387)

Correlation matrix

	Phosphine concentration	Exposure time	Per cent dead
Phosphine concentration	1.000		
Exposure time	0.785	1.000	
Per cent dead	0.844	0.871	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-8.37	1.52	-5.52
Exposure time	2.072	0.346	5.99

The regression coefficient indicates that $e^{2.072} = 7.94$, i.e. for every $g.m^{-3}$ increase in exposure time; it is most likely that the sixth instar larvae of leaf rolling moth mortality will increase by a factor of 7.94.

A.3.4 Key features of the statistical analysis for the results presented in the Chapter 4

A.3.4.1 Larvae of *S. ejectana* (all instar stages) (Chapter 4, Table 4.3, Page 153)

Correlation matrix

	Initial phosphine	Final phosphine	Exposure time	Temperature	Per cent dead
Initial phosphine	1.000				
Final phosphine	0.879	1.000			
Exposure time	-0.206	-0.521	1.000		
Temperature	0.304	-0.171	0.494	1.000	
Per cent dead	0.355	0.104	0.094	0.699	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-1.87	2.97	-0.63
Temperature	0.203	0.161	1.26

The regression coefficient indicates that $e^{0.203} = 1.2$, i.e. for every °C increase of fumigation temperature, it is most likely that the larvae of the leaf rolling moth mortality will increase by a factor of 1.2.

A.3.4.2 Eggs of *E. postvittana* (Chapter 4, Table 4.10, Page 160)

Correlation matrix

	Initial phosphine	Final phosphine	Exposure time	Temperature	Relative humidity	Per cent dead
Initial phosphine	1.000					
Final phosphine	0.323	1.000				
Exposure time	-0.154	0.784	1.000			
Temperature	-0.117	-0.221	0.278	1.000		
Relative humidity	0.832	0.504	0.312	0.338	1.000	
Per cent dead	-0.482	-0.175	0.444	0.914	0.029	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-9.77	8.07	-1.21
Temperature	0.533	0.448	1.19

The regression coefficient indicates that $e^{0.533} = 1.7$, i.e. for every °C increase of fumigation temperature; odds of the eggs of lightbrown apple moth mortality increased by a factor of 1.7.

A.3.4.3 Third instar larvae of *E. postvittana* (Chapter 4, Table 4.15, Page 165)

Correlation matrix

	Phosphine (kg)	Initial phosphine	Final phosphine	Exposure time	Temperature	Relative humidity	Per cent dead
Phosphine (kg)	1.000						
Initial phosphine	0.167	1.000					
Final phosphine	0.188	0.110	1.000				
Exposure time	0.073	-0.330	0.097	1.000			
Temperature	0.133	0.404	-0.245	0.130	1.000		
Relative humidity	0.444	0.209	0.011	0.306	0.112	1.000	
Per cent dead	0.002	0.539	0.088	0.225	0.521	-0.199	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-32.1	13.0	-2.47
Temperature	2.084	0.778	2.68

The regression coefficient indicates that $e^{2.084} = 8.03$, i.e. for every °C increase of fumigation temperature, it is likely that the third instar larvae of lightbrown apple moth mortality will increase by a factor of 8.03.

A.3.4.4 Fourth instar larvae of *E. postvittana* (Chapter 4, Table 4.20, Page 169) (fumigated in the 900 L chamber at Frankston)

Correlation matrix

	Initial phosphine	Final phosphine	Exposure time	Temperature	Relative humidity	Per cent dead
Initial phosphine	1.000					
Final phosphine	0.119	1.000				
Exposure time	-0.371	0.267	1.000			
Temperature	0.404	-0.254	0.138	1.000		
Relative humidity	0.271	0.310	0.026	0.142	1.000	
Per cent dead	0.161	-0.366	0.333	0.448	-0.145	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-25.10	6.59	-3.81
Temperature	1.596	0.392	4.07

The regression coefficient indicates that $e^{1.596} = 4.93$, i.e. for every °C increase of fumigation temperature, odds of the fourth instar larvae of lightbrown apple moth mortality increased by a factor of 4.93.

A.3.4.5 Fourth instar larvae of *E. postvittana* (Chapter 4, Table 4.22, Page 170) (fumigated in a 27 m^3 chamber - Emerald).

Correlation matrix

	Initial phosphine	Final phosphine	Exposure time	Temperature	Per cent dead
Initial phosphine	1.000				
Final phosphine	0.935	1.000			
Exposure time	-0.557	-0.754	1.000		
Temperature	0.406	0.137	0.298	1.000	
Per cent dead	0.389	0.359	0.250	0.411	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-68.2	76.8	-0.89
Temperature	3.94	4.20	0.94

The regression coefficient indicates that $e^{3.94} = 51$, i.e. for every °C increase of fumigation temperature, it is likely that the fourth instar larvae of lightbrown apple moth mortality will increase by a factor of 51.

A.3.4.6 Fifth instar larvae of *E. postvittana* (Chapter 4, Table 4.25, Page 172) (900 L chamber at Frankston)

Correlation matrix

	Phosphine (Kg)	Initial phosphine	Final phosphine	Exposure time	Temperature	Relative humidity	Per cent dead
Phosphine (Kg)	1.000						
Initial phosphine	0.167	1.000					
Final phosphine	0.188	0.110	1.000				
Exposure time	0.073	-0.330	0.097	1.000			
Temperature	0.133	0.404	-0.245	0.130	1.000		
Relative humidity	0.444	0.209	0.011	0.306	0.112	1.000	
Per cent dead	0.181	-0.232	-0.236	0.336	0.336	0.050	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	2.779	0.626	4.44
Phosphine (final conc)	-1.277	0.876	-1.46

The regression coefficient indicates that $e^{-1.277} = 0.27$, i.e. for every $g.m^{-3}$ increase in final phosphine concentration, it is likely that the fifth instar larvae of lightbrown apple moth mortality will increase by a factor of 0.27.

A.3.4.7 Fifth instar larvae of *E. postvittana* (Chapter 4, Table 4.26, Page 173) (27 m^3 chamber at Emerald)

Correlation matrix

	Phosphine (Kg)	Initial phosphine	Final phosphine	Exposure time	Temperature	Per cent dead
Phosphine (Kg)	1.000					
Initial phosphine	0.400	1.000				
Final phosphine	0.334	0.935	1.000			
Exposure time	0.066	-0.557	-0.754	1.000		
Temperature	-0.171	0.406	0.137	0.298	1.000	
Per cent dead	0.360	-0.089	-0.346	0.874	0.547	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-107.8	52.2	-2.07
Temperature	6.02	2.85	2.11

The regression coefficient indicates that $e^{6.02} = 411$, i.e. for every °C increase of fumigation temperature; it is most likely that the fifth instar larvae of lightbrown apple moth mortality will increase by a factor of 411.

A.3.4.8 Sixth instar larvae of *E. postvittana* (Chapter 4, Table 4.31, Page 176) (900 L chamber at Frankston)

Correlation matrix

	Phosphine (Kg)	Initial phosphine	Final phosphine	Exposure time	Temperature	Relative humidity	Per cent dead
Phosphine (Kg)	1.000						
Initial phosphine	0.167	1.000					
Final phosphine	0.188	0.110	1.000				
Exposure time	0.073	-0.330	0.097	1.000			
Temperature	0.085	0.390	-0.269	0.131	1.000		
Relative humidity	0.444	0.209	0.011	0.306	0.056	1.000	
Per cent dead	0.154	-0.019	-0.376	0.652	0.389	0.318	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-18.22	4.41	-4.13
Exposure time	1.177	0.272	4.32

The regression coefficient indicates that $e^{1.177} = 3.2$, i.e. for every hour increase of exposure time, it is most likely that the sixth instar larvae of the lightbrown apple moth mortality will increase by a factor of 3.2.

A.3.4.9 Sixth instar larvae of *E. postvittana* (Table 4.33, Page 178) (27 m^3 chamber at Emerald)

Correlation matrix

	Phosphine (Kg)	Initial phosphine	Final phosphine	Exposure time	Temperature	Per cent dead
Phosphine (Kg)	1.000					
Initial phosphine	0.400	1.000				
Final phosphine	0.334	0.935	1.000			
Exposure time	0.066	-0.557	-0.754	1.000		
Temperature	-0.171	0.406	0.137	0.298	1.000	
Per cent dead	0.577	-0.436	-0.489	0.739	-0.238	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-15.2	10.1	-1.50
Exposure time	1.141	0.646	1.77

The regression coefficient indicates that $e^{1.141} = 3.126$, i.e. for every hour increase of exposure period, it is most likely that the sixth instar larvae of the lightbrown apple moth mortality will increase by a factor of 3.126.

A.3.4.10 Pre-pupae of *E. postvittana* (Chapter 4, Table 4.39, Page 181) (900 L chamber at Frankston)

Correlation matrix

	Phosphine (Kg)	Initial phosphine	Final phosphine	Exposure time	Temperature	Relative humidity	Per cent dead
Phosphine (Kg)	1.000						
Initial phosphine	-0.008	1.000					
Final phosphine	0.059	0.836	1.000				
Exposure time	0.095	-0.276	-0.249	1.000			
Temperature	-0.036	0.243	-0.168	0.288	1.000		
Relative humidity	0.086	-0.062	-0.226	0.477	-0.021	1.000	
Per cent dead	-0.343	0.118	-0.344	0.065	0.673	0.425	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-3.04	3.58	-0.85
Temperature	0.264	0.198	1.33

The regression coefficient indicates that $e^{0.264} = 1.302$, i.e. for every °C increase of fumigation temperature, it is most likely that the pre-pupae of lightbrown apple moth mortality will increase by a factor of 1.302.

A.3.4.11 Pupae of *E. postvittana* (Table 4.43, Page 183) (900 L chamber at Frankston)

Correlation matrix

	Initial phosphine	Final phosphine	Exposure time	Temperature	Relative humidity	Per cent dead
Initial phosphine	1.000					
Final phosphine	0.110	1.000				
Exposure time	-0.330	0.097	1.000			
Temperature	0.404	-0.245	0.130	1.000		
Relative humidity	0.209	0.011	0.306	0.112	1.000	
Per cent dead	0.521	0.205	0.268	0.426	-0.139	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-57.8	17.8	-3.24
Temperature	3.61	1.08	3.35

The regression coefficient indicates that $e^{3.61} = 36.9$, i.e. for every °C increase of fumigation temperature, it is most likely that the pupae of lightbrown apple moth mortality will increase by a factor of 36.9.

A.3.4.12 Eggs of *T. urticae* (Table 4.48 &4.49, Page 188) (27 m^3 chamber at Emerald and Gembrook)

Correlation matrix

	Phosphine	Initial phosphine	Final phosphine	Exposure time	Temperature	Relative humidity	Per cent dead
Phosphine	1.000						
Initial phosphine	0.388	1.000					
Final phosphine	0.346	0.314	1.000				
Exposure time	0.150	0.025	0.377	1.000			
Temperature	-0.058	0.534	-0.083	0.281	1.000		
Relative humidity	0.428	0.166	0.268	-0.003	0.108	1.000	
Per cent dead	0.386	0.803	0.587	0.490	0.627	0.300	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-0.663	0.206	-3.22
Phosphine (initial)	3.486	0.232	15.01

The regression coefficient indicates that $e^{3.486} = 32.6$, i.e. for every $g.m^{-3}$ increase of initial phosphine concentration, it is most likely that the eggs of *T. urticae* mortality will increase by a factor of 32.6.

A.3.4.13 Eggs of *T. urticae* (Table 4.50, Page 189) (900 L chamber at Frankston)

Correlation matrix

	Phosphine (kg)	Initial phosphine	Final phosphine	Exposure time	Temperature	Per cent dead
Phosphine (Kg)	1.000					
Initial phosphine	0.406	1.000				
Final phosphine	0.331	0.942	1.000			
Exposure time	0.066	-0.546	-0.763	1.000		
Temperature	-0.171	0.405	0.132	0.298	1.000	
Per cent dead	0.122	-0.652	-0.819	0.977	0.086	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	24.57	4.28	5.74
Phosphine (Initial con)	-19.29	3.58	-5.39

A.3.4.14 Larvae of the two-spotted mite *T. urticae* (Chapter 4, Table 4.56, Page 195-196) - fumigated at Gembrook and Emerald

Correlation matrix

	Phosphine	Initial phosphine	Final phosphine	Exposure time	Temperature	Per cent dead
Phosphine	1.000					
Initial phosphine	0.406	1.000				
Final phosphine	0.331	0.942	1.000			
Exposure time	0.066	-0.546	-0.763	1.000		
Temperature	-0.171	0.405	0.132	0.298	1.000	
Per cent dead	0.146	-0.254	-0.552	0.929	0.574	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-50.0	36.2	-1.38
Temperature	2.98	1.97	1.51

The regression coefficient indicates that $e^{2.98}$ = 19.6, i.e. for every °C increase of fumigation temperature, it is most likely that the larvae of two-spotted mite mortality will increase by a factor of 19.6.

A.3.4.15 Adults of *T. urticae* (Chapter 4, Table 4.60, Page 199) (900 L chamber at Frankston)

Correlation matrix

	Phosphine	Initial phosphine	Final phosphine	Exposure time	Temperature	Per cent dead
Phosphine	1.000					
Initial phosphine	0.406	1.000				
Final phosphine	0.331	0.942	1.000			
Exposure time	0.066	-0.546	-0.763	1.000		
Temperature	-0.171	0.405	0.132	0.298	1.000	
Per cent dead	0.077	0.026	-0.297	0.771	0.818	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-18.2	34.9	-0.52
Temperature	1.29	1.90	0.68

The regression coefficient indicates that $e^{1.29} = 3.63$, i.e. for every °C increase of fumigation temperature; it is most likely that the adults of two-spotted mite mortality will increase by a factor of 3.63.

A.3.4.16 Adults of *T. urticae* (Chapter 4, Table 4.59, Page 198) (27 m^3 chamber at Emerald and Gembrooke)

Correlation matrix

	Phosphine	Initial phosphine	Final phosphine	Exposure time	Temperature	Per cent dead
Phosphine	1.000					
Initial phosphine	0.388	1.000				
Final phosphine	0.346	0.314	1.000			
Exposure time	0.150	0.025	0.377	1.000		
Temperature	-0.058	0.534	-0.083	0.281	1.000	
Per cent dead	0.539	0.681	0.338	0.430	0.510	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-20.88	6.55	-3.19
Temperature	1.523	0.394	3.87

The regression coefficient indicates that $e^{1.523} = 4.5$, i.e. for every °C increase of fumigation temperature; it is most likely that the adults of two-spotted mite mortality will increase by a factor of 4.5.

A.3.4.17 Larvae of *M. persicae* (Chapter 4, Table 4.63, Page 201) - Fumigated in 900 L chamber at Frankston.

Correlation matrix

	Phosphine	Initial phosphine	Final phosphine	Exposure time	Temperature	Per cent dead
Phosphine	1.000					
Initial phosphine	0.781	1.000				
Final phosphine	0.554	0.332	1.000			
Exposure time	0.011	-0.199	-0.046	1.000		
Temperature	0.351	-0.132	-0.250	0.278	1.000	
Per cent dead	0.543	0.438	-0.083	-0.592	0.513	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	33.45	4.93	6.78
Exposure time	-1.987	0.292	-6.80

The regression coefficient indicates that $e^{-1.987} = 0.13$, i.e. for every hour increase of exposure time; it is most likely that the larvae of green peach aphid mortality will increase by a factor of 0.13.

A.3.4.18 Adults of *M. persicae* (Chapter 4, Table 4.65, Page 202) - Fumigated in the 900 L chamber at Franston.

Correlation matrix

	Phosphine	Initial phosphine	Final phosphine	Exposure time	Temperature	Per cent dead
Phosphine	1.000					
Initial phosphine	0.781	1.000				
Final phosphine	0.554	0.332	1.000			
Exposure time	0.011	-0.199	-0.046	1.000		
Temperature	0.351	-0.132	-0.250	0.278	1.000	
Per cent dead	-0.224	-0.254	-0.446	-0.719	0.313	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	28.90	4.84	5.98
Exposure time	-1.703	0.289	-5.89

The regression coefficient indicates that $e^{-1.703} = 0.182$, i.e. for every hour increase of exposure time; it is most likely that the adults of green peach aphid mortality will increase by a factor of 0.182.

A.3.4.19 Larvae of *M. ornatus* (Chapter 4, Table 4.66, Page 203) - Fumigated in 900 L and 27 m^3 chamber at Emerald.

Correlation matrix

	Phosphine	Initial phosphine	Final phosphine	Exposure time	Temperature	Per cent dead
Phosphine	1.000					
Initial phosphine	0.441	1.000				
Final phosphine	0.180	0.439	1.000			
Exposure time	0.570	0.165	0.368	1.000		
Temperature	0.551	0.838	0.036	0.343	1.000	
Per cent dead	0.488	0.625	0.244	0.775	0.806	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-37.42	5.86	-6.38
Temperature	2.240	0.348	6.44

The regression coefficient indicates that $e^{2.240} = 9.39$, i.e. for every °C increase of fumigation temperature; it is most likely that the larvae of ornate aphid mortality will increase by a factor of 9.39.

A.3.4.19 Adults of *M. ornatus* (Chapter 4, Table 4.69, Page 206)

Correlation matrix

	Phosphine	Initial phosphine	Final phosphine	Exposure time	Temperature	Per cent dead
Phosphine	1.000					
Initial phosphine	0.441	1.000				
Final phosphine	0.180	0.439	1.000			
Exposure time	0.570	0.165	0.368	1.000		
Temperature	0.551	0.838	0.036	0.343	1.000	
Per cent dead	0.076	0.603	0.484	0.552	0.628	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-23.38	6.88	-3.40
Temperature	1.501	0.420	3.57

The regression coefficient indicates that $e^{1.501} = 4.526$, i.e. for every °C increase of fumigation temperature; it is most likely that the adults of ornate aphid mortality will increase by a factor of 4.26.

A.3.4.20 Larvae of *B. brassicae* (Chapter 4, Table 4.72, Page 208) - Fumigated in a 900 L chamber at Frankston.

Correlation matrix

	Phosphine	Initial phosphine	Final phosphine	Exposure time	Temperature	Per cent dead
Phosphine	1.000					
Initial phosphine	0.087	1.000				
Final phosphine	0.023	0.247	1.000			
Exposure time	0.059	-0.312	0.221	1.000		
Temperature	0.243	0.748	-0.192	0.047	1.000	
Per cent dead	0.386	0.372	-0.007	0.433	0.760	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-33.84	3.92	-8.63
Temperature	1.959	0.226	8.68

The regression coefficient indicates that $e^{1.959} = 7$, i.e. for every °C increase of fumigation temperature; it is most likely that the larvae of cabbage aphid mortality will increase by a factor of 7.

A.3.4.21 Adults of *B. brassicae* (Chapter 4, Table 4.75, Page 210)

Correlation matrix

	Phosphine	Initial phosphine	Final phosphine	Exposure time	Temperature	Per cent dead
Phosphine	1.000					
Initial phosphine	0.087	1.000				
Final phosphine	0.023	0.247	1.000			
Exposure time	0.059	-0.312	0.221	1.000		
Temperature	0.243	0.748	-0.192	0.047	1.000	
Per cent dead	0.360	0.345	0.034	0.401	0.733	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-33.75	3.37	-10.00
Temperature	1.952	0.195	10.03

The regression coefficient indicates that $e^{1.952} = 7.04$, i.e. for every °C increase of fumigation temperature; it is most likely that the adults of cabbage aphid mortality will increase by a factor of 7.04.

A.3.4.22 Adults of *I. Purpureus* (Chapter 4, Table 4.78, Page 212) - Fumigated in the 900 L chamber at Frakston.

Correlation matrix

	Phosphine	Initial phosphine	Final phosphine	Exposure time	Temperature	Per cent dead
Phosphine	1.000					
Initial phosphine	0.262	1.000				
Final phosphine	-0.039	0.134	1.000			
Exposure time	-0.494	-0.262	0.143	1.000		
Temperature	0.309	0.831	-0.347	-0.152	1.000	
Per cent dead	-0.253	0.703	0.378	0.319	0.524	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-114.7	18.3	-6.26
Temperature	6.95	1.10	6.31

The regression coefficient indicates that $e^{6.95}$ = 1043, i.e. for every °C increase of fumigation temperature; it is most likely that the adults of meat ants mortality will increase by a factor of 1043.

A.3.4.23 Larvae of *P. persimilis* (Chapter 4, Table 4.81, Page 214-215)

Correlation matrix

	Phosphine	Initial phosphine	Final phosphine	Exposure time	Temperature	Per cent dead
Phosphine	1.000					
Initial phosphine	0.173	1.000				
Final phosphine	0.087	0.110	1.000			
Exposure time	0.150	-0.330	0.097	1.000		
Temperature	-0.226	0.404	-0.245	0.130	1.000	
Per cent dead	0.546	0.434	0.154	0.116	0.366	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-1.06	1.98	-0.54
Phosphine (Kg)	0.1955	0.0820	2.38

The regression coefficient indicates that $e^{0.1955} = 1.2$, i.e. for every kg increase of Phosfume®; it is most likely that the larvae of predatory mite mortality increased by a factor of 1.2. As it is mentioned earlier that Phosfume® contains 98 per cent of carbon dioxide, thus these suggest that the larvae of predatory mites are highly susceptible to carbon dioxide, rather other variables.

A.3.4.24 Adults of *P. persimilis* (Chapter 4, Table 4.84, Page 216)

Correlation matrix

	Phosphine	Initial phosphine	Final phosphine	Exposure time	Temperature	Relative humidity	Per cent dead
Phosphine	1.000						
Initial phosphine	0.173	1.000					
Final phosphine	0.087	0.110	1.000				
Exposure time	0.150	-0.330	0.097	1.000			
Temperature	-0.226	0.404	-0.245	0.130	1.000		
Relative humidity	0.603	0.209	0.011	0.306	0.112	1.000	
Per cent dead	0.565	0.492	0.102	0.237	0.420	0.770	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	2.611	0.417	6.26
Relative humidity	0.02036	0.00610	3.34

The regression coefficient indicates that $e^{0.02036} = 1.02$, i.e. for every per cent increase in relative humidity; it is most likely that the adults of predatory mite mortality will increase by a factor of 1.02.

A.3.5 Key features of the statistical analysis for the results presented in the Chapter 5

A.3.5.1 Larvae of *S. ejectana* (Table 5.1, Page 234-235)

Correlation matrix

	Pyrethrum	Phosphine	Initial phosphine	Final phosphine	Exposure time	Temperature	Per cent dead
Pyrethrum	1.000						
Phosphine	0.071	1.000					
Initial phosphine	-0.169	0.740	1.000				
Final phosphine	-0.141	0.009	0.508	1.000			
Exposure time	0.242	0.045	-0.354	-0.354	1.000		
Temperature	-0.154	-0.018	0.320	0.350	-0.325	1.000	
Per cent dead	0.100	0.202	-0.021	-0.305	0.538	-0.439	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-0.025	0.211	-0.12
Exposure time	0.1845	0.0177	10.44

The regression coefficient indicates that $e^{0.1845} = 1.2$, i.e. for every hour increase in exposure time; it is most likely the larvae of leaf rolling moth mortality will increase by a factor of 1.2.

A.3.5.2 Eggs of *E. postvittana* (Table 5.7, Page 242)

Correlation matrix

	Pyrethrum	Phosphine	Initial phosphine	Final phosphine	Exposure time	Temperature	Per cent dead
Pyrethrum	1.000						
Phosphine	-0.949	1.000					
Initial phosphine	-0.149	-0.007	1.000				
Final phosphine	-0.108	0.061	0.061	1.000			
Exposure time	-0.541	0.697	-0.479	-0.096	1.000		
Temperature	0.319	-0.400	0.355	0.141	-0.371	1.000	
Per cent dead	0.759	-0.757	-0.229	-0.173	-0.283	0.719	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	0.0000	0.0626	0.00
Pyrethrum	0.04505	0.00481	9.36

The regression coefficient indicates that $e^{0.04505} = 1.046$, i.e. for every $g.m^{-3}$ increase of pyrethrum concentration; it is most likely that the eggs of lightbrown apple moth mortality will increase by a factor of 1.046.

A.3.5.3Fourth instar larvae of *E. postvittana* (Table 5.11, Page 246-247)

Correlation matrix

	Pyrethrum	Phosphine	Initial phosphine	Final phosphine	Exposure time	Temperature	Per cent dead
Pyrethrum	1.000						
Phosphine	0.486	1.000					
Initial phosphine	0.177	0.818	1.000				
Final phosphine	0.253	0.805	0.927	1.000			
Exposure time	-0.195	-0.125	-0.049	-0.010	1.000		
Temperature	-0.130	-0.214	0.101	-0.103	0.116	1.000	
Per cent dead	-0.162	-0.152	0.010	-0.169	0.216	0.491	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-4.06	1.49	-2.73
Temperature	0.4382	0.0918	4.77

The regression coefficient indicates that $e^{0.4382} = 1.5$, i.e. for every hour increase in exposure time; it is most likely that the fourth instar larvae of lightbrown apple moth mortality will increase by a factor of 1.5.

A.3.5.4Fifth instar larvae of *E. postvittana* (Table 5.15, Page 250-251)

Correlation matrix

	Pyrethrum	Phosphine	Initial phosphine	Exposure time	Temperature	Per cent dead
Pyrethrum	1.000					
Phosphine	0.489	1.000				
Initial phosphine	0.160	0.825	1.000			
Exposure time	-0.194	-0.125	-0.047	1.000		
Temperature	-0.137	-0.215	0.096	0.117	1.000	
Per cent dead	-0.419	-0.281	-0.017	0.309	0.654	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-5.398	0.869	-6.21
Temperature	0.4700	0.0555	8.47

The regression coefficient indicates that $e^{0.47} = 1.6$, i.e. for every °C increase in exposure time; it is most likely that the fifth instar larvae of lightbrown apple moth mortality will increase by a factor of 1.6.

A.3.5.5Sixth instar larvae of *E. postvittana* (Table 5.19, Page 254-255).

Correlation matrix

	Pyrethrum	Phosphine	Initial phosphine	Exposure time	Temperature	Per cent dead
Pyrethrum	1.000					
Phosphine	0.504	1.000				
Initial phosphin	0.176	0.827	1.000			
Exposure time	0.104	0.192	0.107	1.000		
Temperature	-0.130	-0.199	0.100	0.068	1.000	
Per cent dead	-0.235	-0.115	0.049	0.712	0.541	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-4.687	0.785	-5.97
Temperature	0.4044	0.0474	8.53

The regression coefficient indicates that $e^{0.4044} = 1.5$, i.e. for every C° increase in temperature; it is most likely that the sixth instar larvae of lightbrown apple moth mortality will increase by a factor of 1.5.

A.3.5.6Eggs of *T. urticae* (Table 5.25, Page 260-261)

Correlation matrix

	Pyrethrum	Phosphine	Initial phosphine	Exposure time	Temperature	Per cent dead
Pyrethrum	1.000					
Phosphine	0.190	1.000				
Initial phosphine	-0.017	0.877	1.000			
Exposure time	0.062	-0.361	-0.384	1.000		
Temperature	-0.202	0.172	0.365	-0.472	1.000	
Per cent dead	0.040	-0.147	-0.282	0.853	-0.383	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-57.00	2.12	-26.87
Exposure time	3.922	0.140	27.99

The regression coefficient indicates that $e^{3.922} = 50.5$, i.e. for every hour increase in exposure time; it is most likely that the eggs of two-spotted mite mortality will increase by a factor of 50.5.

A.3.5.7 Adults of *T. urticae* (Table 5.33, Page 269-270)

Correlation matrix

	Pyrethrum	Initial phosphine	Final phosphine	Exposure time	Temperature	Per cent dead
Pyrethrum	1.000					
Initial Phosphine	-0.096	1.000				
Final phosphine	0.060	0.437	1.000			
Exposure time	0.162	-0.631	-0.292	1.000		
Temperature	-0.198	0.311	0.269	-0.387	1.000	
Per cent dead	0.277	0.192	0.196	0.193	0.087	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	4.972	0.442	11.24
Phosphine Final	7.38	2.96	2.49

The regression coefficient indicates that $e^{7.138} = 1603$, i.e. for every $g.m^{-3}$ increase in final phosphine concentration; it is most likely that the adults of two-spotted mites mortality will increase by a factor of 1603.

A.3.5.8Larvae of *M. ornatus* (Table 5.39, Page 276)

Correlation matrix

	Pyrethrum	Phosphine	Initial phosphine	Final phosphine	Exposure time	Temperature	Per cent dead
Pyrethrum	1.000						
Phosphine	0.412	1.000					
Initial phosphine	0.500	0.368	1.000				
Final phosphine	0.428	0.084	0.606	1.000			
Exposure time	0.616	0.186	-0.118	0.423	1.000		
Temperature	0.149	0.279	0.401	-0.033	0.028	1.000	
Per cent dead	0.584	0.031	0.350	0.860	0.669	-0.240	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-1.793	0.376	-4.77
Phosphine Final	17.72	2.62	6.77

The regression coefficient indicates that $e^{17.72} = 49624737$, i.e. for every $g.m^{-3}$ increase in final phosphine concentration; it is most likely that the larvae of ornate aphid mortality will increase by a factor of 49624737.

A.3.5.9 Adults of *M. ornatus* (Table 5.40, Page 277)

Correlation matrix

	Pyrethrum	Phosphine	Initial phosphine	Final phosphine	Exposure time	Temperature	Per cent dead
Pyrethrum	1.000						
Phosphine	0.603	1.000					
Initial phosphine	0.574	0.480	1.000				
Final phosphine	0.519	0.307	0.651	1.000			
Exposure time	0.479	0.042	-0.148	0.356	1.000		
Temperature	0.284	0.421	0.467	0.084	-0.011	1.000	
Per cent dead	0.278	0.692	0.419	0.384	0.258	0.602	1.000

Estimates of regression coefficients

	Estimate	Standard error	t(*)
Constant	-14.14	2.48	-5.69
Temperature	0.805	0.139	5.79

The regression coefficient indicates that $e^{0.805} = 2.2$, i.e. for every °C increase in temperature; it is most likely that the adults of ornate aphid mortality will increase by a factor of 2.2.

A.3.6 Key features of the statistical analysis for the results presented in the Chapter 7

A.3.6.1.1 Concentration of phosphine (ppm) achieved in the 27 m^3 chamber at Emerald by using predetermined time and mass of Phosfume®.

Various factors (weight of the cylinder, time added and mass added) influencing the amount of phosphine gas attained in the chamber was analysed. Relationship among different variables is given in the correlation matrix. The most important factor that influencing the amount of phosphine gas delivered in the chamber is given in the regression coefficients.

Correlation matrix

	Ambient Temp	Weight of cylinder	Time added	Mass of Phosfume added
Ambient Temp	1.000			
Weight of cylinder	-0.559	1.000		
Time added	0.225	-0.483	1.000	
Mass of Phosfume added	0.085	-0.224	0.130	1.000

Estimates of regression coefficients

	Estimate	**Standard error**	**t(23)**
Constant	2.68	1.35	1.99
Weight of cylinder	-0.0251	0.0227	-1.10

The statistical analysis indicates that the amount of active ingredient achieved in the chamber was positively correlated with the weight of the cylinder. The regression coefficient indicates that $e^{-0.0251} = 0.97$, i.e. for every kg decrease in weight of phosphine; it is most likely that the concentration of phosphine attained in the chamber will increase by a factor of 0.97.

