A method for replacing serrated tussock (*Nassella trichotoma*) with kangaroo grass (*Themeda triandra*) in lowland native grassland remnants

Alistair Phillips

Master of Science

2000

Victoria University

Contents

Abstract

A 'spray and hay' method was developed which replaces the noxious weed serrated tussock (*Nassella trichotoma* (Nees) Arech.) with indigenous kangaroo grass (*Themeda triandra* Forrsk.) in partly disturbed remnants of western (basalt) plains indigenous grassland, a community listed as vulnerable to extinction. Investigations were carried out on Melbourne's north-west urban fringe. Refinements of the 'spray and hay' method resulted in low densities of other weeds amongst the newly established *T. triandra* swards. First steps in the method involved removal of weeds by slashing and follow-up treatment with either of the herbicides, glyphosate (as Monsanto Roundup®) or atrazine (as Nufarm Nutrazine®). These steps resulted in close to 100% kill of mature *N. trichotoma* plants. Greater than 98% replacement of *N. trichotoma* with *T. triandra* was achieved by thatching over herbicide-treated plots with seed-bearing *T. triandra* hay harvested from remnant grasslands and then removal of the hay several months later by either burning or physical removal.

The type and timing of herbicide application, thatching and removal of thatch were found to be central for successfully establishing competitive densities of *T. triandra* seedlings and minimizing re-establishment of *N. trichotoma* and other weed seedlings. A treatment set involving: slashing weed biomass in summer (January), herbicide application in autumn (April) followed by thatching with seed-bearing *T. triandra* hay in winter (July) and removal of thatch in spring (October) produced the best results. Assessing the seed content of hay and germinability of seed prior to revegetation were also important for calculating the amount of hay laid and subsequent seedling densities established. The seed content and germinability of seed-bearing hay was found to vary markedly in samples harvested in three different years, across discrete remnant grassland sites and even within undisturbed grassland sites.

Reasons for the success of the method, and why other variations are less successful are discussed, as is the wider application of the method for weed control and replacement with indigenous grasses in lowland grassland remnants.

Acknowledgments

During this research my supervisor Dr Colin Hocking provided insightful comments on my methodology, statistical assessment and critical comment on this thesis.

I also thank a number of other people who kindly assisted me in the following tasks:

- Critical comment on experimental design — Dr Malcolm Goodall (*dec.*) and Robyn Watson,
- Assistance in establishing of many hundreds of quadrats in the field — Barrie Richardson,
- Engineering and construction of the steel burn box — Tom Hocking,
- Harvesting and storage of *Themeda triandra* hay — Dr Colin Hocking, Bram Mason, Meagan O'Shea, Ian Shears, and Park Rangers from Organ Pipes National Park,
- Burning trial plots — Patrick Lai, Barrie Richardson, Bram Mason, Dr Colin Hocking, Dr Golam Kibria, Meagan O'Shea, Ian Shears, Dr Fiona Bird and officers from the Metropolitan Fire Brigade North-west Region,
- Advice on statistical assessment — Bram Mason and Neil Diamond,
- Editorial comments on the final draft of this thesis — Dr Sue Lewis,
- The personal support needed to carry out this research — my partner Janine Le Couteur.

Thesis style

Note: the presentation style of this thesis follows requirements set out for the journal Plant Protection Quarterly (Appendix 1). Chapters 2, 3, 4 and 5 have either been submitted to Plant Protection Quarterly for publication or are in the process of being prepared for submission to Plant Protection Quarterly.

1. Introduction

1.1 Overall aim of the study and framework for research

The aim of this study has been to investigate and refine a reliable set of treatments that allow replacement of exotic serrated tussock (*Nassella trichotoma* (Nees) Arech.), a cool-season small to medium tussock forming species from South America, with the indigenous kangaroo grass (*Themeda triandra* Forrsk.) (syn *Themeda australis*), a warm-season larger tussock forming species using a minimum of steps.

T. triandra is commonly the dominant plant in the floristic structure of many of the surviving remnants of indigenous grasslands in the western basalt plains of Victoria (Stuwe and Parsons, 1977). It is proposed that techniques to control invading weeds, and in particular, to establish weed-free *T. triandra* swards are vital to the ongoing structural integrity of grassland remnants (Department of Conservation & Natural Resources 1994, Hocking 1998). According to McDougall (1989):

> **A re-established sward of *Themeda triandra* should be self-perpetuating and capable of effectively competing with weeds if it is to provide suitable conditions for the introduction of other grassland species.**

Trialing the method used in this study, referred to here as the 'spray and hay' method, has shown many factors require careful consideration if the method is to be applied with any measurable level of success. The essential elements of what has been developed here as the *T. triandra* 'spray and hay' revegetation method, were first proposed by McDougall (1989), and can be summarised as follows:

- Harvest and store seed bearing hay from a weed-free *T. triandra* site,
- Reduce weed biomass on site to be treated, by fire or slashing,
- Apply herbicide treatment selectively to resprouting weeds,
- Introduce *T. triandra* seeds to the site by thatching over with seed-bearing *T. triandra* hay - the thatch also acts to minimize weed seedling establishment,

- Leave hay in place for sufficient time to allow *T. triandra* seed to fall through and lodge in the soil, a process facilitated by seed awn movement,
- Remove hay, by hand or by setting fire to the thatch.

The study reported in this thesis has assessed the 'spray and hay' method for *T. triandra* establishment with the aim of answering questions recently raised about the effectiveness of, and methodologies used in, the method (Carr and Todd 1991, Carr and Muir 1993, Carr and Muir 1994, Craigie and Ross 1995).

Data from the research reported in this thesis may also assist public open space managers and agricultural land holders to control *N. trichotoma* and other weeds, and at the same time retain and use indigenous grasses.

1.1.1 Ecological management context of this study

Recent approaches to grassy vegetation restoration in Europe and the United Kingdom have been based on stimulating recruitment through *in-situ* propagules located in seed banks (Gurgerli 1993, Dutoit and Alard 1994, Willems 1995). Other approaches in the management of extant grassland populations involve biomass disturbance, for example through slashing or hydrological manipulation with the aim of broadening species diversity (Best *et al.*1995, Ryser *et al*. 1995).

Restoration and conservation management of grassy ecosystems in other parts of the world are often frustrated by the rapid colonisation of revegetation sites by a range of alien plants following disturbance (Anderson 1995). Literature on the management and restoration of grasslands has a clear focus on manipulation of *in-situ* resources. The study reported in this thesis focuses on a combination of the manipulation of *in-situ* resources (i.e. disturbance by slashing for biomass reduction and herbicide removal of exotic flora) with introduction of *ex-situ* resources (indigenous grass seed in hay).

This study is proposed to be a component of the field of ecological research generally known as 'restoration ecology'. Restoration ecology is described here as: observing, assessing and understanding ecological processes and using these data in the rehabilitation of damaged or degraded ecosystems. Restoration ecology is referred to as applied ecology (Jordan 1995), and as a maintenance device for ecosystems (Cairns Jr 1995).

It is asserted here that the fundamental value of restoration ecology is when such operations produce a vegetation system free of invasive plants in at least part of a natural ecosystem. If such an outcome is achieved the potential exists for ongoing recolonisation by other complementary organisms of that ecosystem. Such an outcome, however promising, might be assessed as merely replacing a small piece of what are mostly very complex 'puzzles'. Katz (1991) sets out a caution that restoration ecology is becoming understood as the 'technological fix' for all ecosystem damage and argues for prudence in accepting such tenets. Restoration ecology presently focuses on plant re-establishment— re-establishing a small component, of a larger community, which itself is part of a much larger ecosystem. In a review of restoration work Anderson (1995) suggests that re-establishment of particular plant species is the level of 'restoration' that restoration ecology has reached. It is proposed here that restoration ecology should not be described as capable of achieving more than a moderate level of replacement of species and complexity. In short, ecosystem restoration should be recognised as a 'tool' in ecosystem conservation and not an 'alternative' to ecosystem conservation. Conway (1988) summarises: "Most losses of biological diversity, to say nothing of lost ecological services, are quite beyond human ability to repair."

1.1.2 Grassland: A cosmopolitan vegetation system

Every continent possesses grassland communities (Estes *et al*. 1989). These grassy ecosystems were, and are important to the economies of indigenous people of various continents. Examples of prominent grassland ecosystems include; prairies (North America), chalk grasslands (United Kingdom and Europe), pampas grasslands (South America), savanna grasslands (Africa) and the steppes grasslands (northern Asia) (Miller 1992). The grass family (Poaceae) contains worldwide some 10,000 species classified within 650 genera (Scarlett *et al*. 1992).

Grassland definitions

Worldwide, grasslands are considered to "...include a wide variety of plant communities; some are related to savannas, others to deserts and still others to temperate deciduous forests. Grasslands generally occur over large areas of the interior portions of the continents. (Raven *et al*. 1981). According to Risser (1988) "...grasslands can be described as types of vegetation that are subjected to periodic drought and have a canopy dominated by grass and grass-like species...". Also, "...grasslands are maintained both by grazing and periodic fires. In their absence, litter accumulation, which ties up nutrients and blocks seedling growth, limits their net productivity." (Raven and Johnson 1989).

For the purposes of this research indigenous lowland grassland in south-east Australia is defined as: a vegetation type with few or no trees, in which the dominant species are indigenous grasses (Poaceae) (Department of Conservation & Environment 1992). Typical Australian plant taxa such as Mimosaceae, Myrtaceae, Proteaceae and Epacridaceae are generally absent (Willis 1984). Western (basalt) plains indigenous grassland meets the general definition of indigenous grassland with the addition that western (basalt) plains indigenous grassland is commonly dominated by *T. triandra* (Groves 1965).

Grassy woodland

The vegetation type most closely related to grassland is grassy woodland. Grassy woodland also occurs as remnant vegetation in Victoria and often has many species in common with lowland grassland. Grassy woodland, as a vegetation type has been described as follows: the floristic composition of grasslands and grassy woodlands (in Victoria) is often very similar. Grassy woodland is a vegetation system with scattered trees (generally of less than 30% projective foliage cover) above a ground layer which is dominated by indigenous grasses. (Department of Conservation & Environment 1992).

The revegetation techniques reported in this thesis have relevance to the repair and removal of weeds from grassy woodlands as well as open grasslands (Hitchmough 1994).

1.1.3 Grasslands in Australia and their representation in Victoria

Australia has a number of grassland communities including; alpine, lowland and upland. The two primary classifications of low altitude (lowland) indigenous grassland communities are:

'Tropical grasslands' situated north of a line running between central western Queensland through to the Kimberly Ranges in Western Australia and 'Temperate grasslands' which are generally located south of that line and have various remnant distributions in Queensland, New South Wales, ACT, Victoria, South Australia and Western Australia (Scarlett *et al*. 1992). These grassland communities support a wide variety of grass and forb species (Trèmont and McIntyre 1994).

In south-east Australia twenty-six distinct lowland grassland communities are considered to exist across approximately 300 sites. These communities are reported to support 711 indigenous plant taxa (McDougall 1994). A number of species from the Liliaceae and Asteraceae of lowland grassland communities are reported to have provided food sources for local aboriginal tribes (Gott 1993). The study reported in this thesis was located on a small remnant of the Victorian lowland grassland type commonly known as, 'western basalt plains *T. triandra*-dominated grassland' (Department of Conservation & Environment 1992).

Grassland in Victoria

It is estimated the combined areas of indigenous grassland and grassy woodland once covered 34% of Victoria (Department of Conservation & Environment 1992). Victoria is considered to have once contained the largest continuous tract of grassland in Australia. This grassland is generally associated with Victoria's volcanic plains (western basalt plains) located between the west of Melbourne and the state's Western District (Gibbons and Rowan 1993). The coverage of this area of grassland was considered to be 20,000 km^{-2}, or 12% of the state (Scarlett *et al*. 1992). This distribution of temperate grassland in Victoria is generally referred to as the western (basalt) plains grassland. On the eastern sector of its distribution (Melbourne region) the soils are shallow, rocky and poorly drained cracking clays with an annual rainfall of 450 mm. To the west (Hamilton region) the soils are deeper grey-black rich clays with a rainfall of 750 mm per annum (Scarlett *et al*. 1992).

The catastrophic depletion and fragmentation of lowland indigenous grasslands which has occurred since European colonisation in Victoria has been paralleled by similar reductions in other states (McDougall and Kirkpatrick 1994, Barr and Cary 1992). The dominant reasons for this are believed to be linked to changes in grazing patterns brought about by the introduction of European agricultural practices. Indigenous herbivores of the grasslands were replaced by the herbivores introduced by the colonialists. Unlike the indigenous grazers, the new herbivores had cloven hooves which created new trampling pressures on the flora as well as general soil disturbance leading to degradation through invasion by introduced plants (Barr and Cary 1992). In addition, pasture improvement (involving comprehensive introductions of exotic grasses) undertaken by pastoralists this century (Adamson and Fox 1982, Barr and Cary 1992) and weed invasion (Carr *et al*. 1992) has resulted in broad-scale damage to the indigenous grasslands.

Today 99.5% of Victoria's indigenous grassland communities have been either eliminated or degraded (Department of Conservation & Environment 1990). In 1986 it was estimated that 0.16% of the original 21,000 km^{-2} distribution of western basalt plains grassland remained (Stuwe 1986), and by 1992 it was estimated that remaining expanse had been reduced to 0.1% of the original area (Craigie and Stuwe 1992), although recently this has been revised to around 1.0% (J. Ross 1999, pers. comm.). Very little of what remains is presently protected in reserves under State legislation (Department of Conservation & Environment 1992). The Derrimut Grassland Reserve, eight kilometres south of the St Albans research site for this work, represents 80% of the total remnant area of basalt plains grassland protected in reserves. The Derrimut Reserve approximates 0.00007% of the original extent of western basalt plains grassland (Craigie and Stuwe 1992).

The basalt plains to the west of Melbourne are considered to have always been virtually treeless (Lunt 1992), or interspersed with woody groves (open woodland) in river and creek valleys or wetland margins (McDougall 1989). The surviving remnants of the west Melbourne, or Keilor plains grassland ecosystems collectively support approximately 40 species of indigenous grasses and 150 species of small perennial and annual herbs (flowering non-grass species) (Willis 1992). The Keilor plains areas of grassland remnants are considered to contain some of the highest quality remnant grasslands in the state, in terms of species richness and ecologically viable size (Department of Conservation & Environment *et. al.* 1992). One major structural characteristic of this part of the western plains grassland is the dominant grass species *T. triandra* (kangaroo grass).

1.1.4 The importance of remnant indigenous grasslands

The consequences arising from the loss and degradation of Victoria's unique western (basalt) plains grasslands, as with other natural ecosystems, have now been recognised. In many instances, remedial actions have been proposed or implemented (Craigie and Hocking 1999).

In addition to the loss of plant genetic diversity, fragmentation of grassland has led to breaks in the important nexus between flora and fauna, leading to widespread changes and further losses in both species diversity and extent of distribution. The Victorian Draft Flora and Fauna Guarantee Strategy (1992), which has been prepared pursuant to the Flora and Fauna Guarantee Act (1988), outlines four main reasons for maintaining genetic diversity. These are:

- A moral obligation based on our power to change ecosystems,
- Self-interest in maintaining ecosystems that keep our environment habitable,
- Current and potential benefits deriving from uses of natural products,

- Intrinsic subjective values such as beauty, cultural identity and the sense of place that contact with local nature offers.

1.1.5 *Themeda triandra*

T. triandra, the indigenous species under investigation in this study is a medium to large tussock forming grass which has a wide distribution across Australia (Groves 1965, Groves 1975), New Guinea (Evans and Knox 1969), parts of Asia and southern Africa (Coetzee *et al*. 1995a). For example *T. triandra* in Africa is recorded as a dominant or co-dominant species in grassy ecosystems in the Transvaal Region of South Africa (Coetzee *et al*. 1995b). *T. triandra* is a warm season (C_4 photosynthesis) grass which can be found in a variety of Australian vegetation systems including: coastal vegetation, sub-alpine forest and woodland, lowland open woodland, lowland open scrubland, a range of riparian vegetation and open sub-tropical and temperate grassland. *T. triandra* is recorded as the dominant species in Victoria's western basalt plains grassland (Groves 1965).

1.1.6 *Nassella trichotoma*

The principal weed species under investigation in this study is *N. trichotoma.* This perennial, medium sized tussock grass with a C_3 photosynthetic pathway, is a native species of Peru, Chilè, Uruguay and Argentina (Campbell 1982). *N. trichotoma* is now established, and classified as a weed species, in New Zealand, South Africa and Australia. In Australia it has various distributions in NSW, ACT, Victoria and Tasmania (Lamp and Collet 1989). Reasons for its presence in Australia are unclear, but *N. trichotoma* was first positively identified in the north-west Melbourne region in 1935 (Lamp *et al.* 1990). *N. trichotoma* is an effective coloniser of disturbed soil, with 4000 seedlings m^{-2} recorded as establishing on bared ground in the Yass region (Campbell 1958). Campbell (1985), reports heavy infestations of *N. trichotoma* are capable of producing 930 million seeds annually per hectare, or 93,000 seeds m^{-2} annually. It is considered one of the most significant pasture weeds in NSW (Lamp *et al*. 1990). *N. trichotoma*, a declared noxious weed was recently recognised in Victoria as a pest plant requiring particular government programs to assist in its control (Aberdeen 1995). In 1996 the Victorian Government established a Serrated Tussock Task Force.

Many remnant grassland populations west of Melbourne either contain, or are adjacent to sites occupied by *N. trichotoma*. The weed appears to be actively invading indigenous grassland remnants (Craigie and Stuwe 1992). Where soil disturbance has occurred invasion is rapid. Circumstantial evidence suggests a slower rate of invasion into high quality, well managed remnants (Lunt and Morgan 1998). Developing effective methods to replace *N. trichotoma* with indigenous species is crucial to the long-term viability of these grassland remnants. *N. trichotoma* produces large numbers of seeds in late spring, at the end of its cool-season growth phase (Lamp *et al.* 1990). *N. trichotoma* has great potential to spread into disturbed land. Seed bearing panicles break off and are wind dispersed in spring and summer. Panicles have been noted to have been blown distances of 16 kilometres (Campbell 1982). It is noteworthy that *N. trichotoma* is in a group of grasses collectively referred to in South America as 'paja voladora', the flying straw (Campbell 1982). In summary, *N. trichotoma* is considered to represent one of the most significant threats to remnant basalt plains grassland and agricultural land in south-east Australia (Craigie and Stuwe 1992).

1.2 Grassland management and weed control

The world's grassland systems are often referred to by two very general categories: managed (agricultural) or unmanaged (natural) grasslands. The former contains land used and modified at various levels of intensity for agriculture. The latter, while the origin of most of the managed grassland species and western agricultural systems, is considered to be declining as a set of natural ecosystems (Alard *et al*, 1994, Wedin 1992). As the unmanaged grasslands continue to diminish in distribution, a need exists to commence and refine management of these unmanaged (natural) grasslands for successful conservation outcomes.

In the State of Victoria, the western basalt plains is a local representative case of the global change in natural grasslands. With approximately 0.5% of Victoria's original distribution of natural grasslands remaining in some form (J. Ross 1999, pers. comm.), active management is highly desirable if this ecosystem is to survive at any level of integrity (Department of Conservation & Environment *et. al.* 1992). Major threats to the western plains community and its sub-communities include:

- soil disturbance (Craigie 1992),
- poorly planned urban expansion (Craigie and Ross 1995),
- reduction in biodiversity through removal or modification of natural processes such as fire and marsupial grazing, (Stuwe and Parsons 1977, Lunt 1991, Craigie and Stuwe 1992),
- invasion by alien (weed) plant species (Govanstone *et al*. 1992).

All of these threats are summarised in the Victorian Flora and Fauna Guarantee Act (1988) Action Statement Nº 53 Western (Basalt) Plains Grassland (Department of Conservation & Natural Resources 1994). Weed control, a key component in the management of Victoria's western basalt plains remnants is the focus of the research reported in this thesis. In particular, weed control through replacement of the dominant weed species serrated tussock (*Nassella trichotoma* (Nees) Arech.) and sub-dominant introduced grass and broad-leaf species with an undisturbed, continuous and competitive kangaroo grass (*Themeda triandra* Forrsk.) sward.

1.3 The necessity for this research

Weed control in indigenous grasslands is a complex issue. Many variables are thought to affect the success of weed colonisation of remnants of the western basalt plains grassland community. To date the measurement, assessment, and control of many of those variables remain mostly unstudied. Research into practical management actions for western (basalt) plains indigenous grassland remnants is still in its infancy. Research literature on south-east Australian lowland grassland management in general is limited but a number of key studies are in progress (for examples see Craigie and Hocking 1999).

Only in the last decade has the importance of biological research into the ecology of indigenous grasslands been recognised. Some research had occurred prior to this period. For example, Groves (1965) carried out a botanical survey of indigenous grasslands and studied the growth characteristics of *T. triandra* in the St Albans region, north-west of Melbourne. Twelve years later Stuwe and Parsons (1977) carried out botanical surveys across Victoria's basalt plains. Preliminary management concepts were also discussed in this paper.

Excepting McDougall (1989), few, if any formal studies into management techniques for the flora of western (basalt) plains grasslands had commenced prior to 1990. One notable exception is Robertson (1986), but this work has never been published. To this point much of the literature available was in the form of government policies and strategies which were often not based on specific studies of basalt plains grassland. Recommendations for management of weeds and other threats frequently used information from pasture management studies carried out by the agricultural science community (see for example, Campbell 1958, Campbell 1982, Campbell 1990a, Campbell 1990b, Mitchell and Johnston 1990). This was particularly the case when recommendations for herbicide use were being made (Department of Conservation & Environment *et. al.* 1992, Department of Conservation & Environment 1992).

At the commencement of this study in 1993 some research was under way to investigate management actions for the conservation of remnant basalt plains grassland. Topics of early studies included; herbicide use (Hitchmough *et al*. 1994), revegetation with *T. triandra* (Stafford 1990), the use of fire as a tool in remnant indigenous grassland management (Lunt 1990), indigenous forb re-establishment (Morgan 1995a, Morgan 1995b, Shears 1998) and seed ecology (Delpratt in progress).

Anecdotally, it was reported that the use of fire as a 'fuel reduction' management tool for roadside verges containing indigenous grassland has contributed incidentally to the maintenance of high species diversity in these linear, remnant populations (Scarlet *et al*. 1993). Investigations carried out by

McDougall (1989) and Stafford (1990) suggested that fire may be a useful component in a set of treatments employed to re-establish *T. triandra* in weed degraded areas of remnant indigenous grasslands. Most other studies under way in the early 1990's were essentially flora surveys of remnant basalt plains grassland.

1.3.1 Weed control issues in lowland grassland

Natural or simulated natural vegetation disturbance for biomass reduction, such as caused by fire or natural grazing regimes, is considered to be potentially an important tool in the management of remnants of lowland grasslands (Lunt 1990, Lunt 1991, McIntyre 1993). It is proposed here that the use of fire or other biomass reduction techniques in the management of remnant grasslands must also incorporate considerations of weed recruitment ecology.

Anthropogenic vegetation disturbance in lowland grasslands, in particular mechanical soil tillage, is considered an undesirable form of disturbance which often advantages exotic weed species over indigenous species (McIntyre 1993, Wijesuriya and Hocking 1999). Replacement of indigenous species by weed species is thought to be rapidly promoted when soil is disturbed in western basalt plains grassland remnants (Craigie and Stuwe 1992).

Methods for removal of weed flora in or adjacent to remnant grasslands which involve low levels of soil disturbance are likely to be achieved successfully through a combination of biomass reduction (slashing or burning), with follow-up herbicide treatment of regrowth. There are however, issues of herbicide selection as to date very little rigorous field-based assessment of herbicides has been carried out. Hitchmough *et al*. (1994), used glasshouse-based trials with some field assessment to investigate the effects of three grass-specific herbicides on a number of exotic and indigenous grasses. Although detailed in many respects the 1994 Hitchmough *et al*. study raised as many questions about herbicide selection and usage as were answered.

The literature reviewed above suggests that the most effective strategy for weed management in western basalt plains grassland remnants is positioned between a need for no soil disturbance, and a critical need for major vegetation disturbance in the form of weed flora removal. In addition, it is proposed here that there is a need for rapid and dense replacement with desired indigenous flora in the nichès created by removal of weeds. In short, techniques are required for weed flora removal and replacement by indigenous species with minimum, or nil disturbance to the soil surface.

Avoiding soil disturbance

Soil disturbance in remnant indigenous vegetation adjacent or near areas colonised by weeds is generally considered a threat to that remnant vegetation as it results in the creation of a nichè for weed establishment (Hitchmough 1994, Buchanan 1989, Bradley 1986). Soil disturbance and associated alien plant colonisation have been described as two of the five serious threats to the existence of remnant basalt plains indigenous grasslands (Craigie and Stuwe 1992). Carr and Todd (1991) report on recommendations for soil disturbance in the form of ripping or cultivation being prescribed for *T. triandra* direct seeding operations in Royal Park, Melbourne. This *T. triandra* revegetation project was assessed as having low levels of *T. triandra* establishment and high densities of weeds (Carr and Todd 1991). Recent studies by Morgan (in progress), C. Hocking (1997 pers. comm.) and, Wijesuriya and Hocking (1999), suggest that soil nutrients may play a key part in the process of colonisation of disturbed soil by weeds in indigenous grasslands. Because of the importance of avoiding soil disturbance, it was decided to investigate a *T. triandra* reinstatement method that would not require disruption to the soil surface.

Assessing the 'spray and hay' method

The 'spray and hay' method for *T. triandra* establishment as described by McDougall (1989) required no visible disturbance of the soil surface. In the 'spray and hay' method soil is only disturbed to the point that existing vegetation cover is destroyed through the application of herbicide. Although McDougall's 1989 research and subsequent recommendations are regarded in this thesis as seminal a number of questions about the original method have arisen as a result of attempts to apply it to practical revegetation projects. However, little or no recording of methods and results has been carried out by land managers using the 'spray and hay' method and no rigorous testing had been carried out on many aspects of the method. Stafford (1991) carried out some testing at Cleland Conservation Park and roadside sites, Adelaide Hills, South Australia. Stafford (1990) concludes that a continuous sward of *T. triandra* is capable of resisting weed colonisation but the correlation between seeding rates and sward density or other key parameters was not documented. Carr and Todd (1991) make the point that seeding rates in this technique depend on the quality of the hay. They suggest that a method to predict germination rates for seed-bearing hay would be a useful component of the 'spray and hay' method. Carr and Muir (1993), and Muir and Carr (1994), note the problem of establishing *T. triandra* germinants by the 'spray and hay' method in such a way that they out-compete weed species. This issue would appear to relate to both the density of the *T. triandra* sward being established, and the ability of the pre-treatment herbicide to suppress competitive growth of weeds long enough for *T. triandra* to successfully establish.

The timing of treatments applied across a season was perceived as important to evaluate when investigating the reproducibility and efficacy of the original 'spray and hay' method. Issues of timing to investigate included:

- Timing and types of pre-thatch weed control (eg. burning or slashing),
- Herbicide type and timing of application,
- Timing of thatching,
- Timing and method of thatch removal (eg. burning or physical removal).

1.3.2 Herbicide selection

Selection of the herbicides to be trialed in this study were primarily based on recommendations in the Derrimut Grassland Reserve Draft Management Plan, Laverton North (Craigie and Stuwe 1992). Derrimut Grassland Reserve is four kilometres south of the study site. These recommendations were for the herbicides atrazine, glyphosate and flupropionate to be used on another South American *Nassella* species, *Nassella neesiana* (Chilean Needle Grass) Based on the generic level relationship between *N. neesiana* and *N. trichotoma* it was decided to use the same herbicides recommended by Craigie and Stuwe (1992), for the first year trials of this research. In addition, glyphosate and flupropionate had been documented by Campbell (1990a, 1990b) as effective herbicides against *N. trichotoma* and atrazine was known to be selective against C_3 grasses growing amongst *T. triandra*.

Atrazine

McDougall (1989), observed that atrazine, a C_3 (cool-season plant) specific herbicide had no perceivable effect on C_4 (warm-season) *T. triandra* seedlings when applied at standard rates. Based on recommendations from McDougall (1989), K. McDougall (1997 pers. comm.) and Craigie and Stuwe (1992), proposed the trialing of atrazine at Derrimut Grassland Reserve for control of *N. neesiana*. a cool-season (C_3) grass. *N. trichotoma*, a member of the same genus, is also a C_3 (cool-season) plant, and it was therefore decided to include atrazine, a root and foliar translocating herbicide, in the range finding trials.

Glyphosate

Craigie and Stuwe (1992), recommended glyphosate for control of *N. trichotoma* based on previous (undocumented) trial work which revealed this herbicide as capable of killing *N. neesiana* at Derrimut Grassland Reserve and Organ Pipes National Park, Keilor. Glyphosate is a commonly used (non-selective) knockdown herbicide. Based on these two pieces of information it was determined that glyphosate should be includes in the trials.

Fluproponate

Campbell, who has extensively studied the biology of *N. trichotoma* (1958, 1982, 1985, 1990a, 1990b), recommends fluproponate, a non-selective root translocating herbicide, for controlling *N. trichotoma* in agricultural environments. Fluproponate is also prescribed for the control of *N. trichotoma* on agricultural land by Lamp *et al*. (1990). Craigie and Stuwe (1992) recommend fluproponate for trialing in grassland weed management particularly as it is often recommended for use on *N. trichotoma* in agricultural situations.

Note: The grass-specific herbicide fluazifop was also considered. Hitchmough *et al*. (1994) assessed fluazifop as potentially useful for weedy grass management in grassy ecosystems. However questions remained regarding the toxicity of fluazifop to non-target organisms and this herbicide was therefore not included in this study.

1.3.3 Re-establishing *Themeda triandra* on weed-colonised sites

The re-establishment of indigenous grasses on degraded land has been shown to be achievable in a variety of situations. In South Australia, Stafford (1990) used a procedure involving removal of alien species by application of fire, or fire and herbicide, followed by an overlaying or thatching of the treated area with seed-bearing *T. triandra* hay — in essence the 'spray and hay' method described above. The use of this method has been reported as achieving 'inconsistent outcomes' in Royal Park, Melbourne (Carr and Todd 1991), Organ Pipes National Park, Keilor (Carr and Muir 1993), and Altona North, Victoria (Muir and Carr 1994).

Stafford (1990) advises that when using *T. triandra* seed in direct seeding revegetation it is important to leave the seed and floret complete to maintain the integrity of the awn. The awn has a hygroscopically activated segment which, when exposed to fluctuating levels of humidity, such as moisture retention provided by thatched hay, twists one way and then the other acting to move the seed across the soil surface. This movement eventually forces the seed head into small cracks in readiness for germination. Cleaning the seed can damage or remove the awn (Sindel *et al*. 1991).

T. triandra was noted in all literature reviewed in this study as being the dominant plant on western basalt plains grassland. It was therefore proposed in the planning of this project that the re-establishment of a robust *T. triandra* sward might act as a critical first step in controlling the movement of alien species into grassland remnants, and returning these remnants toward a more intact state.