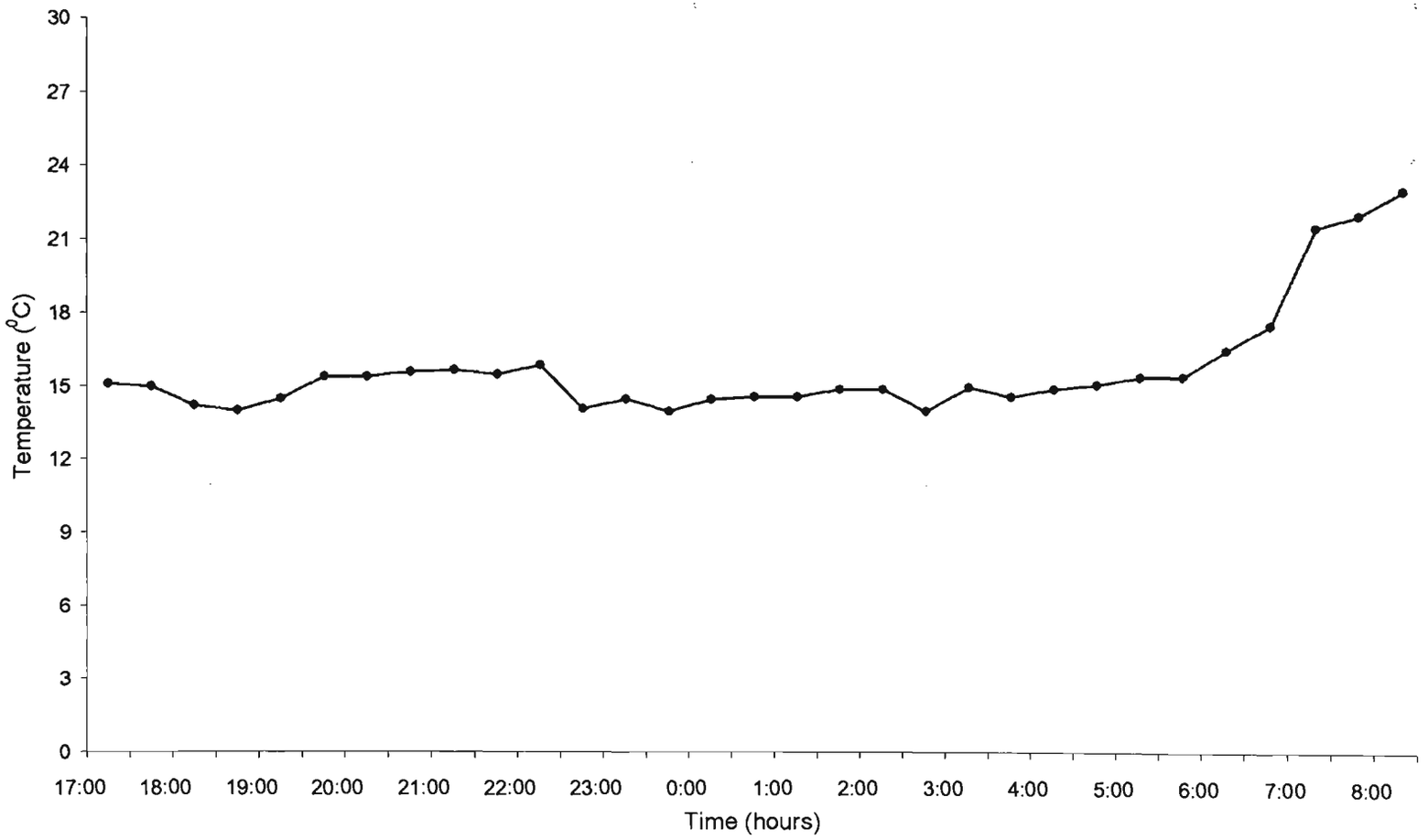
Appendix - 4

Tempeature - Time Trajectories (Chapter 4)

Appendix 4 Temperature-Time Trajectories (Chapter 4 Fumigation Trials)

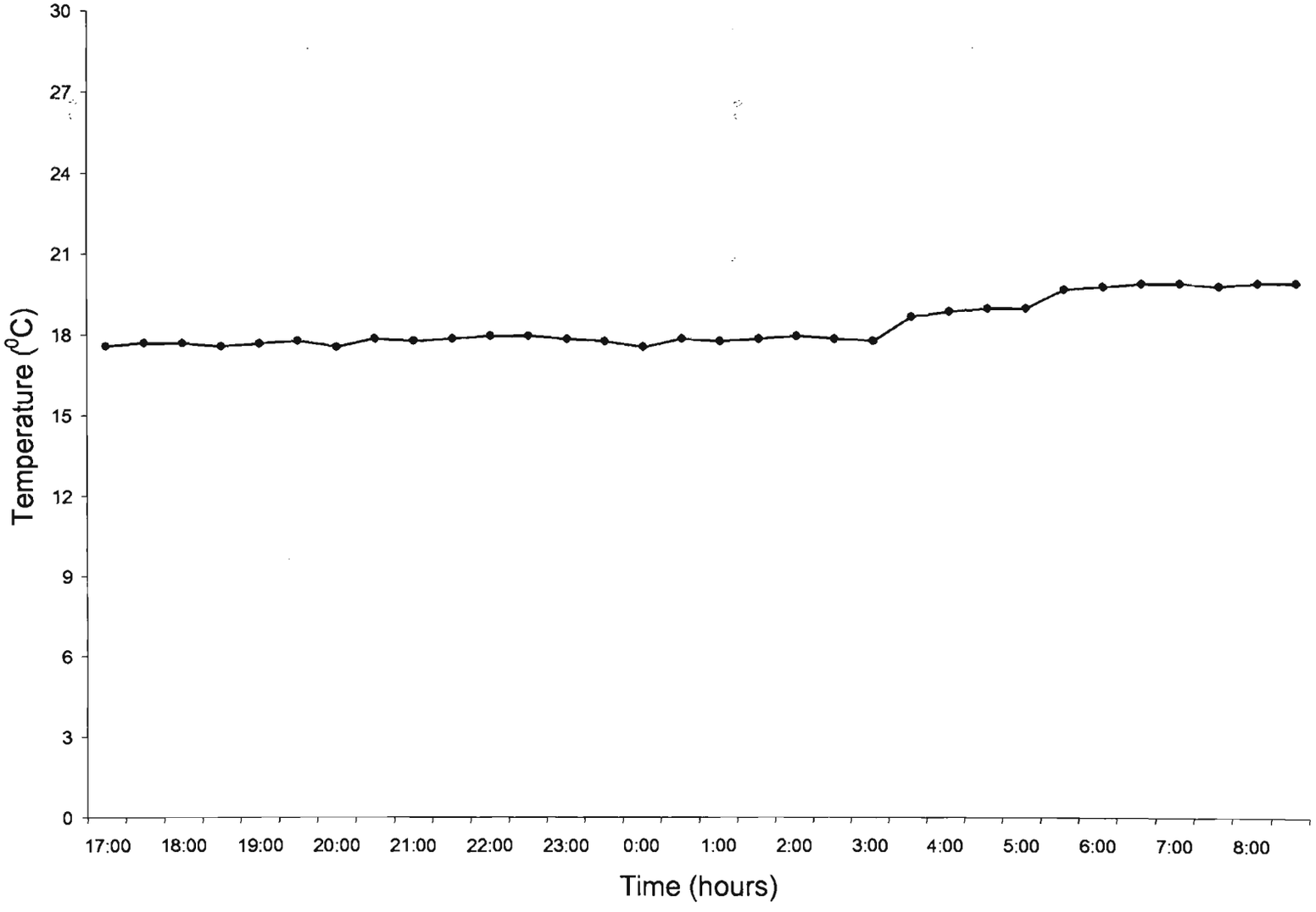
Date of fumigation: 3/12/1997

Time (hrs	Temp (^{0}C)
17:00:00	15.1
17:30:00	15
18:00:00	14.2
18:30:00	14
19:00:00	14.5
19:30:00	15.4
20:00:00	15.4
20:30:00	15.6
21:00:00	15.7
21:30:00	15.5
22:00:00	15.9
22:30:00	14.1
23:00:00	14.5
23:30:00	14
0:00:00	14.5
0:30:00	14.6
1:00:00	14.6
1:30:00	14.9
2:00:00	14.9
2:30:00	14
3:00:00	15
3:30:00	14.6
4:00:00	14.9
4:30:00	15.1
5:00:00	15.4
5:30:00	15.4
6:00:00	16.5
6:30:00	17.5
7:00:00	21.5
7:30:00	22
8:00:00	23
Average:	**15.7**



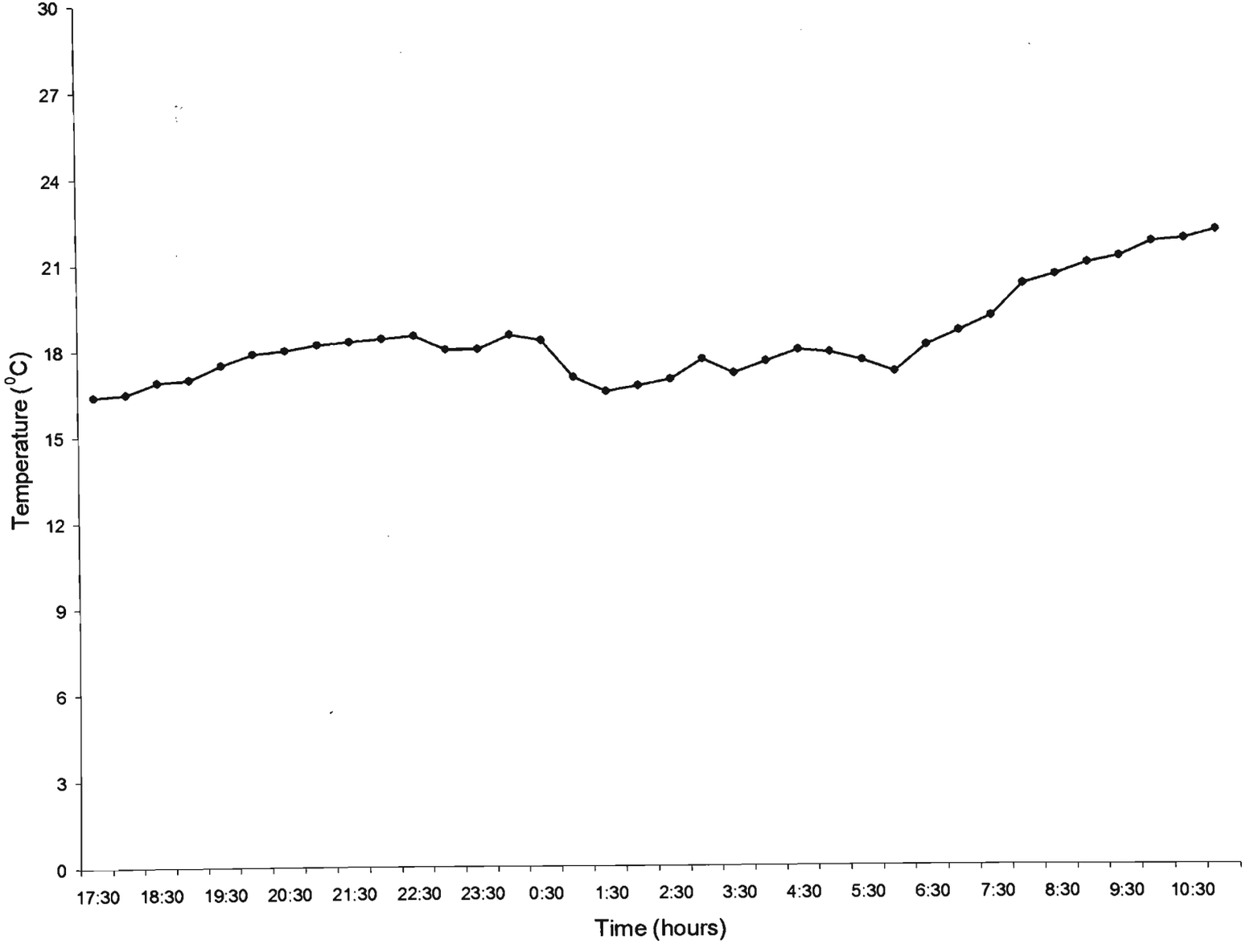
Date of fumigation: 30/12/1996

Time (hrs	Temp (^{0}C)
17:00:00	17.6
17:30:00	17.7
18:00:00	17.7
18:30:00	17.6
19:00:00	17.7
19:30:00	17.8
20:00:00	17.6
20:30:00	17.9
21:00:00	17.8
21:30:00	17.9
22:00:00	18
22:30:00	18
23:00:00	17.9
23:30:00	17.8
0:00:00	17.6
0:30:00	17.9
1:00:00	17.8
1:30:00	17.9
2:00:00	18
2:30:00	17.9
3:00:00	17.8
3:30:00	18.7
4:00:00	18.9
4:30:00	19
5:00:00	19
5:30:00	19.7
6:00:00	19.8
6:30:00	19.9
7:00:00	19.9
7:30:00	19.8
8:00:00	19.9
8:30:00	19.9
Average:	**18.4**



Date of fumigation: 5/05/97

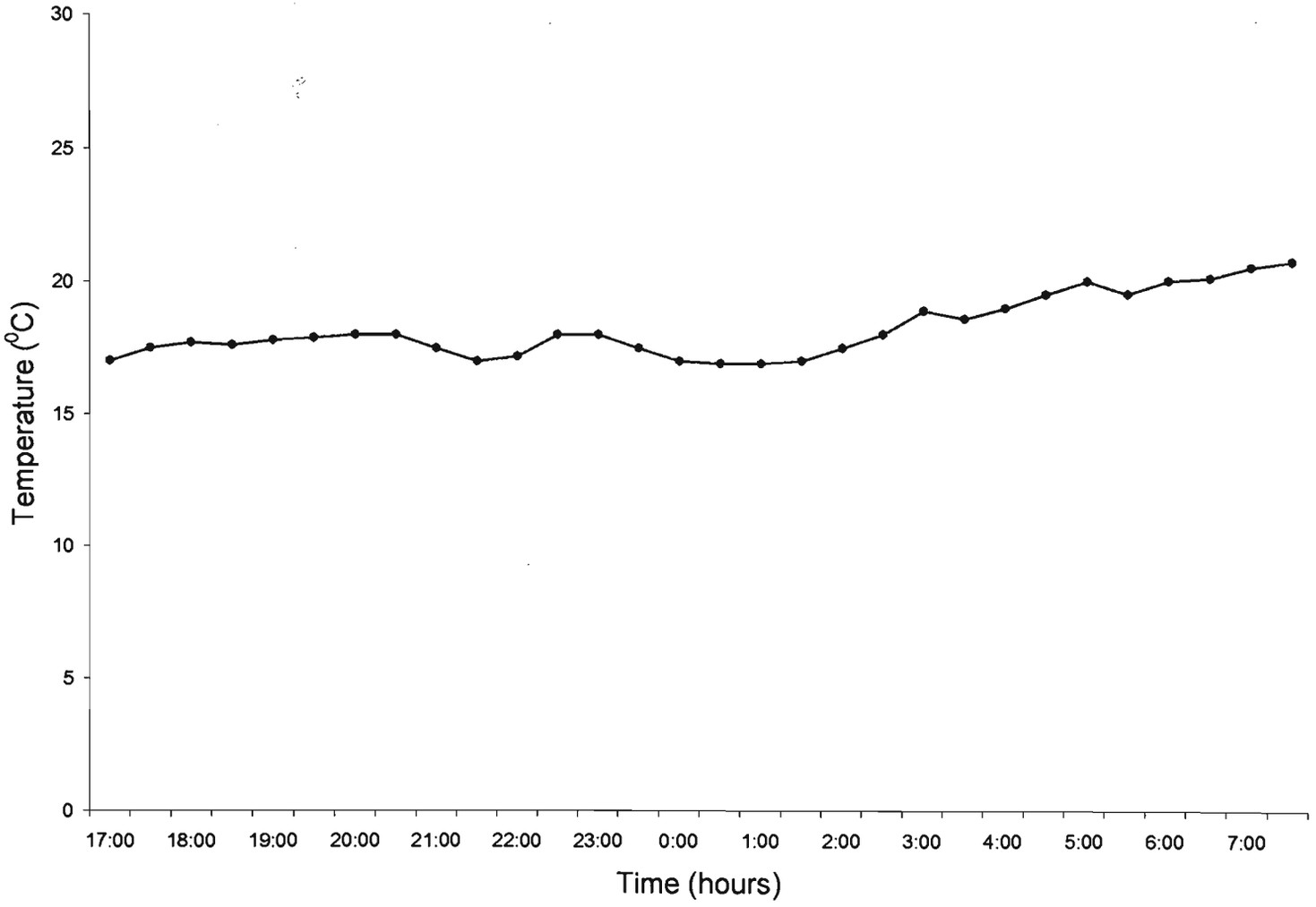
Time (hrs	Temp (^{0}C)
17:30:00	16.4
18:00:00	16.5
18:30:00	16.9
19:00:00	17
19:30:00	17.5
20:00:00	17.9
20:30:00	18
21:00:00	18.2
21:30:00	18.3
22:00:00	18.4
22:30:00	18.5
23:00:00	18
23:30:00	18
0:00:00	18.5
0:30:00	18.3
1:00:00	17
1:30:00	16.5
2:00:00	16.7
2:30:00	16.9
3:00:00	17.6
3:30:00	17.1
4:00:00	17.5
4:30:00	17.9
5:00:00	17.8
5:30:00	17.5
6:00:00	17.1
6:30:00	18
7:00:00	18.5
7:30:00	19
8:00:00	20.1
8:30:00	20.4
9:00:00	20.8
9:30:00	21
10:00:00	21.5
10:30:00	21.6
11:00:00	21.9
Average:	**18.3**



Date of fumigation: 23/01/97

Time (hrs	Temp (^{0}C)
17:00:00	17
17:30:00	17.5
18:00:00	17.7
18:30:00	17.6
19:00:00	17.8
19:30:00	17.9
20:00:00	18
20:30:00	18
21:00:00	17.5
21:30:00	17
22:00:00	17.2
22:30:00	18
23:00:00	18
23:30:00	17.5
0:00:00	17
0:30:00	16.9
1:00:00	16.9
1:30:00	17
2:00:00	17.5
2:30:00	18
3:00:00	18.9
3:30:00	18.6
4:00:00	19
4:30:00	19.5
5:00:00	20
5:30:00	19.5
6:00:00	20
6:30:00	20.1
7:00:00	20.5
7:30:00	20.7

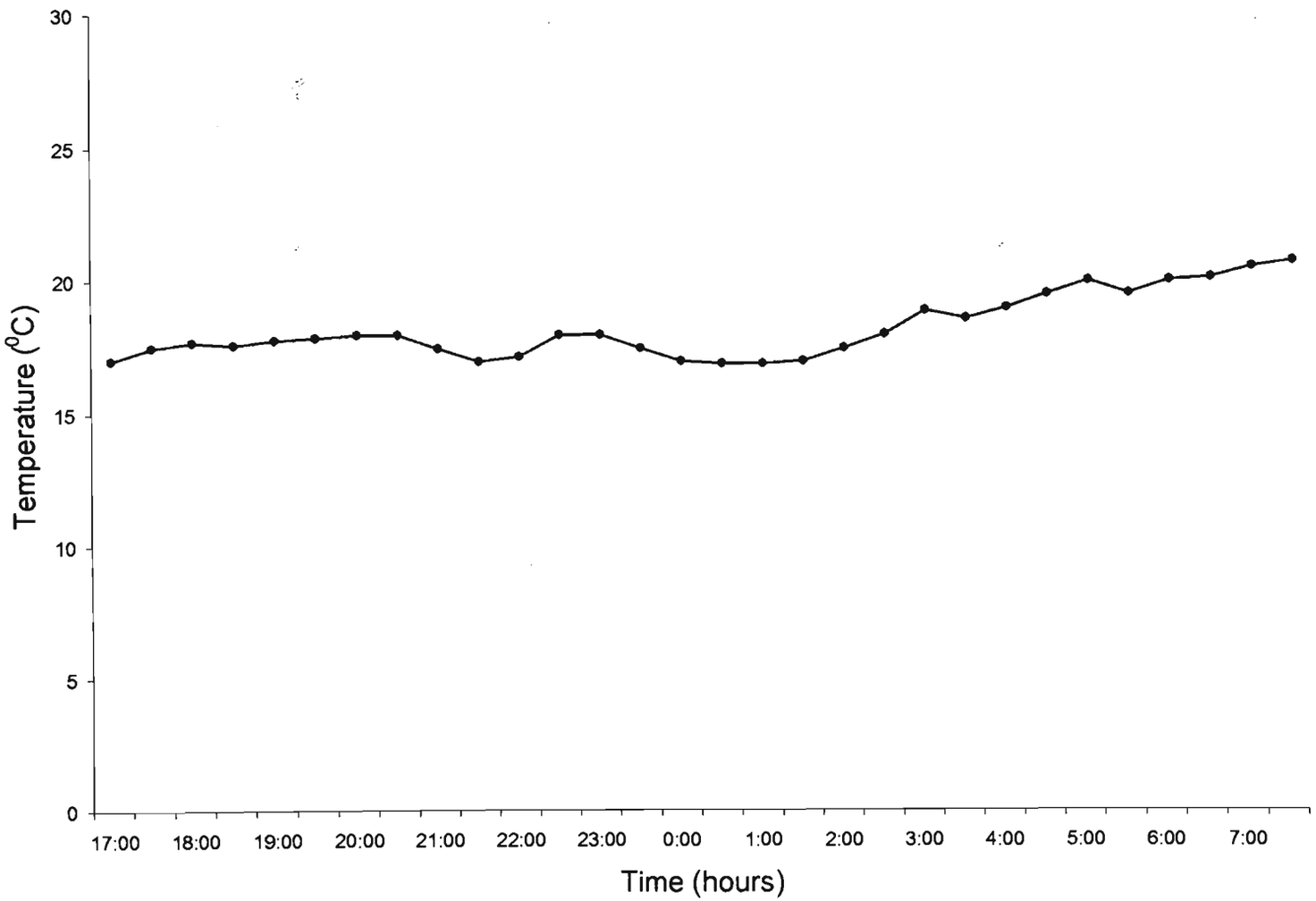
Average: 18.2



Date of fumigation: 23/01/97

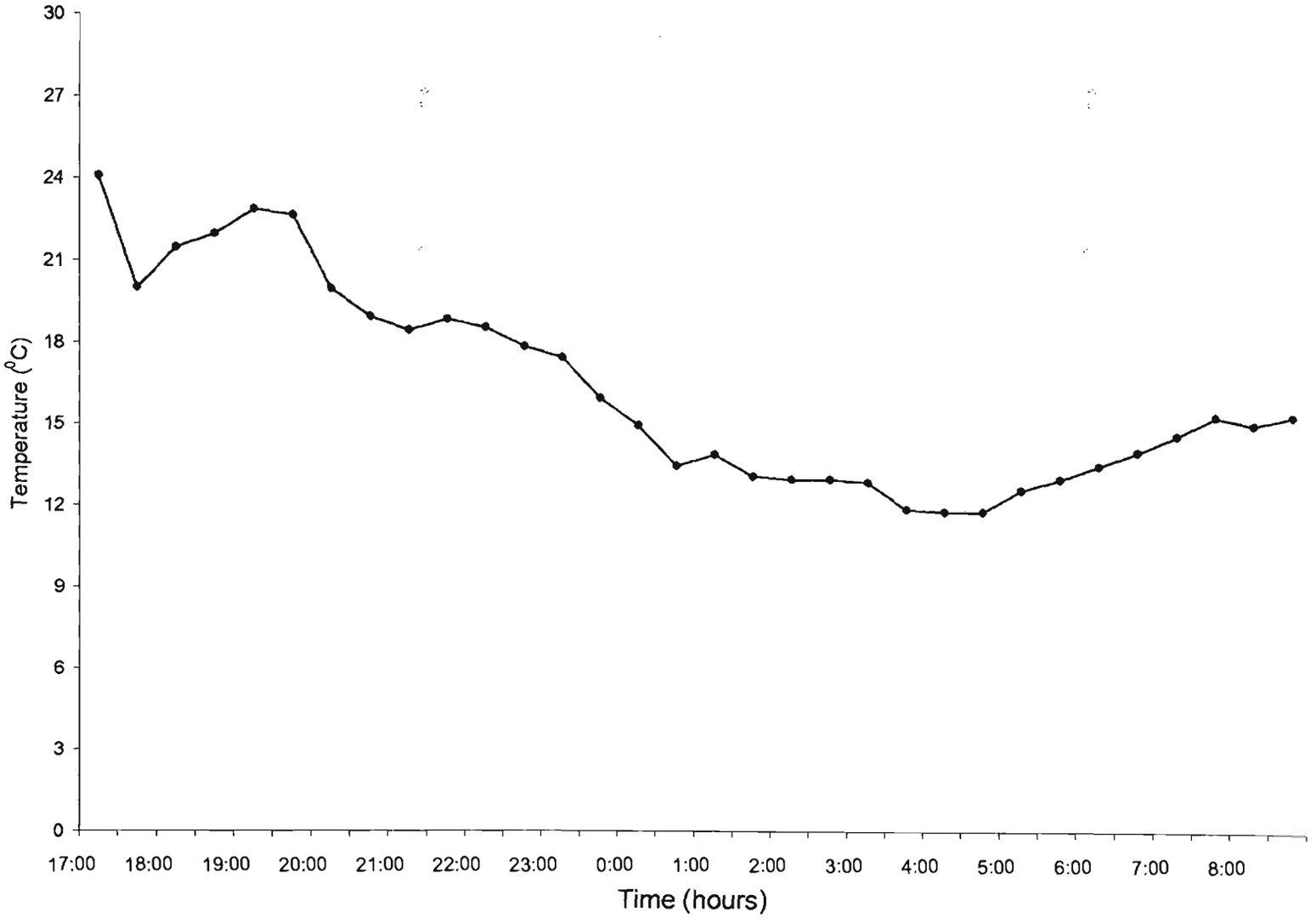
Time (hrs	Temp (^{0}C)
17:00:00	17
17:30:00	17.5
18:00:00	17.7
18:30:00	17.6
19:00:00	17.8
19:30:00	17.9
20:00:00	18
20:30:00	18
21:00:00	17.5
21:30:00	17
22:00:00	17.2
22:30:00	18
23:00:00	18
23:30:00	17.5
0:00:00	17
0:30:00	16.9
1:00:00	16.9
1:30:00	17
2:00:00	17.5
2:30:00	18
3:00:00	18.9
3:30:00	18.6
4:00:00	19
4:30:00	19.5
5:00:00	20
5:30:00	19.5
6:00:00	20
6:30:00	20.1
7:00:00	20.5
7:30:00	20.7

Average: 18.2



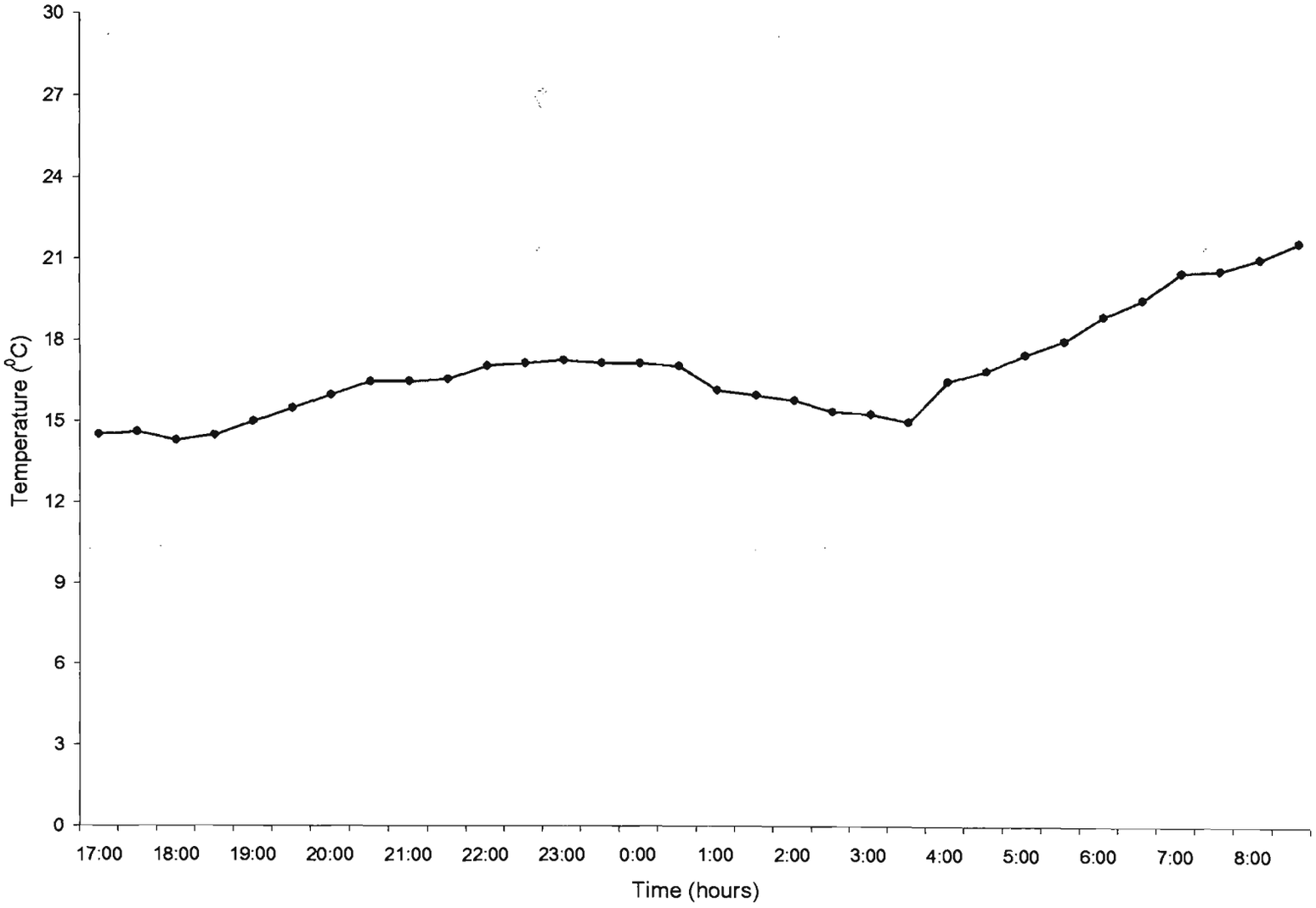
Date of fumigation: 17/03/97

Time (hrs	Temp (^{0}C)
17:00:00	24.1
17:30:00	20
18:00:00	21.5
18:30:00	22
19:00:00	22.9
19:30:00	22.7
20:00:00	20
20:30:00	19
21:00:00	18.5
21:30:00	18.9
22:00:00	18.6
22:30:00	17.9
23:00:00	17.5
23:30:00	16
0:00:00	15
0:30:00	13.5
1:00:00	13.9
1:30:00	13.1
2:00:00	13
2:30:00	13
3:00:00	12.9
3:30:00	11.9
4:00:00	11.8
4:30:00	11.8
5:00:00	12.6
5:30:00	13
6:00:00	13.5
6:30:00	14
7:00:00	14.6
7:30:00	15.3
8:00:00	15
8:30:00	15.3
Average:	**16.3**



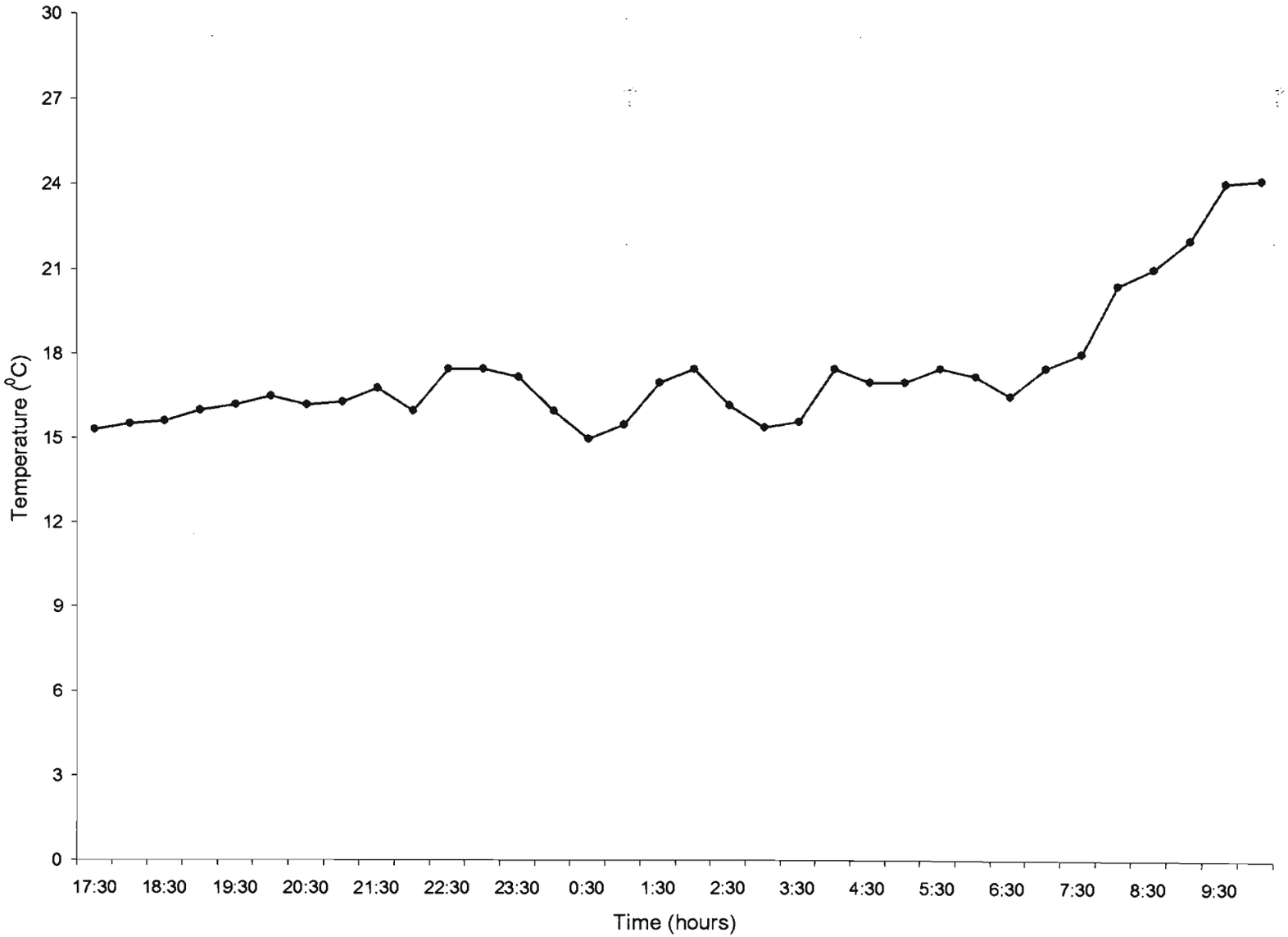
Date of fumigation: 20/03/97

Time (hrs	Temp (^{0}C)
17:00:00	14.5
17:30:00	14.6
18:00:00	14.3
18:30:00	14.5
19:00:00	15
19:30:00	15.5
20:00:00	16
20:30:00	16.5
21:00:00	16.5
21:30:00	16.6
22:00:00	17.1
22:30:00	17.2
23:00:00	17.3
23:30:00	17.2
0:00:00	17.2
0:30:00	17.1
1:00:00	16.2
1:30:00	16
2:00:00	15.8
2:30:00	15.4
3:00:00	15.3
3:30:00	15
4:00:00	16.5
4:30:00	16.9
5:00:00	17.5
5:30:00	18
6:00:00	18.9
6:30:00	19.5
7:00:00	20.5
7:30:00	20.6
8:00:00	21
8:30:00	21.6
Average:	**16.9**



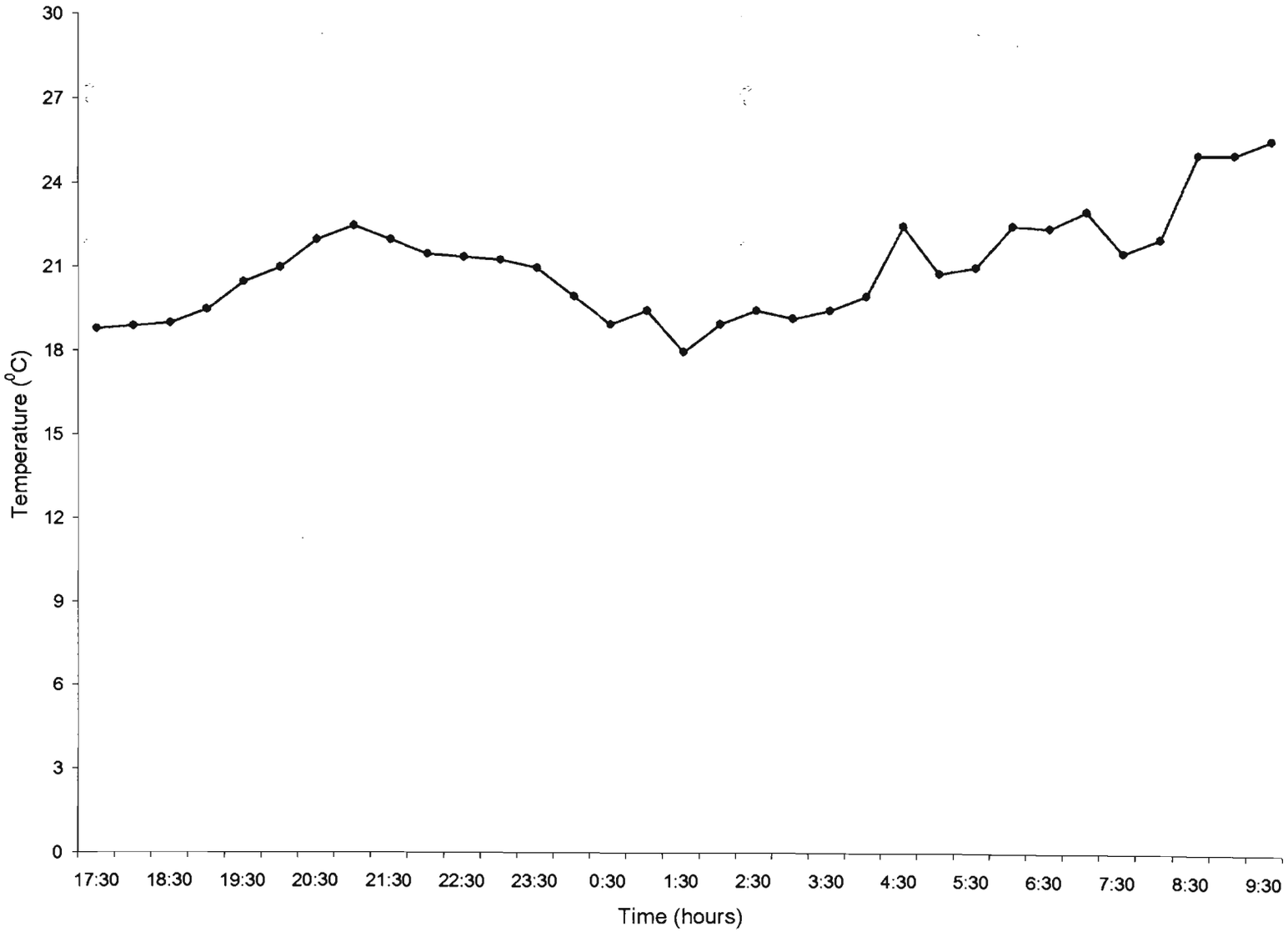
Date of fumigation: 24/03/97

Time (hrs	Temp (^{0}C)
17:30:00	15.3
18:00:00	15.5
18:30:00	15.6
19:00:00	16
19:30:00	16.2
20:00:00	16.5
20:30:00	16.2
21:00:00	16.3
21:30:00	16.8
22:00:00	16
22:30:00	17.5
23:00:00	17.5
23:30:00	17.2
0:00:00	16
0:30:00	15
1:00:00	15.5
1:30:00	17
2:00:00	17.5
2:30:00	16.2
3:00:00	15.4
3:30:00	15.6
4:00:00	17.5
4:30:00	17
5:00:00	17
5:30:00	17.5
6:00:00	17.2
6:30:00	16.5
7:00:00	17.5
7:30:00	18
8:00:00	20.4
8:30:00	21
9:00:00	22
9:30:00	24
10:00:00	24.1
Average:	**17.3**



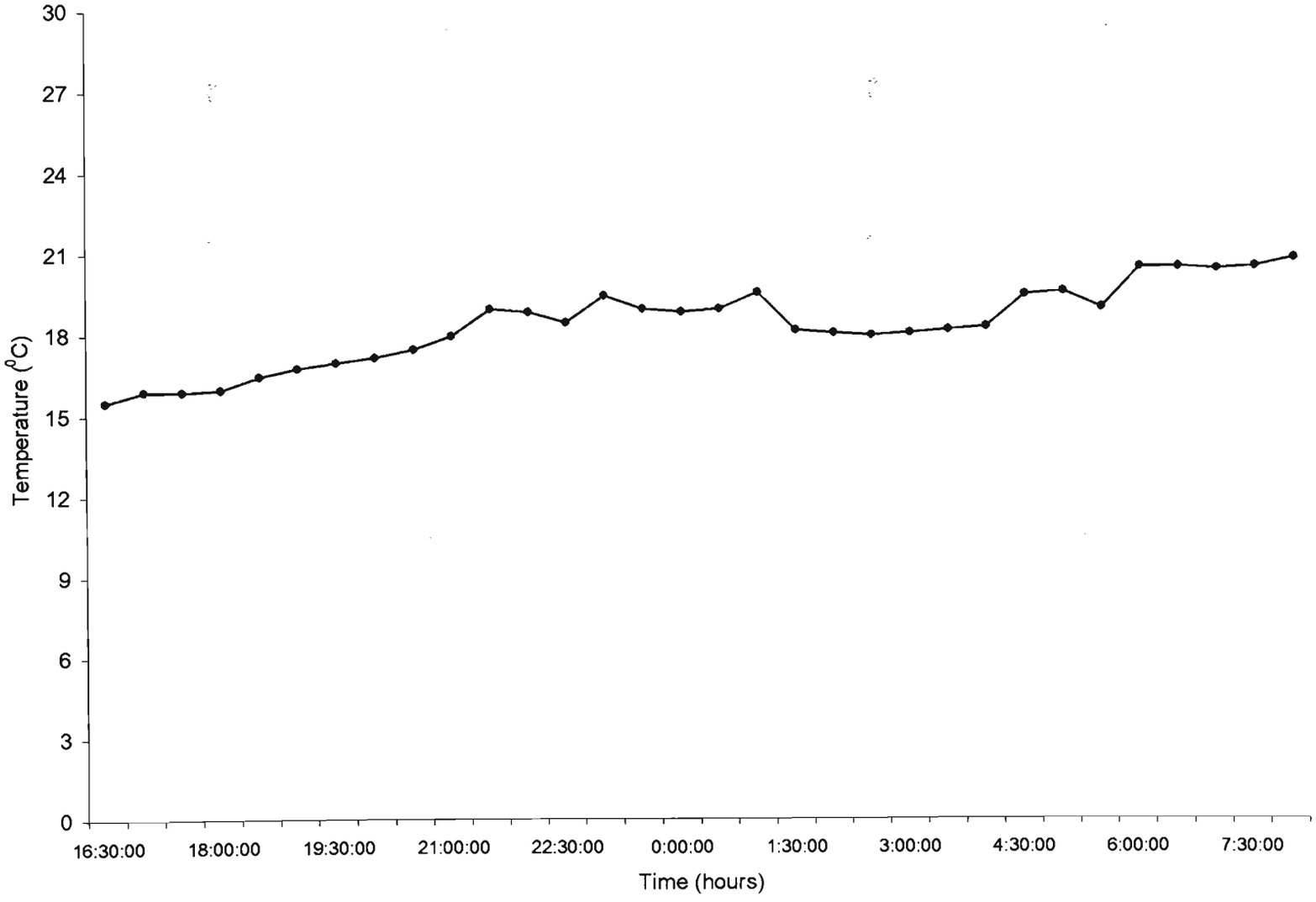
Date of fumigation: 4/09/97

Time (hrs	Temp (^{0}C)
17:30:00	18.8
18:00:00	18.9
18:30:00	19
19:00:00	19.5
19:30:00	20.5
20:00:00	21
20:30:00	22
21:00:00	22.5
21:30:00	22
22:00:00	21.5
22:30:00	21.4
23:00:00	21.3
23:30:00	21
0:00:00	20
0:30:00	19
1:00:00	19.5
1:30:00	18
2:00:00	19
2:30:00	19.5
3:00:00	19.2
3:30:00	19.5
4:00:00	20
4:30:00	22.5
5:00:00	20.8
5:30:00	21
6:00:00	22.5
6:30:00	22.4
7:00:00	23
7:30:00	21.5
8:00:00	22
8:30:00	25
9:00:00	25
9:30:00	25.5
Average:	**21.1**



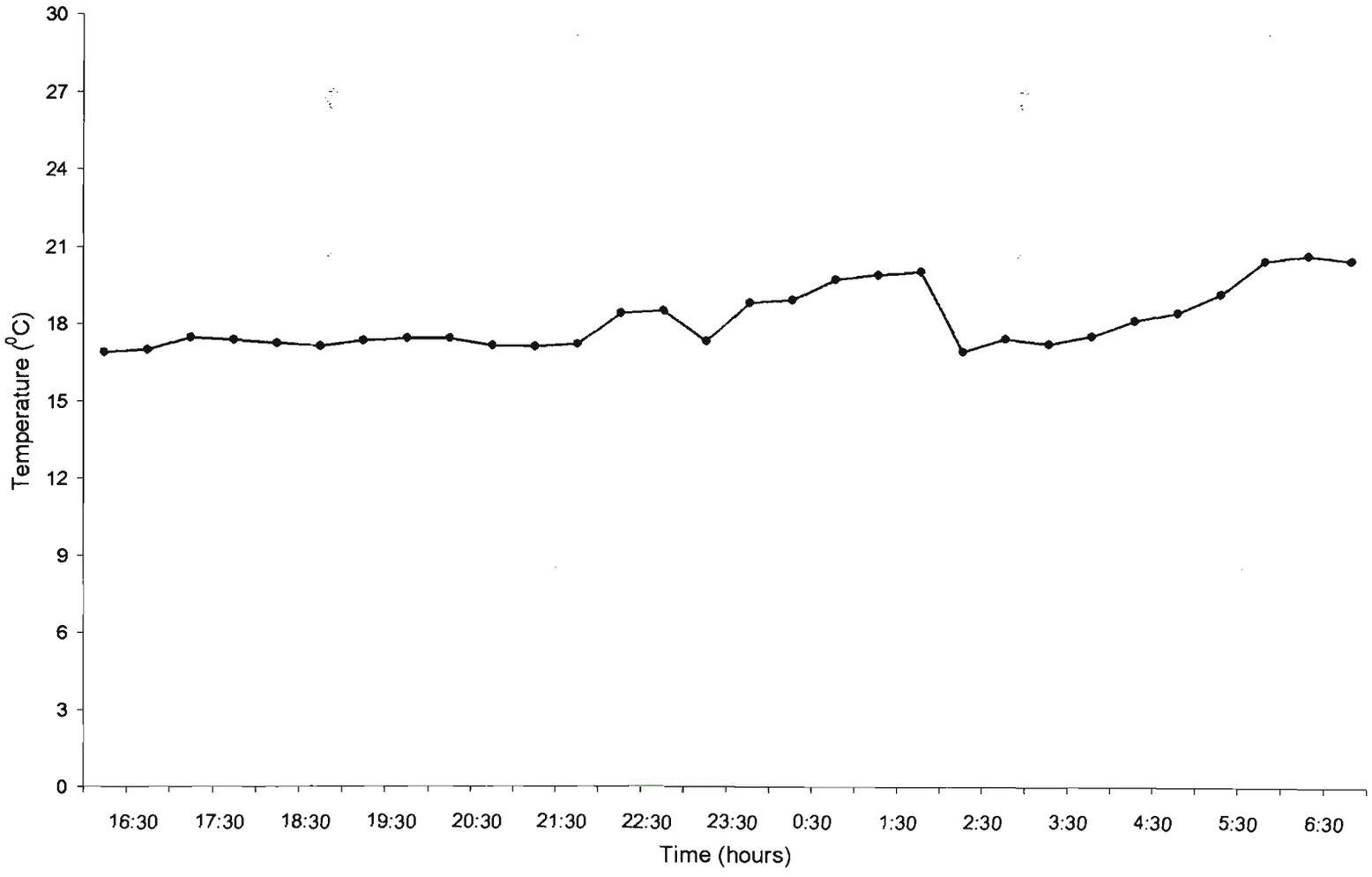
Date of fumigation: 30/09/96

Time (hrs	Temp (^{0}C)
16:30:00	15.5
17:00:00	15.9
17:30:00	15.9
18:00:00	16
18:30:00	16.5
19:00:00	16.8
19:30:00	17
20:00:00	17.2
20:30:00	17.5
21:00:00	18
21:30:00	19
22:00:00	18.9
22:30:00	18.5
23:00:00	19.5
23:30:00	19
0:00:00	18.9
0:30:00	19
1:00:00	19.6
1:30:00	18.2
2:00:00	18.1
2:30:00	18
3:00:00	18.1
3:30:00	18.2
4:00:00	18.3
4:30:00	19.5
5:00:00	19.6
5:30:00	19
6:00:00	20.5
6:30:00	20.5
7:00:00	20.4
7:30:00	20.5
8:00:00	20.8
Average:	**18.4**



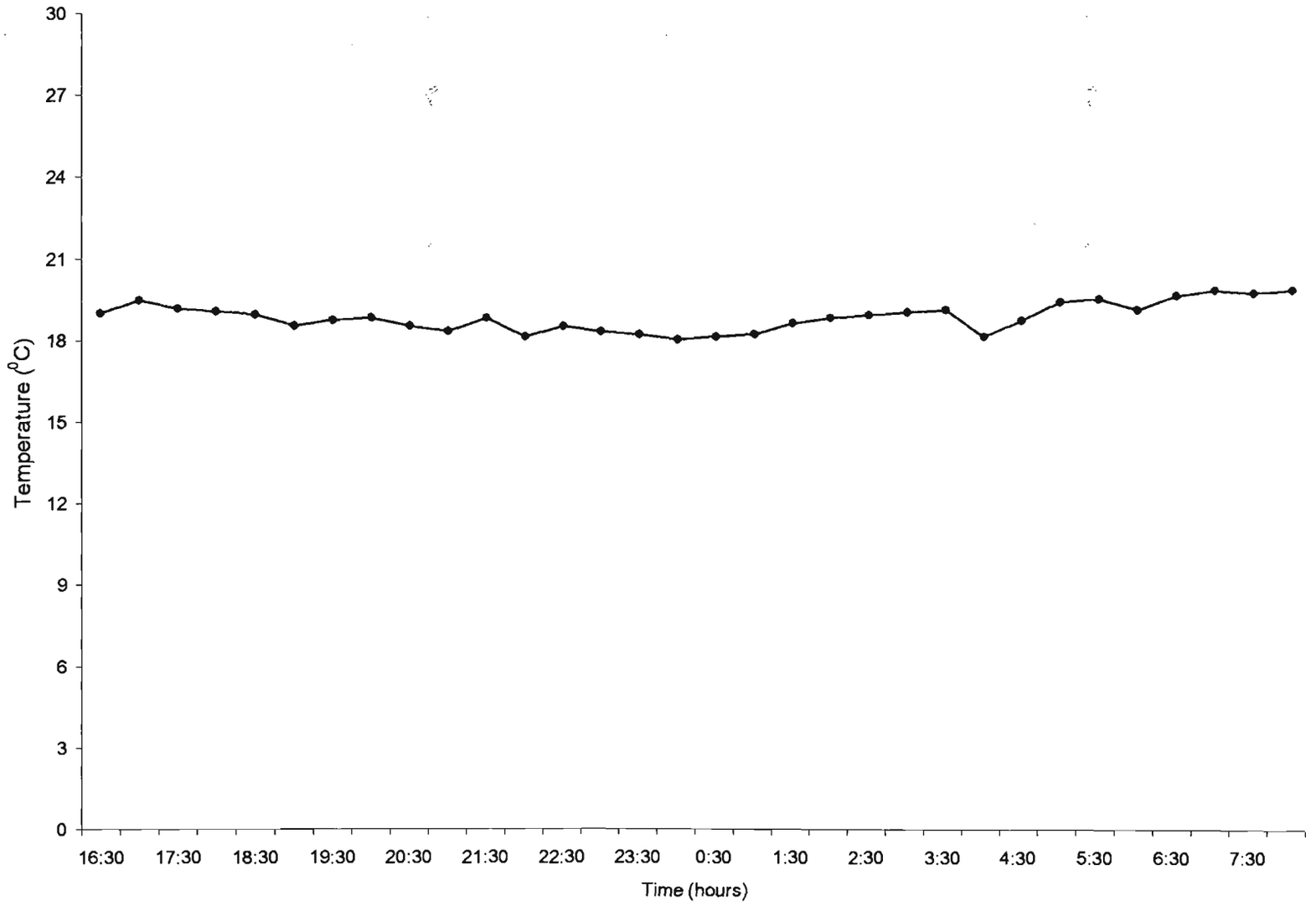
Date of fumigation: 18/01/97

Time (hrs	Temp (^{0}C)
16:30:00	16.9
17:00:00	17
17:30:00	17.5
18:00:00	17.4
18:30:00	17.3
19:00:00	17.2
19:30:00	17.4
20:00:00	17.5
20:30:00	17.5
21:00:00	17.21
21:30:00	17.2
22:00:00	17.3
22:30:00	18.5
23:00:00	18.6
23:30:00	17.4
0:00:00	18.9
0:30:00	19
1:00:00	19.8
1:30:00	20
2:00:00	20.1
2:30:00	17
3:00:00	17.5
3:30:00	17.3
4:00:00	17.6
4:30:00	18.2
5:00:00	18.5
5:30:00	19.2
6:00:00	20.5
6:30:00	20.7
7:00:00	20.5
Average:	**18.2**



Date of fumigation: 26/02/97

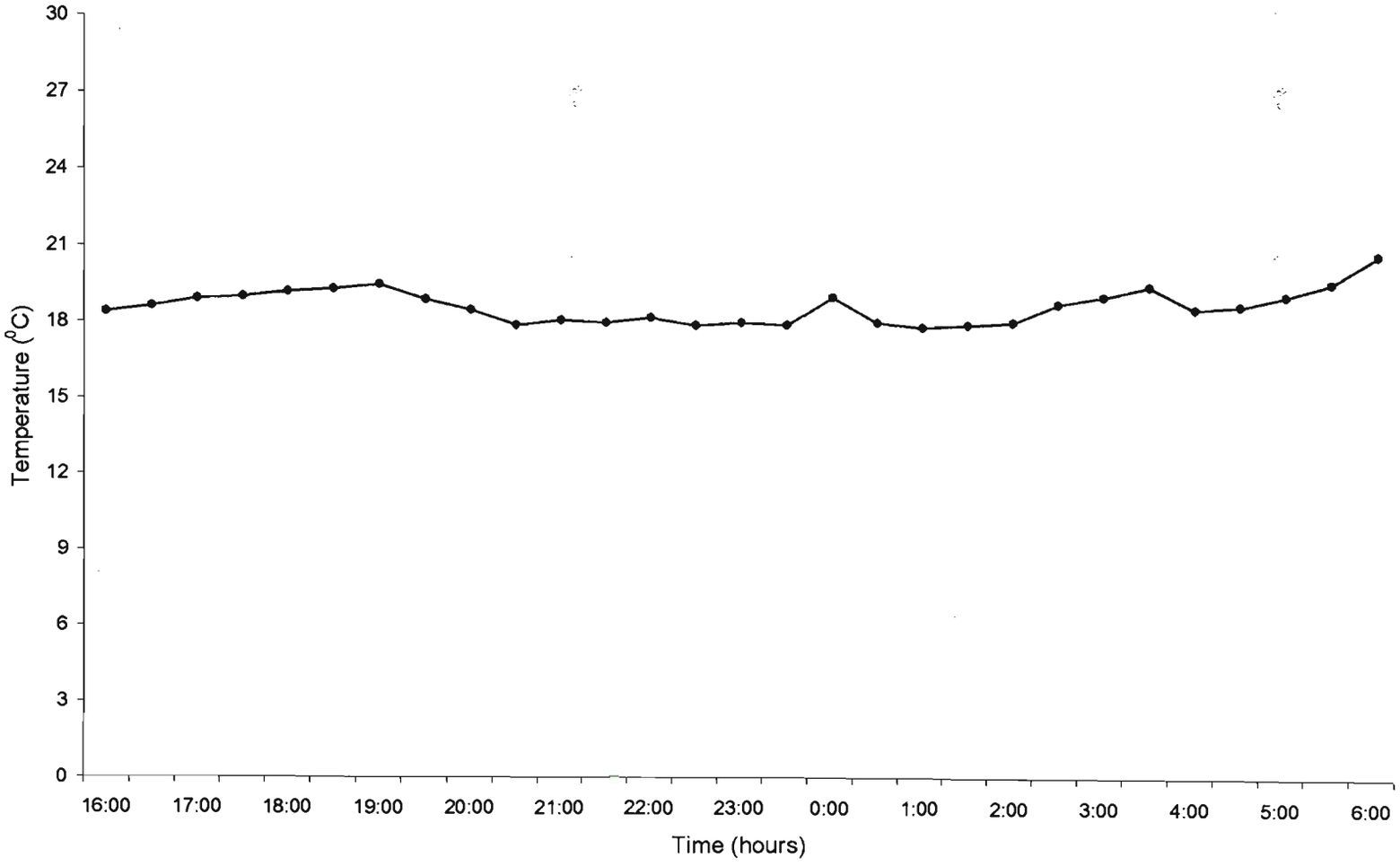
Time (hrs	Temp (^{0}C)
16:30:00	19
17:00:00	19.5
17:30:00	19.2
18:00:00	19.1
18:30:00	19
19:00:00	18.6
19:30:00	18.8
20:00:00	18.9
20:30:00	18.6
21:00:00	18.4
21:30:00	18.9
22:00:00	18.2
22:30:00	18.6
23:00:00	18.4
23:30:00	18.3
0:00:00	18.1
0:30:00	18.2
1:00:00	18.3
1:30:00	18.7
2:00:00	18.9
2:30:00	19
3:00:00	19.1
3:30:00	19.2
4:00:00	18.2
4:30:00	18.8
5:00:00	19.5
5:30:00	19.6
6:00:00	19.2
6:30:00	19.7
7:00:00	19.9
7:30:00	19.8
8:00:00	19.9
Average:	**18.9**



Date of fumigation: 2/10/97

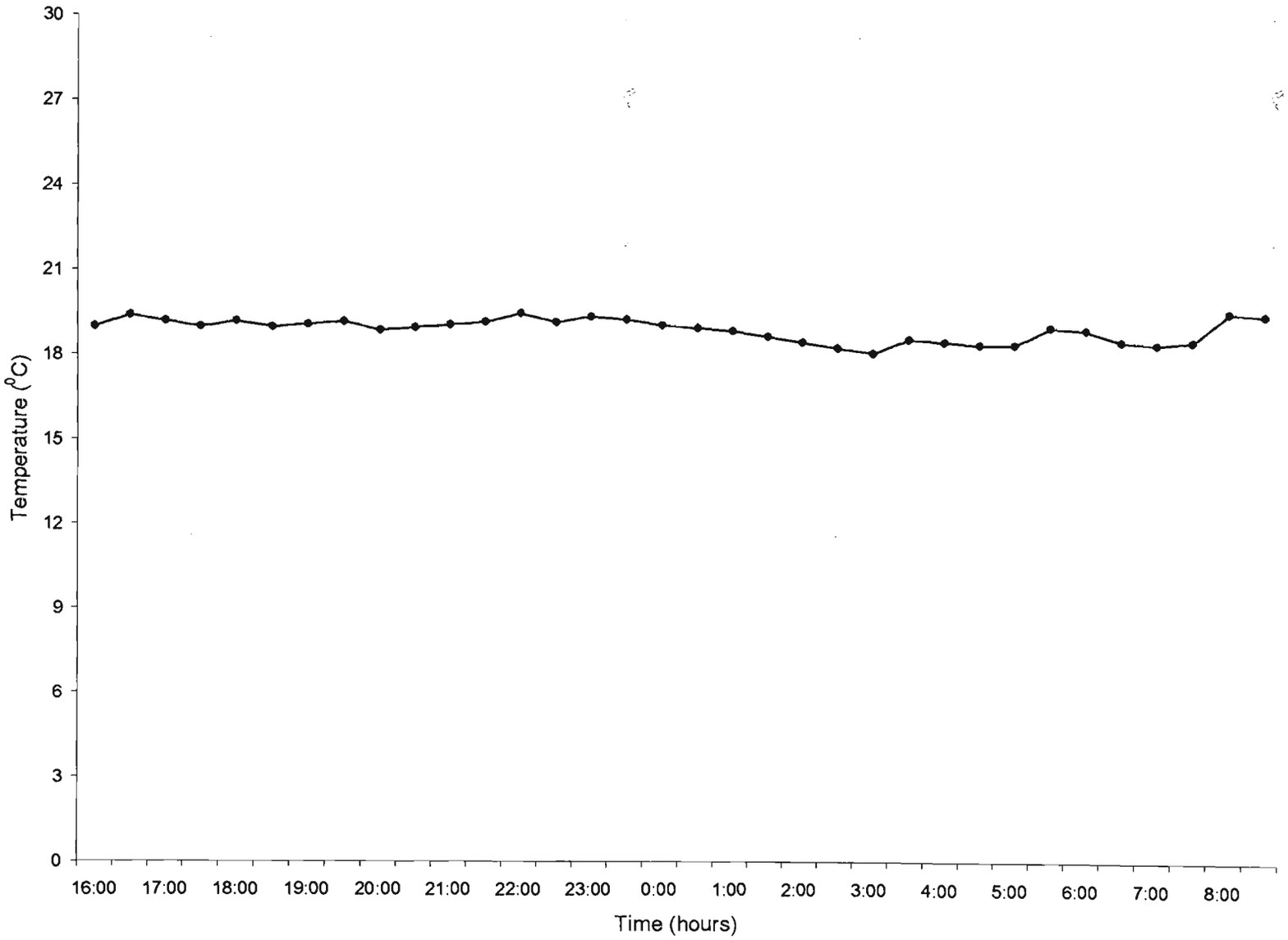
Time (hrs	Temp (^{0}C)
16:00:00	18.4
16:30:00	18.61
17:00:00	18.91
17:30:00	19
18:00:00	19.2
18:30:00	19.3
19:00:00	19.5
19:30:00	18.9
20:00:00	18.5
20:30:00	17.9
21:00:00	18.1
21:30:00	18
22:00:00	18.2
22:30:00	17.9
23:00:00	18
23:30:00	17.9
0:00:00	19
0:30:00	18
1:00:00	17.8
1:30:00	17.9
2:00:00	18
2:30:00	18.7
3:00:00	19
3:30:00	19.4
4:00:00	18.5
4:30:00	18.6
5:00:00	19
5:30:00	19.5
6:00:00	20.6

Average: **18.6**



Date of fumigation: 14/04/97

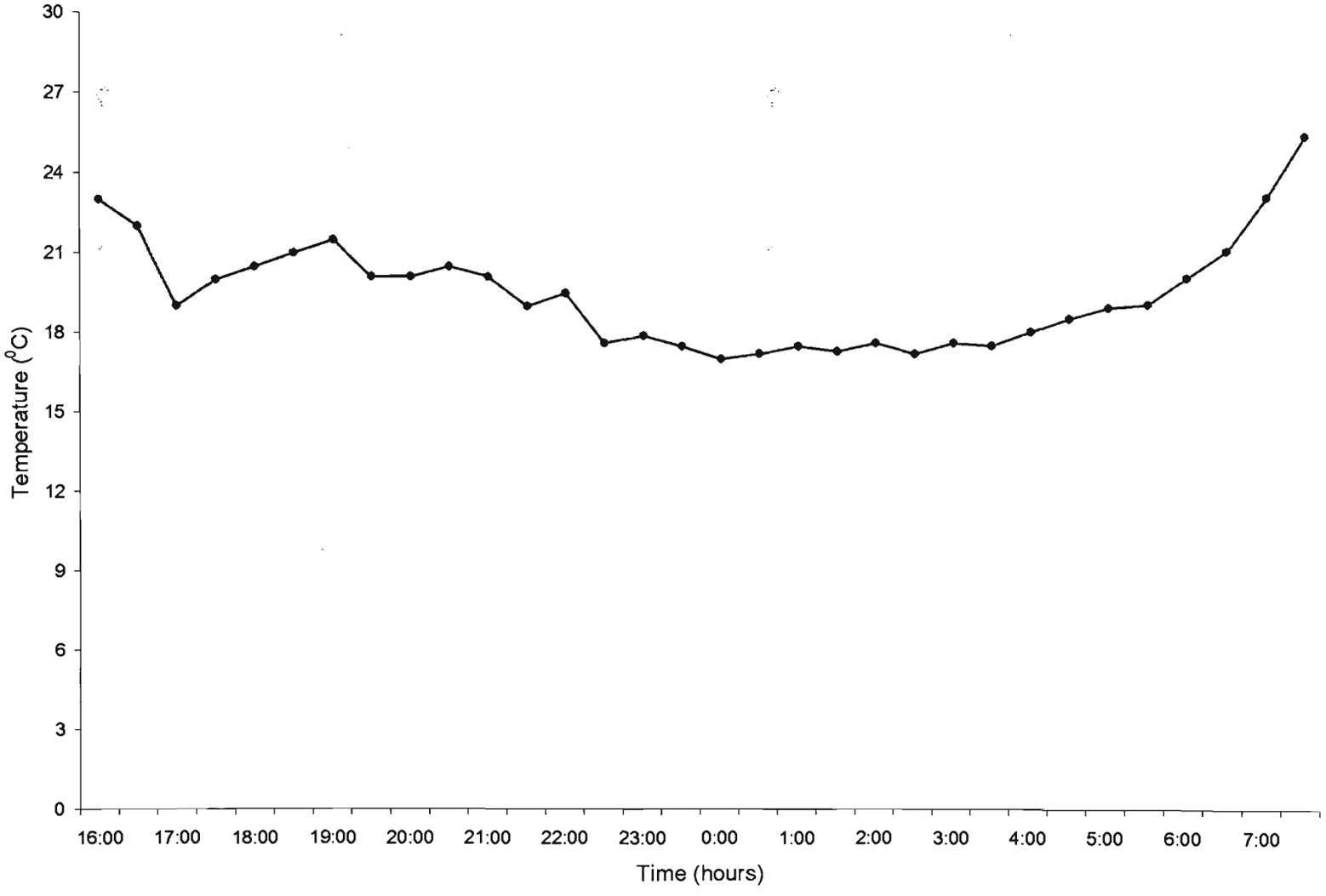
Time (hrs	Temp (^{0}C)
16:00:00	19
16:30:00	19.4
17:00:00	19.2
17:30:00	19
18:00:00	19.2
18:30:00	19
19:00:00	19.1
19:30:00	19.2
20:00:00	18.9
20:30:00	19
21:00:00	19.1
21:30:00	19.2
22:00:00	19.5
22:30:00	19.2
23:00:00	19.4
23:30:00	19.3
0:00:00	19.1
0:30:00	19
1:00:00	18.9
1:30:00	18.7
2:00:00	18.5
2:30:00	18.3
3:00:00	18.1
3:30:00	18.6
4:00:00	18.5
4:30:00	18.4
5:00:00	18.4
5:30:00	19
6:00:00	18.9
6:30:00	18.5
7:00:00	18.4
7:30:00	18.5
8:00:00	19.5
8:30:00	19.4
Average:	**18.9**



Date of fumigation: 21/04/97

Time (hrs	Temp (^{0}C)
16:00:00	23
16:30:00	22
17:00:00	19
17:30:00	20
18:00:00	20.5
18:30:00	21
19:00:00	21.5
19:30:00	20.1
20:00:00	20.1
20:30:00	20.5
21:00:00	20.1
21:30:00	19
22:00:00	19.5
22:30:00	17.6
23:00:00	17.9
23:30:00	17.5
0:00:00	17
0:30:00	17.2
1:00:00	17.5
1:30:00	17.3
2:00:00	17.6
2:30:00	17.2
3:00:00	17.6
3:30:00	17.5
4:00:00	18
4:30:00	18.5
5:00:00	18.9
5:30:00	19
6:00:00	20
6:30:00	21
7:00:00	23
7:30:00	25.3

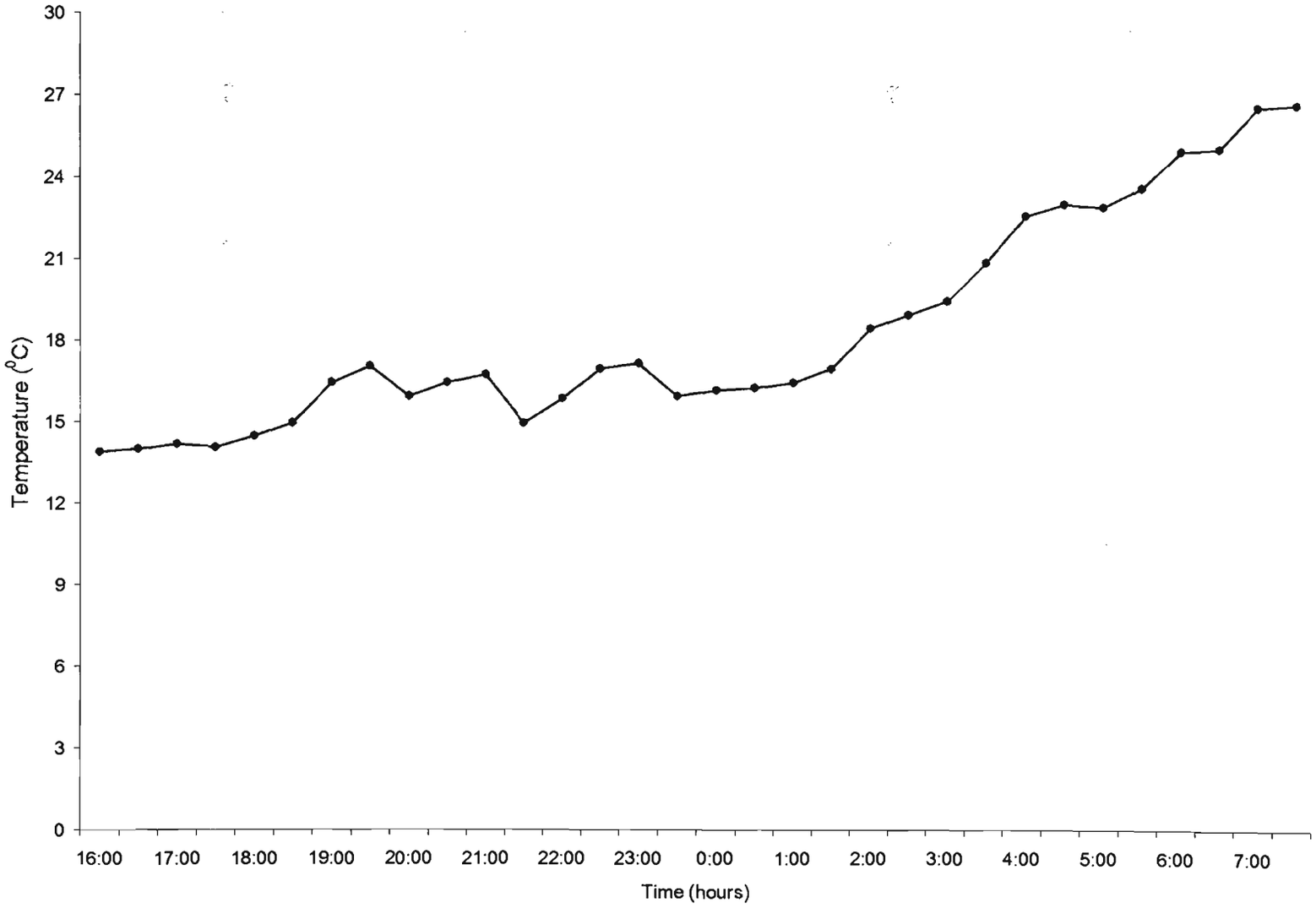
Average **19.4**



Date of fumigation: 29/04/97

Time (hrs	Temp (^{0}C)
16:00	13.9
16:30	14
17:00	14.2
17:30	14.1
18:00	14.5
18:30	15
19:00	16.5
19:30	17.1
20:00	16
20:30	16.5
21:00	16.8
21:30	15
22:00	15.9
22:30	17
23:00	17.2
23:30	16
0:00	16.2
0:30	16.3
1:00	16.5
1:30	17
2:00	18.5
2:30	19
3:00	19.5
3:30	20.9
4:00	22.6
4:30	23
5:00	22.9
5:30	23.6
6:00	24.9
6:30	25
7:00	26.5
7:30	26.6