1.4 Key elements of the methodology

The research reported in this thesis extended over three seasons and the techniques used in each year were developed in response to findings arising from the year previous. The general methodology in all cases was to pre-treat the *N. trichotoma* by reducing weed biomass and introducing *T. triandra* propagules by thatching sites cleared using one of the herbicides with seed-bearing *T. triandra* hay. The hay was subsequently removed and quantitative data on *T. triandra* seedling densities and weed establishment was collected and assessed. Details on specific trial methods are presented in Chapters 2, 3, 4 and 5.

1.5 The research site location and characteristics

The primary research site was located on a three hectare area of remnant western (basalt) plains indigenous grassland, situated in the grounds of the St Albans campus of Victoria University, 16 kilometres north-west of central Melbourne, Victoria, Australia (Figure 1).

Figure 1
Location of research site

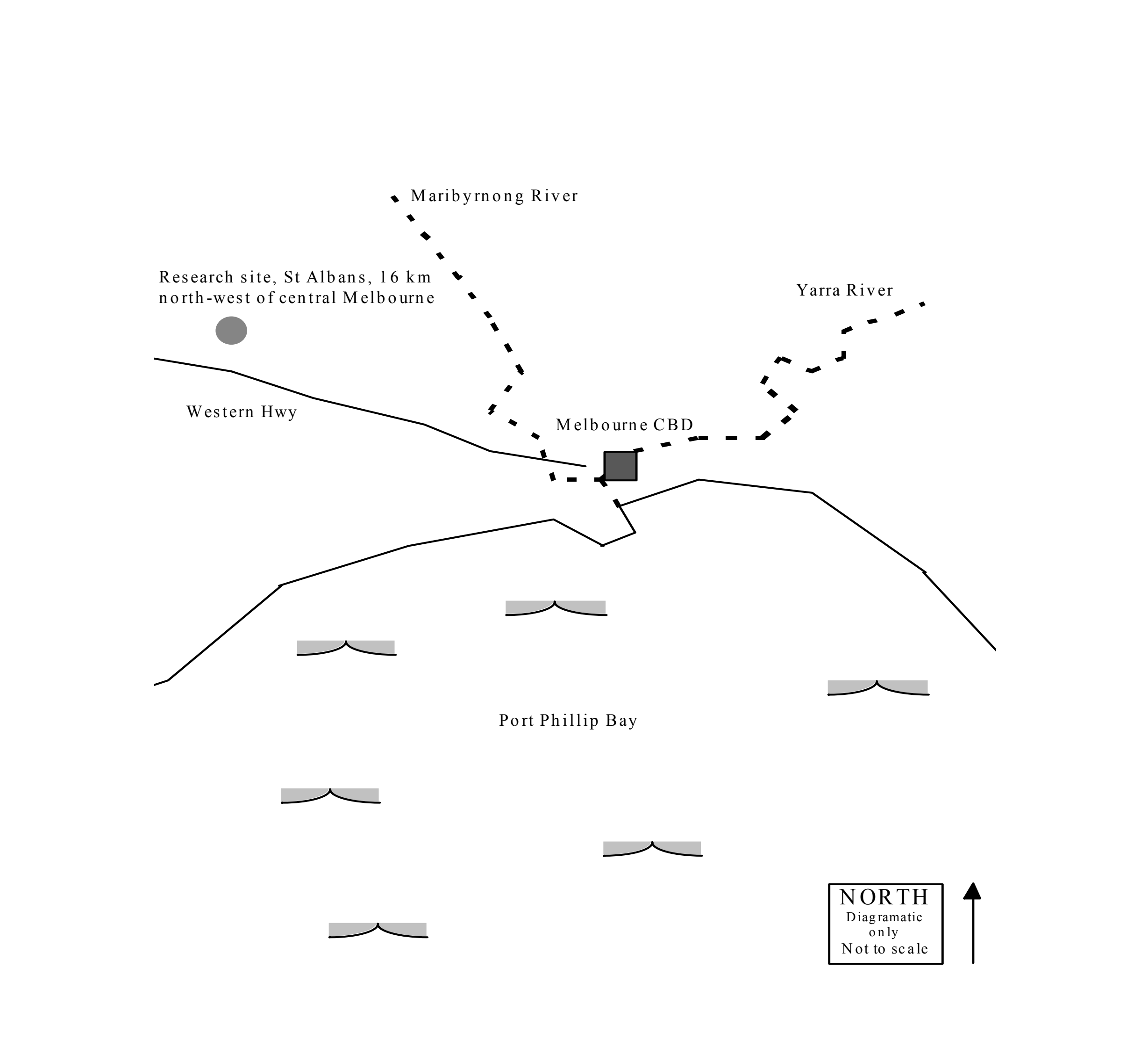
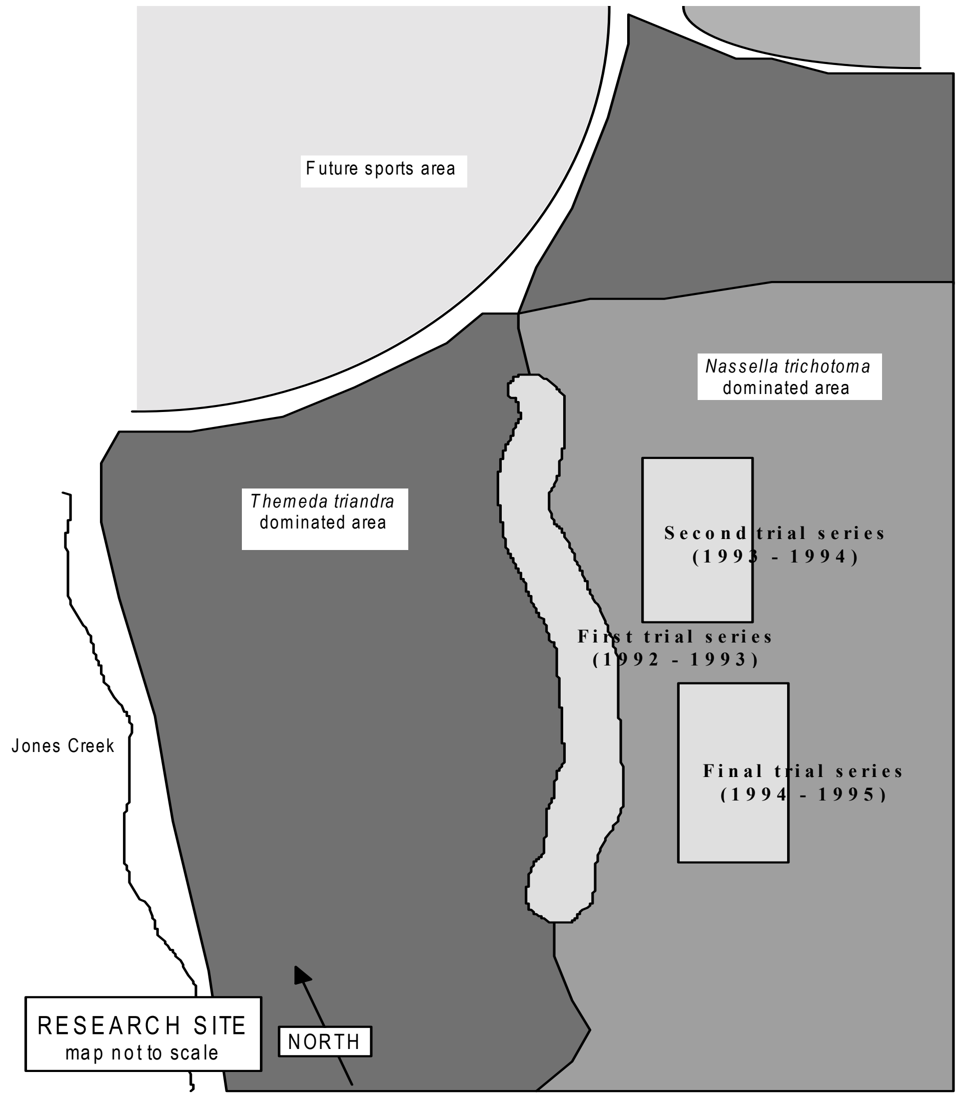


Figure 2
Approximate dominant species distribution on the 3.0 ha research site at Victoria University (St Albans)



The research site is dominated by the indigenous *T. triandra* with the exotic grass species *N. trichotoma* as co-dominant, occupying discrete areas within the site (Figure 2). *N. trichotoma* is thought to have been introduced to the region in the 1930's. Aerial photographs revealed that the *N. trichotoma* dominated area was lightly ploughed (ripped) at some time between 1965 and 1969. Because the methods proposed to be trialed addressed issues of indigenous grassland management there was a need to locate the research within a site that provided commonly encountered management problems, such as fringe presence of *N. trichotoma* and a large number of other weed species common to basalt plains grasslands. The site at St Albans provided these requirements. The proclaimed noxious weed *N. trichotoma* dominated disturbed areas on approximately forty five percent of the research site and the balance consisted of *T. triandra* dominated remnant grassland. The research site had a clear line between the *N. trichotoma* and the *T. triandra* dominated areas.

Parts of the research site had been originally proposed for development as sports facilities in the longer term. Nowhere on the site were any species listed as rare or threatened detected (see section 1.6.1). This meant the site presented a unique opportunity to investigate a range of methods for accomplishing a weed-free replacement of *N. trichotoma* by *T. triandra* without the constraints imposed by conservation imperatives on most other remnant grassland sites. Locating the research site at the St Albans site placed it in a region containing important western (basalt) plains grassland remnants including:

- Derrimut Grassland Reserve, 5.5 kilometres south of the study site,
- Laverton North remnant grassland, 6.5 kilometres south of the study site,
- Albion remnant grassland, 0.4 kilometre west and adjacent to the study site,
- Organ Pipes National Park, 10.6 kilometres north of the study site.

1.5.1 Flora of the research site

The site appears to have been progressively colonised by *N. trichotoma* as a result of periodic disturbances. The main pattern of colonisation of *N. trichotoma* has resulted in a clear boundary transecting the site between the co-dominant species *T. triandra* and *N. trichotoma*. *N. trichotoma* is dominant on the soil disturbed side of this boundary, on which large rocks have been lifted out of the ground as a result of ploughing (Figure 2). The site is also under pressure from invasion by various thistle species and other weeds that have colonised recent earthworks adjacent to the site. There are smaller, discrete *N. trichotoma* incursions into the *T. triandra*-dominated western half of the site. In February 1993 the site was burned by a wildfire of unknown origin.

Soil at the site is newer volcanic red basaltic clay of the upper Cainozoic (quaternary) period (Douglas 1993). A range of annual grasses, thistle species and other broadleaf weeds occurred on and adjacent to the trial plots, along with a range of common indigenous forb species at low levels of abundance (Phillips 1994).

A flora survey on the research site revealed thirty-five indigenous grasses, herbs and sedges and thirty-six plant species classified as either environmental or noxious weeds (Phillips 1994). Species diversity in this *T. triandra* grassland is at a level expected for an urban fringe land area which has been subjected to general prolonged agricultural uses. The list of plants compiled from the flora survey did not contain any indigenous species that are individually listed as rare or endangered on state or federal legislation. The parcel of land and the vegetation can be classified as western (basalt) plains grassland (K. McDougall 1997, pers. comm.). This community has been listed under Victorian legislation (Schedule 2 [1991] of Flora and Fauna Guarantee Act [1988]) as vulnerable to extinction. This means the whole community is considered to be only two steps away from extinction, the final step prior to extinction being the 'endangered' classification. All indigenous species recorded by Phillips (1994) were also recorded thirty years earlier by Groves (1965) from a nearby St Albans site.

2. Control of *Nassella trichotoma* by the herbicides atrazine, glyphosate and flupropionate in indigenous grassland conservation contexts

2.1 Summary

Field trials over three years examined the efficacy of three herbicides, applied at label strength and backpack rates, in controlling serrated tussock (*Nassella trichotoma* (Nees) Arech.) and other weeds commonly found in temperate lowland indigenous grassland remnants in Victoria. Glyphosate (as Monsanto Roundup®) and atrazine (as Nufarm Nutrazine®) applied in winter (June-July) were effective in killing mature *N. trichotoma* plants, and resulted in no seedling establishment for at least six months after application. Atrazine was the most effective herbicide, with applications in either February or July maintaining largely weed-free plots for over ten months. Flupropionate (as ICI Frenock®) was also effective in killing mature *N. trichotoma* plants and preventing seedling establishment, but compared with the other two treatments resulted in invasion at much higher levels by a range by other exotic weeds.

2.2 Introduction

Nassella trichotoma is one of the most serious current and potential agricultural and environmental weeds in south-eastern Australia (McLaren *et al* 1998). It has been proclaimed a noxious weed in the Australian Capital Territory, New South Wales, Victoria, South Australia and Tasmania. Due to its potential impact on lowland indigenous grassland biodiversity and the productivity of agricultural systems, *N. trichotoma* is listed as either a regionally prohibited or regionally controlled weed in all but one of Victoria's Catchment Management Areas (V. Craigie 1999, pers. comm.). Recently, *N. trichotoma* has been listed as a weed of national significance.

At the national '*Nassella* Workshop' held at Victoria University in 1998 methods of control across agricultural and conservation contexts were compared. It was concluded that: Methods for eradication and control on agricultural land may have relevance for eradication and control on non-arable land or conservation reserves, and vica versa, but applicability of these methods from one context to the other cannot be automatically assumed (Shepherd and Richardson 1998).

Extensive research has now been conducted for control of *N. trichotoma* in agricultural contexts (Campbell 1998, Miller 1998) but few studies have focussed on control in conservation contexts (Gardener and Sindel 1998, Hocking 1998).

The use of herbicides to remove *N. trichotoma* and other South American stipoid grasses from indigenous grasslands without physically disturbing the soil would appear to be a useful strategy (Hocking 1998), considering the rapid and major invasion by a wide range of weeds following soil disturbance of indigenous grassland areas (Department of Conservation & Natural Resources 1994, Craigie and Stuwe 1992). Hocking (1998) proposes that approaches to using herbicides for *N. trichotoma* control in conservation contexts are likely to be different to methods for control in agricultural contexts in the following ways:

- the scale of use will usually be smaller in conservation contexts and herbicides more often applied on a highly selective basis – the volumes and costs of the actual herbicides used will usually be minimal compared with the labour costs required for sensitive application within conservation contexts,
- the species used for competitive replacement will be different to those common for agricultural situations,
- the need for control of a range of exotic species will be high in many cases, and which exotic species need to be controlled will sometimes differ from agricultural contexts.

Two of the herbicides chosen for this study, glyphosate and flupropionate (tetrapion), are in common usage for *N. trichotoma* control in agricultural contexts (Campbell 1998). The third herbicide included in the study, atrazine, is in common use for control of C_3 photosynthetic pathway exotic grasses in conservation areas (Craigie and Stuwe 1992). Here the main indigenous tussock grasses possess C_4 photosynthetic pathways, because atrazine is selective for C_3 pathway species.

For conservation purposes, the type, pattern and timing of establishment of plant species (indigenous and exotic) on sites following herbicide removal of *N. trichotoma* is at least as important as the efficacy of the herbicide itself. The type of regrowth will affect the overall diversity and seedbank capacity for weed establishment across the site, as well as the potential for revegetation with competitive indigenous species. High levels of cover of weed species soon after herbicide clearance of *N. trichotoma* are likely to impede germination and seedling growth from existing and added indigenous seed. To date there has been no in-depth study in conservation contexts of the effects of the type and timing of herbicide treatment on post-application weed establishment.