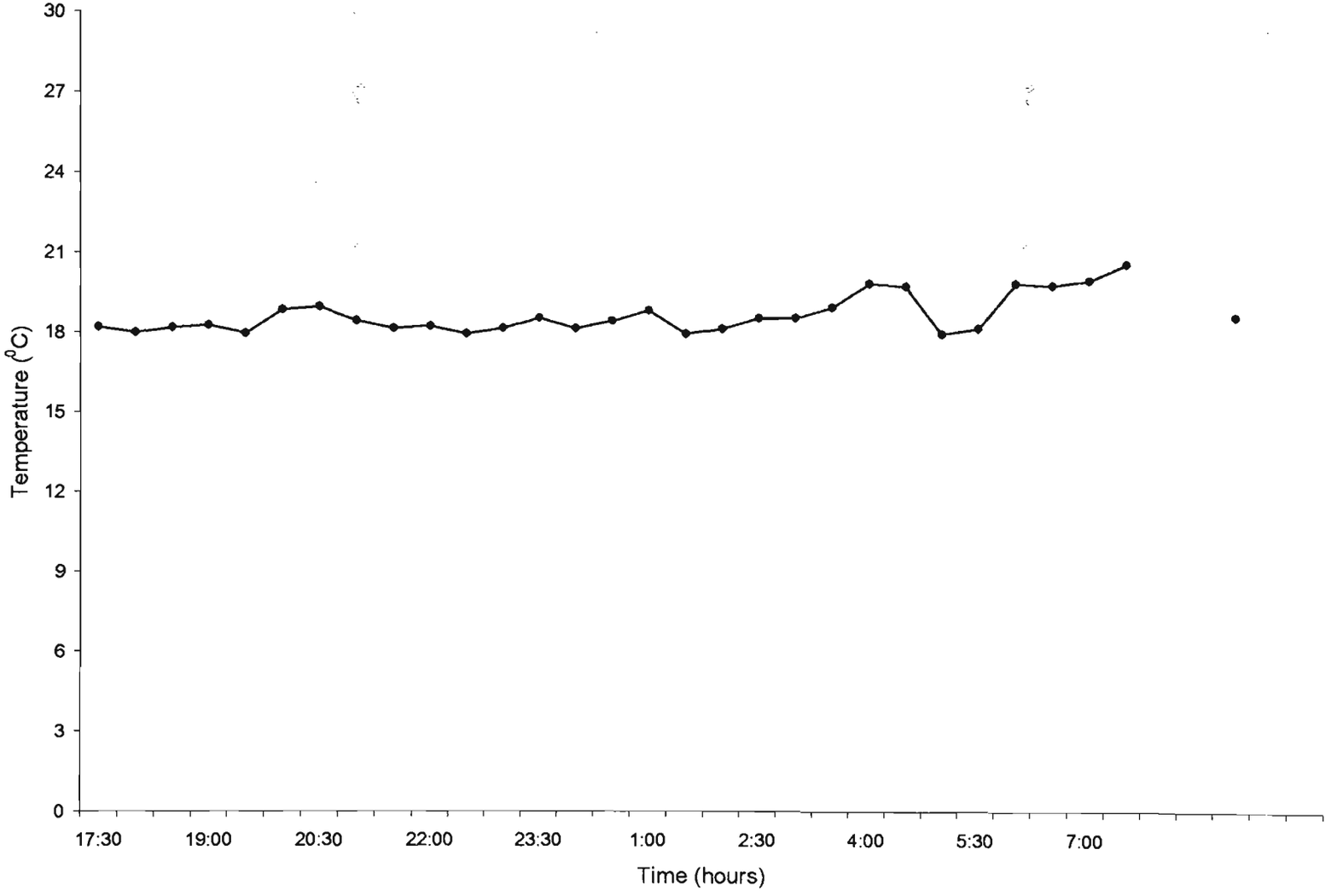
Average: 18.4



Date of fumigation: 2/10/97

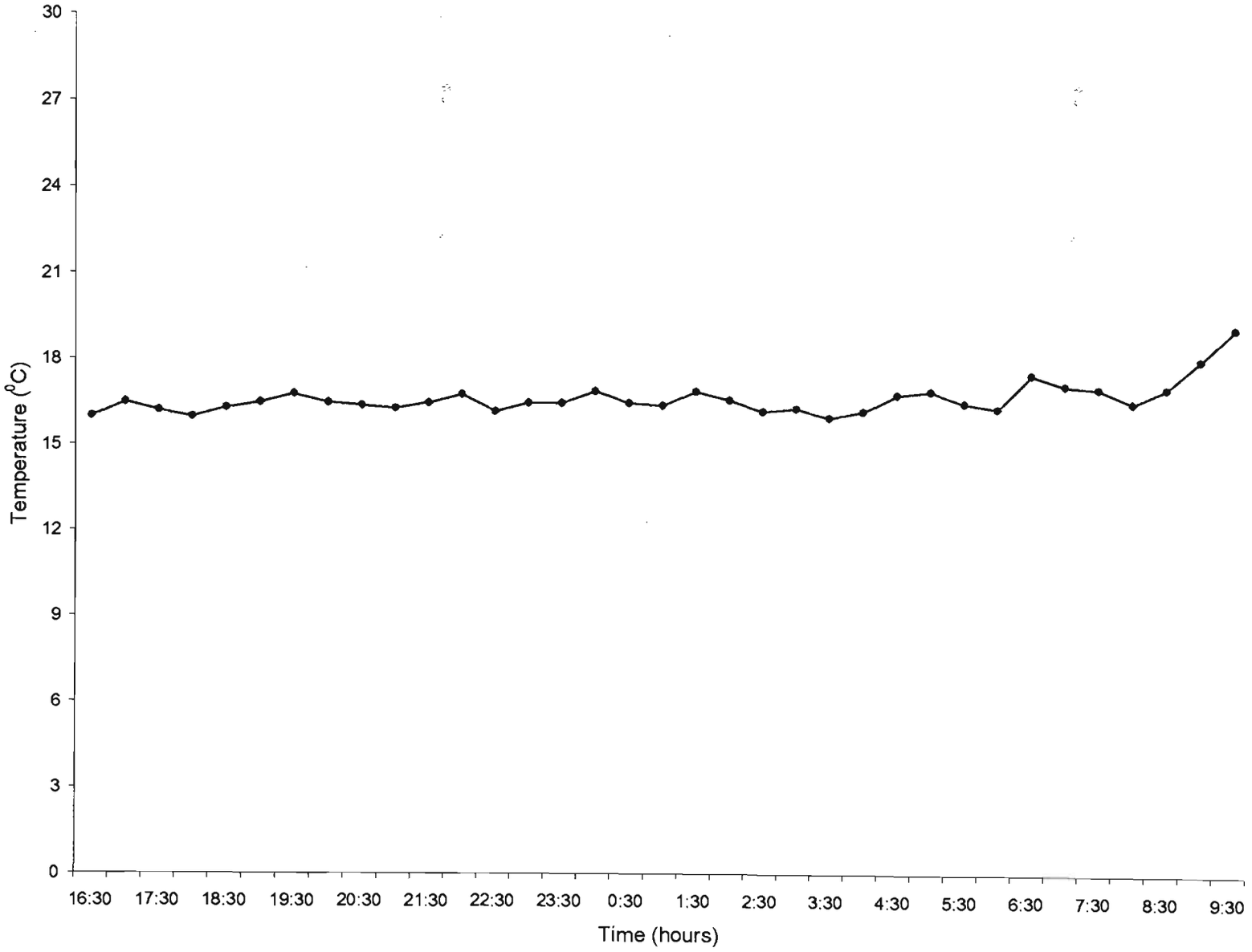
Time (hrs	Temp (^{0}C)
16:30	17.8
17:00	18
17:30	18.2
18:00	18
18:30	18.2
19:00	18.3
19:30	18
20:00	18.9
20:30	19
21:00	18.5
21:30	18.2
22:00	18.3
22:30	18
23:00	18.2
23:30	18.6
0:00	18.2
0:30	18.5
1:00	18.9
1:30	18
2:00	18.2
2:30	18.6
3:00	18.6
3:30	19
4:00	19.9
4:30	19.8
5:00	18
5:30	18.2
6:00	19.9
6:30	19.8
7:00	20
7:30	20.6

Average: **18.6**



Date of fumigation: 26/05/97

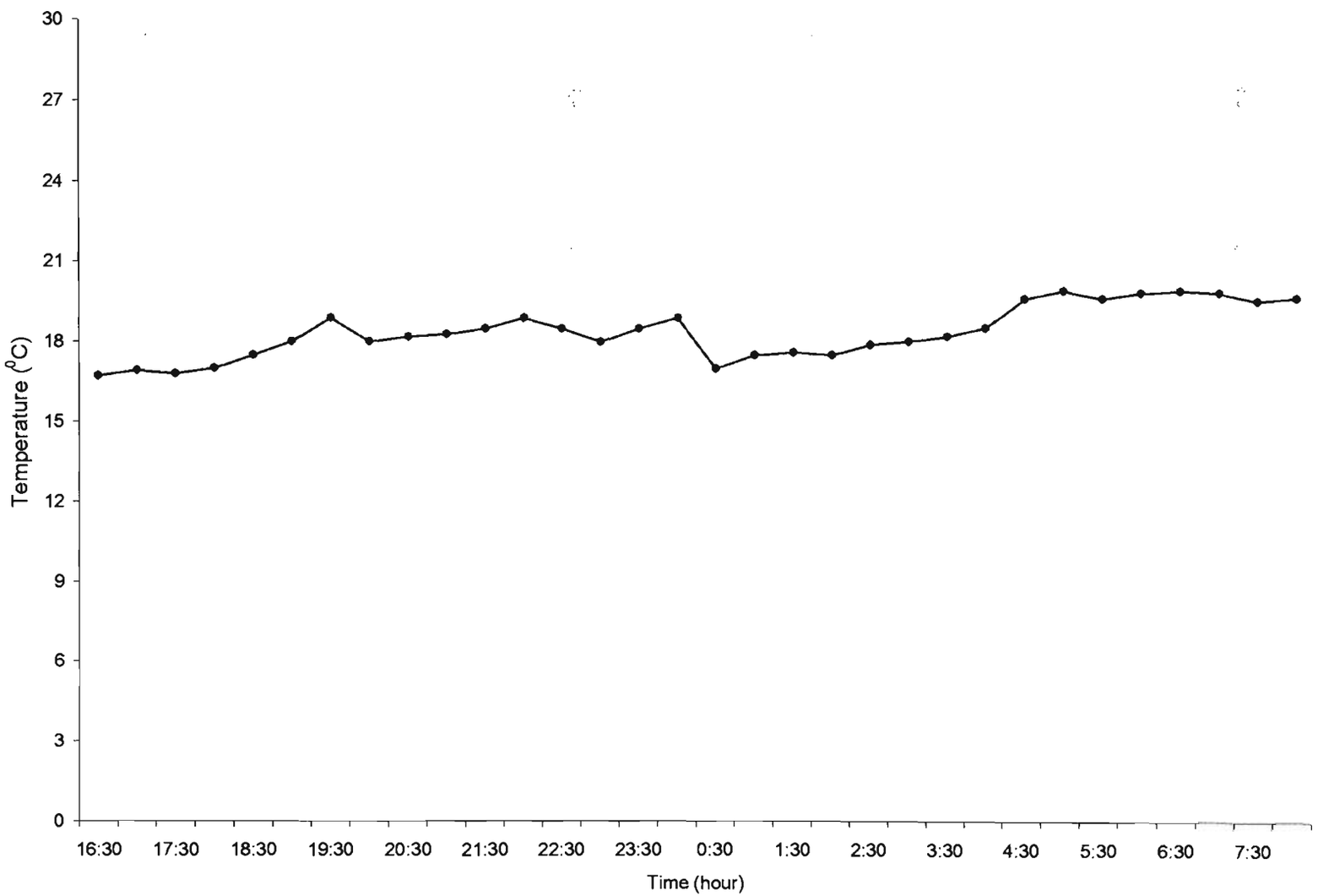
Time (hrs	Temp (^{0}C)
16:30	16
17:00	16.5
17:30	16.2
18:00	16
18:30	16.3
19:00	16.5
19:30	16.8
20:00	16.5
20:30	16.4
21:00	16.3
21:30	16.5
22:00	16.8
22:30	16.2
23:00	16.5
23:30	16.5
0:00	16.9
0:30	16.5
1:00	16.4
1:30	16.9
2:00	16.6
2:30	16.2
3:00	16.3
3:30	16
4:00	16.2
4:30	16.8
5:00	16.9
5:30	16.5
6:00	16.3
6:30	17.5
7:00	17.1
7:30	17
8:00	16.5
8:30	17
9:00	18
9:30	19.1
Average:	**17.5**



Date of fumigation: 6/03/97

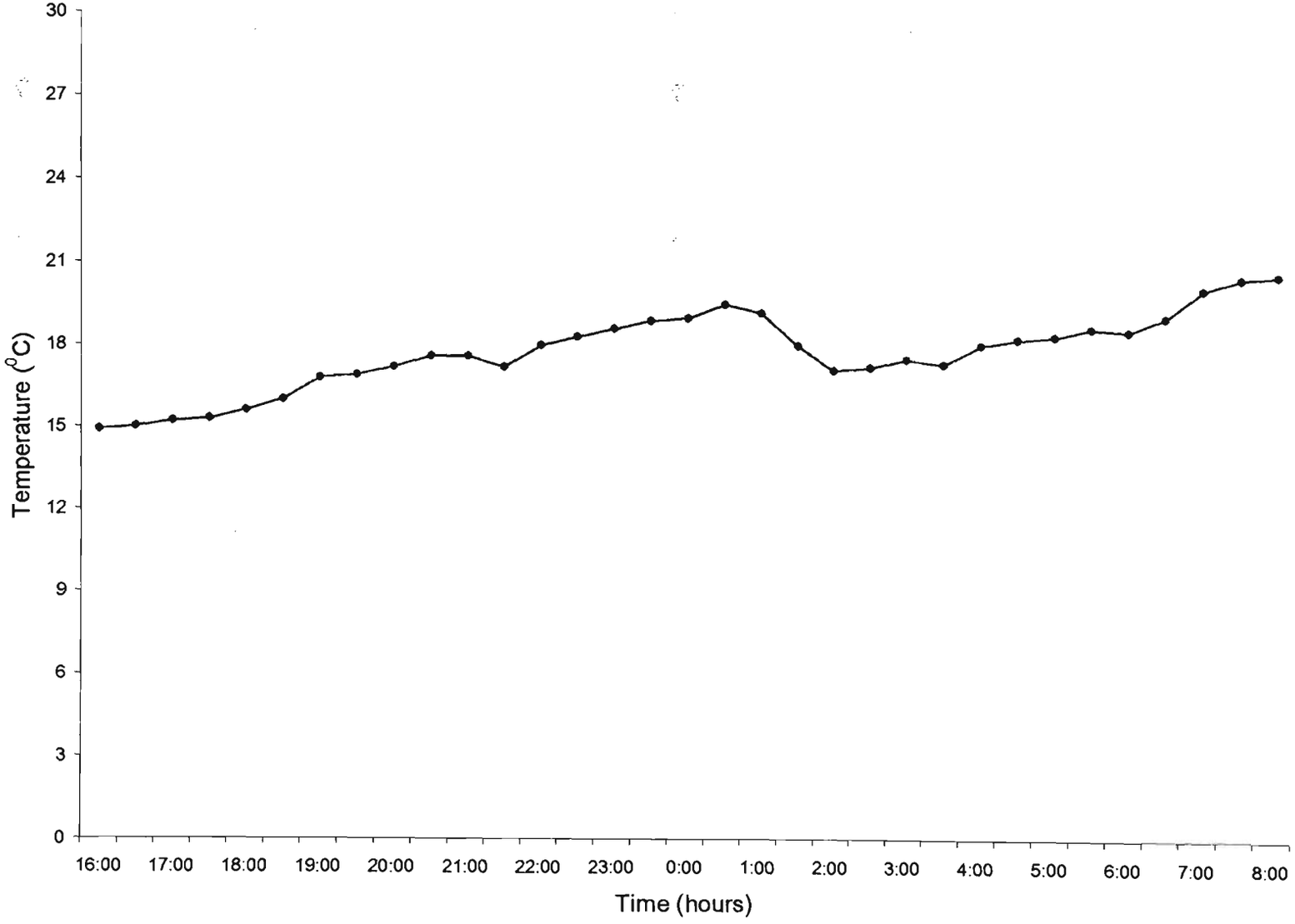
Time (hrs	Temp (^{0}C)
16:30	16.7
17:00	16.9
17:30	16.8
18:00	17
18:30	17.5
19:00	18
19:30	18.9
20:00	18
20:30	18.2
21:00	18.3
21:30	18.5
22:00	18.9
22:30	18.5
23:00	18
23:30	18.5
0:00	18.9
0:30	17
1:00	17.5
1:30	17.6
2:00	17.5
2:30	17.9
3:00	18
3:30	18.2
4:00	18.5
4:30	19.6
5:00	19.9
5:30	19.6
6:00	19.8
6:30	19.9
7:00	19.8
7:30	19.5
8:00	19.6

Average: 18.4



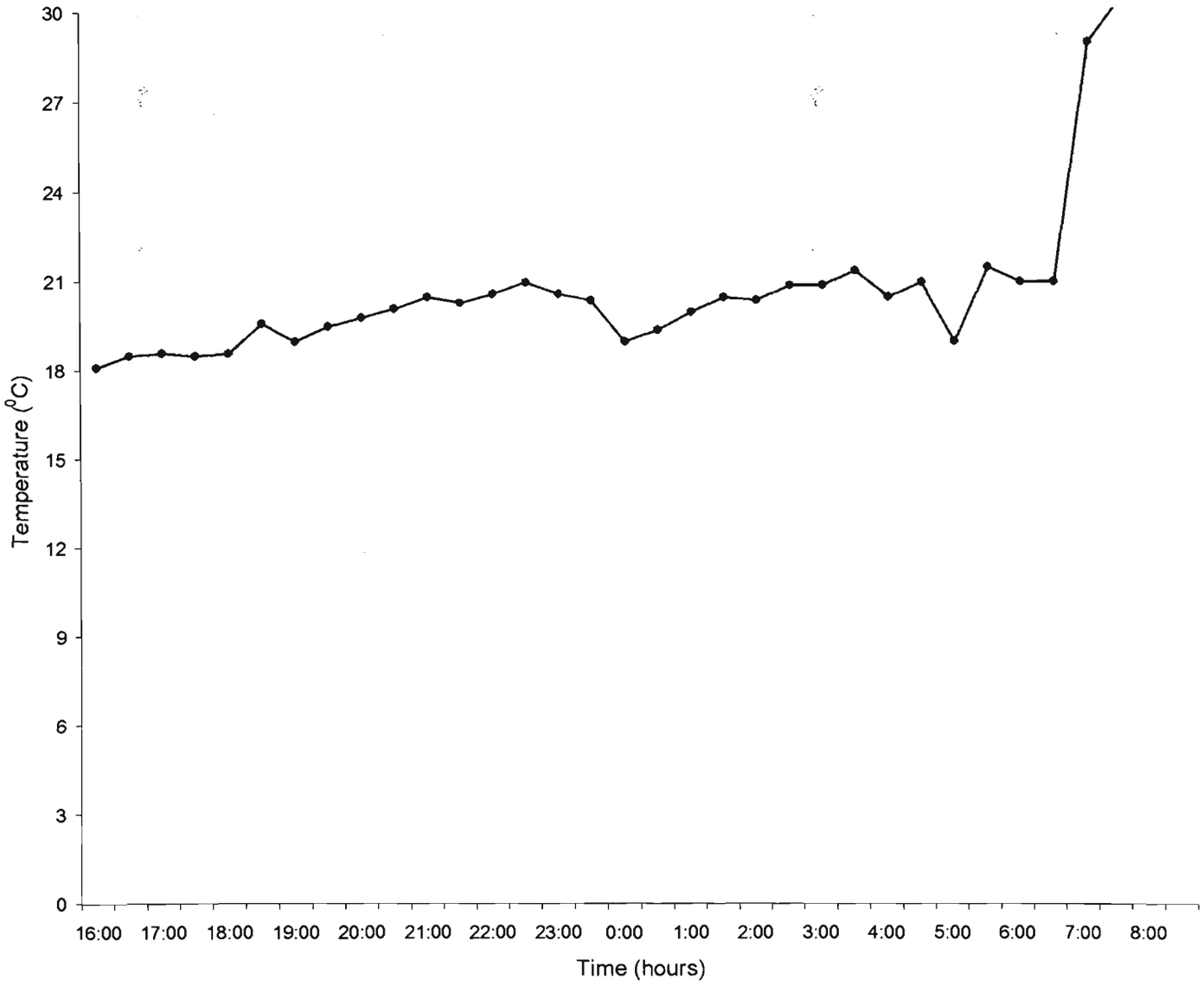
Date of fumigation: 15/07/97

Time (hrs	Temp (^{0}C)
16:00	14.9
16:30	15
17:00	15.2
17:30	15.3
18:00	15.6
18:30	16
19:00	16.8
19:30	16.9
20:00	17.2
20:30	17.6
21:00	17.6
21:30	17.2
22:00	18
22:30	18.3
23:00	18.6
23:30	18.9
0:00	19
0:30	19.5
1:00	19.2
1:30	18
2:00	17.1
2:30	17.2
3:00	17.5
3:30	17.3
4:00	18
4:30	18.2
5:00	18.3
5:30	18.6
6:00	18.5
6:30	19
7:00	20
7:30	20.4
8:00	20.5
Average:	**17.7**



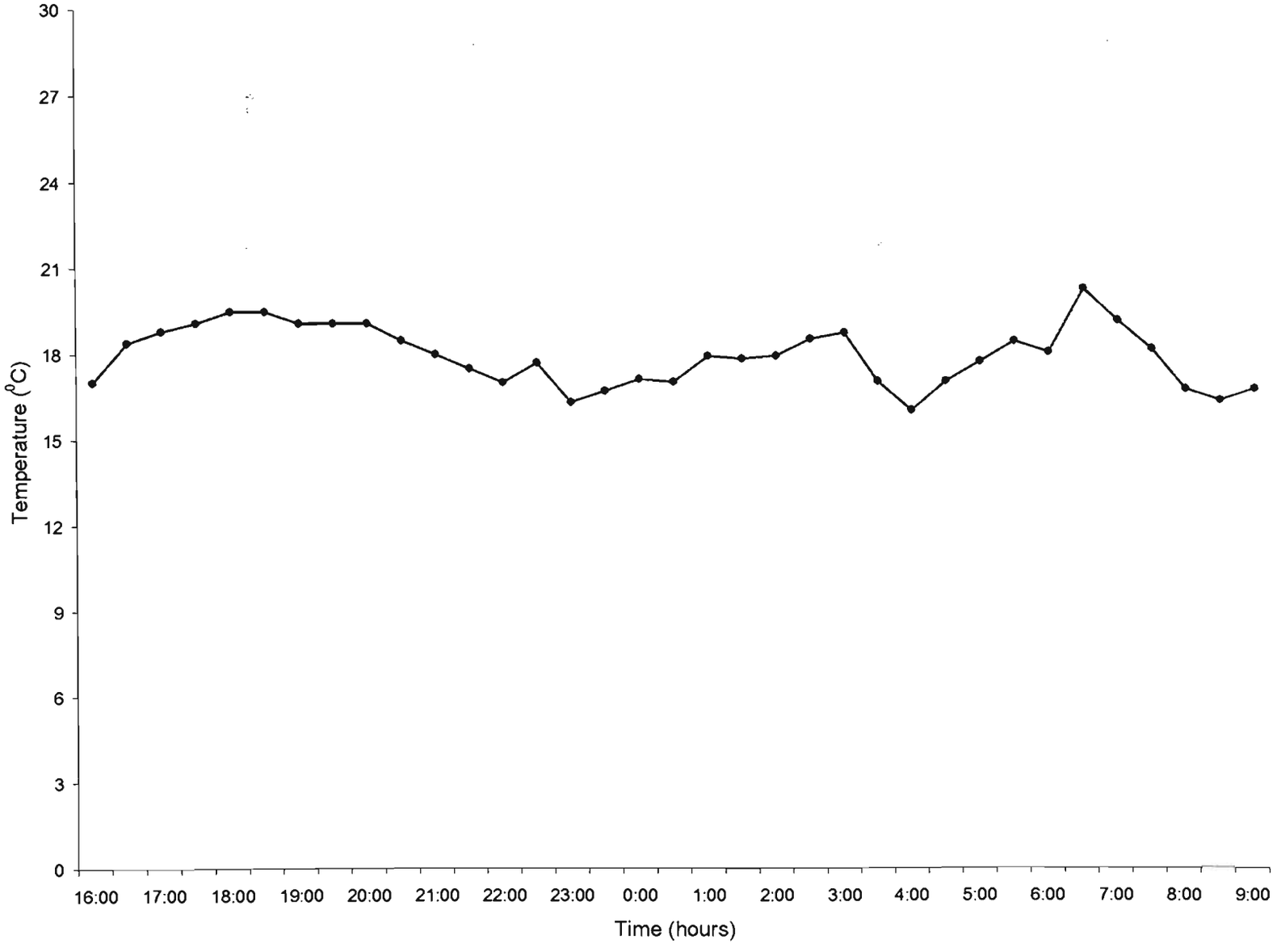
Date of fumigation: 2/12/97

Time (hrs	Temp (^{0}C)
16:00	18.1
16:30	18.5
17:00	18.6
17:30	18.5
18:00	18.6
18:30	19.6
19:00	19
19:30	19.5
20:00	19.8
20:30	20.1
21:00	20.5
21:30	20.3
22:00	20.6
22:30	21
23:00	20.6
23:30	20.4
0:00	19
0:30	19.4
1:00	20
1:30	20.5
2:00	20.4
2:30	20.9
3:00	20.9
3:30	21.4
4:00	20.5
4:30	21
5:00	19
5:30	21.5
6:00	21
6:30	21
7:00	29
7:30	30.5
8:00	31.5
8:30	34.8
Average:	**21.3**



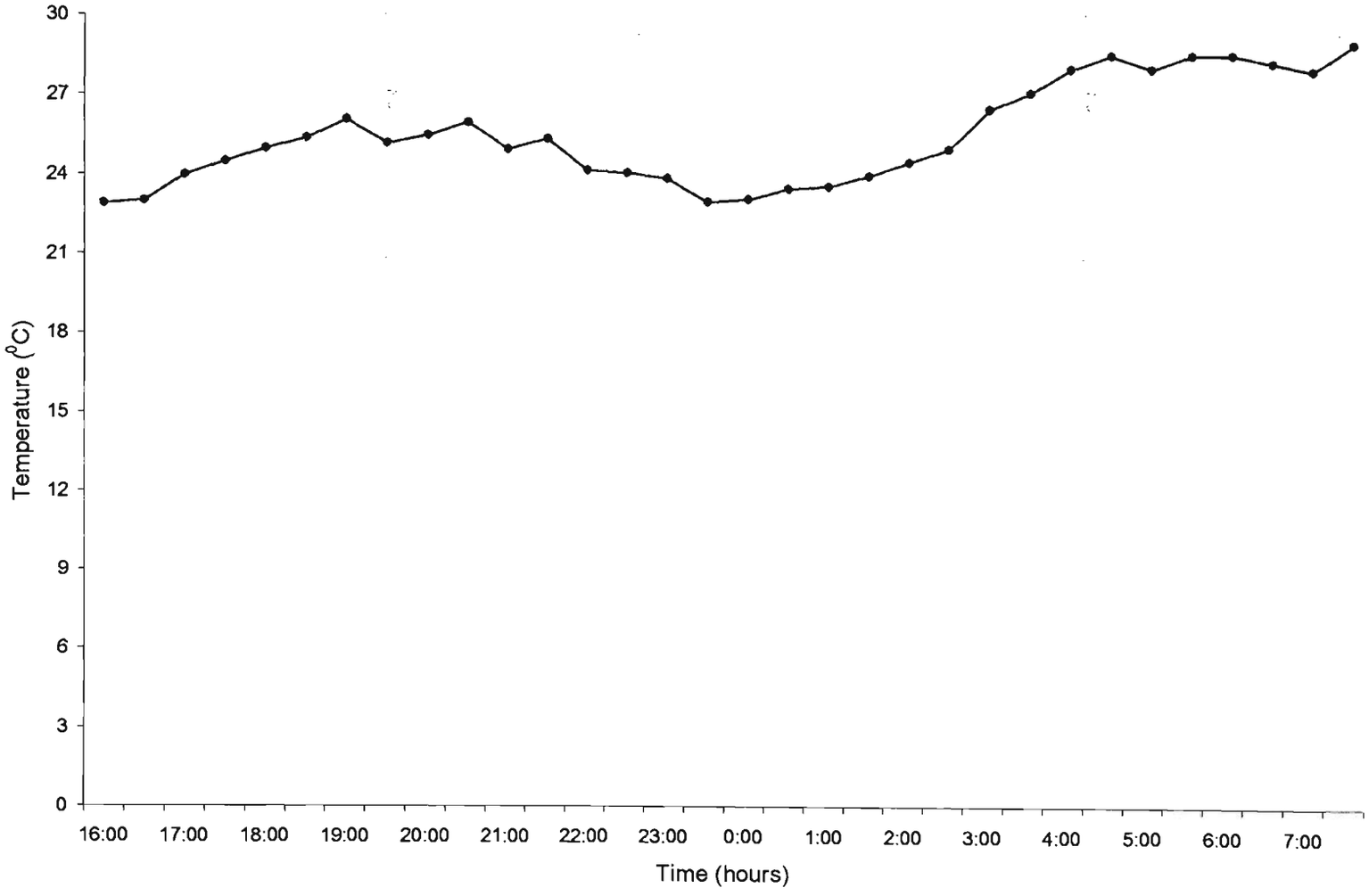
Date of fumigation: 25/06/97

Time (hrs	Temp (^{0}C)
16:00	17
16:30	18.4
17:00	18.8
17:30	19.1
18:00	19.5
18:30	19.5
19:00	19.1
19:30	19.1
20:00	19.1
20:30	18.5
21:00	18
21:30	17.5
22:00	17
22:30	17.7
23:00	16.3
23:30	16.7
0:00	17.1
0:30	17
1:00	17.9
1:30	17.8
2:00	17.9
2:30	18.5
3:00	18.7
3:30	17
4:00	16
4:30	17
5:00	17.7
5:30	18.4
6:00	18
6:30	20.2
7:00	19.1
7:30	18.1
8:00	16.7
8:30	16.3
9:00	16.7
Average:	**17.9**



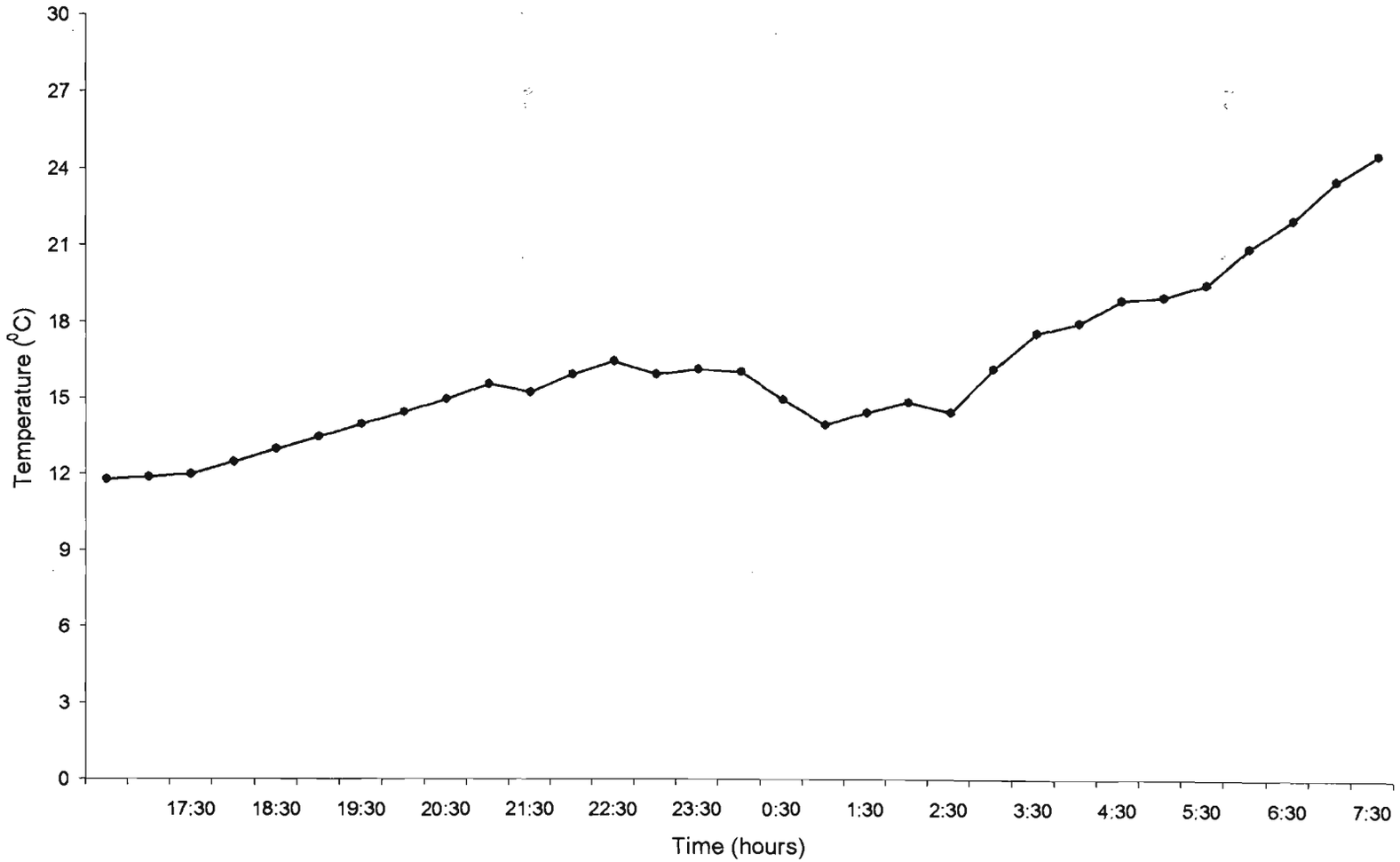
Date of fumigation: 14/01/97

Time (hrs	Temp (^{0}C)
16:00	22.9
16:30	23
17:00	24
17:30	24.5
18:00	25
18:30	25.4
19:00	26.1
19:30	25.2
20:00	25.5
20:30	26
21:00	25
21:30	25.4
22:00	24.2
22:30	24.1
23:00	23.9
23:30	23
0:00	23.1
0:30	23.5
1:00	23.6
1:30	24
2:00	24.5
2:30	25
3:00	26.5
3:30	27.1
4:00	28
4:30	28.5
5:00	28
5:30	28.5
6:00	28.5
6:30	28.2
7:00	27.9
7:30	28.9
Average:	**25.5**