2.3 Materials and Methods

2.3.1 Quadrat size and shape

The trials were carried out at Victoria University (St Albans) within the indigenous grassland conservation reserve (Figure 2), with *T. triandra* as the dominant indigenous tussock grass and *N. trichotoma* scattered across the site in dominant areas where soil disturbance (ploughing, sheep encampments) has occurred (Chapter 1). Quadrats in all trials measured 1.3 m x 1.5 m (1.95 m^{-2}), and this was the standard area subjected to the different treatments. Only the central 1.0 m x 1.0 m (1.0 m^2), within the greater quadrat was used for data collection. In this way biases generally attributed to 'edge effect' (discussed by Scougall *et al*. 1993 in the context of larger scales) were minimised. This format also allowed maximum access for inspection from the sides of the plots with minimal disturbance of ground adjacent to quadrats and provided a total treatment area approximating 2.0 m^{-2}, which simplified calculations for rates of herbicide application.

2.3.2 Quadrat treatment

Initial Weed Biomass Reduction

Prior to herbicide application, the biomass of mature *N. trichotoma* plants was reduced by either burning or mowing, or a combination of these at different times (as detailed in individual trials below). The weed regrowth following the biomass reduction treatment was sprayed with one of three herbicides. In the first year of the project, immediately prior to the year 1 pilot trials being set up a wild fire passed through the research site at the end of summer substantially reducing the plant biomass. In the following two years of trials initial weed biomass reduction was carried out by slashing the sites at the end of summer. A domestic lawn mower was used at a setting to cut weeds to a height averaging 80 mm (almost exclusively *N. trichotoma* in all plots). All slashed grass was removed by manually raking the site with a domestic garden rake. Contact between the rake teeth and the soil surface was minimised.

Herbicide Weed Control

Treatment plots were randomised across the site prior to herbicide application. The three herbicides used, atrazine (as Nufarm Nutrazine®), glyphosate (as Monsanto Roundup®) and flupropanate or tetrapion (as ICI Frenock®), were applied at label strength recommended for backpack application (Table 1). Herbicide was applied using a manually operated pressure pump from a 15 litre (Solo®) backpack spray unit calibrated for standard delivery of a set volume/unit time. Rates of application were adjusted to deliver an average of 770 ml m^{-2} which equates in each case to about five times the agricultural rate. These are the rates that would normally be applied in a conservation setting (J. Bush 1997, pers. comm.).

Table 1
Herbicides label recommended dilution (1993)

Atrazine as Nutrazine®	(130 ml/15 l)
Glyphosate as Roundup®	(150 ml/15 l)
Flupropanate as Frenock®	(30 ml/15 l)

Trials were carried out early winter (July) to late autumn (May) over the growing season of three consecutive years: year 1: 1993-94, year 2: 1994-95 and year 3: 1995-96. In year 1, following winter application of herbicide, plots were assessed in spring and late summer for percentage cover using 100 point intercept frames. In years 2 and 3, plots were assessed for number of plants m^{-2} of each major species at periodic intervals after herbicide application, depending on the year of application and the specific trial. All data collection commenced at the first observation of cotyledons on trial plots.

2.4 Results

2.4.1 Year 1 Pilot Trials (1993 – 1994)

Weed growth following burning in February and each of three herbicides applied in June

In 1993-94 trials, herbicides were applied in June 1993 to plots burnt in the previous February. Plots were assessed for species composition and percentage cover in November 1993 and February 1994, five and eight months respectively after herbicide application. In each plot treated with one of the three herbicides used, no mature *N. trichotoma* plants survived. Careful on-site inspection revealed that *N. trichotoma* plants, observed on herbicide treated plots after the application of herbicide, were all seedlings established from the soil seedbank.

In control plots *N. trichotoma* averaged 30% cover in November 1993 (Figure 3) and this increased to 53% m^{-2} in February 1994, as a result of seedling recruitment following gaps created by the fire in February 1993. A variety of other weeds commonly found in disturbed lowland grassland remnants, including the broadleaf *Picris echioides*, two thistle species *Carthamus lanatus* and *Cirsium vulgare* and the annual grasses *Briza minor*, *Vulpia bromoides* and *Aira caryophyllea* (included as part of 'other weeds' in Figure 3) also occurred in significant numbers on each plot at each assessment.

On plots treated with atrazine in June 1993, no living *N. trichotoma* plants were found at either the November 1993 or February 1994 assessments (Figure 4). Nor were there any other weeds on any of the plots at either of the assessments.

On glyphosate treated plots, an average *N. trichotoma* cover of 2% had established by the November 1993 assessment (Figure 5) and this had increased to an average of 14% by the February 1994 assessment, one year after herbicide application. In addition to *N. trichotoma*, a range of other weeds had established in low numbers on glyphosate treated plots by November 1993 (Figure 5). Of these, *C. vulgare* exhibited the highest average percentage cover, being approximateley 2% at the second assessment – a very low level of invasion.

On flupropanate treated plots *N. trichotoma* had established at less than 1% cover by the November 1993 assessment. All these plants had died by the February 1994 assessment (Figure 6). However, significant cover of thistle plants, mainly of *C. lanatus* and *C. vulgare*, as well as the broadleaf weed *P. echioides* had established on plots by November 1993. After another three months (February 1994) the cover of *C. lanatus* plants had increased significantly to an average of 39% (single factor ANOVA $p<0.005$). At the same three month assessment (February 1994) *P. echioides* had decreased in number but the plants remaining on plots had increased in size, resulting in no significant change in percentage cover (single factor ANOVA $p>0.26$), while *C. vulgare* cover decreased significantly (single factor ANOVA $p<0.04$) (Figure 6).

Figure 3 CONTROL IN 1993 (N=8)

Cover in November 1993 and February 1994 of major weeds on quadrats burnt in February 1993, with no further treatment.

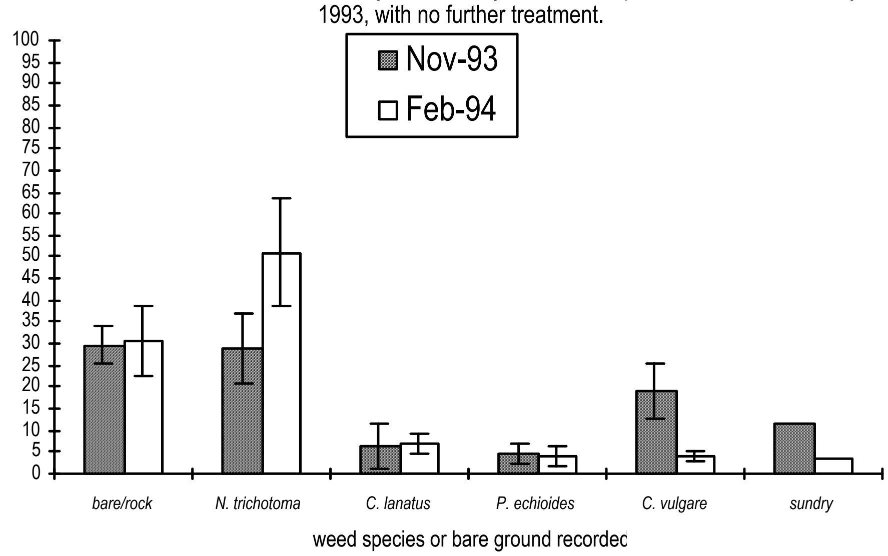


Figure 4 ATRAZINE IN 1993 (N=4)

Cover in November 1993 and February 1994 of major weeds on quadrats burnt in February 1993, sprayed atrazine June 1993.

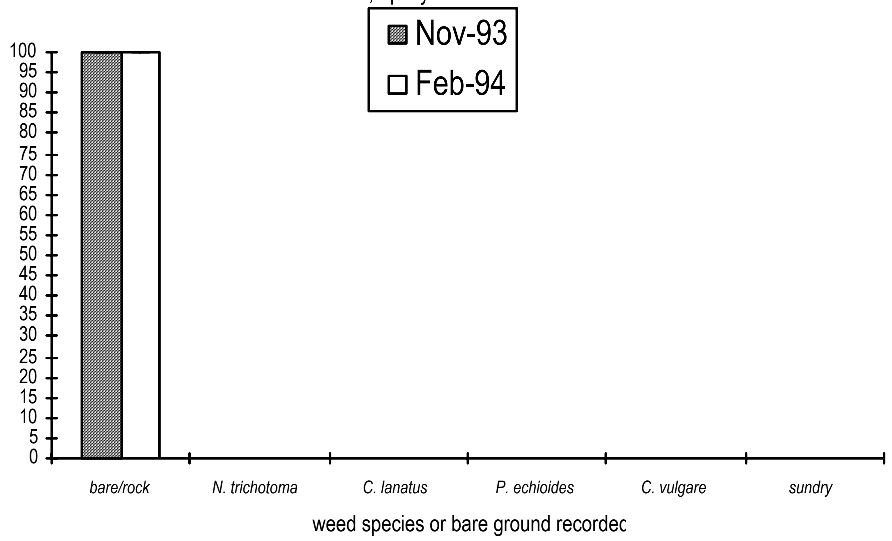
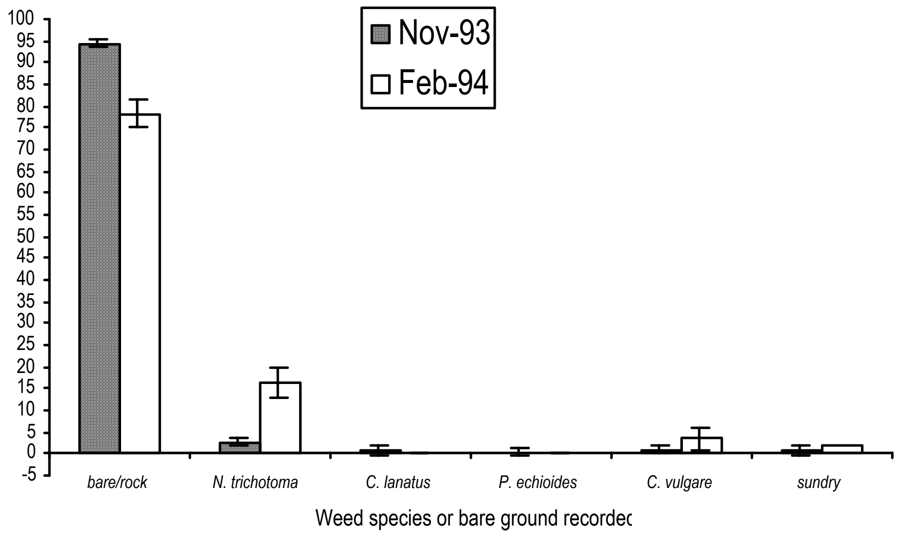
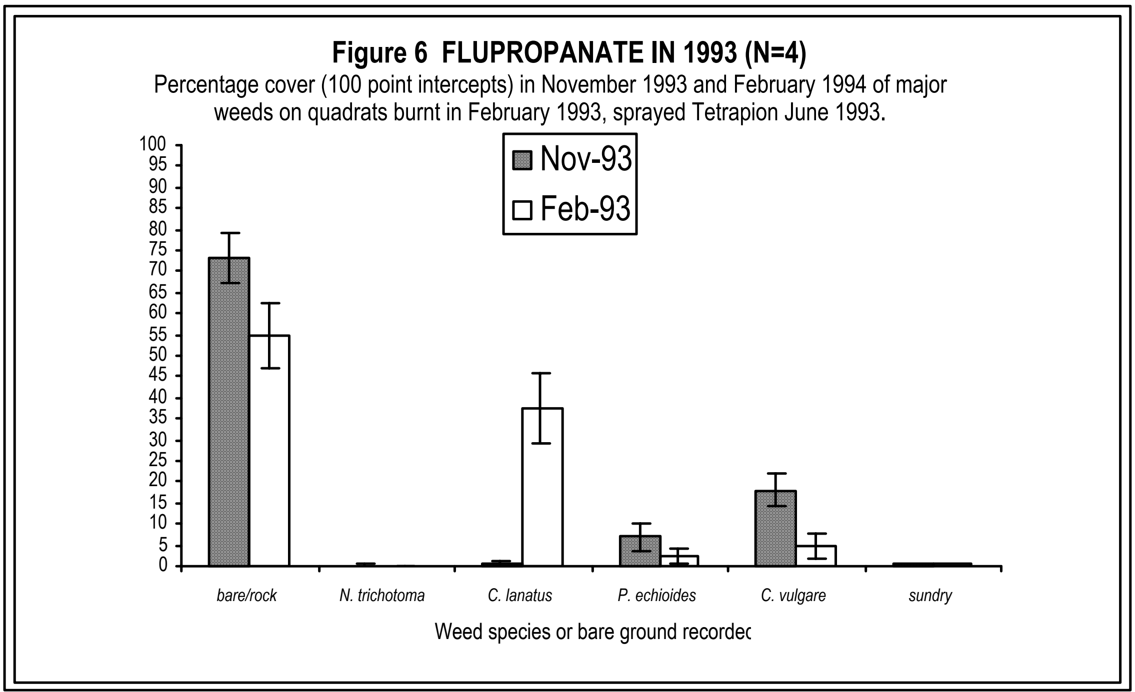


Figure 5 GLYPHOSATE IN 1993 (N=6)

Cover in November 1993 and February 1994 of major weeds on quadrats burnt in February 1993, sprayed Glyphosate June 1993.





2.4.2 Year 2 Trials (1994 – 1995)

Weed growth following mowing in March and glyphosate application in March & July

In 1994-95 trials, the herbicide glyphosate was applied at one of two times, in late March 1994 or July 1994, to plots burnt in February 1993 and mown and raked in early March 1994. Unmown control plots were also included. Plots were assessed for species composition (plant densities) in October 1994 and April 1995. In each year 1 trial plot treated with glyphosate, no mature *N. trichotoma* plants survived. *N. trichotoma* plants observed on glyphosate treated plots were all seedlings established from the soil seedbank.

In year 2 unmown control plots the number of mature *N. trichotoma* averaged 44 plants m^{-2} in October 1994 (Figure 7) and this density was maintained in April 1995. Little seedling recruitment was observed at either of the assessments, despite heavy onset of flowering and seed fall of surrounding mature *N. trichotoma* plants in the spring of 1994. Other weeds were observed at low numbers on plots, including *C. lanatus*, *C. vulgare* and *P. echioides.*

In year 2 mown control plots, the number of mature *N. trichotoma* plants (Figure 8) was very similar to those on unmown control plots, with an average of just below 40 plants m^{-2} at the October 1994 and April 1995 assessments. After mowing, however, the number of other weeds was higher on mown than on unmown control plots, with *C. lanatus* at an average of 9 plants m^{-2} at the October 1994 assessment and not observed on unmown plots. At the April 1995 assessment *C. lanatus* was higher on mown plots, although not significantly, at 8 plants m^{-2} than on unmown plots at 5 plants m^{-2} (single factor ANOVA $p>0.17$) (Figures 7 and 8). The other main weed to show a significant response to mowing was *Romulea rosea*, an exotic geophyte common in lowland grassland remnants. The average density of *R. rosea* on mown plots in October 1994 was 18 plants m^{-2}, consisting entirely of regrowth from underground corms. This density was significantly higher than for the unmown plots in October 1994 with *R. rosea* at 5 plants m^{-2} (single factor ANOVA $p<0.002$). Reflecting the autumn-winter seasonal appearance of *R. rosea*, this species had decreased in density to 10 plants m^{-2} by April 1995 on mown plots while it was not observed on umown plots at this time (Figures 7 and 8).

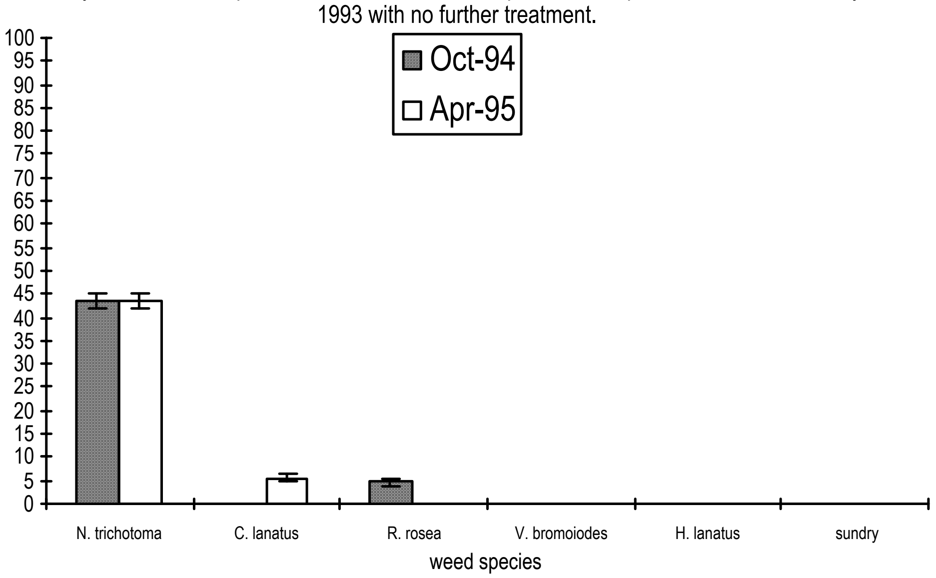
In plots treated with glyphosate (July 1994), very similar patterns of post-herbicide weed establishment were observed at the October assessment (Figure 9) compared with those found after a similar time interval following the application of glyphosate in June 1993 (in year 1 trials Figure 5). Few *N. trichotoma* seedlings had established by October, and the densities of other weeds, including thistle species and *P. echioides* were low. The average *N. trichotoma* density was also low at the April assessment, as were the majority of broadleaf weeds. The notable exception was *C. lanatus* which had average seedling densities of 60 plants m^{-2}. There were also notable densities of *R. rosea* in both October 1994 and April 1995 on plots treated with glyphosate in July 1994 (Figure 9).

In plots treated with glyphosate in March 1994 (Figure 10), few *N. trichotoma* seedlings had established by October. An average density of 7 *N. trichotoma* seedlings had become established by April 1995. In addition, the patterns of establishment of other weeds in these early autumn glyphosate treated plots were very different, when assessed in either October 1994 or April 1995, compared with weed establishment patterns observed at the same times on plots treated with glyphosate in winter (July) 1994 (Figure 9). There were high densities of the annual weeds *Holcus lanatus* and *V. bromoides* (Figure 10) and notable densities of the thistle *C. lanatus* autumn treated plot by October 1994. As might be expected at the end of the growing season the annual weeds had disappeared but *C. lanatus* was at high densities, as it was in the July 1994 glyphosate treatment plots (Figure 9). The exotic geophyte *R. rosea* was also in high numbers in October 1994 and April 1995.

In a separate set of trials on the same site in year 2 (details of method and data not shown), fluproponate was applied at the backpack rate to plots in July 1994, at three concentrations: label strength; twice label strength; and three times label strength. The plots were assessed for plant density and cover in November 1994. These plots produced essentially the same result as in year 1 (1993-94). All of the mature *N. trichotoma* plants on plots were killed, but high numbers and cover of thistles species, especially *C. lanatus* and *C. vulgare*, as well as *P. echioides* had established within 5 months of herbicide application. Increasing the concentration of fluproponate did not reduce recruitment and growth of these thistle, or of other broadleaf species.

Figure 7 UNMOWN CONTROL IN 1994 (N=5)

Density of main weed species in October 1994 and April 1995 on quadrats burnt in February 1993 with no further treatment.



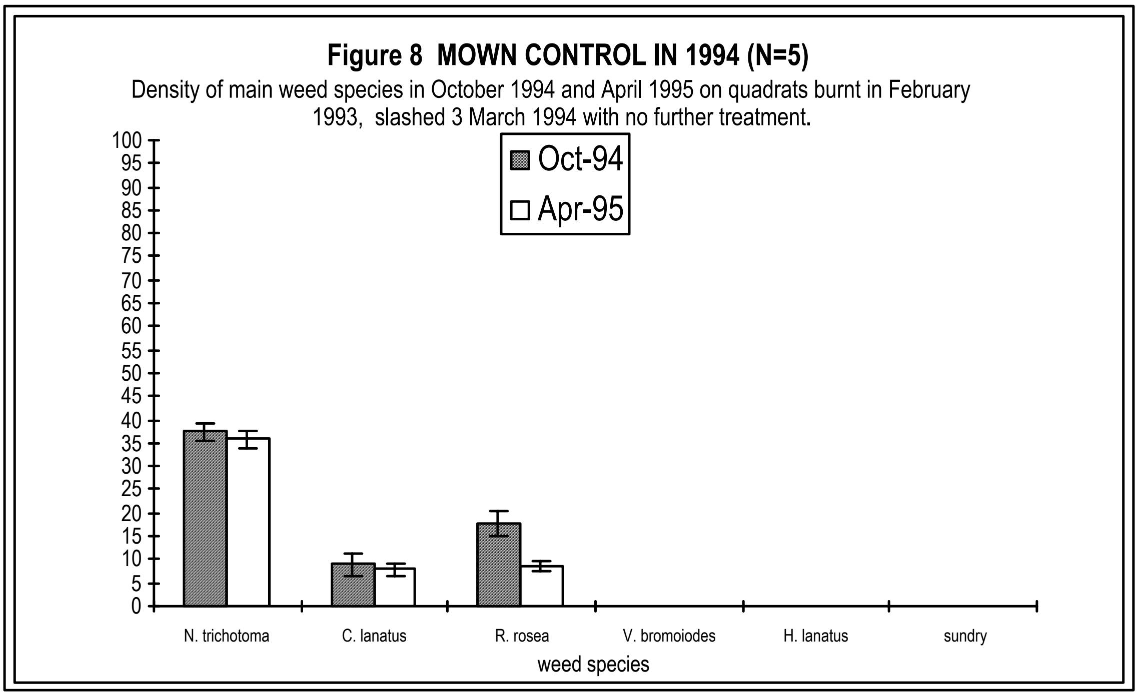


Figure 8 MOWN CONTROL IN 1994 (N=5)
Density of main weed species in October 1994 and April 1995 on quadrats burnt in February 1993, slashed 3 March 1994 with no further treatment.
Oct-94
Apr-95
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
N. trichotoma
C. lanatus
R. rosea
V. bromoiodes
H. lanatus
sundry
weed species

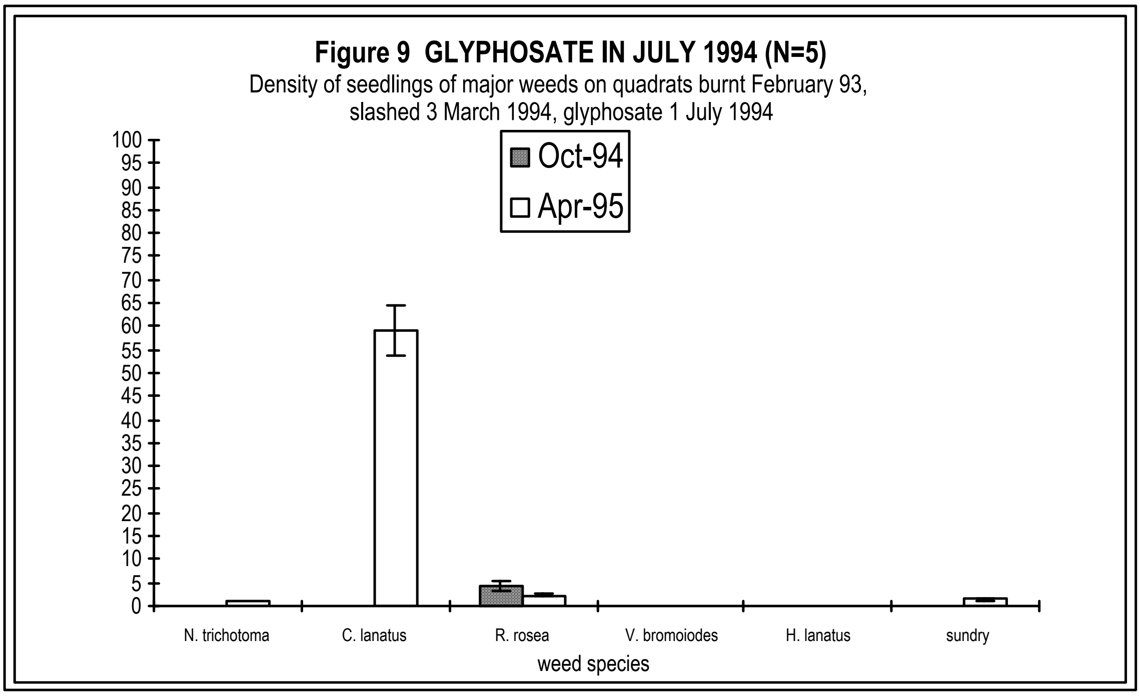
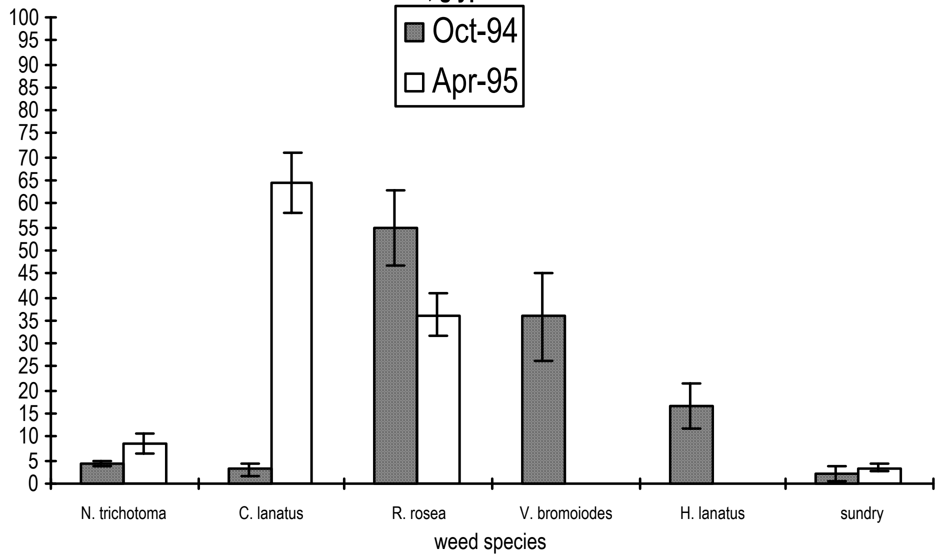


Figure 9 GLYPHOSATE IN JULY 1994 (N=5)
Density of seedlings of major weeds on quadrats burnt February 93, slashed 3 March 1994, glyphosate 1 July 1994
Oct-94
Apr-95
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
N. trichotoma
C. lanatus
R. rosea
V. bromoiodes
H. lanatus
sundry
weed species

Figure 10 GLYPHOSATE IN MARCH 1994 (N=5)
Density of seedlings of major weeds on quadrats burnt February 93, slashed 3 March 1994, glyphosate 30 March 1994 .



2.4.3 Year 3 Trials (1995 – 1996)

Glyphosate treatments

In 1995-96 trials, glyphosate was applied at one of four times in 1995: February, May, July or October to plots burnt in February 1993 and mown and raked in January 1995. Atrazine was applied in February and July to other plots integrated into the overall random block design of the trial. Plots were regularly assessed for species composition at one or two month intervals after herbicide application. This was increased to monthly assessments during spring (October to December). In each plot treated with one of the herbicides, no mature *N. trichotoma* plants survived. As for previous trials, the only *N. trichotoma* plants observed on herbicide treated plots were as seedlings established from the soil seedbank.

Control plots maintained the same densities of mature *N. trichotoma* plants as observed in the previous two years; around 40 plants m^{-2} (Figure 11). *C. lanatus* and *R. rosea* exhibited patterns of appearance consistent with year 2 results for the mow and collect controls (Figure 8), although in 1995 *R. rosea* rose to an average density of 52 plants m^{-2} in October (Figure 11), compared with 17 plants m^{-2} in October 1994 (Figure 8).

Plots treated with glyphosate in February 1995 (Figure 12) exhibited many similar patterns of growth of weed species to plots treated with glyphosate in March 1994 (Figure 10). Densities of *R. rosea* were high in winter (Figure 12), became very low by late spring (1995 data only) and began to re-appear in the following autumn. Densities of *C. lanatus* were high across autumn and winter (Figure 12), but became low across spring (1995 data only) and in the autumn of the year following herbicide treatment. Densities of annual weeds such as *V. bromoides* and *Anagallis arvensis* became significant in the spring following herbicide application, and were still persistent on the site in the autumn of the next year (Figures 10 and 12). Small but significant densities of *N. trichotoma* seedlings began establishing on plots in the spring following herbicide application, and by the autumn of the following year had reached an average of 17 plants m^{-2} (Figure 12).

By comparison, weed levels on plots sprayed with glyphosate in either late Autumn (May) or mid Winter (July) in 1995 remained comparatively low over the following 10 to 12 months (Figures 13 and 14). Low densities of *C. lanatus, R. rosea* and annual grasses were observed on all plots in these two treatments across the year following herbicide application. The patterns of weeds appearing on plots treated with glyphosate in July 1995 (Figure 14) were similar to those for plots treated with glyphosate in July 1994 (Figure 9). The exception was *C. lanatus* which occurred at much higher levels in April 1995 (57 plants m^{-2}) on 1994 treated plots than in May 1996 (3 plants m^{-2}) on 1995 treated plots.

Ten months after treatment, *N. trichotoma* seedling densities had also increased to higher levels on July 1995 treated plots, when compared with May 1995 treated plots (Figures 13 and 14) or July 1994 herbicide treated plots (Figure 9). It would appear that different years of treatment affect how long glyphosate treatment can prevent *N. trichotoma* seedlings from establishing on herbicide cleared sites.

Glyphosate sprayed in October 1995 (Figure 15) cleared plots of *N. trichotoma* until May 1996. At this time *N. trichotoma* had returned to plots at 2.4 plants m^{-2}. Other weeds noted were: *C. lanatus* at 4.2 plants m^{-2}; *R. rosea* at 8.0 plants m^{-2};

and three weed species assessed under the 'sundry weeds' category at 10.2 plants m^{-2}. This was considered a favourable outcome in terms of site clearance of *N. trichotoma* and other weeds. It is worth noting however, that application of herbicide in October is not thought to be advantageous in overall revegetation programs which involve thatching with seed-bearing *T. triandra* hay and thatch removal for *T. triandra* revegetation (see Chapters 4 and 5).

On plots treated with glyphosate in October of year 3 (Figure 15), establishment of *N. trichotoma* seedlings was at lower densities seven months later than any of the other glyphosate treatments, as judged by comparing densities of weeds at the May 1995 assessment across treatments (Figures 11 – 15). Densities of other weeds in the spring (October) glyphosate treatment were also low at the seven month assessment and comparable with those of the autumn (May) and winter (July) glyphosate treatments.