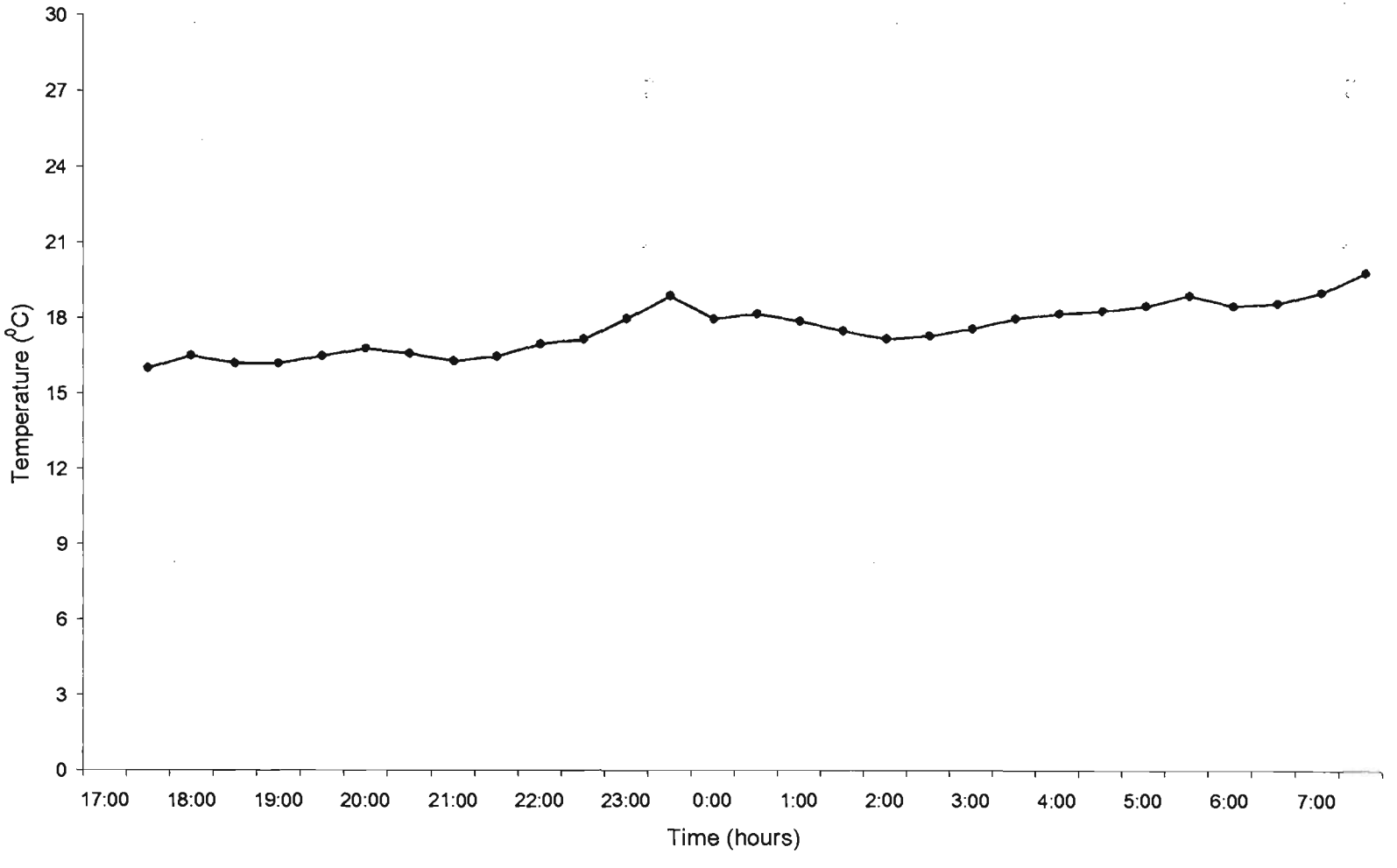
Date of fumigation: 17/03/97

Time (hrs	Temp (^{0}C)
17:00	11.8
17:30	11.9
18:00	12
18:30	12.5
19:00	13
19:30	13.5
20:00	14
20:30	14.5
21:00	15
21:30	15.6
22:00	15.3
22:30	16
23:00	16.5
23:30	16
0:00	16.2
0:30	16.1
1:00	15
1:30	14
2:00	14.5
2:30	14.9
3:00	14.5
3:30	16.2
4:00	17.6
4:30	18
5:00	18.9
5:30	19
6:00	19.5
6:30	20.9
7:00	22
7:30	23.5
8:00	24.5
Average:	**16.2**



Date of fumigation: 07/08/97

Time (hrs	Temp (^{0}C)
17:00	16
17:30	16.5
18:00	16.2
18:30	16.2
19:00	16.5
19:30	16.8
20:00	16.6
20:30	16.3
21:00	16.5
21:30	17
22:00	17.2
22:30	18
23:00	18.9
23:30	18
0:00	18.2
0:30	17.9
1:00	17.5
1:30	17.2
2:00	17.3
2:30	17.6
3:00	18
3:30	18.2
4:00	18.3
4:30	18.5
5:00	18.9
5:30	18.5
6:00	18.6
6:30	19
7:00	19.8
Average:	**17.6**



Appendix - 5

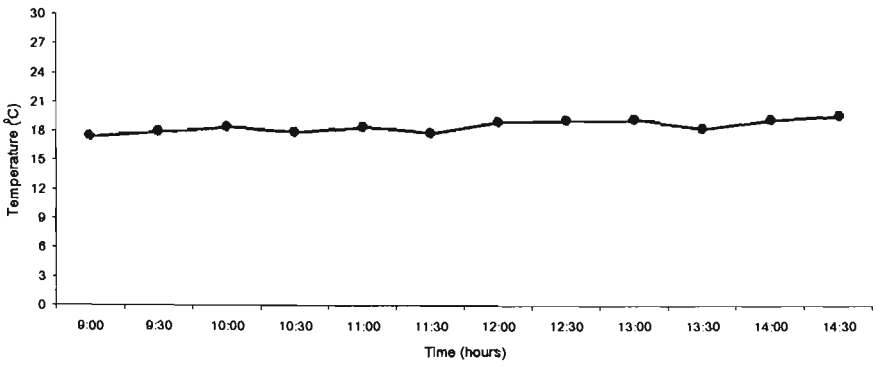
Tempeature - Time Trajectories (Chapter 5)

Appendix 5 Temperature-Time Trajectories (Chapter 5)

The data presented here are the temperatures recorded among the flowers.

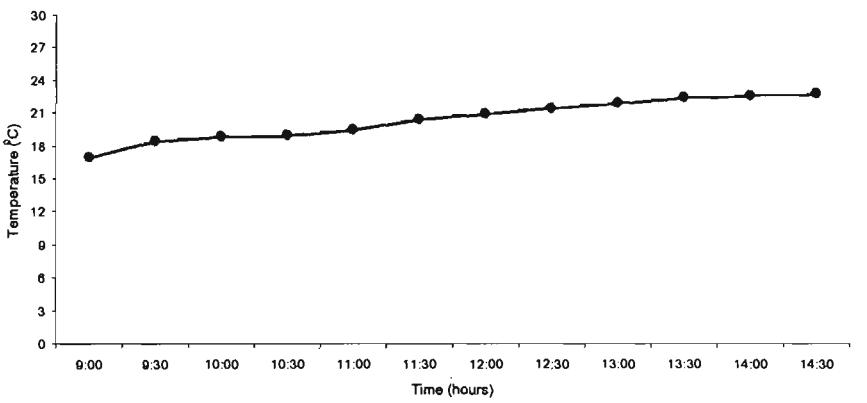
Date: 28/6/95

Time	Temperature
9:00	17.5
9:30	18
10:00	18.5
10:30	18
11:00	18.6
11:30	18
12:00	19.2
12:30	19.3
13:00	19.5
13:30	18.5
14:00	19.5
14:30	19.9
Average	**18.7**



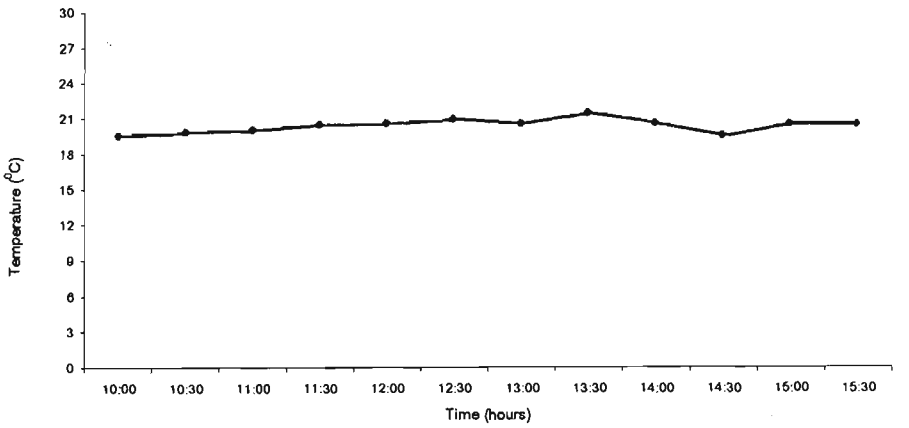
Date: 19/10/95

9:00	17
9:30	18.5
10:00	18.9
10:30	19
11:00	19.5
11:30	20.5
12:00	21
12:30	21.5
13:00	22
13:30	22.5
14:00	22.7
14:30	22.9
Average	**20.5**



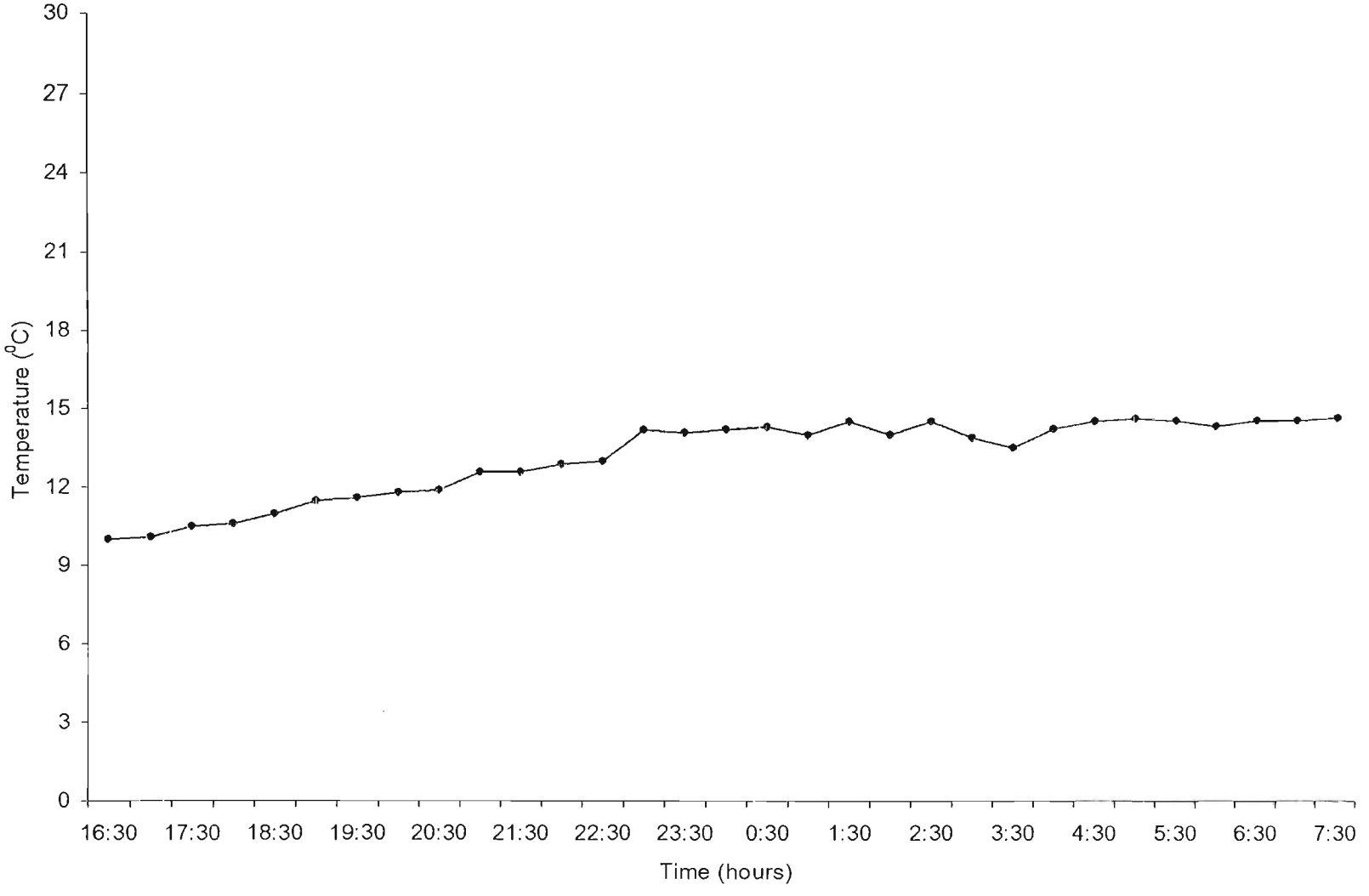
Date: 26/10/95

10:00	19.5
10:30	19.8
11:00	20
11:30	20.4
12:00	20.5
12:30	20.9
13:00	20.5
13:30	21.4
14:00	20.6
14:30	19.5
15:00	20.5
15:30	20.5
Average	**20.3**



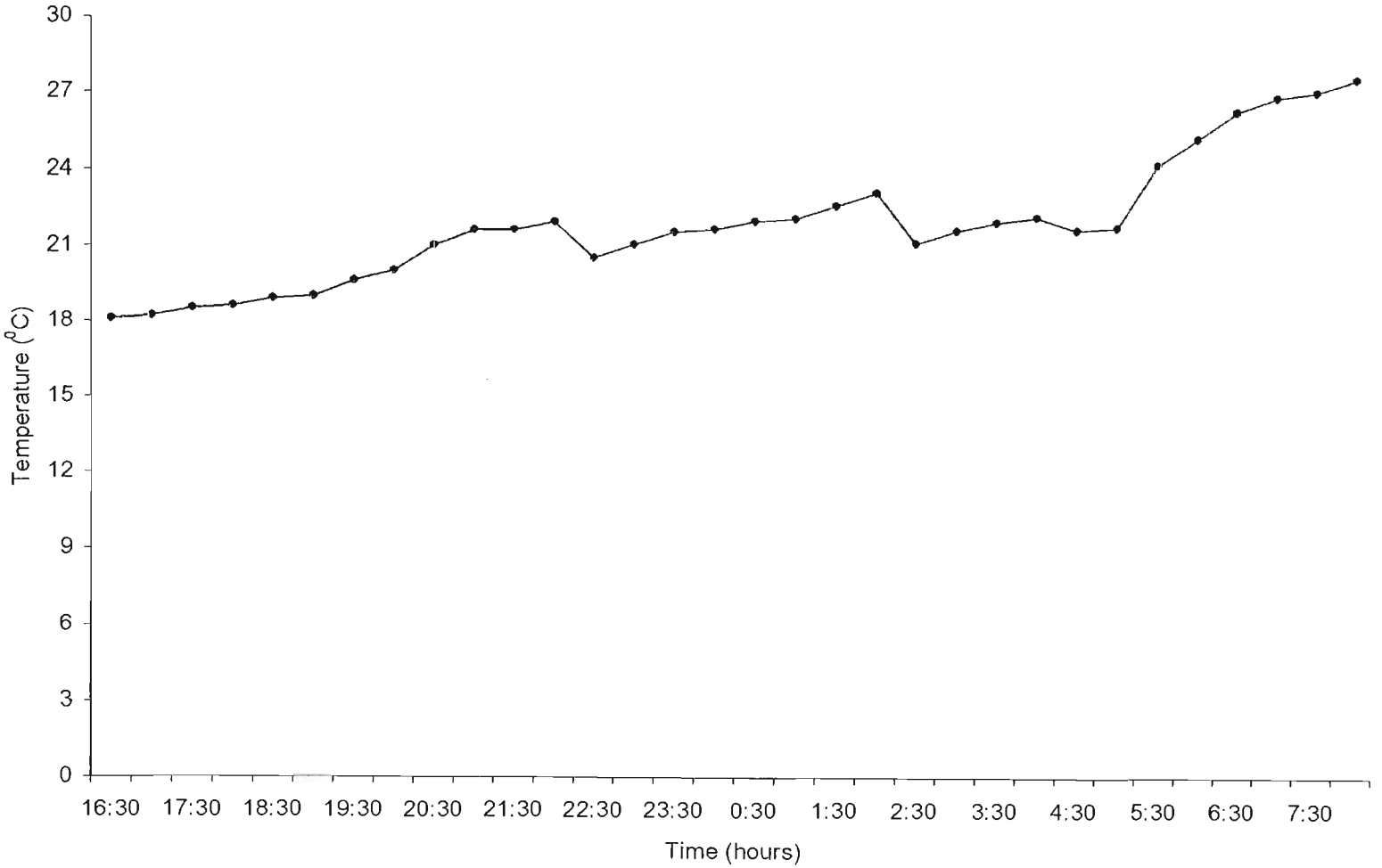
Date of fumigation: 18/3/96

Time (hrs)	Temp (^{0}C)
16:30	10
17:00	10.1
17:30	10.5
18:00	10.6
18:30	11
19:00	11.5
19:30	11.6
20:00	11.8
20:30	11.9
21:00	12.6
21:30	12.6
22:00	12.9
22:30	13
23:00	14.2
23:30	14.1
0:00	14.2
0:30	14.3
1:00	14
1:30	14.5
2:00	14
2:30	14.5
3:00	13.9
3:30	13.5
4:00	14.2
4:30	14.5
5:00	14.6
5:30	14.5
6:00	14.3
6:30	14.5
7:00	14.5
7:30	14.6
Average	**13.1**



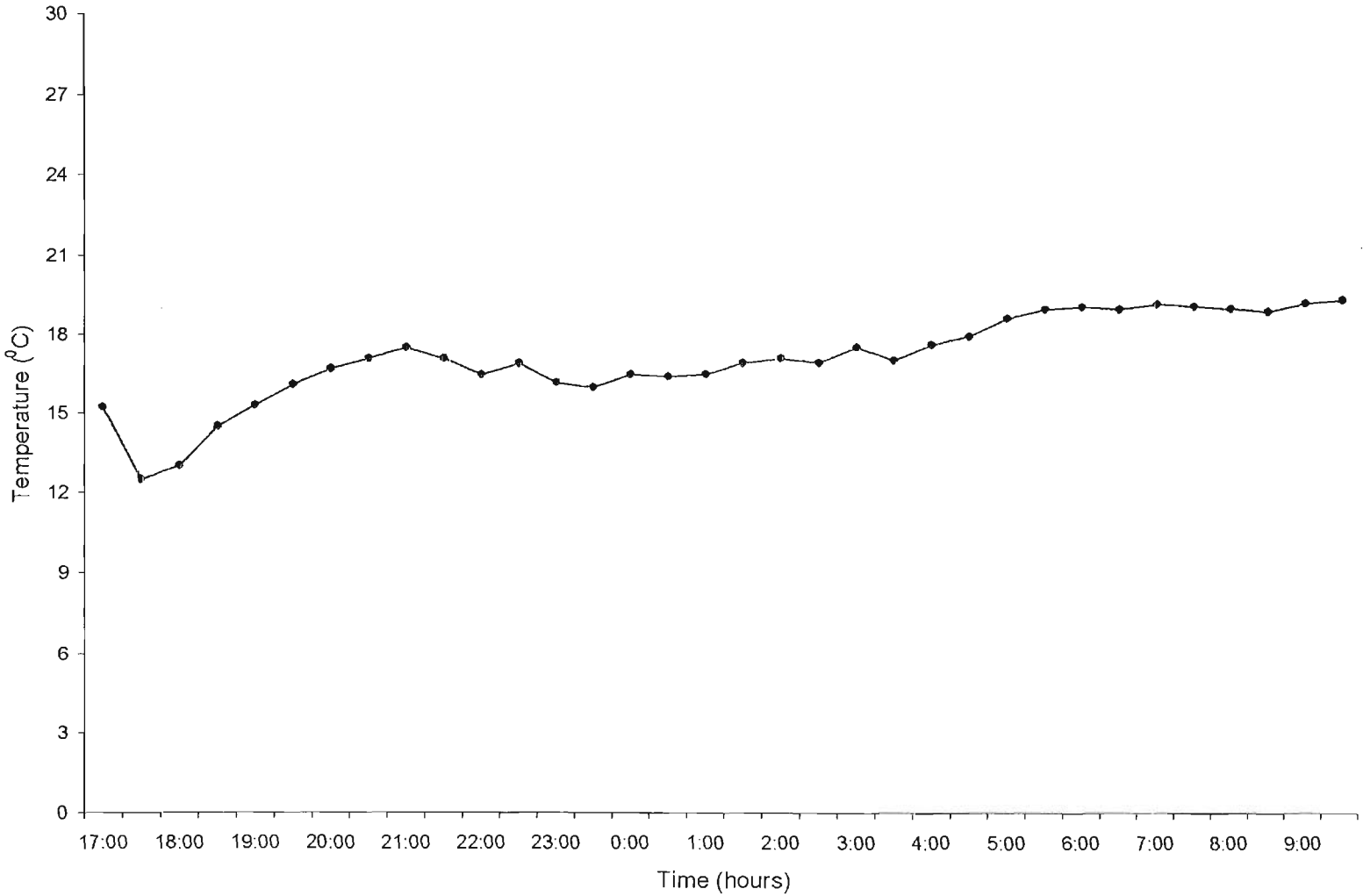
Date of fumigation: 25/3/96

Time (hrs)	Temp (^{0}C)
16:30	18.1
17:00	18.2
17:30	18.5
18:00	18.6
18:30	18.9
19:00	19
19:30	19.6
20:00	20
20:30	21
21:00	21.6
21:30	21.6
22:00	21.9
22:30	20.5
23:00	21
23:30	21.5
0:00	21.6
0:30	21.9
1:00	22
1:30	22.5
2:00	23
2:30	21
3:00	21.5
3:30	21.8
4:00	22
4:30	21.5
5:00	21.6
5:30	24
6:00	25
6:30	26
7:00	26.5
7:30	26.7
8:00	27.2
Average	**21.7**



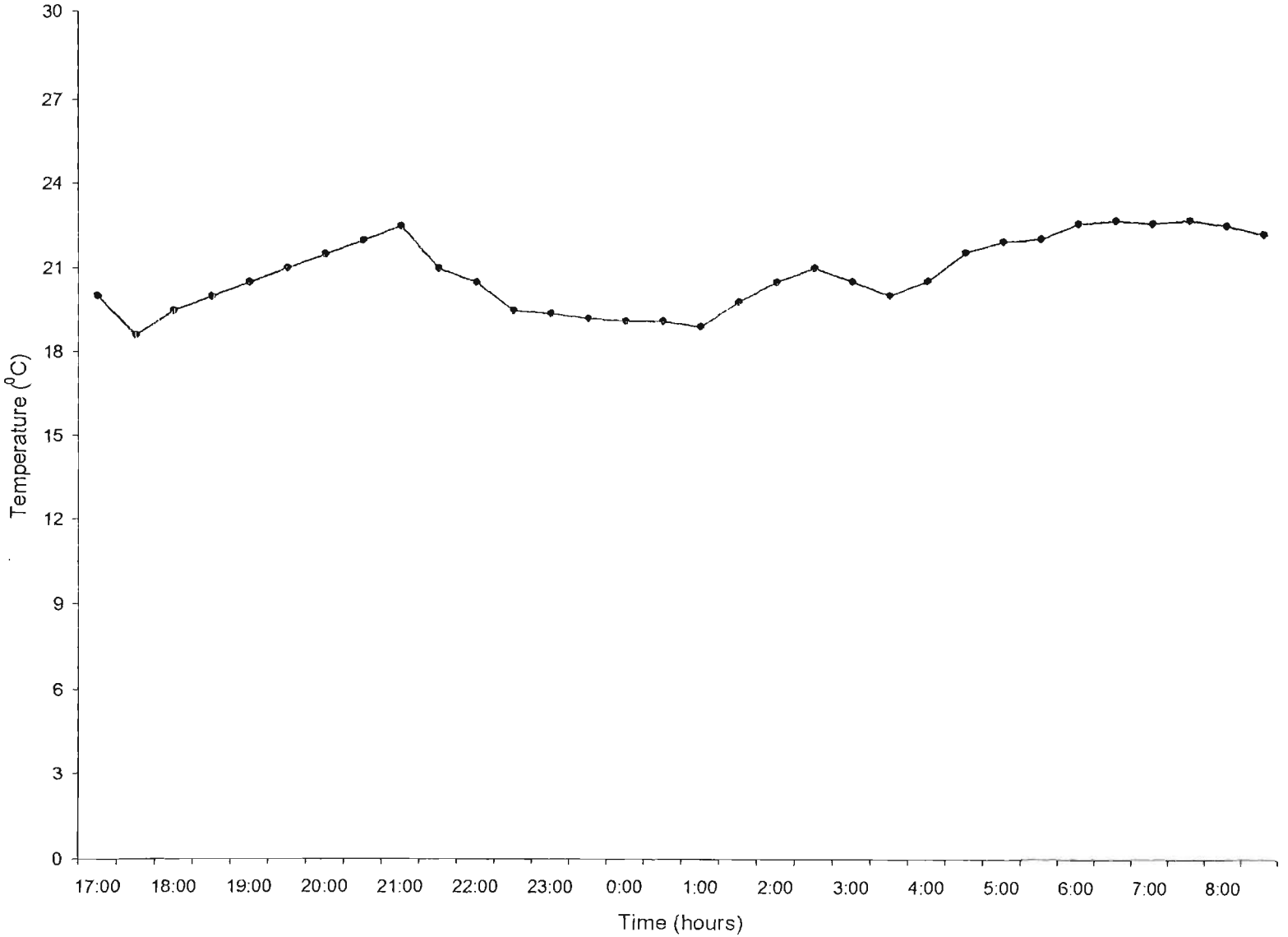
Date of fumigation: 16/10/96

Time (hrs)	Temp (^{0}C)
17:00	15.2
17:30	12.5
18:00	13
18:30	14.5
19:00	15.3
19:30	16.1
20:00	16.7
20:30	17.1
21:00	17.5
21:30	17.1
22:00	16.5
22:30	16.9
23:00	16.2
23:30	16
0:00	16.5
0:30	16.4
1:00	16.5
1:30	16.9
2:00	17.1
2:30	16.9
3:00	17.5
3:30	17
4:00	17.6
4:30	17.9
5:00	18.6
5:30	18.9
6:00	19
6:30	18.9
7:00	19.1
7:30	19
8:00	18.9
8:30	18.8
9:00	19.1
9:30	19.2
Average	**17.1**



Date of fumigation: 2/12/96

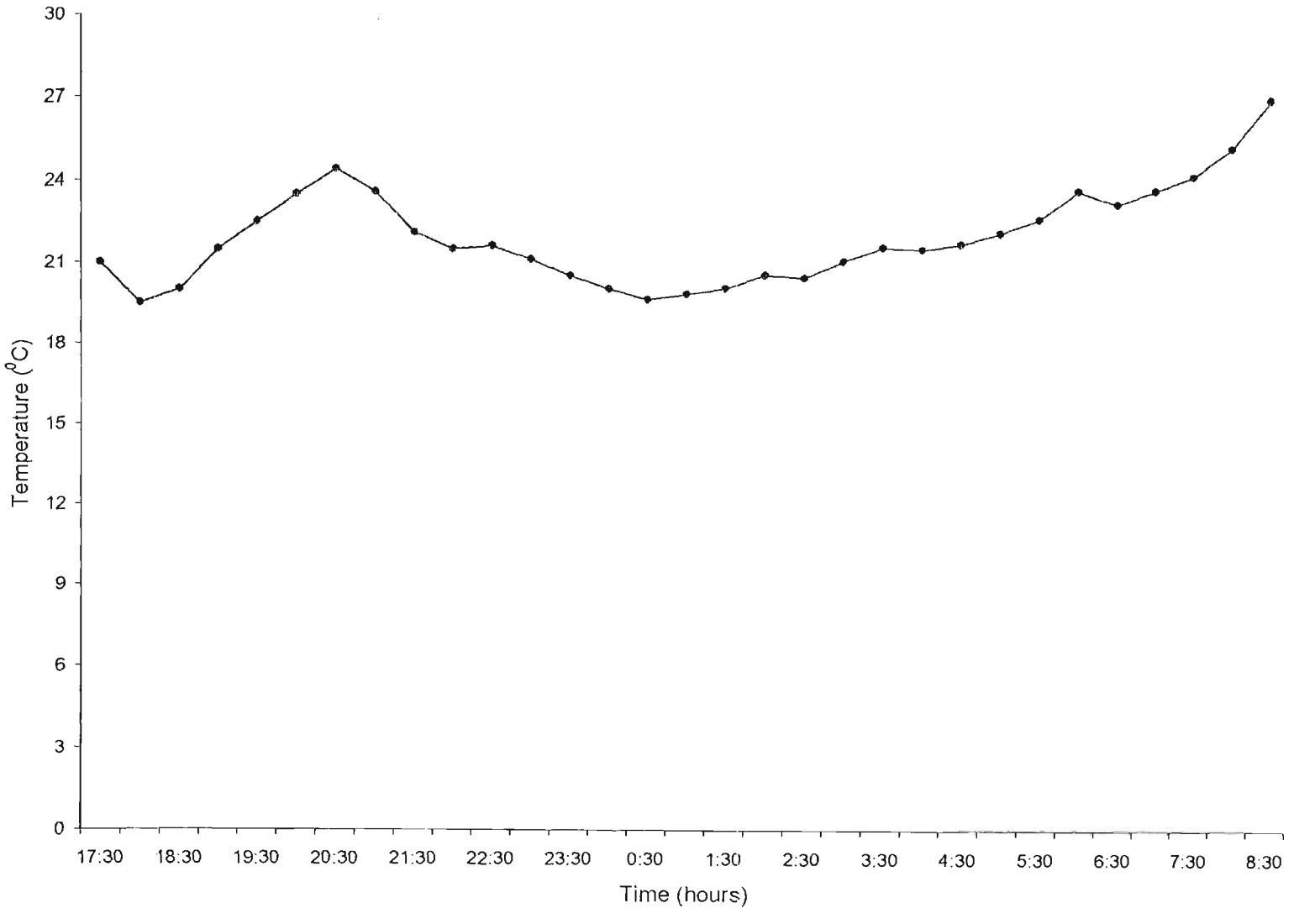
Time (hrs)	Temp (^{0}C)
17:00	20
17:30	18.6
18:00	19.5
18:30	20
19:00	20.5
19:30	21
20:00	21.5
20:30	22
21:00	22.5
21:30	21
22:00	20.5
22:30	19.5
23:00	19.4
23:30	19.2
0:00	19.1
0:30	19.1
1:00	18.9
1:30	19.8
2:00	20.5
2:30	21
3:00	20.5
3:30	20
4:00	20.5
4:30	21.5
5:00	21.9
5:30	22
6:00	22.5
6:30	22.6
7:00	22.5
7:30	22.6
8:00	22.4
8:30	22.1
Average:	**20.7**



Date of fumigation: 26/2/96

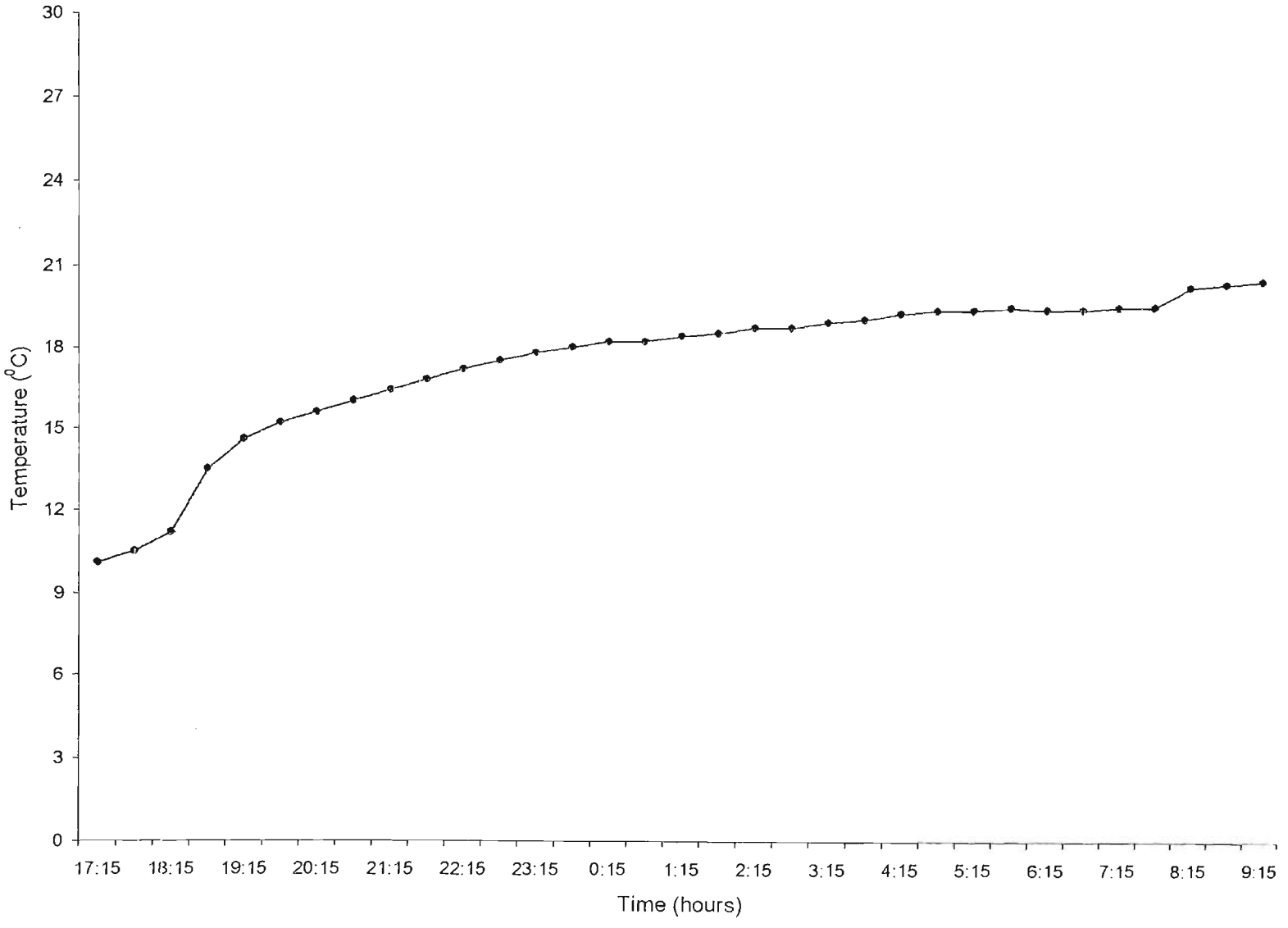
Time (hrs)	Temp (^{0}C)
17:30	21
18:00	19.5
18:30	20
19:00	21.5
19:30	22.5
20:00	23.5
20:30	24.4
21:00	23.6
21:30	22.1
22:00	21.5
22:30	21.6
23:00	21.1
23:30	20.5
0:00	20
0:30	19.6
1:00	19.8
1:30	20
2:00	20.5
2:30	20.4
3:00	21
3:30	21.5
4:00	21.4
4:30	21.6
5:00	22
5:30	22.5
6:00	23.5
6:30	23
7:00	23.5
7:30	24
8:00	25
8:30	26.7

Average: **21.9**



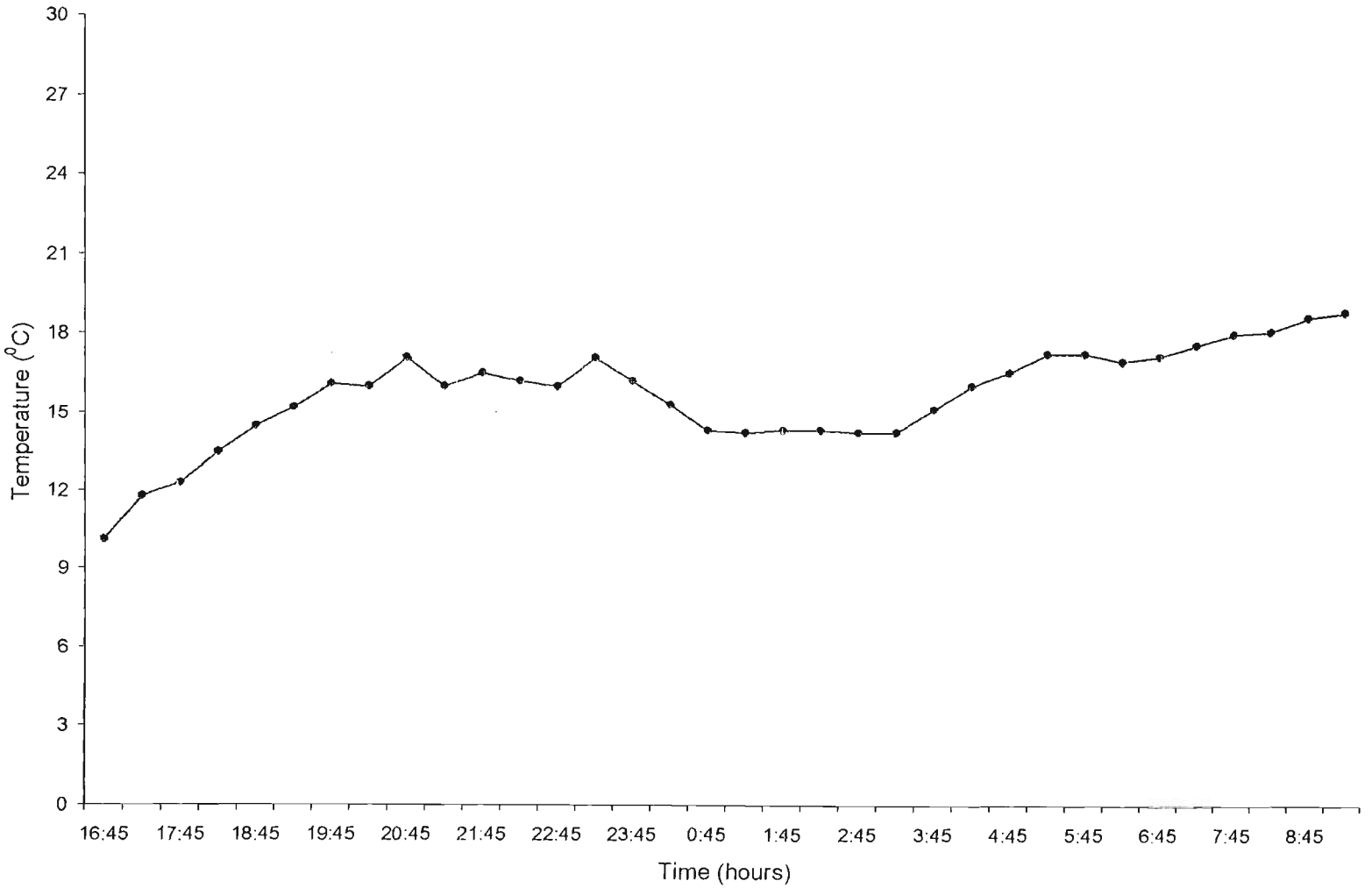
Date of fumigation: 18/7/96

Time (hrs)	Temp (^{0}C)
17:15	10.1
17:45	10.5
18:15	11.2
18:45	13.5
19:15	14.6
19:45	15.2
20:15	15.6
20:45	16
21:15	16.4
21:45	16.8
22:15	17.2
22:45	17.5
23:15	17.8
23:45	18
0:15	18.2
0:45	18.2
1:15	18.4
1:45	18.5
2:15	18.7
2:45	18.7
3:15	18.9
3:45	19
4:15	19.2
4:45	19.3
5:15	19.3
5:45	19.4
6:15	19.3
6:45	19.3
7:15	19.4
7:45	19.4
8:15	20.1
8:45	20.2
9:15	20.3
Average:	**17.4**



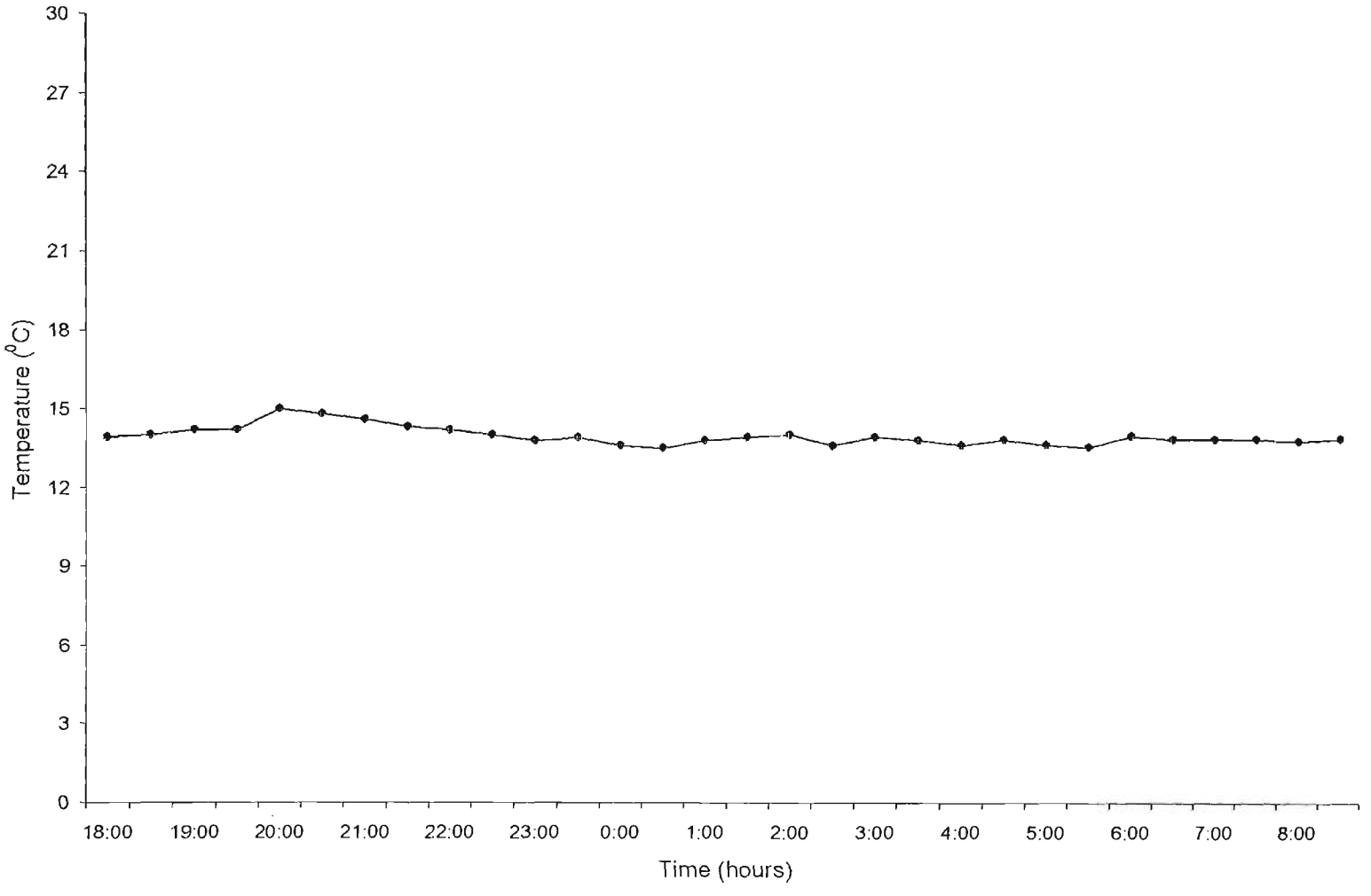
Date of fumigation: 7/10/96

Time (hrs)	Temp (^{0}C)
16:45	10.1
17:15	11.8
17:45	12.3
18:15	13.5
18:45	14.5
19:15	15.2
19:45	16.1
20:15	16
20:45	17.1
21:15	16
21:45	16.5
22:15	16.2
22:45	16
23:15	17.1
23:45	16.2
0:15	15.3
0:45	14.3
1:15	14.2
1:45	14.3
2:15	14.3
2:45	14.2
3:15	14.2
3:45	15.1
4:15	16
4:45	16.5
5:15	17.2
5:45	17.2
6:15	16.9
6:45	17.1
7:15	17.5
7:45	17.9
8:15	18
8:45	18.5
9:15	18.7
Average:	**15.6**



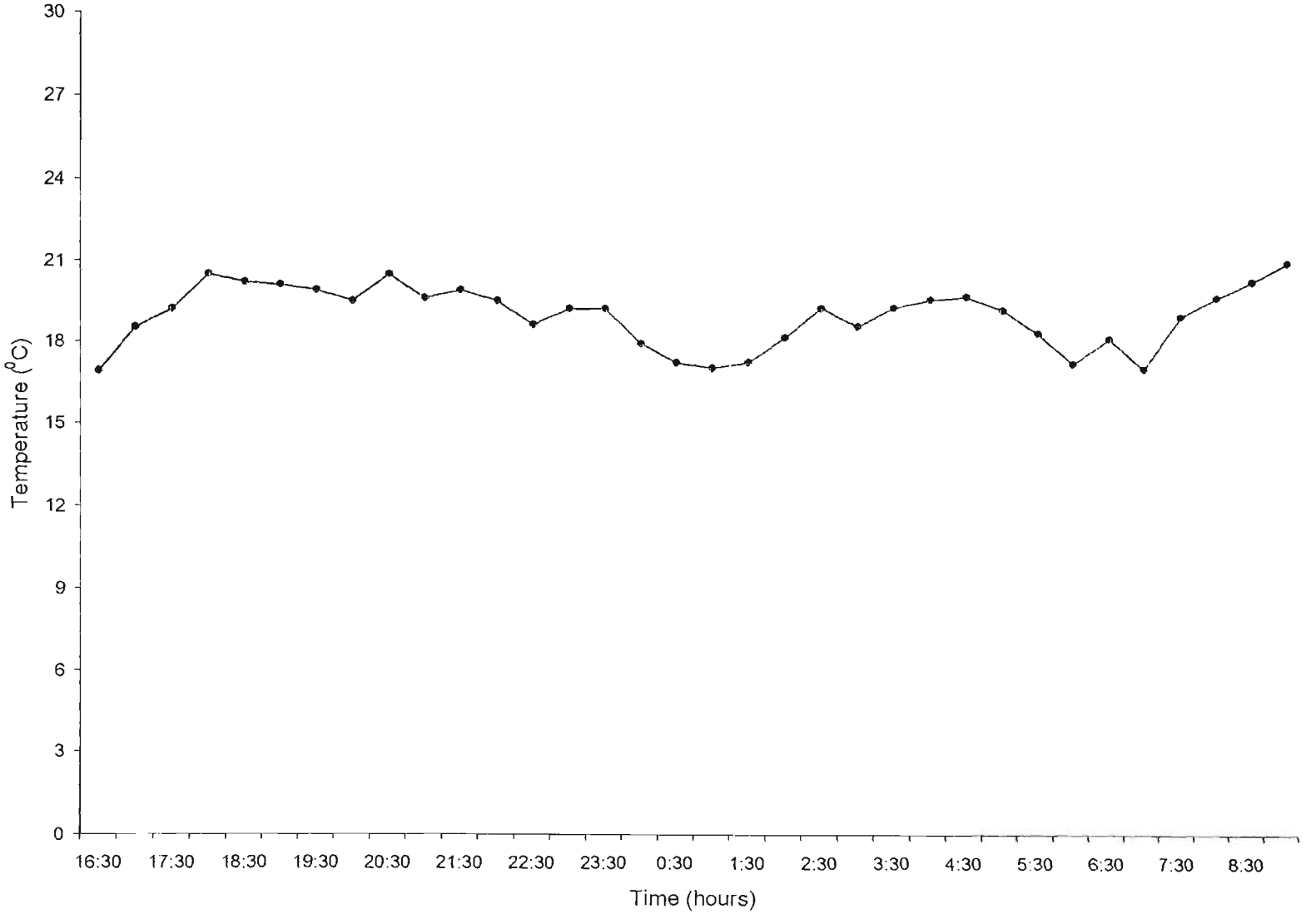
Date of fumigation: 4/2/96

Time (hrs)	Temp (^{0}C)
18:00	13.9
18:30	14
19:00	14.2
19:30	14.2
20:00	15
20:30	14.8
21:00	14.6
21:30	14.3
22:00	14.2
22:30	14
23:00	13.8
23:30	13.9
0:00	13.6
0:30	13.5
1:00	13.8
1:30	13.9
2:00	14
2:30	13.6
3:00	13.9
3:30	13.8
4:00	13.6
4:30	13.8
5:00	13.6
5:30	13.5
6:00	13.9
6:30	13.8
7:00	13.8
7:30	13.8
8:00	13.7
8:30	13.8
Average	**13.9**



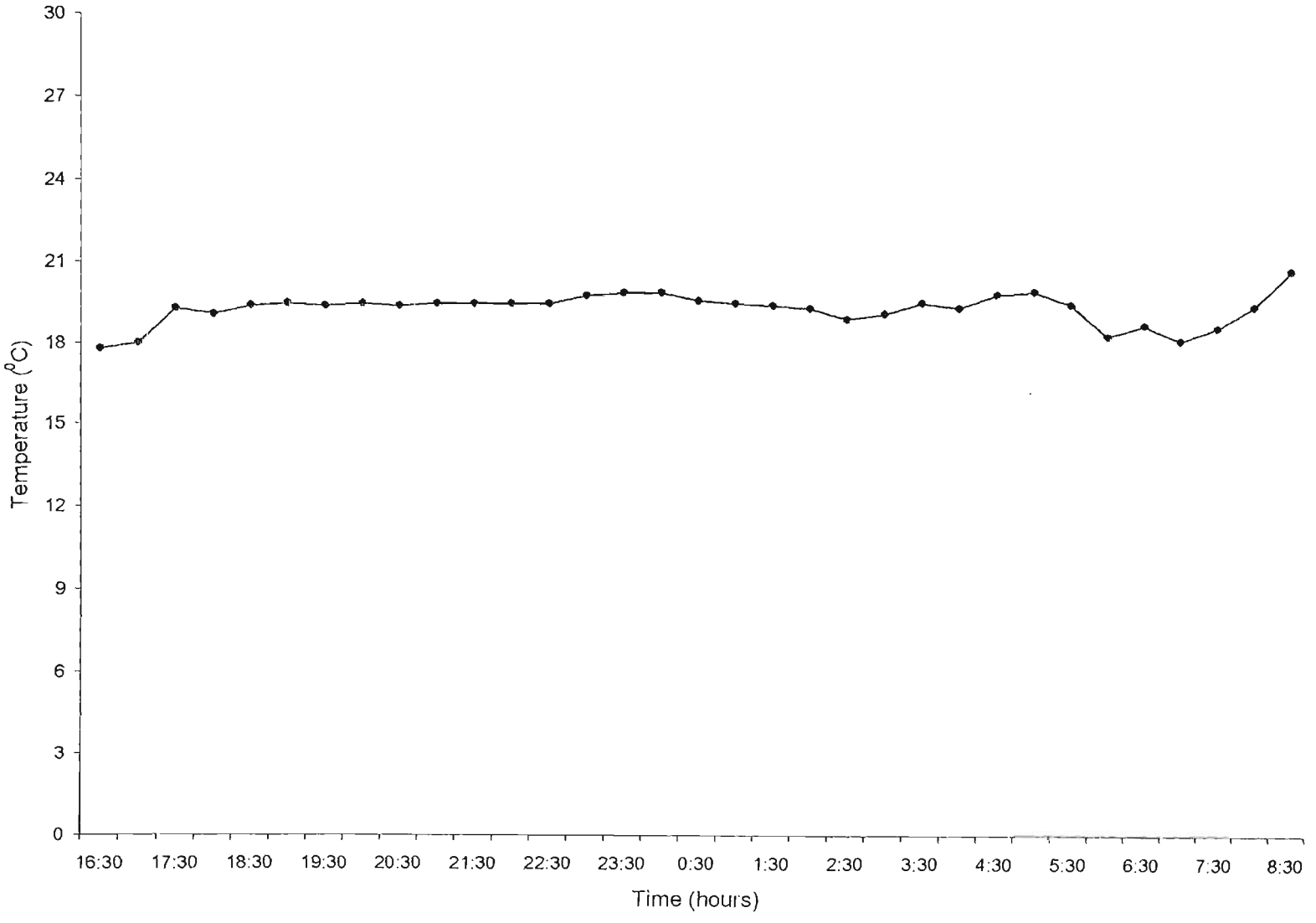
Date of fumigation: 23/9/96

Time (hrs)	Temp (^{0}C)
16:30	16.9
17:00	18.5
17:30	19.2
18:00	20.5
18:30	20.2
19:00	20.1
19:30	19.9
20:00	19.5
20:30	20.5
21:00	19.6
21:30	19.9
22:00	19.5
22:30	18.6
23:00	19.2
23:30	19.2
0:00	17.9
0:30	17.2
1:00	17
1:30	17.2
2:00	18.1
2:30	19.2
3:00	18.5
3:30	19.2
4:00	19.5
4:30	19.6
5:00	19.1
5:30	18.2
6:00	17.1
6:30	18
7:00	16.9
7:30	18.8
8:00	19.5
8:30	20.1
9:00	20.8
Average:	**18.9**



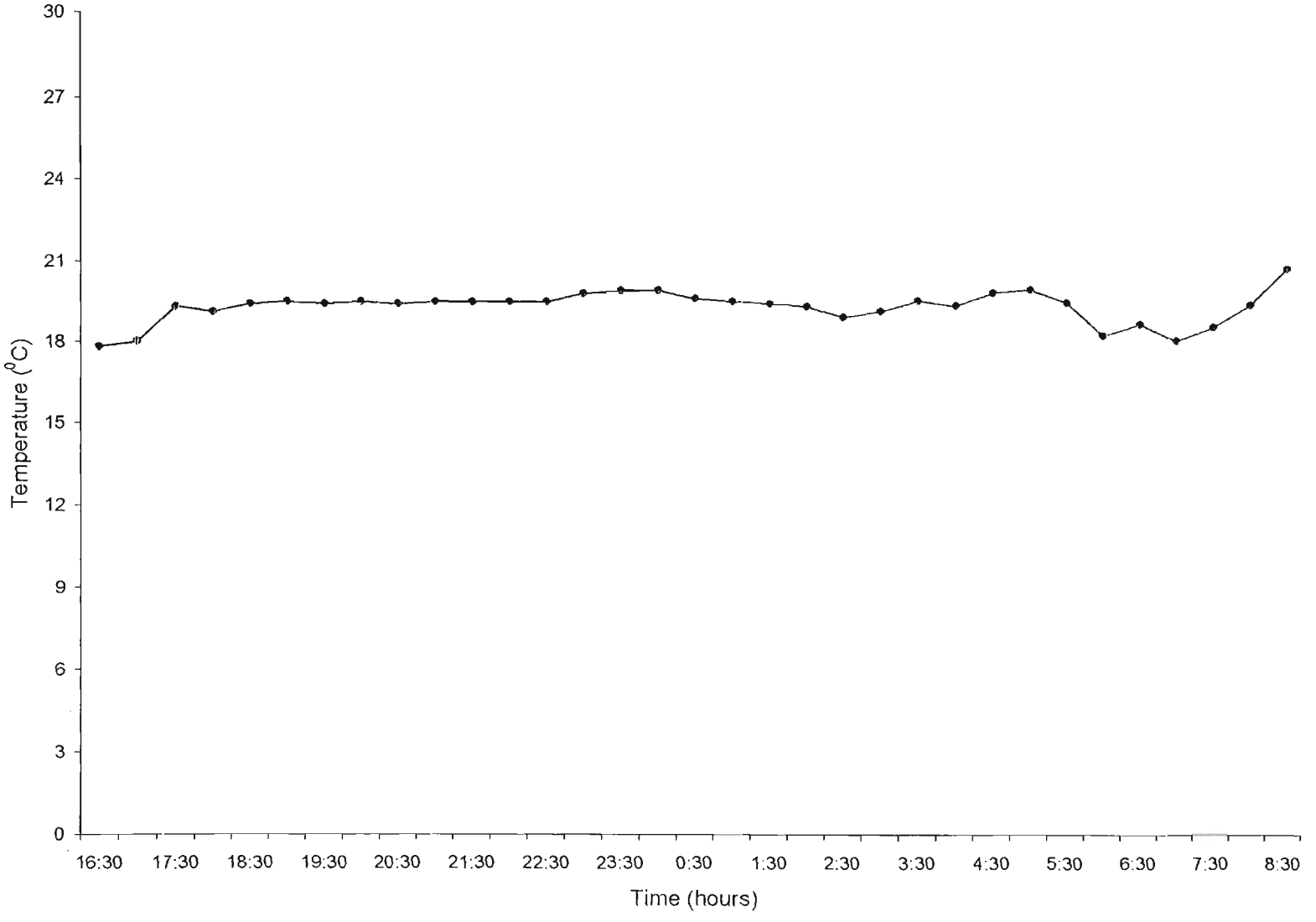
Date of fumigation: 18/9/96

Time (hrs)	Temp (^{0}C)
16:30	17.8
17:00	18
17:30	19.3
18:00	19.1
18:30	19.4
19:00	19.5
19:30	19.4
20:00	19.5
20:30	19.4
21:00	19.5
21:30	19.5
22:00	19.5
22:30	19.5
23:00	19.8
23:30	19.9
0:00	19.9
0:30	19.6
1:00	19.5
1:30	19.4
2:00	19.3
2:30	18.9
3:00	19.1
3:30	19.5
4:00	19.3
4:30	19.8
5:00	19.9
5:30	19.4
6:00	18.2
6:30	18.6
7:00	18
7:30	18.5
8:00	19.3
8:30	20.6
Average:	**19.2**



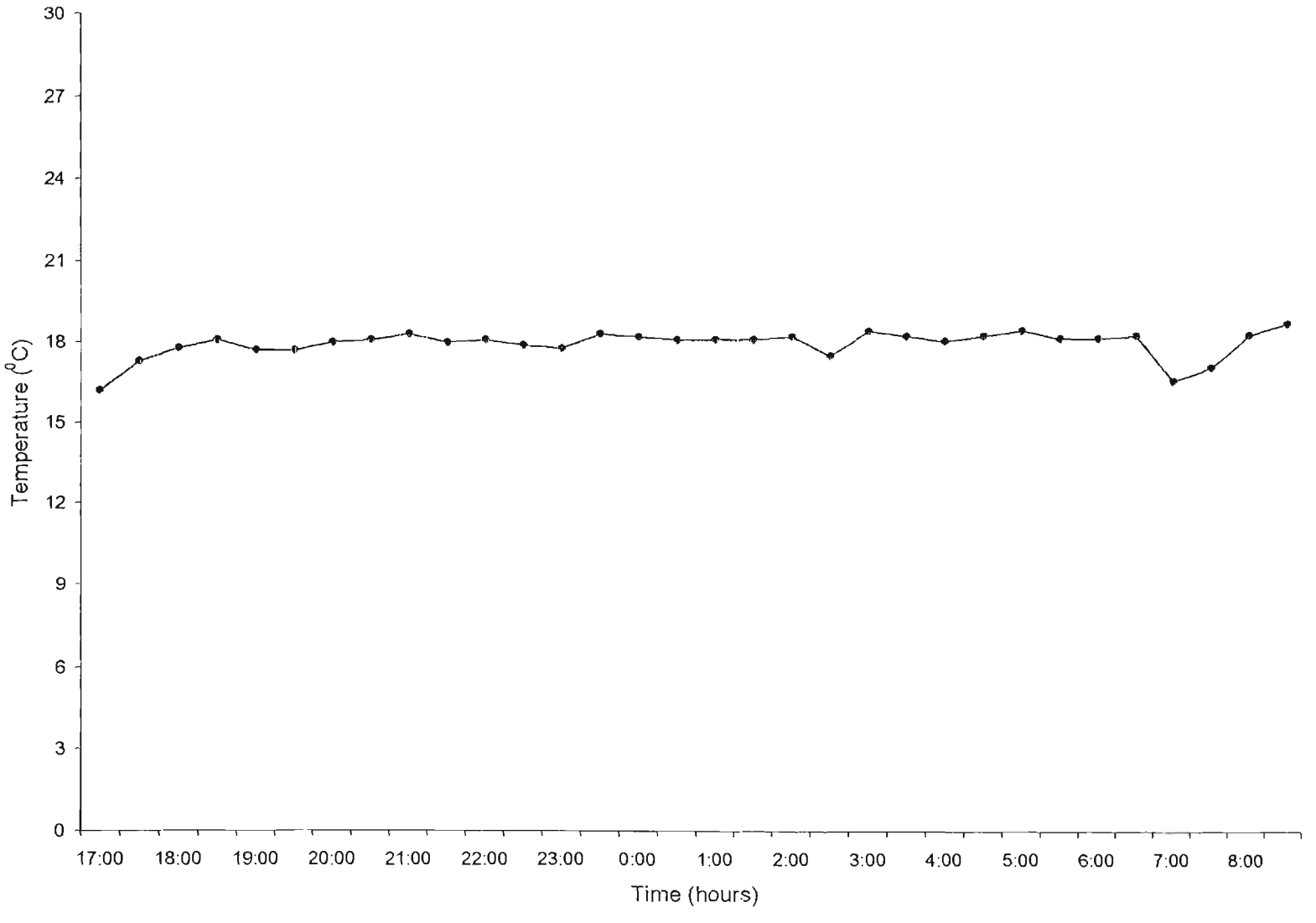
Date of fumigation: 29/8/96

Time (hrs)	Temp (^{0}C)
16:30	16.6
17:00	16.8
17:30	17.1
18:00	17.4
18:30	17.4
19:00	17.7
19:30	17.6
20:00	17.6
20:30	17.3
21:00	17.5
21:30	17.4
22:00	17.3
22:30	17.5
23:00	17.4
23:30	17
0:00	16.9
0:30	17
1:00	18
1:30	18.5
2:00	18.2
2:30	17.5
3:00	18
3:30	18.2
4:00	18.5
4:30	18.3
5:00	18.9
5:30	19.5
6:00	19.6
6:30	20
7:00	19.5
7:30	18.1
8:00	18.6
8:30	20
9:00	20.1
Average:	**18**



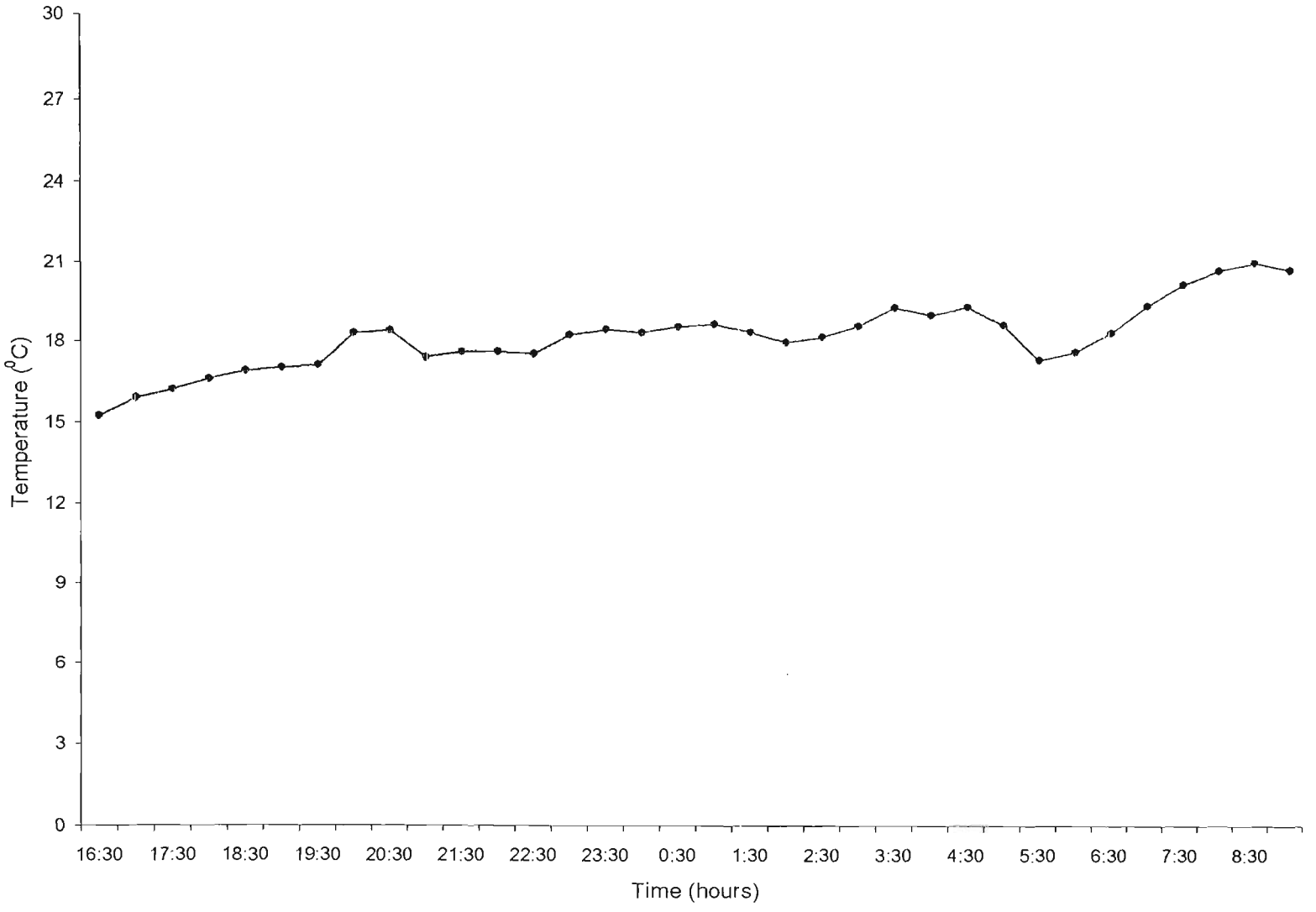
Date of fumigation: 9/9/96

Time (hrs)	Temp (^{0}C)
17:00	16.2
17:30	17.3
18:00	17.8
18:30	18.1
19:00	17.7
19:30	17.7
20:00	18
20:30	18.1
21:00	18.3
21:30	18
22:00	18.1
22:30	17.9
23:00	17.8
23:30	18.3
0:00	18.2
0:30	18.1
1:00	18.1
1:30	18.1
2:00	18.2
2:30	17.5
3:00	18.4
3:30	18.2
4:00	18
4:30	18.2
5:00	18.4
5:30	18.1
6:00	18.1
6:30	18.2
7:00	16.5
7:30	17
8:00	18.2
8:30	18.6
Average:	**17.9**



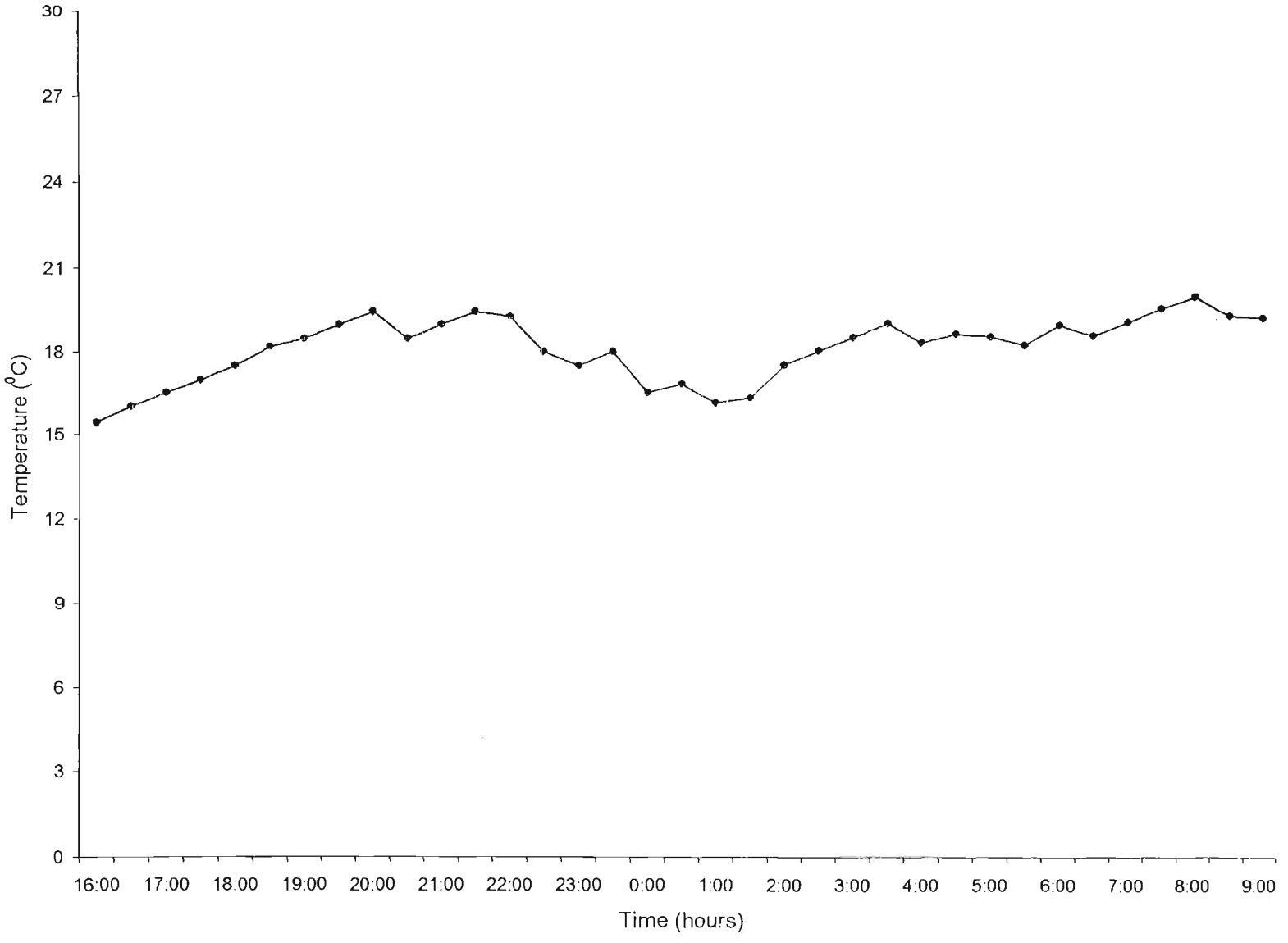
Date of fumigation: 9/4/96

Time (hrs)	Temp (^{0}C)
16:30	15.2
17:00	15.9
17:30	16.2
18:00	16.6
18:30	16.9
19:00	17
19:30	17.1
20:00	18.3
20:30	18.4
21:00	17.4
21:30	17.6
22:00	17.6
22:30	17.5
23:00	18.2
23:30	18.4
0:00	18.3
0:30	18.5
1:00	18.6
1:30	18.3
2:00	17.9
2:30	18.1
3:00	18.5
3:30	19.2
4:00	18.9
4:30	19.2
5:00	18.5
5:30	17.2
6:00	17.5
6:30	18.2
7:00	19.2
7:30	20
8:00	20.5
8:30	20.8
9:00	20.5
Average:	**18.1**