Atrazine treatments

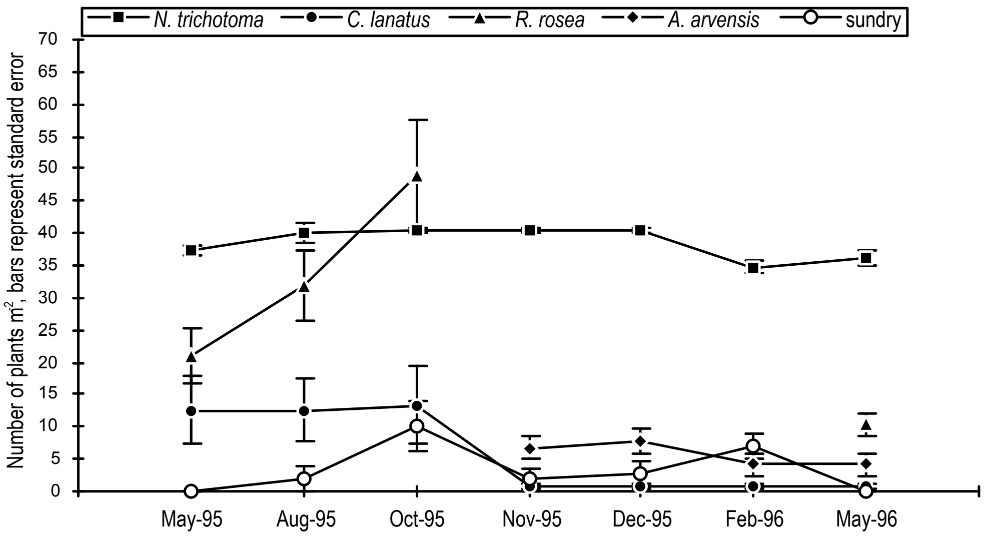
Plots treated with atrazine in either February 1995 (Figure 16) or July 1995 (Figure 17) had the lowest densities of weeds across the following 10-12 months when compared with all other treatments. On plots to which atrazine had been applied in February, the densities of *N. trichotoma* seedling remained at below 3 plants m^{-2} for the 12 month duration of the subsequent monitoring period (Figure 16). Other weed species which had established at high densities at various times on February glyphosate treatments, such as *C. lanatus* , *A. arvensis* and other annual weeds (Figure 12) were virtually non-existent on February atrazine treatment plots (Figure 16). The only significant weed presence after 12 months (May 1996 assessment) on plots treated with atrazine in either February 1995 or July 1995 were small annual weeds (included as other weeds in Figure 16) and a few *C. lanatus* plants.

The only exotic species that was at high densities at any time following February atrazine treatment in year 3 trials was *R. rosea.* On February treatment plots *R. rosea* was at high densities by May 1995 (40 plants m^{-2}), comparable with the densities in May of *R. rosea* on February glyphosate treatment plots (Figure 12). On atrazine plots *R. rosea* numbers fell quickly and did not re-appear in the following autumn - winter period (Figure 16), in contrast to their increase in the same period on glyphosate treated plots (Figure 12).

Application of atrazine in July 1995 produced a similar outcome for weed control (Figure 17) compared with the application of atrazine in February 1995. Densities of all weeds observed as major invaders in other trials remained at low levels. Seedlings of *N. trichotoma* never rose higher than 2 plants m^{-2} across the 10 months of post application monitoring. As for February atrazine treatments, the only significant weed presence on July atrazine plots after 12 months (May 1996) were small annual weeds (included as other weeds in Figure 16) and a few *C. lanatus* plants. At the final data collection February atrazine plots showed *R. rosea* below 0.5 plant m^{2} while the July atrazine plots showed *R. rosea* at 13 plants m^{-2} (Figures 16 and 17).

Figure 11 CONTROL IN 1995 (N=5)

Density of major weed species on quadrats slashed January 1995, no further treatment.



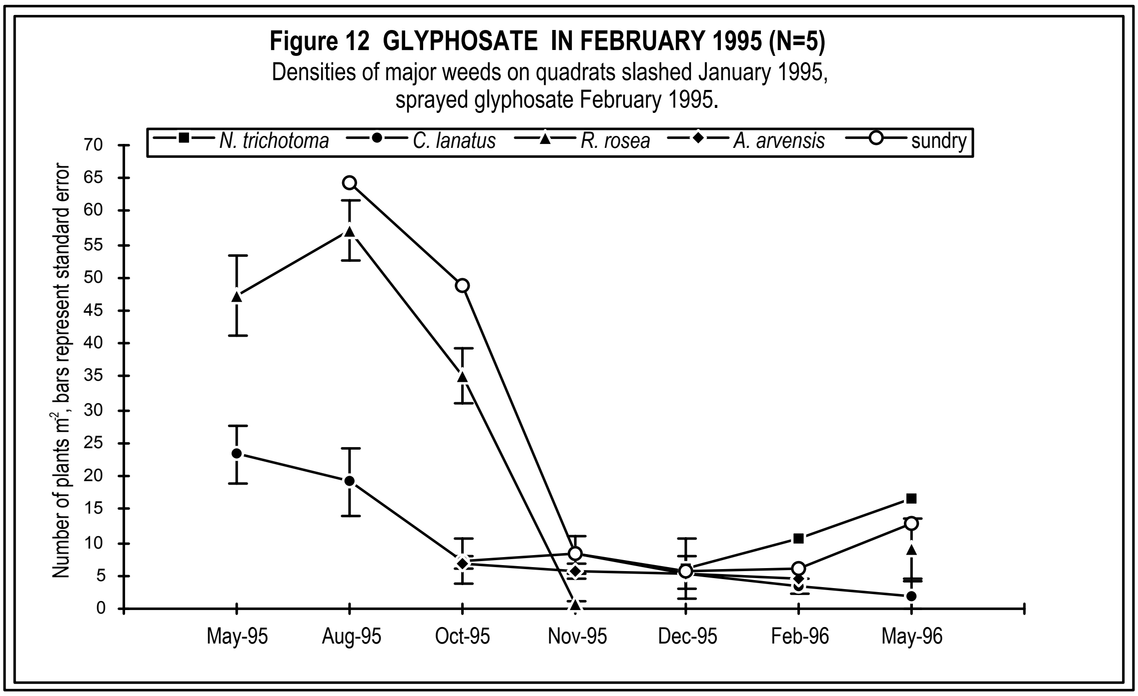


Figure 12 GLYPHOSATE IN FEBRUARY 1995 (N=5)
Densities of major weeds on quadrats slashed January 1995, sprayed glyphosate February 1995.
N. trichotoma
C. lanatus
R. rosea
A. arvensis
sundry
Number of plants m2, bars represent standard error
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
May-95
Aug-95
Oct-95
Nov-95
Dec-95
Feb-96
May-96

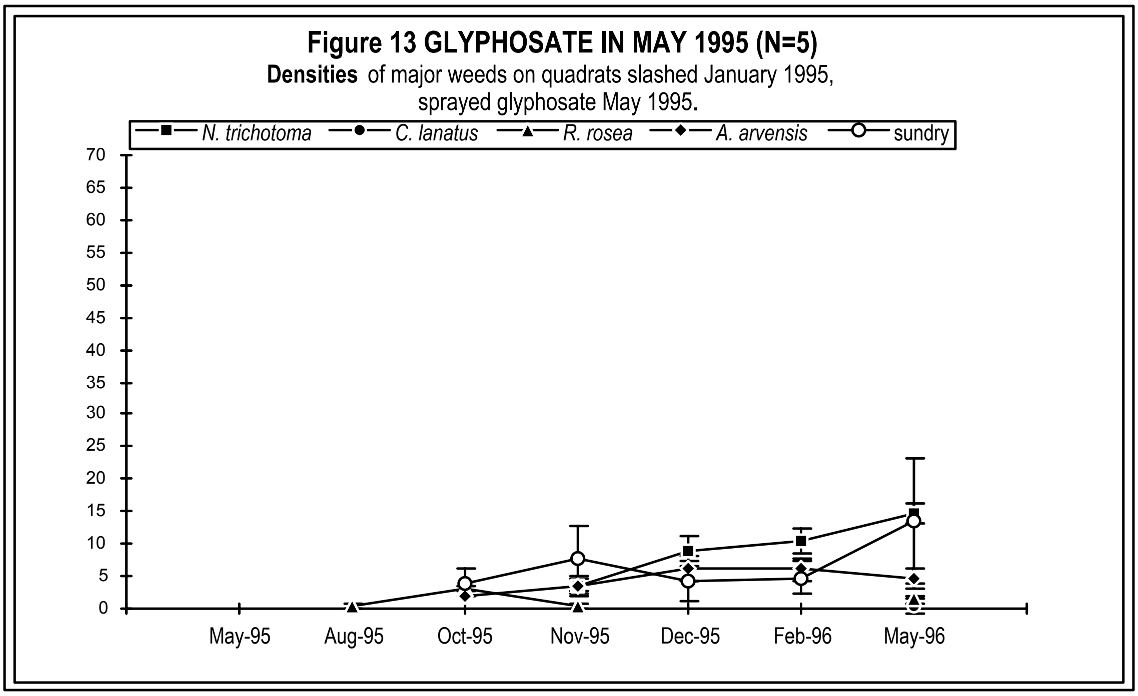


Figure 13 GLYPHOSATE IN MAY 1995 (N=5)
Densities of major weeds on quadrats slashed January 1995, sprayed glyphosate May 1995.
N. trichotoma
C. lanatus
R. rosea
A. arvensis
sundry
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
May-95
Aug-95
Oct-95
Nov-95
Dec-95
Feb-96
May-96

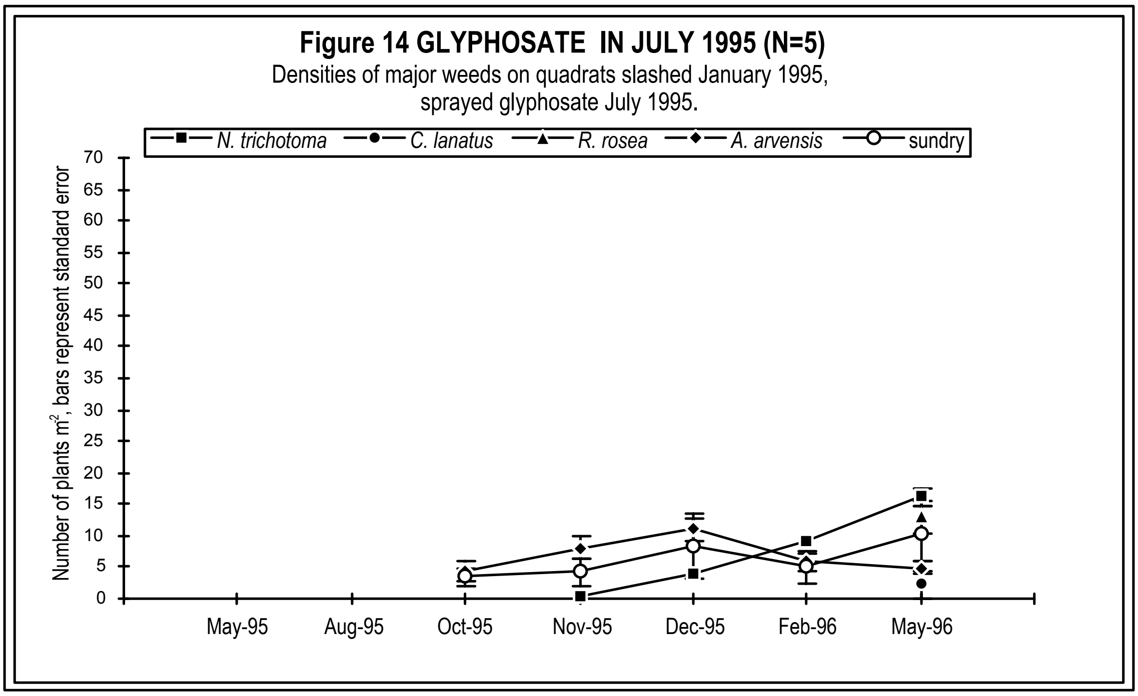


Figure 14 GLYPHOSATE IN JULY 1995 (N=5)
Densities of major weeds on quadrats slashed January 1995, sprayed glyphosate July 1995.
N. trichotoma
C. lanatus
R. rosea
A. arvensis
sundry
Number of plants m2, bars represent standard error
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
May-95
Aug-95
Oct-95
Nov-95
Dec-95
Feb-96
May-96