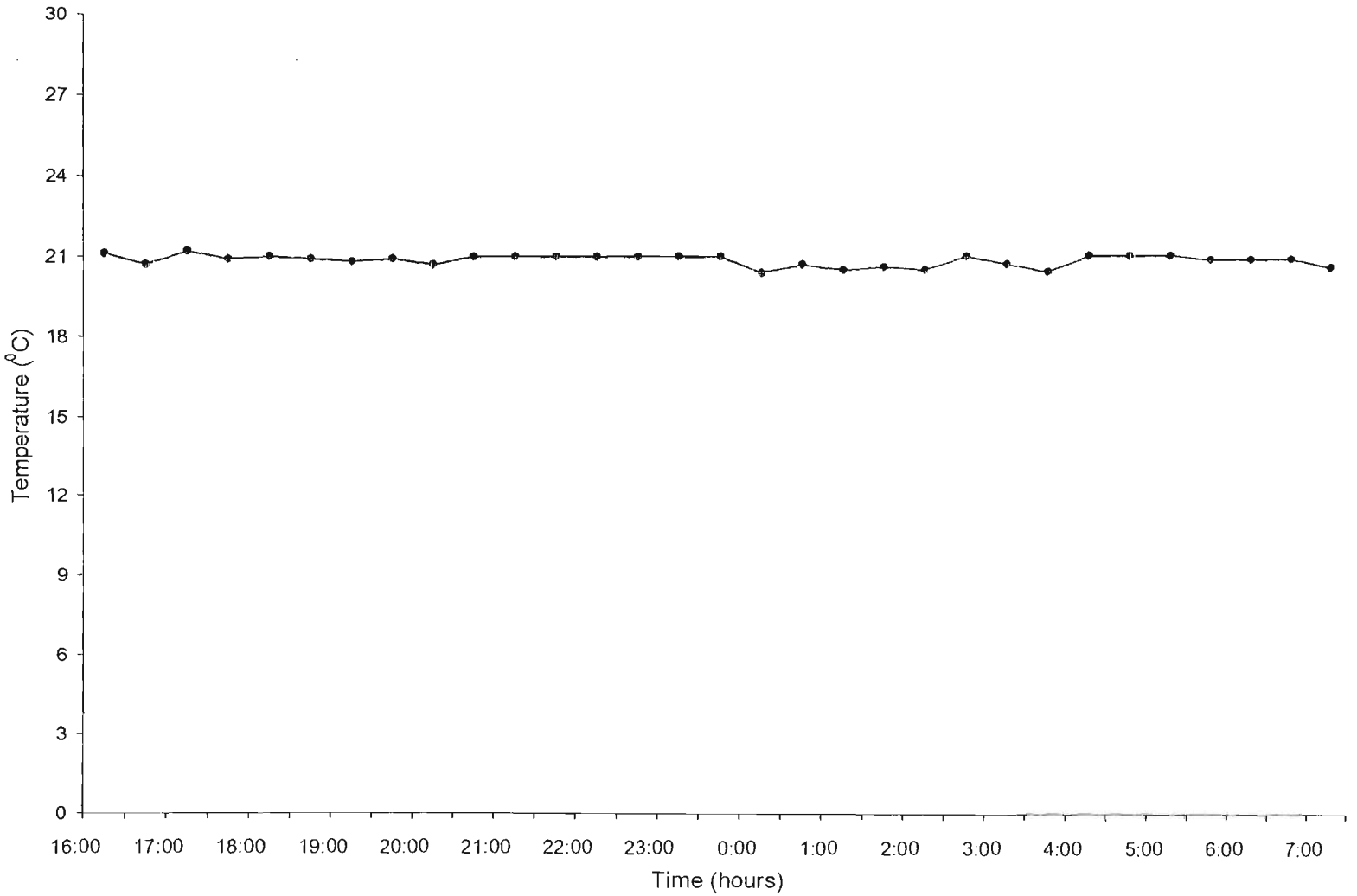
Date of fumigation: 29/5/96

Time (hrs)	Temp (^{0}C)
16:00	15.4
16:30	16
17:00	16.5
17:30	17
18:00	17.5
18:30	18.2
19:00	18.5
19:30	19
20:00	19.5
20:30	18.5
21:00	19
21:30	19.5
22:00	19.3
22:30	18
23:00	17.5
23:30	18
0:00	16.5
0:30	16.8
1:00	16.1
1:30	16.3
2:00	17.5
2:30	18
3:00	18.5
3:30	19
4:00	18.3
4:30	18.6
5:00	18.5
5:30	18.2
6:00	18.9
6:30	18.5
7:00	19
7:30	19.5
8:00	19.9
8:30	19.2
9:00	19.1
Average:	**18.1**



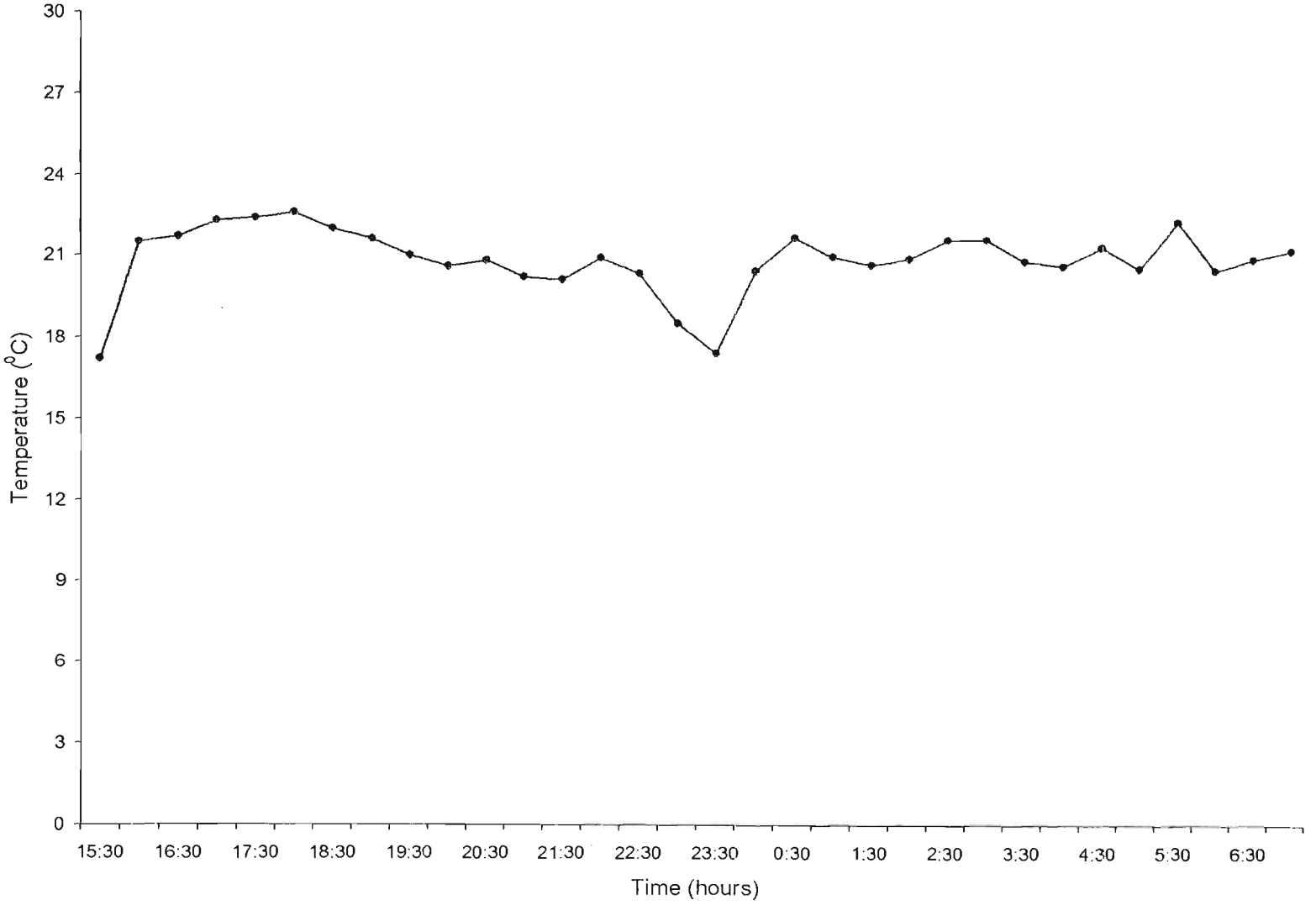
Date of fumigation: 23/11/95

Time (hrs)	Temp (^{0}C)
16:00	21.1
16:30	20.7
17:00	21.2
17:30	20.9
18:00	21
18:30	20.9
19:00	20.8
19:30	20.9
20:00	20.7
20:30	21
21:00	21
21:30	21
22:00	21
22:30	21
23:00	21
23:30	21
0:00	20.4
0:30	20.7
1:00	20.5
1:30	20.6
2:00	20.5
2:30	21
3:00	20.7
3:30	20.4
4:00	21
4:30	21
5:00	21
5:30	20.8
6:00	20.8
6:30	20.8
7:00	20.5
Average:	**20.8**



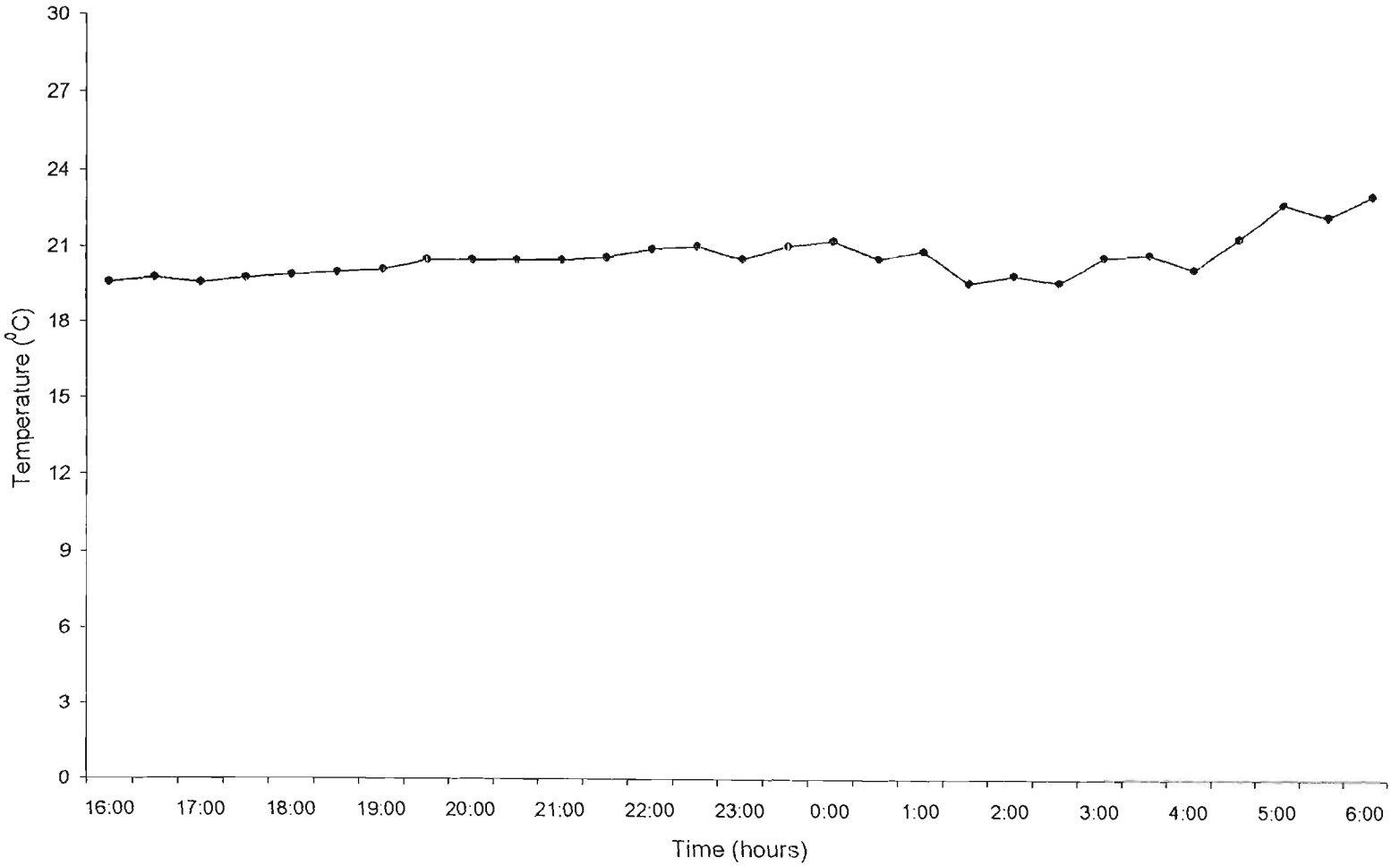
Date of fumigation: 19/2/96

Time (hrs)	Temp (^{0}C)
15:30	17.2
16:00	21.5
16:30	21.7
17:00	22.3
17:30	22.4
18:00	22.6
18:30	22
19:00	21.6
19:30	21
20:00	20.6
20:30	20.8
21:00	20.2
21:30	20.1
22:00	20.9
22:30	20.3
23:00	18.5
23:30	17.4
0:00	20.4
0:30	21.6
1:00	20.9
1:30	20.6
2:00	20.8
2:30	21.5
3:00	21.5
3:30	20.7
4:00	20.5
4:30	21.2
5:00	20.4
5:30	22.1
6:00	20.3
6:30	20.7
7:00	21
Average:	**20.8**



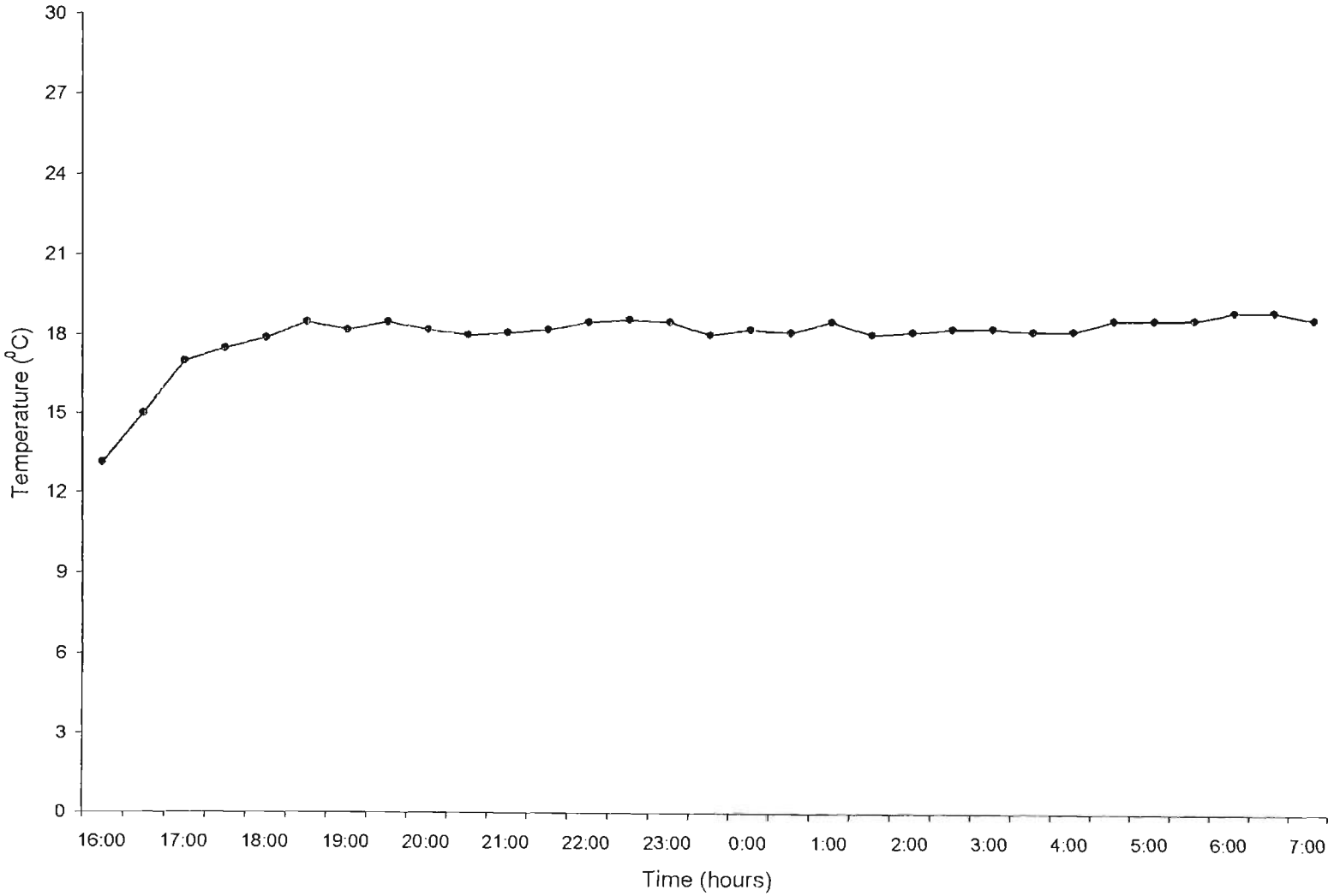
Date of fumigation: 30/11/95

Time (hrs)	Temp (^{0}C)
16:00	19.6
16:30	19.8
17:00	19.6
17:30	19.8
18:00	19.9
18:30	20
19:00	20.1
19:30	20.5
20:00	20.5
20:30	20.5
21:00	20.5
21:30	20.6
22:00	20.9
22:30	21
23:00	20.5
23:30	21
0:00	21.2
0:30	20.5
1:00	20.8
1:30	19.5
2:00	19.8
2:30	19.5
3:00	20.5
3:30	20.6
4:00	20
4:30	21.2
5:00	22.5
5:30	22
6:00	22.8
Average:	**20.5**



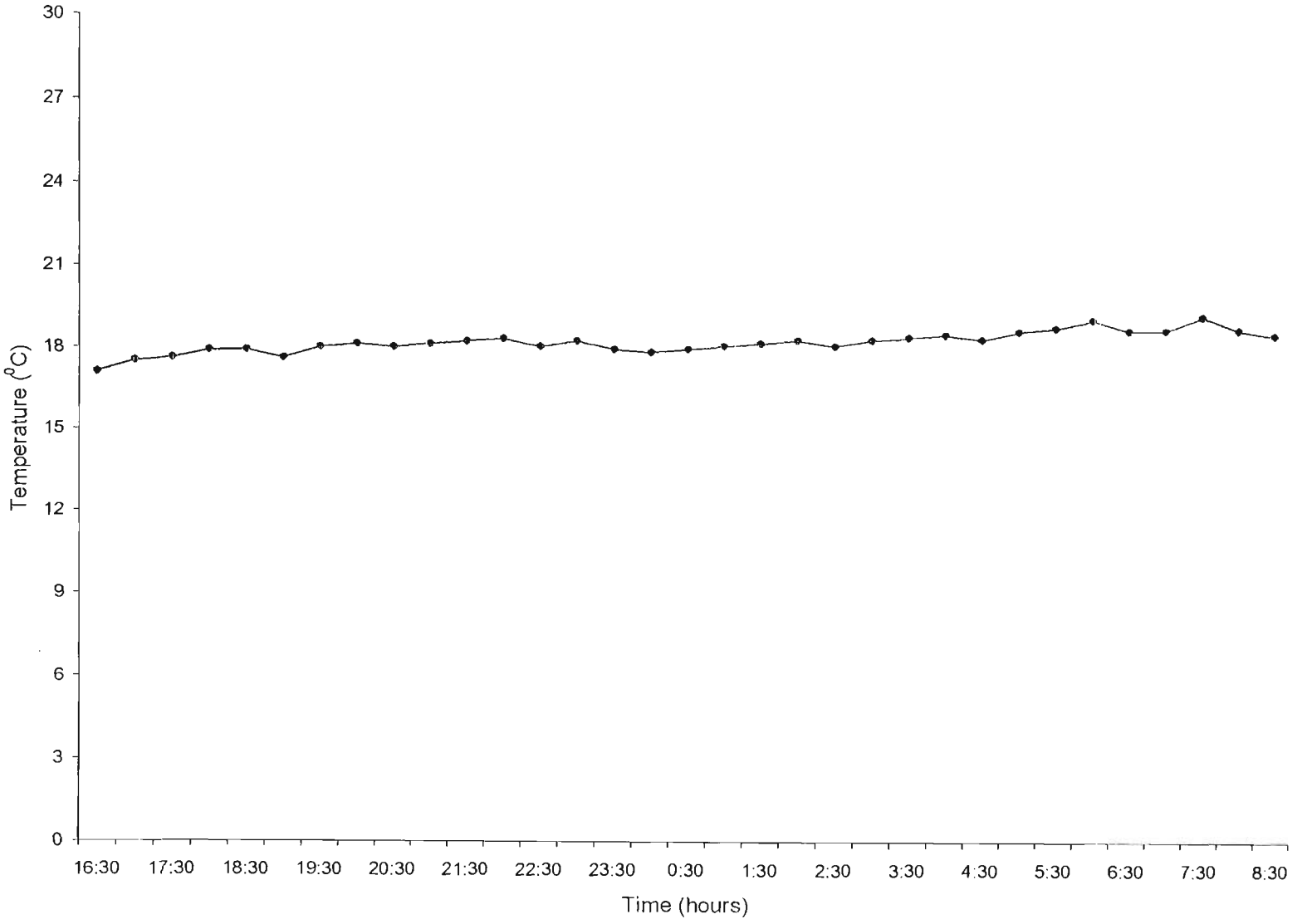
Date of fumigation: 10/7/96

Time (hrs)	Temp (^{0}C)
16:00	13.1
16:30	15
17:00	17
17:30	17.5
18:00	17.9
18:30	18.5
19:00	18.2
19:30	18.5
20:00	18.2
20:30	18
21:00	18.1
21:30	18.2
22:00	18.5
22:30	18.6
23:00	18.5
23:30	18
0:00	18.2
0:30	18.1
1:00	18.5
1:30	18
2:00	18.1
2:30	18.2
3:00	18.2
3:30	18.1
4:00	18.1
4:30	18.5
5:00	18.5
5:30	18.5
6:00	18.8
6:30	18.8
7:00	18.5
Average:	**18**



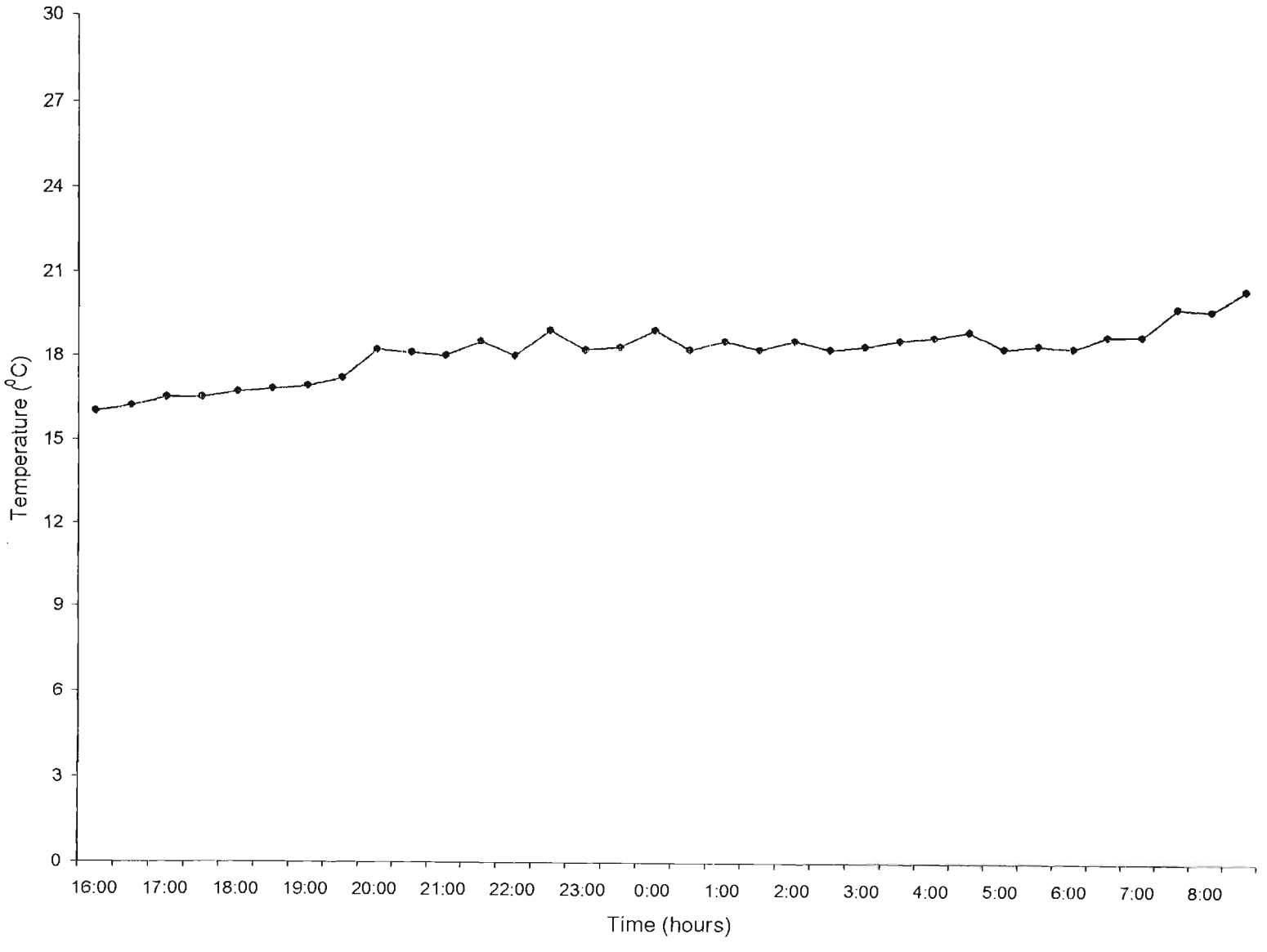
Date of fumigation: 30/10/96

Time (hrs)	Temp (^{0}C)
16:30	17.1
17:00	17.5
17:30	17.6
18:00	17.9
18:30	17.9
19:00	17.6
19:30	18
20:00	18.1
20:30	18
21:00	18.1
21:30	18.2
22:00	18.3
22:30	18
23:00	18.2
23:30	17.9
0:00	17.8
0:30	17.9
1:00	18
1:30	18.1
2:00	18.2
2:30	18
3:00	18.2
3:30	18.3
4:00	18.4
4:30	18.2
5:00	18.5
5:30	18.6
6:00	18.9
6:30	18.5
7:00	18.5
7:30	19
8:00	18.5
8:30	18.3
Average:	**18.1**



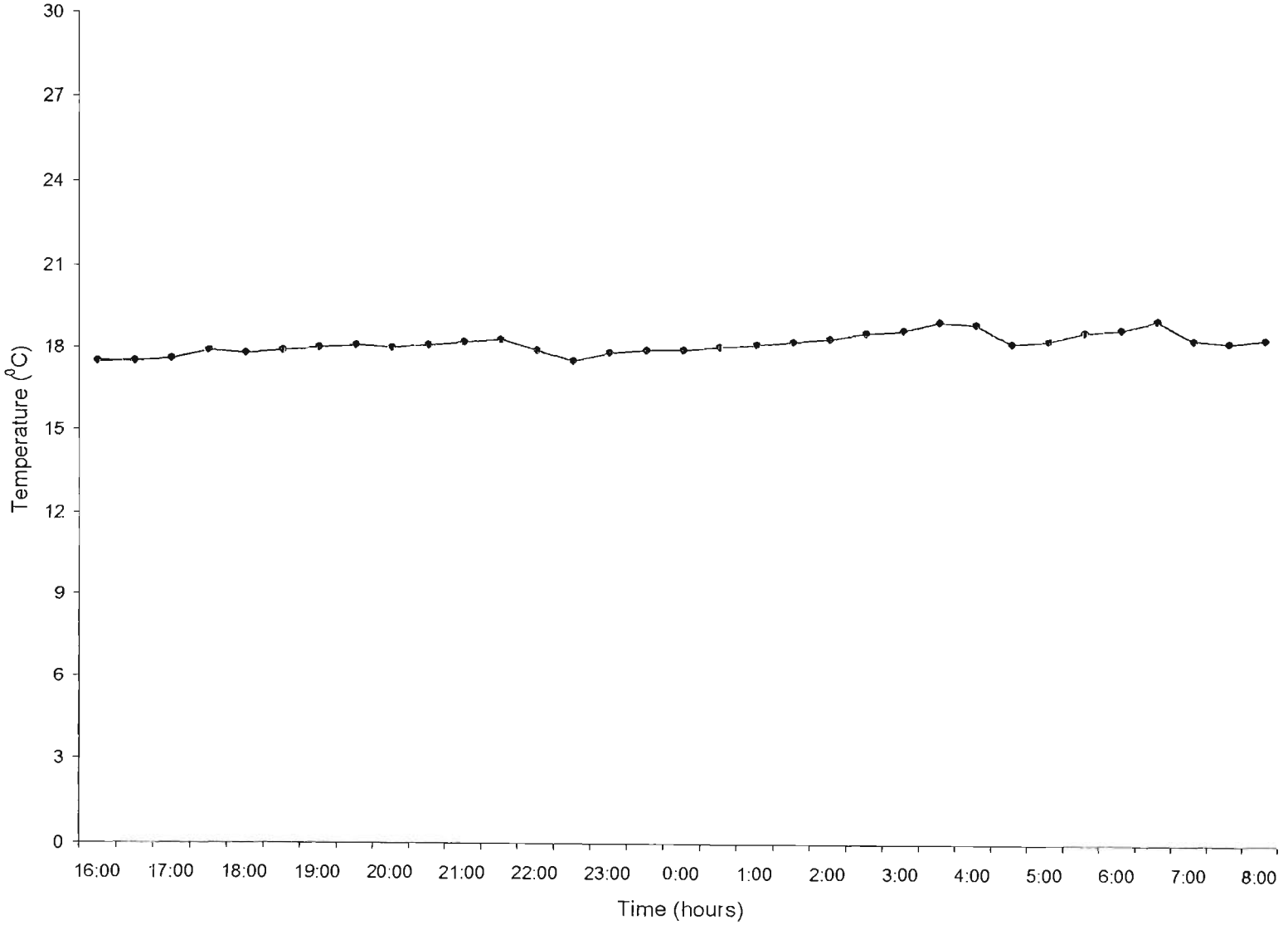
Date of fumigation: 25/6/97

Time (hrs)	Temp (^{0}C)
16:00	16
16:30	16.2
17:00	16.5
17:30	16.5
18:00	16.7
18:30	16.8
19:00	16.9
19:30	17.2
20:00	18.2
20:30	18.1
21:00	18
21:30	18.5
22:00	18
22:30	18.9
23:00	18.2
23:30	18.3
0:00	18.9
0:30	18.2
1:00	18.5
1:30	18.2
2:00	18.5
2:30	18.2
3:00	18.3
3:30	18.5
4:00	18.6
4:30	18.8
5:00	18.2
5:30	18.3
6:00	18.2
6:30	18.6
7:00	18.6
7:30	19.6
8:00	19.5
8:30	20.2
Average:	**18.1**