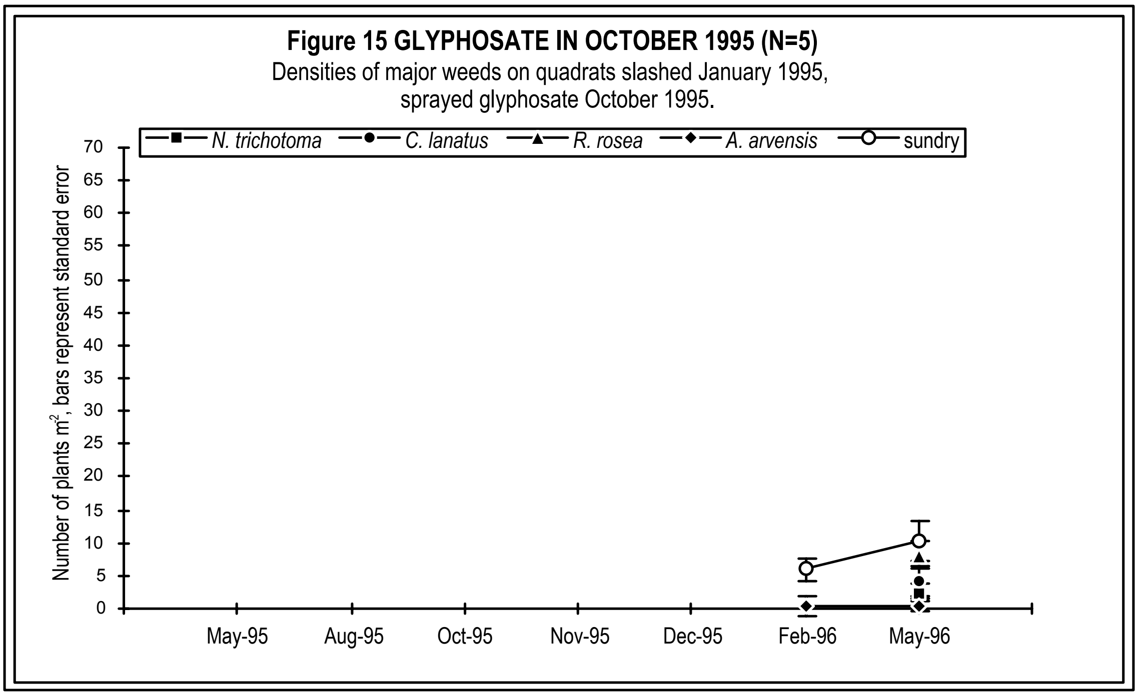
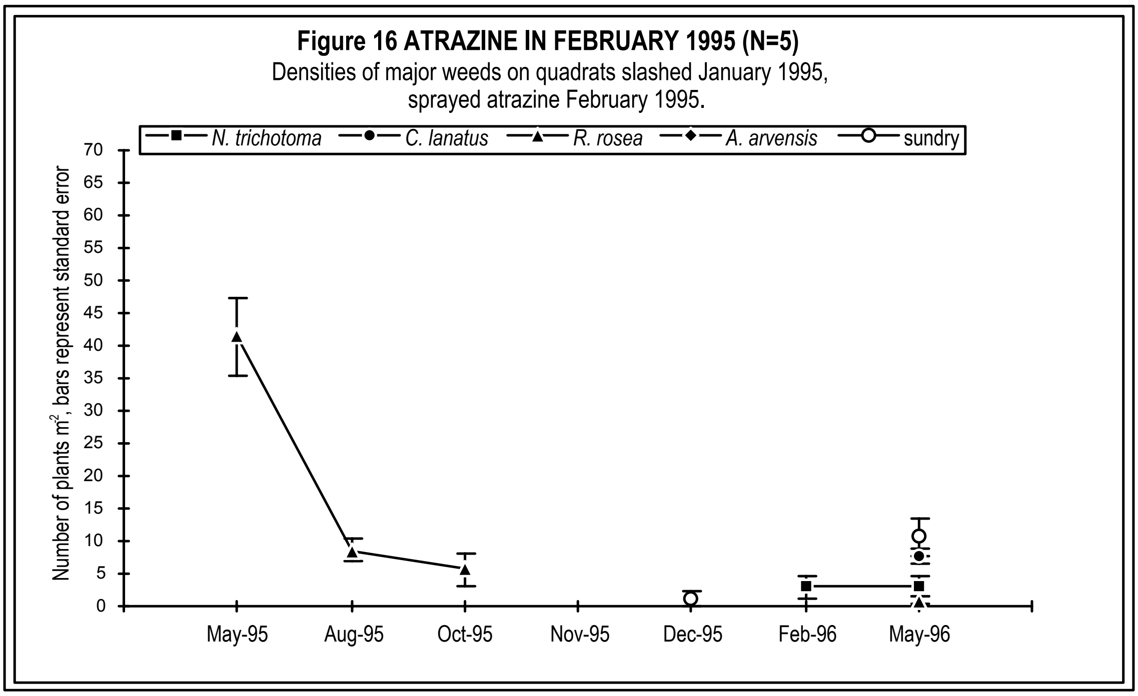
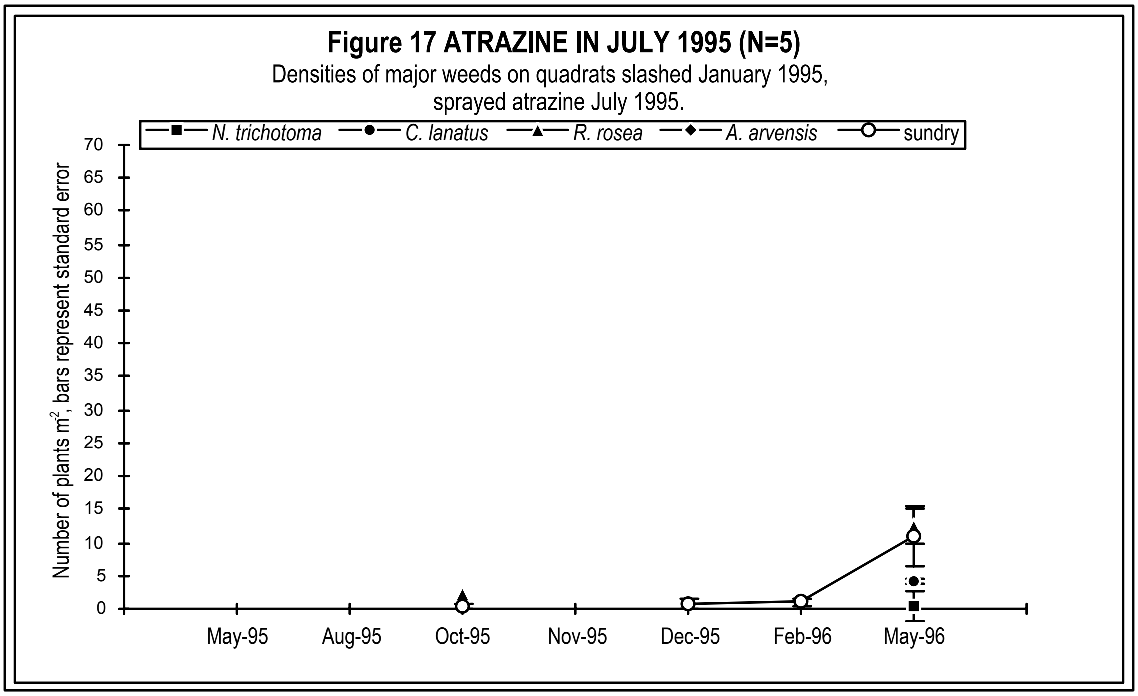


Figure 15 GLYPHOSATE IN OCTOBER 1995 (N=5)
Densities of major weeds on quadrats slashed January 1995, sprayed glyphosate October 1995.
N. trichotoma
C. lanatus
R. rosea
A. arvensis
sundry
Number of plants m2, bars represent standard error
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
May-95
Aug-95
Oct-95
Nov-95
Dec-95
Feb-96
May-96





2.5 Discussion

Each of the herbicides atrazine and glyphosate killed all mature *N. trichotoma* plants on all plots when applied across a range of seasons and years. Flupropanate also killed all mature *N. trichotoma* plants in each of the two application seasons tested (in February and June) as well as when applied in winter (June or July) of two separate years. It would appear that the removal of mature *N. trichotoma* tussocks from indigenous grassland remnants on the Keilor Plains is relatively easy, using any of the three herbicides tested at the label strength recommended for back-pack use and application rates commonly employed in conservation contexts (i.e. foliage sprayed until fully wet). The experience of other land managers of indigenous grassland remnants on the Keilor Plains is consistent with these findings. The key issues for control of *N. trichotoma* in western (basalt) plains indigenous grassland remnants therefore focuses on which species of weed seedlings (including *N. trichotoma*) re-establish on sites cleared of *N. trichotoma* by herbicides, and how re-invasion by weeds can be minimised in the short-term and long term. For a fuller discussion of these issues, see Hocking (1998).

2.5.1 Effectiveness of Atrazine

Of the three herbicides tested, atrazine appears to have provided the best outcomes for the removal of, and mid-term protection from, *N. trichotoma* and the range of other weed species commonly encountered in lowland indigenous grassland contexts. In the ten months following atrazine application in each of the years 1993 and 1995, all mature *N. trichotoma* plants died, and recruitment of seedlings from the seedbank was minimal (Figures 4, 16 and 17). Recruitment of other common indigenous grassland weeds such as *P. echioides*, *C. lanatus*, *C. vulgare*, *R. rosea* and various annual grasses and other weeds were also minimal across the assessment period following atrazine application. These result were obtained in two different years when atrazine was applied in July (Figures 4 and 17) and in one of the years when atrazine was also applied in February (Figure 16).

R. rosea made a brief appearance in high densities in May 1995 following the February application of atrazine, but was controlled across the following ten months and into May of the following year. *R. rosea* is a perennial geophyte that resprouts from underground bulbs during late autumn and early winter. It is likely that the atrazine application in July was too late in the growth cycle of *R. rosea* to be absorbed in the months immediately following application.

However, it also appears likely that atrazine at the rates applied was still present in sufficient residual concentrations in the soil in the following year to kill off most of the *R. rosea* sprouting in the autumn of 1996.

The action of atrazine in providing long term protection from weeds of sites cleared of *N. trichotoma* is no doubt due in part to its residuality in the soil. In addition to its obvious benefits, residuality can also be a disadvantage when attempting to restore natural grassland communities. This issue of the use of herbicides in the replacement of *N. trichotoma* with *T. triandra* is discussed in Chapter 4.

In addition to the benefits of providing a long weed-free window of opportunity for establishment of competitive indigenous vegetation to replace *N. trichotoma* (Hocking 1998), atrazine has the potential advantage of being selective for species with the C_3 photosynthetic pathway. It would therefore be particularly suitable where there is a need to remove *N. trichotoma* from amongst C_4 indigenous grasses – these include: *Themeda triandra*; *Bothriochloa macra*; and *Dichanthium sericeum*, or where there is a need to replace *N. trichotoma* with these species during revegetation (Chapters 4 and 5).

2.5.2 Effectiveness of Glyphosate

Glyphosate applied in autumn, winter or early spring appeared to be effective in removing mature *N. trichotoma* and maintaining low weed densities on treated sites during the following spring period (Figures 5, 10, 13 - 15). By comparison, glyphosate applied in summer (Figure 12) or early autumn (Figure 10) resulted in high densities of both *N. trichotoma* seedlings and other weeds in the following spring. Although not consistent with glyphosate being described as a foliar absorbed herbicide with no soil-borne residual capacity for uptake through root systems (Parsons 1992), one explanation for these results is that glyphosate applied (at label strength and backpack rates) in summer or early autumn is washed out of the soil by autumn and winter rains, and is not at sufficient levels to provide protection from weeds in spring. By comparison, glyphosate applied in May or later was probably still at sufficiently residual levels in the soil at the onset of seedling growth in the following spring to prevent major weed seedling establishment. *N. trichotoma* observed in these trials were individual seedlings and not vegetative recovery from existing tussocks.

It is also likely that the outcomes of application of glyphosate across the summer-autumn period would be affected by the extent of rainfall in autumn and spring in any particular year, as evidenced by the different densities of weed establishment on plots treated with glyphosate in July 1994 (Figure 9) and July 1995 (Figure 14). The lack of effectiveness of glyphosate in preventing establishment of *N. trichotoma* and other weeds by the autumn following application (Figures 5, 9, 10, 12 - 14) and the comparative success of the October application in suppressing weed growth in the following autumn (Figure 15) are additional evidence that the level of residuality of glyphosate in the soil is the key factor in determining how successfully weed establishment is suppressed in the seasons following application. It is also likely that, at other sites, the particular characteristics of soil would affect the rates of leaching and biological break-down of glyphosate, and hence its effectiveness in providing a weed-free window in the months following application.

2.5.3 Effectiveness of Fluproponate

The application of fluproponate in winter prevented any major establishment of *N. trichotoma* seedlings for at least 9 months (Figure 6), but resulted in the growth of high numbers and biomass of the weeds *P. echioides*, *C. vulgare* and *C. lanatus* in the following spring. The mid-term protection from *N. trichotoma* seedling establishment afforded by flupropanate suggests that it is residual at relatively high levels for long periods in the basaltic clay soil. However, it is also apparent that the weed species that grew in high densities on fluproponate treated sites in spring and autumn following treatment are resistant to the effects of fluproponate.

P. echioides, *C. vulgare* and *C. lanatus* are all significant and intractable weeds in degraded indigenous grassland remnants. The promotion of *P. echioides* establishment and growth by the application of fluproponate is of special concern, as it is one of the more difficult weeds to remove from lowland grassland remnants by either herbicide treatment, burning or grazing. In their assessment of Derrimut and Laverton North grassland reserves after 10 years of management, Lunt and Morgan (1998) found that *P. echioides* and *C. vulgare* were a persistent problem in unburnt or infrequently burnt, senescing areas of *T. triandra*.

From the outcomes of the trials reported above, it would appear that the use of flupropionate for *N. trichotoma* control in indigenous grassland remnants is not advisable. Flupropionate application clearly promoted the establishment of unwanted and intractable weeds in the indigenous grassland remnants in the St Albans trials. Flupropionate has other detrimental effects also when used for weed clearance during revegetation with *T. triandra*, which will be discussed in Chapter 4. This work shows that residual soil concentrations of flupropionate in the spring and summer following winter application adversely affect revegetation efforts to establish *T. triandra* as a competitive replacement cover, because *T. triandra* seedlings are killed by flupropionate, and the levels of cover by broadleaf weeds during revegetation hinder growth of *T. triandra* seedlings.

It is possible that lower rates of flupropionate than used in this study might provide effective removal of *N. trichotoma* from indigenous grassland remnants in some circumstances. Flupropionate has been reported to be selective for *N. trichotoma* seedlings establishing amongst several indigenous grass species when used at low rates (Campbell 1998). This possibility is currently under investigation by Victoria University at the St Albans campus grassland reserve. It is difficult however to conceive how flupropionate could be used for replacement of *N. trichotoma* with indigenous grasses, as lower levels of flupropionate than used in this study are still likely to allow rapid, large-scale invasion by *P. echioides*, *C. vulgare* and *C. lanatus* if seeds of these species are present in the seedbank.

Effects of mowing and removing treatment in early March 1994 had a significant effect on the appearance in the following spring and autumn of *C. lanatus* and *R. rosea* on plots (Figure 8) compared with unmown controls (Figure

7). This suggests that the appearance of these weeds is affected by competition from light. The effects of mowing on the availability of below ground resources such as nutrients and water cannot be ruled out because above ground growth and below ground resource utilisation are closely linked. However, it is likely that any major effects of mowing, such as the stimulation of growth of mature *N. trichotoma* plants, would have tended to reduce nutrient and water availability in the soil and therefore the potential of weeds other than *N. trichotoma* to grow. The effects of mowing on the seedbank were not determined but were likely to be minor at the time the mowing was carried out (ie. after seed drop). A large number and diverse range of weed seeds and below ground storage organs were likely to have been present in the seedbank, as evidenced by the rampant growth of *V. bromoides*, *H. lanatus* and *R. rosea* following application of glyphosate in late March (Figure 10).

The mow and remove treatment also had no significant effect on *N. trichotoma* densities, which were slightly lower at around 40 plants m^{-2} in mow and collect plots (Figure 8) than on untreated plots, which recorded densities averaging around 45 plants m^{-2} (Figure 7). This suggests that the density of *N. trichotoma* plants established on plots was not determined as much by competition for light as by competition for below ground resources (for a discussion of these issues, see Wijesuriya and Hocking 1999).

3. Harvesting and assessing *Themeda triandra* seed-bearing hay for revegetation

3.1 Summary

A standard method for assessing the germinable seed content of seed-bearing kangaroo grass (*Themeda triandra* Forrsk.) hay was devised and tested. This method assessed the accuracy of methods used to predict the variation in quantity and germinability of seed in *T. triandra* hay harvested immediately prior to seed fall. The seed content of hay harvested from a range of remnant populations of *T. triandra* on the Keilor Plains, as well as the extent of seed shedding from the hay and rates of germination were found to be highly variable across sites and between years. Seed content of hay harvested in two consecutive days across two remnant areas in 1995 varied by up to two-fold within sites and eight-fold between sites. Percentage germinable seed from hay harvested in 1995 was about double that from hay harvested in 1994. Germination trials on seed from stored hay samples demonstrated a progressive increase in the rate of seed germination up to double initial levels over nine months. This increase suggests significant dormancy for samples from some sites. Trials also revealed an increase in the quantity of available seed with increased time of storage, suggesting that seed drop (*T. triandra* seed per grams of hay), increases progressively post-harvest while *T. triandra* hay is in storage. The implications for revegetation projects of the variability in germinable seed content of *T. triandra* hay harvested from different localities, and the increase in seed availability and germinability with storage, are discussed.

3.2 Introduction

Over the past two decades there has been a marked increase in the use of direct seeding methods for repair and restoration of the ground cover of indigenous grassy ecosystem remnants in Australia (Kilby *et al.* 1993, Sindel *et al*. 1993, Stafford 1991, Hitchmough *et al.* 1989, Hagon and Chan 1977, Hagon 1977, Hagon and Groves 1977, Hagon 1976) and overseas (for example: Knapp and

Rice 1994, Jacobson *et al*. 1994, Wells *et al*. 1993, Wells T. C. E. 1989, Zajicek *et al.* 1986). The most extensive investigation of direct seeding methods for restoration of *Themeda triandra* dominated grasslands was undertaken by McDougall (1989) who looked at levels of *T. triandra* seed production, ripening and availability of seed in samples harvested from remnant sites in Victoria and how these influenced subsequent trials involving *T. triandra* seedlings during restoration. McDougall (1989) reported success in establishing *T. triandra* seedlings on restoration sites using seed-bearing *T. triandra* hay. However, the results arising from the method were not highly reproducible. It is noted here that Zacharek (1994) investigated plant division and direct planting of *T. triandra* tillers into sites treated with herbicide or with topsoil scalped. This study, located in the Tasmanian Midlands, reported limited success in re-establishing *T. triandra* using these methods. Other studies of *T. triandra* seed have looked at percentage germination and the factors that influence this (Sindel *et al*. 1993, Hagon and Groves 1977, Hagon 1976) and dormancy in *T. triandra* seed has been reported by Sindel and Groves (1991) and Sindel *et al.* (1993). Groves *et al.* (1982) and Hagon (1976) documented various levels of dormancy of stored *T. triandra* seed, as measured by increases in percentage germination with increasing storage time. The extent of dormancy of seed harvested from various sites around Australia was found to vary, with a general trend of high dormancy in seed from northern Australian latitudes (New South Wales and Queensland) and low or negligible dormancy in seed harvested from southern states (Victoria and Tasmania). Hagon (1976) reported that dormancy appeared to be imposed by the lemma and was relieved by either gibberellin acid treatment or extended storage, with the decrease in dormancy being more rapid at higher storage temperatures (up to $64^{o}/24^{o}$ C). McDougall (1989) found no detectable continuation in development of *T. triandra* seed from, and up to six weeks after harvest – although he also noted reservations about the methods used to document this.