Date of fumigation: 11/12/96

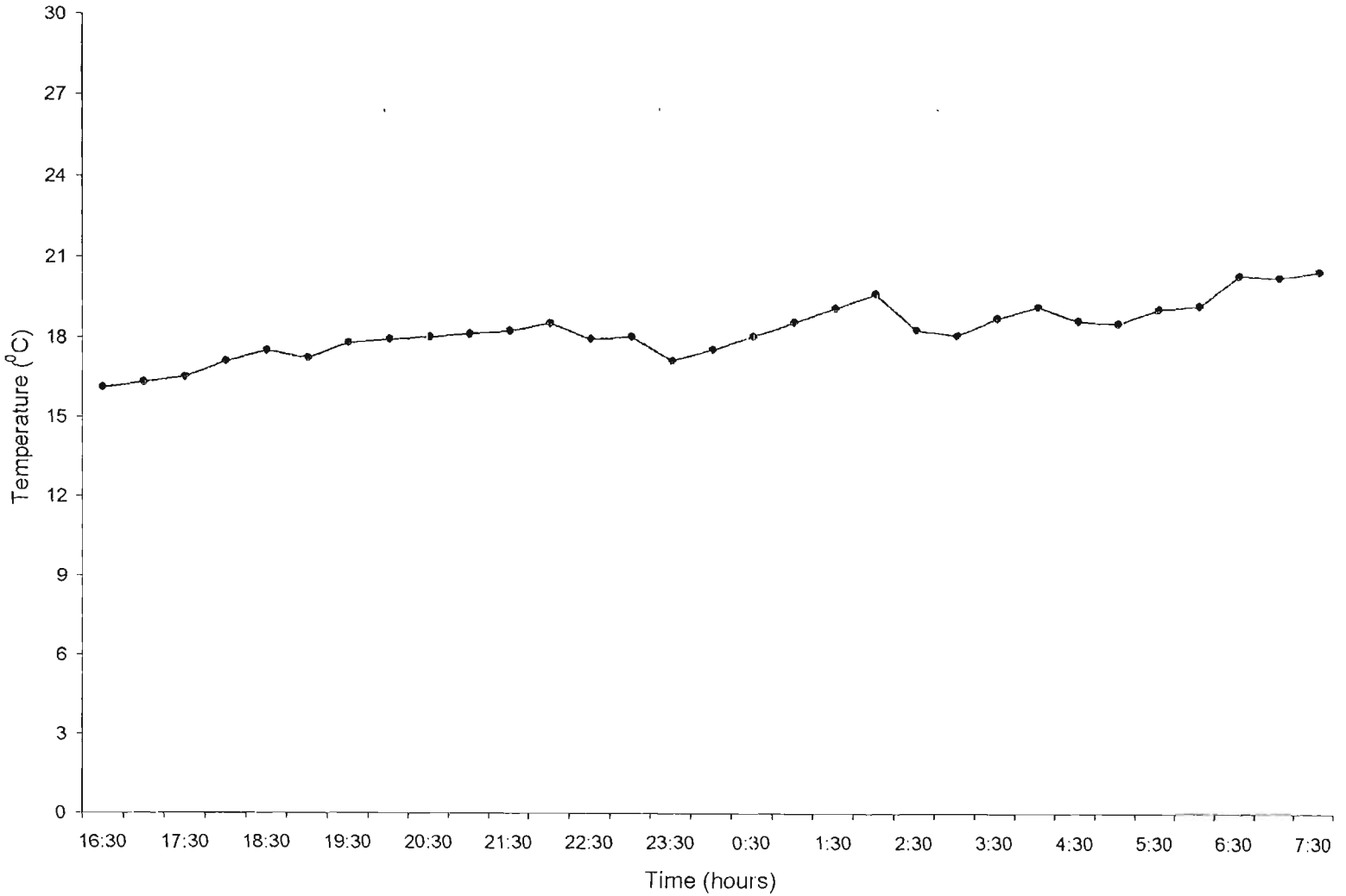
Time (hrs)	Temp (^{0}C)
16:00	17.5
16:30	17.5
17:00	17.6
17:30	17.9
18:00	17.8
18:30	17.9
19:00	18
19:30	18.1
20:00	18
20:30	18.1
21:00	18.2
21:30	18.3
22:00	17.9
22:30	17.5
23:00	17.8
23:30	17.9
0:00	17.9
0:30	18
1:00	18.1
1:30	18.2
2:00	18.3
2:30	18.5
3:00	18.6
3:30	18.9
4:00	18.8
4:30	18.1
5:00	18.2
5:30	18.5
6:00	18.6
6:30	18.9
7:00	18.2
7:30	18.1
8:00	18.2
Average:	**18.1**



Date of fumigation: 13/11/96

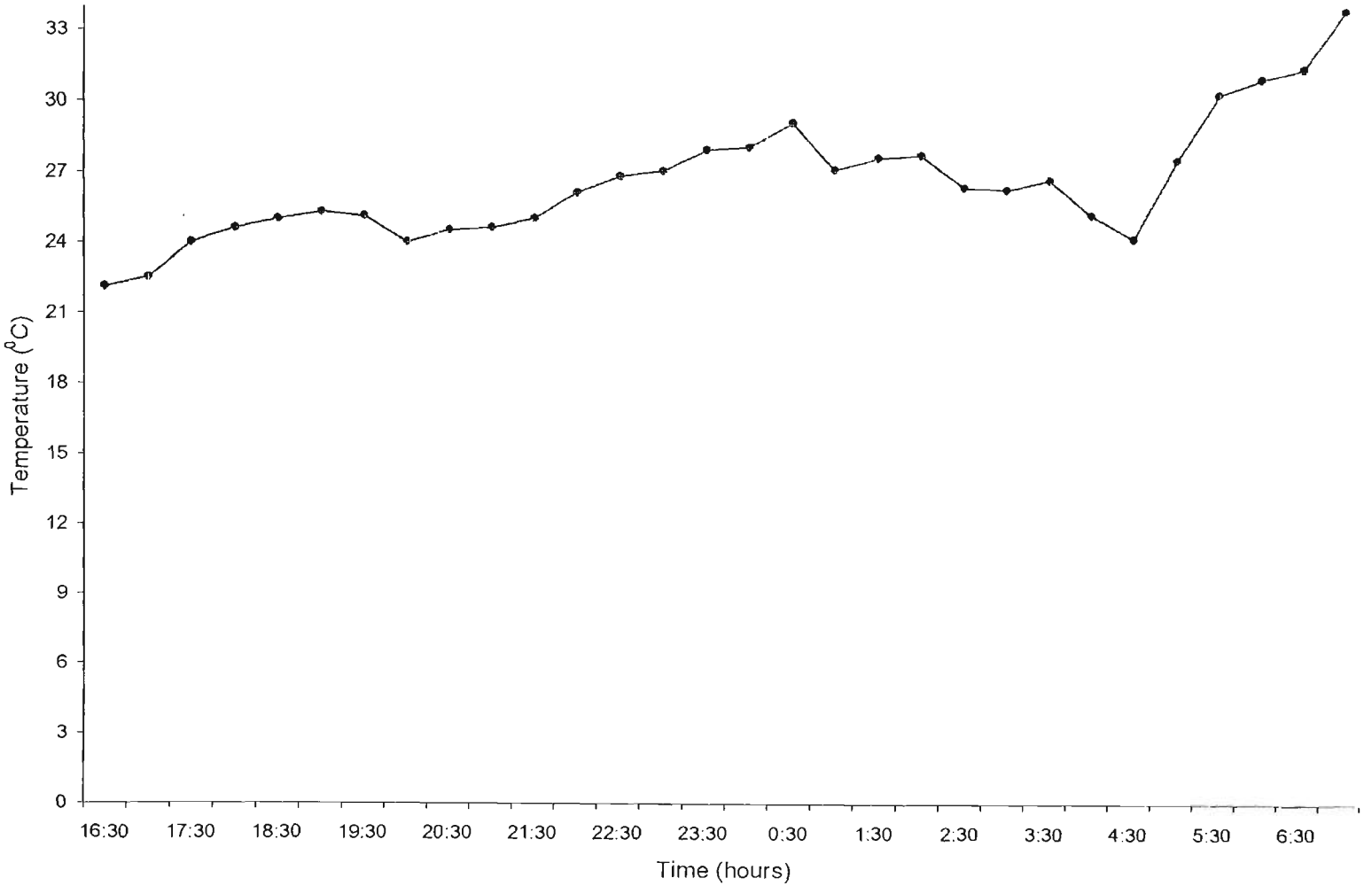
Time (hrs)	Temp (^{0}C)
16:30	16.1
17:00	16.3
17:30	16.5
18:00	17.1
18:30	17.5
19:00	17.2
19:30	17.8
20:00	17.9
20:30	18
21:00	18.1
21:30	18.2
22:00	18.5
22:30	17.9
23:00	18
23:30	17.1
0:00	17.5
0:30	18
1:00	18.5
1:30	19
2:00	19.5
2:30	18.2
3:00	18
3:30	18.6
4:00	19
4:30	18.5
5:00	18.4
5:30	18.9
6:00	19
6:30	20.1
7:00	20
7:30	20.2

Average: 18.9



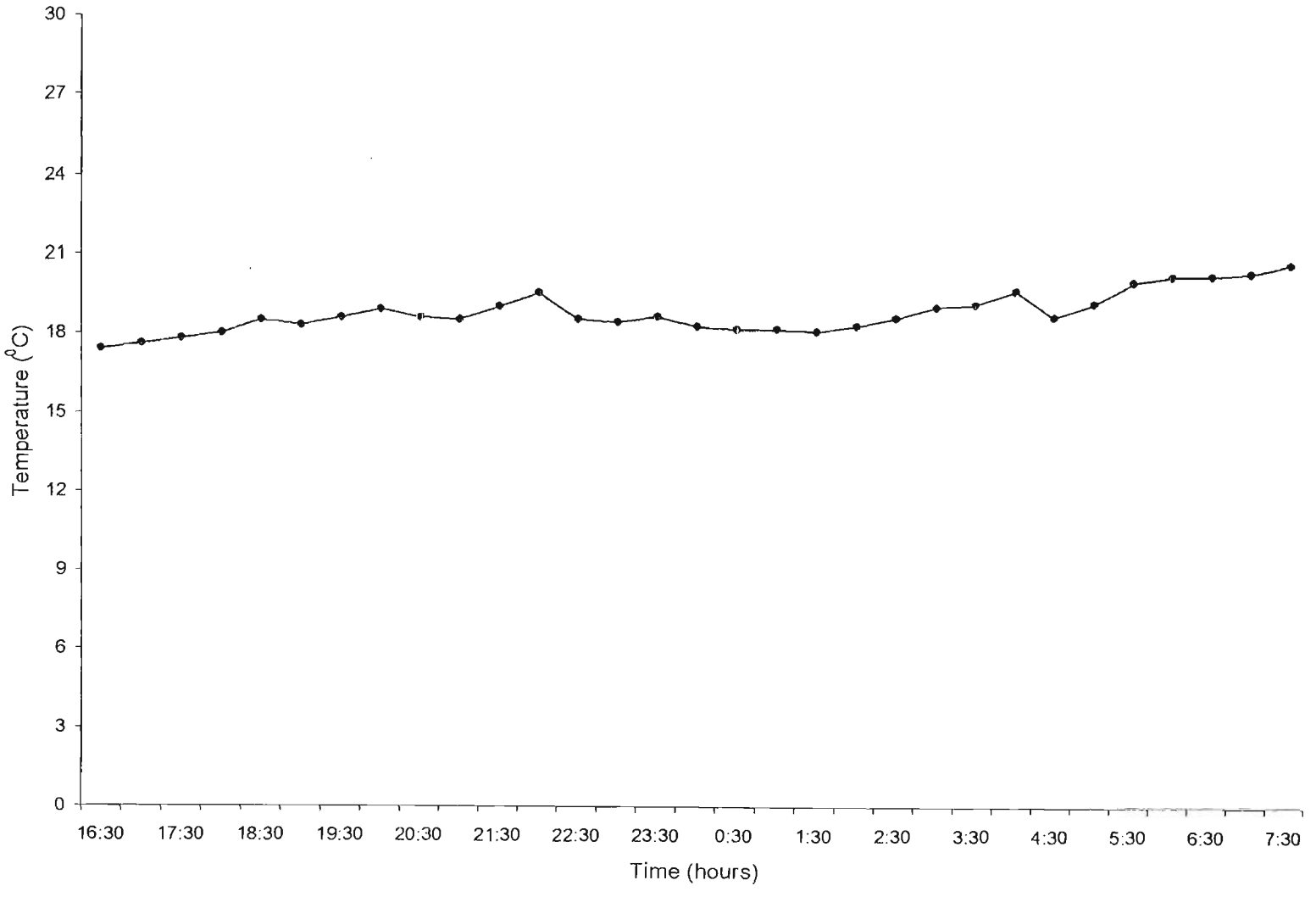
Date of fumigation: 17/2/97

Time (hrs)	Temp (^{0}C)
16:30	22.1
17:00	22.5
17:30	24
18:00	24.6
18:30	25
19:00	25.3
19:30	25.1
20:00	24
20:30	24.5
21:00	24.6
21:30	25
22:00	26.1
22:30	26.8
23:00	27
23:30	27.9
0:00	28
0:30	29
1:00	27
1:30	27.5
2:00	27.6
2:30	26.2
3:00	26.1
3:30	26.5
4:00	25
4:30	24
5:00	27.3
5:30	30
6:00	30.6
6:30	31
7:00	33.4
Average:	**26.4**



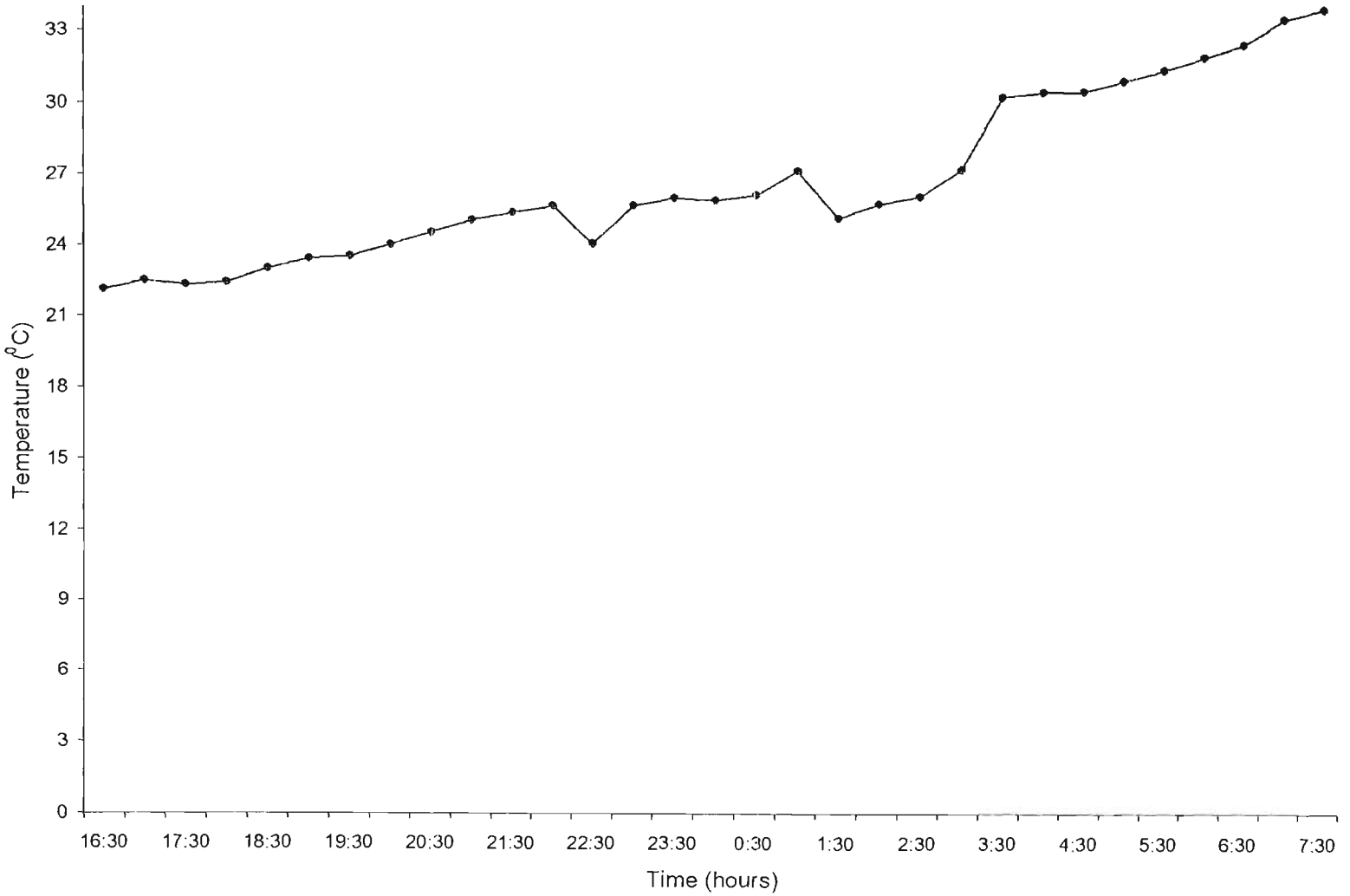
Date of fumigation: 26/2/97

Time (hrs)	Temp (^{0}C)
16:30	17.4
17:00	17.6
17:30	17.8
18:00	18
18:30	18.5
19:00	18.3
19:30	18.6
20:00	18.9
20:30	18.6
21:00	18.5
21:30	19
22:00	19.5
22:30	18.5
23:00	18.4
23:30	18.6
0:00	18.2
0:30	18.1
1:00	18.1
1:30	18
2:00	18.2
2:30	18.5
3:00	18.9
3:30	19
4:00	19.5
4:30	18.5
5:00	19
5:30	19.8
6:00	20
6:30	20
7:00	20.1
7:30	20.4
Average:	**18.7**



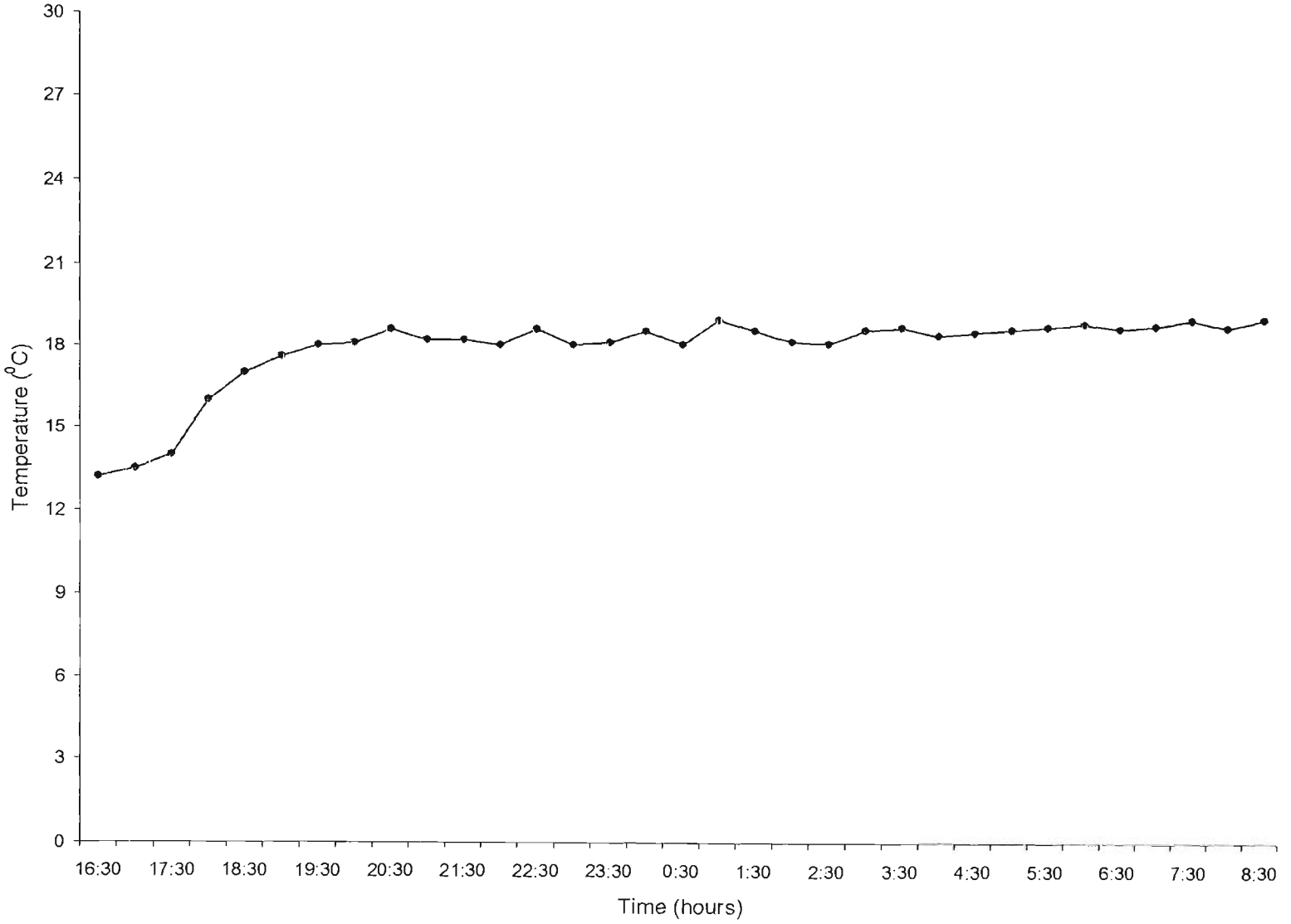
Date of fumigation: 23/1/97

Time (hrs)	Temp (^{0}C)
16:30	22.1
17:00	22.5
17:30	22.3
18:00	22.4
18:30	23
19:00	23.4
19:30	23.5
20:00	24
20:30	24.5
21:00	25
21:30	25.3
22:00	25.6
22:30	24
23:00	25.6
23:30	25.9
0:00	25.8
0:30	26
1:00	27
1:30	25
2:00	25.6
2:30	25.9
3:00	27
3:30	30
4:00	30.2
4:30	30.2
5:00	30.6
5:30	31
6:00	31.5
6:30	32
7:00	33
7:30	33.4
Average:	**26.5**



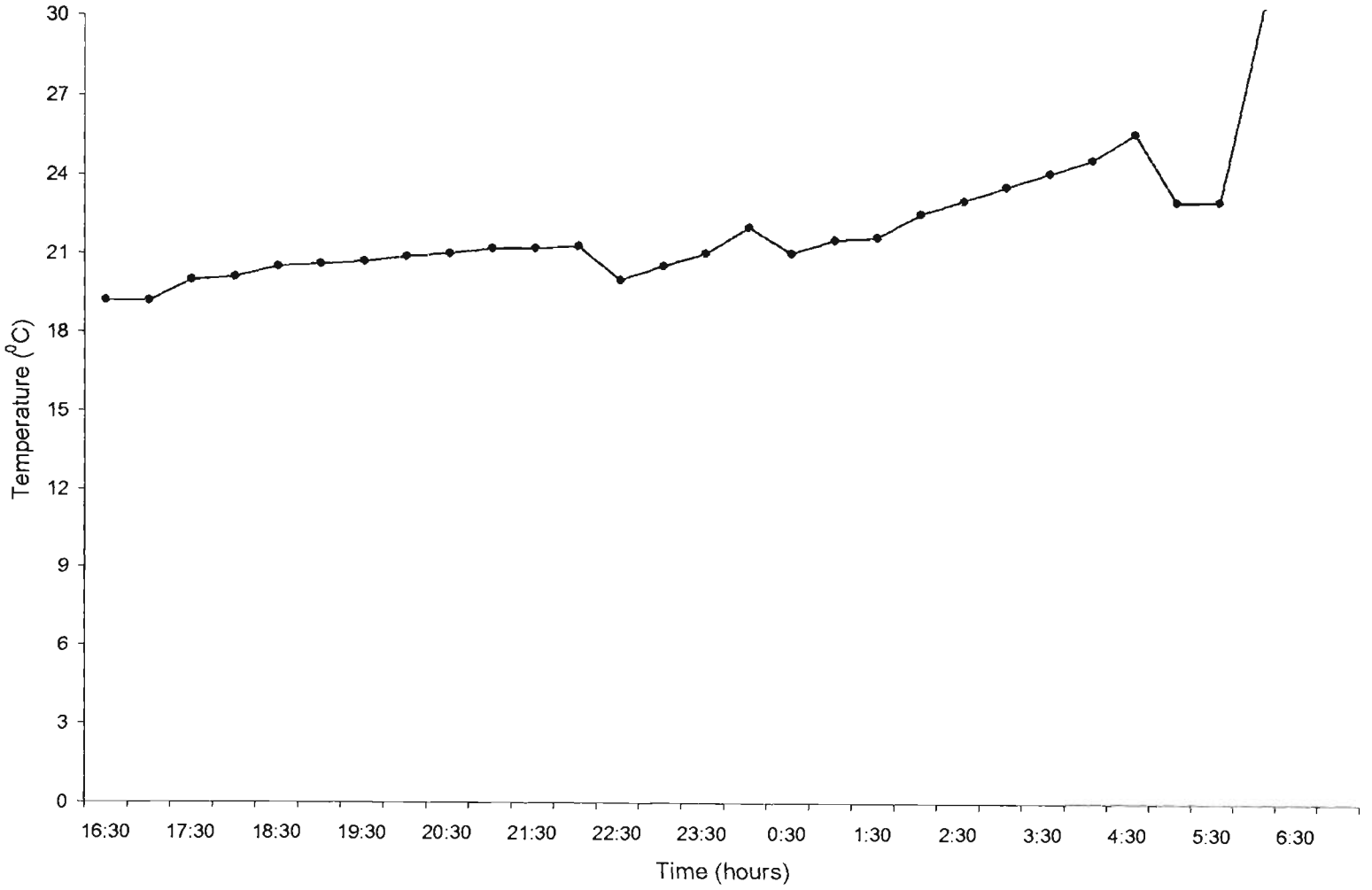
Date of fumigation: 5/1/97

Time (hrs)	Temp (^{0}C)
16:30	13.2
17:00	13.5
17:30	14
18:00	16
18:30	17
19:00	17.6
19:30	18
20:00	18.1
20:30	18.6
21:00	18.2
21:30	18.2
22:00	18
22:30	18.6
23:00	18
23:30	18.1
0:00	18.5
0:30	18
1:00	18.9
1:30	18.5
2:00	18.1
2:30	18
3:00	18.5
3:30	18.6
4:00	18.3
4:30	18.4
5:00	18.5
5:30	18.6
6:00	18.7
6:30	18.5
7:00	18.6
7:30	18.8
8:00	18.5
8:30	18.8
Average:	**17.8**



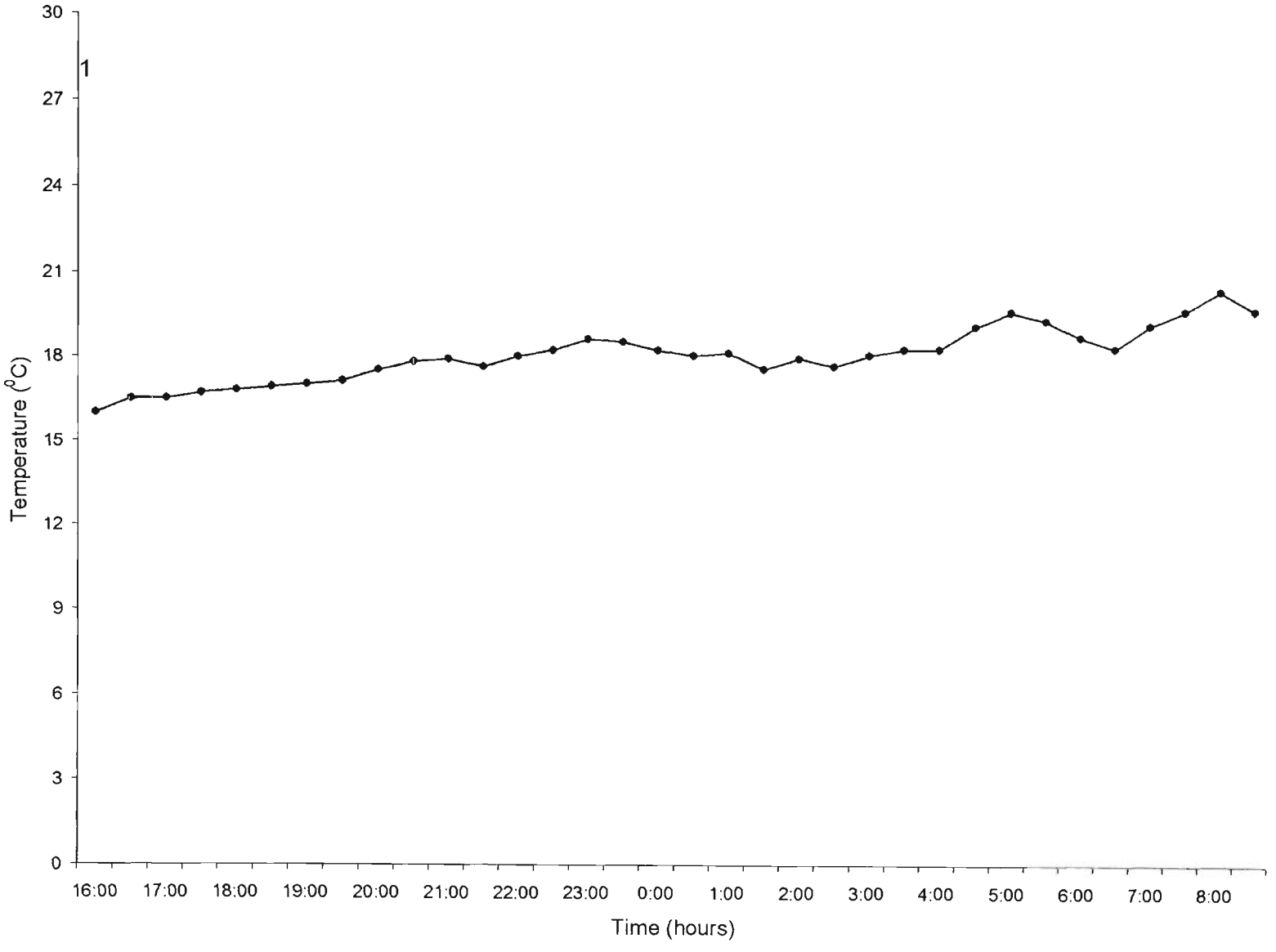
Date of fumigation: 3/5/97

Time (hrs)	Temp (^{0}C)
16:30	19.21
17:00	19.2
17:30	20
18:00	20.1
18:30	20.5
19:00	20.6
19:30	20.7
20:00	20.9
20:30	21
21:00	21.2
21:30	21.2
22:00	21.3
22:30	20
23:00	20.54
23:30	21
0:00	22
0:30	21
1:00	21.5
1:30	21.6
2:00	22.5
2:30	23
3:00	23.5
3:30	24
4:00	24.5
4:30	25.5
5:00	22.9
5:30	22.9
6:00	30.1
6:30	31
7:00	32.4
Average:	**22.5**



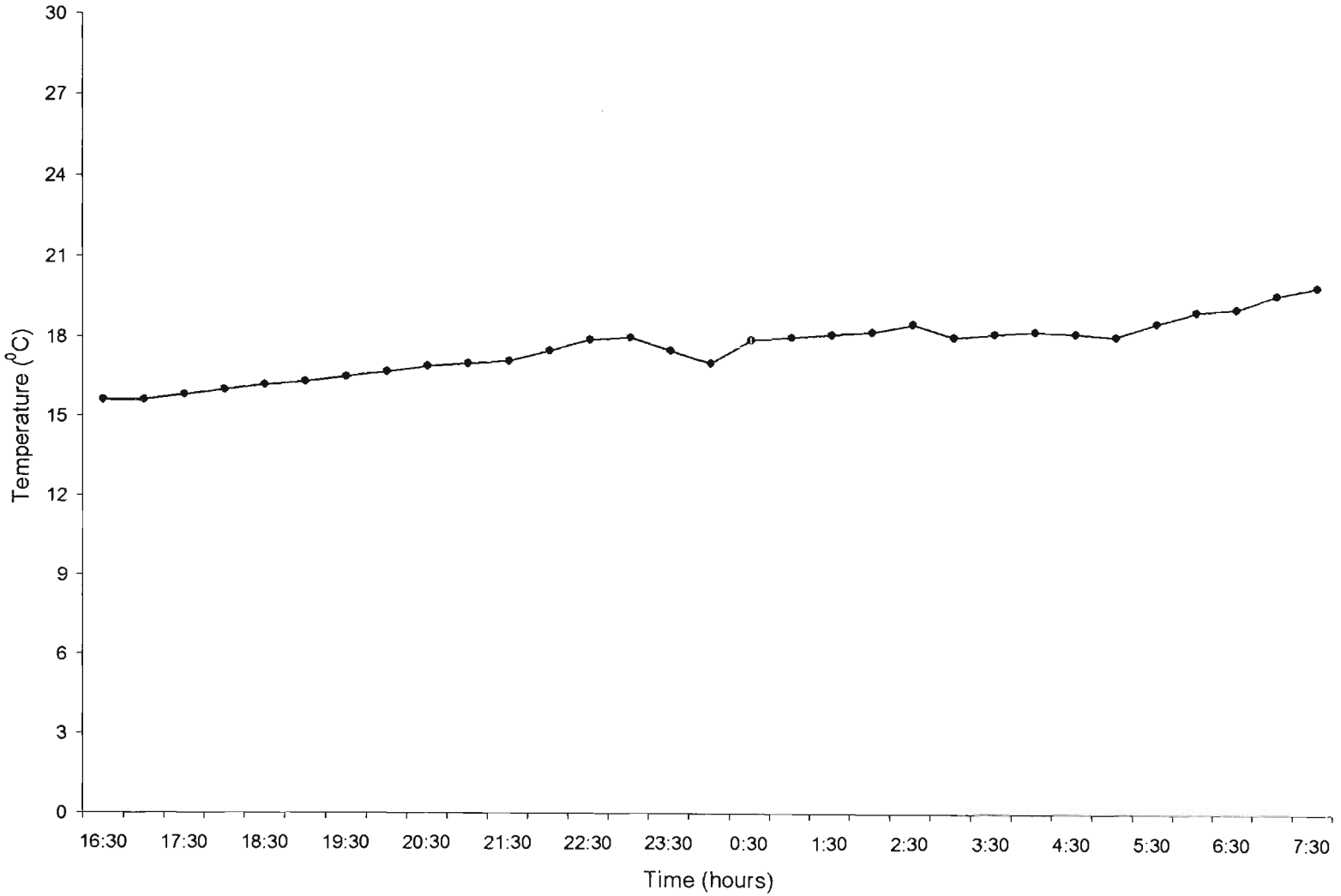
Date of fumigation: 25/6/97

Time (hrs)	Temp (^{0}C)
16:00	16
16:30	16.5
17:00	16.5
17:30	16.7
18:00	16.8
18:30	16.9
19:00	17
19:30	17.1
20:00	17.5
20:30	17.8
21:00	17.9
21:30	17.6
22:00	18
22:30	18.2
23:00	18.6
23:30	18.5
0:00	18.2
0:30	18
1:00	18.1
1:30	17.5
2:00	17.9
2:30	17.6
3:00	18
3:30	18.2
4:00	18.2
4:30	19
5:00	19.5
5:30	19.2
6:00	18.6
6:30	18.2
7:00	19
7:30	19.5
8:00	20.2
8:30	19.5
Average:	**18**



Date of fumigation: 7/8/97

Time (hrs)	Temp (^{0}C)
16:30	15.6
17:00	15.6
17:30	15.8
18:00	16
18:30	16.2
19:00	16.3
19:30	16.5
20:00	16.7
20:30	16.9
21:00	17
21:30	17.1
22:00	17.5
22:30	17.9
23:00	18
23:30	17.5
0:00	17
0:30	17.9
1:00	18
1:30	18.1
2:00	18.2
2:30	18.5
3:00	18
3:30	18.1
4:00	18.2
4:30	18.1
5:00	18
5:30	18.5
6:00	18.9
6:30	19
7:00	19.5
7:30	19.8
Average:	**17.5**



Date of fumigation: 18/3/96

Time (hrs)	Temp (^{0}C)
16:30	10
17:00	10.1
17:30	10.5
18:00	10.6
18:30	11
19:00	11.5
19:30	11.6
20:00	11.8
20:30	11.9
21:00	12.6
21:30	12.6
22:00	12.9
22:30	13
23:00	14.2
23:30	14.1
0:00	14.2
0:30	14.3
1:00	14
1:30	14.5
2:00	14
2:30	14.5
3:00	13.9
3:30	13.5
4:00	14.2
4:30	14.5
5:00	14.6
5:30	14.5
6:00	14.3
6:30	14.5
7:00	14.5
7:30	14.6
Average:	**13.1**