The overall aim of the work reported in this chapter was to clarify key characteristics of *T. triandra* seed production and germinability which might have an effect on the use of seed-bearing *T. triandra* hay in direct seeding revegetation trials of the type pioneered by McDougall (1989). Investigations were designed and carried out to determine:

- how the seed content in *T. triandra* hay (expressed as seeds kg hay) varied within each harvest site and between harvest sites on the Keilor Plains,
- how seed germinability (expressed as a percentage germination rate under laboratory conditions) varied for *T. triandra* seed derived from hay collected in different years,
- what level of dormancy was exhibited by *T. triandra* seed collected from a range of sites and locations within sites,
- whether the volume of *T. triandra* seed shed from harvested hay (i.e. rate of seed dehiscence) changed with time of storage.

3.3 Materials and methods

In the methods and results described below, the term 'available seed' of *T. triandra* is defined as seed with glumes coloured brown through to coal brown which had dehisced from the floret (with callus and awn remaining intact). Seeds were removed from the hay samples by thinly spreading each sample on a laboratory bench, vigorously shaking by hand and collecting each free seed.

The mean and standard error were calculated for results and are depicted in the Figures 18-22. Single or two factor analysis of variance (ANOVA) using Microsoft Excel 5.0 for Macintosh with confidence intervals set at 0.05 were used to determine whether differences between treatments or sampling regimes were significant and the outcomes are reported in the results and discussion section 3.4.

3.3.1 Harvesting seed-bearing *T. triandra* hay

The seed-bearing *T. triandra* hay used in all three trial years was harvested with a sickle bar mower set to cut the hay at approximately 200 mm – 250 mm below the lower seed spikelets. The cut hay, including leaf chaff and flower stalks, was gathered by hand and packed loosely into wool bales in the field (each approximately 1.2 m^3 in volume). Wool bales were then transported to weatherproof storage prior to use in *T. triandra* revegetation trials. Time of harvest was determined when field inspections of spikelets revealed seed dehiscence had commenced. Due to the asynchronous nature of *T. triandra* seed maturation the early phase of seed shedding was used as the indicator for optimum time of harvest. McDougall (1989) suggests that 30% – 40% seed dehiscence can be used as an indicator for optimum time of harvest of seed-bearing *T. triandra* hay.

3.3.2 Sampling seed-bearing hay

All hay samples used to investigate seed availability and germinability parameters weighed 38.5 g which represented 5% of the 770 g measure of hay applied as thatch to *T. triandra* revegetation trial plots (see Chapters 4 and 5).

1994 - 1995 Hay harvest and sampling for seed trials

Seed-bearing *T. triandra* hay was harvested from a remnant *T. triandra* grassland on the Western Highway, Rockbank, six kilometres west of the trial site in mid January 1994 and stored in a non-air-conditioned, unlit storage facility in Laverton. In June 1994, September 1994 and December 1994 two 38.5 g hay samples were collected from each of five wool bales of non-homogenised hay, the bulk of which was to be used in revegetation trials (see Chapter 4). This represented ten random hay samples at each of the three sampling intervals. Available seed was removed from each sample (see section 3.3). Hence germination and seed availability rates were assessed at six, nine and twelve months after harvest.

1995 - 1996 Hay harvest and sampling for seed trials

Seed-bearing *T. triandra* hay was harvested from remnant *T. triandra* grasslands on the Maribyrnong Escarpment (Maribyrnong River, North Sunshine) four kilometres south-east of the trial site, and the Slough Estate (Princes Highway, Laverton) three kilometres south of the trial site. Harvesting took place on two consecutive days early in March 1995. Bales of hay were stored in a cool, dark room without air-conditioning at Victoria University, St Albans. In April 1995 five hay samples were drawn from all stored wool bales and assessed for available seed (Figure 18). Data from this assessment informed the selection of hay to be used in revegetation trials (Chapters 4 and 5). The highest quality seed-bearing *T. triandra* hay from Slough Estate, Laverton, was selected for revegetation trials, laid out on a concrete floor and mixed together in May 1995 (see Chapter 4 for details). At this time 38.5 g samples were drawn randomly from the homogenised hay for use in the seed availability and germinability trials (Figures 19, 21, 22). In total fifty hay samples were taken and randomly allocated into two equal lots.

<u>1995 - 1996 Seed availability and germinability - seed maintained in hay until tested for germination</u>

Twenty-five hay samples (from homogenised hay) were randomised and then stored in groups of five under the same storage conditions as the hay used in *T. triandra* revegetation trials. Seed was removed from each group of five samples at the time that germination rates were determined; in May 1995, July 1995, October 1995, December 1995 and February 1996. At the time when the seed was separated from hay for germination trials, the number of separating seeds was counted and used to assess changes in seed availability over time of storage (Figure 19). Seed for this trial was stored in hay samples in a darkened environment at temperatures ranging from 10^0 Celsius to 35^0 Celsius

<u>1995 - 1996 Seed removed from hay two months after harvesting and stored as separated seed prior to germination trials)</u>

In May 1996 the available seed was removed from the remaining twenty-five hay samples taken from the homogenised hay, randomised and then packaged and stored in twenty five paper bags containing equal numbers of seed. Five of these packets were randomly selected and used for germination trials at each of the following times; May 1995, July 1995, October 1995, December 1995 and February 1996. Seed for this trial was stored as separated seed (dehisced (free) seed collected and awns removed) in a darkened environment at a consistent room temperature of 19^0 Celsius.

3.3.3 Germination trials

For germination trials, the awn of each seed was removed and inserted callus down into (non-nutrient) bacteriological agar[1]. Seeds were inserted to approximately 33% of the overall length of seed (callus plus glumes). The depth of agar in the Petri dishes was three times the overall seed length.

The callus and part of the endosperm was inserted to increase the potential for seeds to imbibe moisture. In the field *T. triandra* seed is driven by its terminal, hygroscopically activated awn into micro-sites (Stafford 1990) which, it is speculated, also increases moisture contact and potential for imbibition during rain.

Petri dishes with plated seeds were placed in a (Thermoline®) germination cabinet illuminated with four 25 watt fluorescent grow lights for twenty-one days. After preliminary trials to determine the optimum conditions for germination of *T. triandra* seed (using the results of Hagon 1977 as a guide), the germination cabinet was set as follows:

- light 12 hours at 30^0 Celsius,
- dark 12 hours at 20^0 Celsius.

Deionised distilled water was added to saturation levels to agar plates showing signs of desiccation at seven and fourteen days after each trial commencement. After twenty-one days in the germination cabinet, trials were ended and all germinated seed counted. Seeds were counted as having germinated if they showed both radicle and plumule emergence. NOTE: In all trials the first radicles were observed at day two, and over 90% of all germinating seed had initiated both radicles and plumules by day fourteen.

3.4 Results and Discussion

3.4.1 Seed availability

Seed availability within and between harvest sites

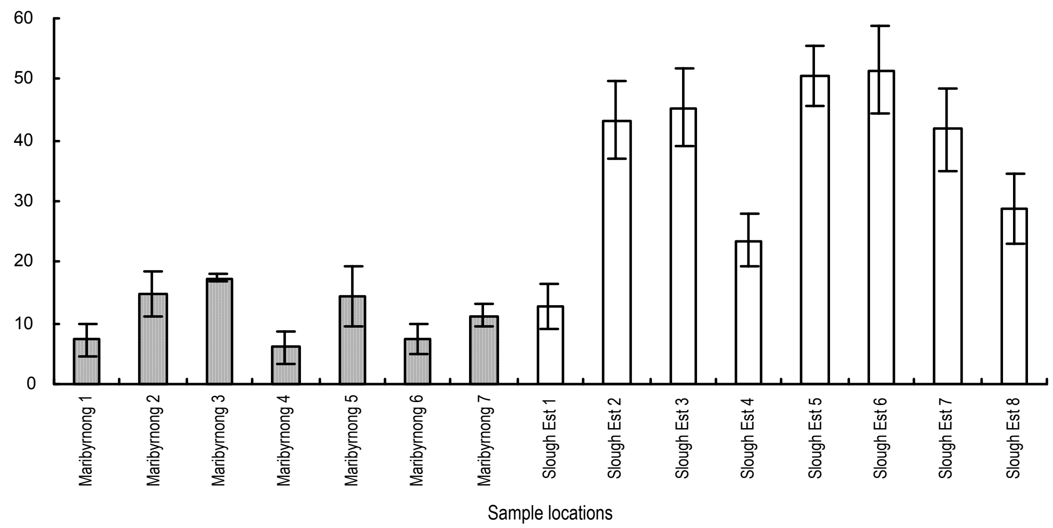
[1] Bacteriological agar prepared as follows: 6.5 grams Oxoid® bacteriological agar combine with one litre deionised water, autoclave agar mix at 120^0 Celsius for 15 minutes, pour 45 millilitres agar mix per Petrie dish in a lamina flow cabinet.

There were highly significant differences in average available seed numbers between hay samples from individual wool bales from each of the Maribyrnong Escarpment and Slough Estate harvest sites. The lowest average available seed from an individual hay bale from the Maribyrnong Escarpment was 6.0 seeds per 38.5 g whilst the highest average available seed, from another hay bale, was 17.4 seeds per 38.5 g hay. The lowest average available seed per bale from Slough Estate was 12.6 seeds per 38.5 g hay whilst the highest average available seed per bale was 51.4 seeds per 38.5 g hay (Figure 18). Although a range in averages existed in the Maribyrnong Escarpment samples, a one-factor ANOVA in data revealed no significant statistical difference between sample means ($p=0.17$). A one-factor ANOVA of Slough Estate data revealed that a significant difference existed between sample means ($p<0.05$). A two-factor ANOVA (with replication) showed a significant difference between overall sample means of the two harvest sites ($p<0.05$). The variability of seed from different localities and years was raised as an issue by McDougall (1989), but the extent of variability documented in this thesis was not realised in earlier investigations. The extent of variability within a harvest site (up to two fold in this study) has not previously been raised as an issue, and has significant consequences for revegetation programs which use *T. triandra* seed-bearing hay (see section 3.5).

Figure 18

1995 Assessment of available seed (N = 5)

Hay harvested March 95. Samples drawn, seeds separated and counted April 95.



Changes over time in seed availability in stored seed-bearing hay

Hay of *T. triandra* harvested in March 1995 and assessed for available seed in May 1995 two months after harvest revealed an average of 37.5 available seed in 38.5g. hay. The amount of available seed from stored hay then increased significantly (linear regression $p<0.03$) over the following nine months (see Figure 19) to be:

- 46.4 available seed 38.5 g. hay four months after harvest,
- 49.6 available seed 38.5 g. hay seven months after harvest,
- 50.8 available seed 38.5 g. hay nine months after harvest and
- 55.6 available seed 38.5 g. hay eleven months after harvest

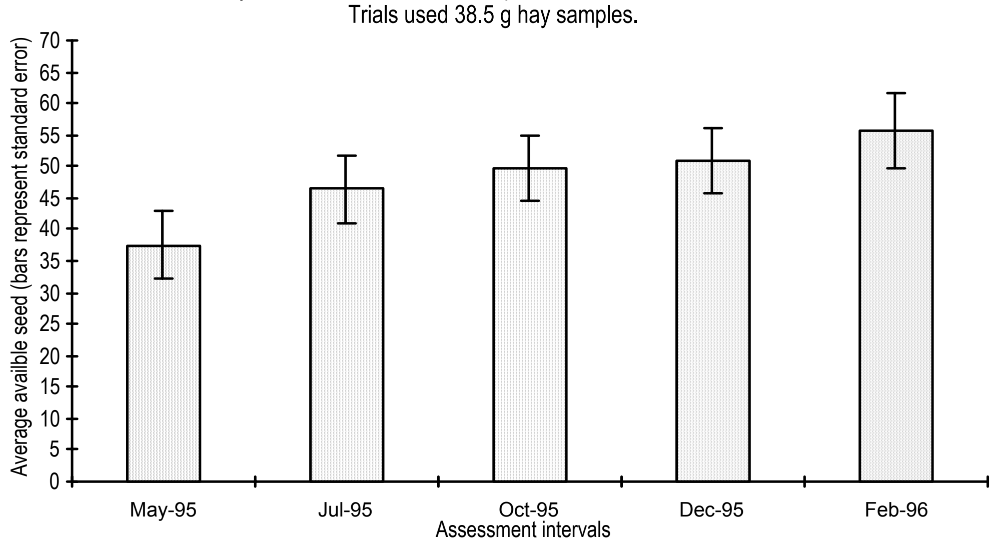
The increase in available seed in *T. triandra* hay with storage time is likely to have significant consequences for revegetation programs which use *T. triandra* seed-bearing hay – see section 3.5. The pattern of seed availability of hay immediately placed onto revegetation sites after harvest is unknown, and needs to be investigated. On-site conditions of temperature and moisture could affect seed dehiscence (Hagon and Chan 1977) and consequently availability in either positive or negative ways. Seed harvesting by animals on-site could also significantly affect the availability of seed for germination in revegetation where hay is layed out soon after summer harvesting.

Figure 19

1995 assessment over time of available seed in *Themeda triandra* hay (N=5)

Hay harvested March 1995 samples stored until assessment.

Trials used 38.5 g hay samples.



3.4.2 Germination rates

Initial germination rates and standard errors of available seed from hay harvested in 1994 and 1995 and homogenised were as follows:

- Hay harvested in 1994: 15.9 (±1.2) % (assessed in June 1994, 5 months after harvest),
- Hay harvested in 1995: 29.5 (±5.2) % (assessed in May 1995, 2 months after harvest).

Germination trials revealed an increase in germination rates over time with seed in hay stored after harvest. For seed in hay harvested from Rockbank in 1994 and stored in the hay (i.e. seed separated at the time of assessment) percentage germinability of available seed was 15.1% five months after harvest (Figure 20). This increase in germinability continued so that eight months after harvest the germination rate had increased by approximately 10% five months after harvest to 25.8%. At eleven months after harvest germinability then levelled off to be 26.1%, 11% and significantly higher than initial germination rates five months after harvest (single factor ANOVA $p<0.02$) (Figure 20).

Hay harvested from Slough Estate in 1995 showed an overall percentage germinability of available seed of 29.5% two months after harvest. In the germinability assessment where seed was separated from hay samples at the time of trials, this increased to 31.2% four months after harvest, then 46.1% seven months after harvest, followed by 51.4% nine months after harvest and finally 57.1% eleven months after harvest. This represented a significant increase (single factor ANOVA $p<0.000004$) in germinability over nine months of storage (Figure 21). In the trial where seed was separated and stored at the time of hay sampling, percentage germinability increased from the shared 29.5% baseline percentage two months after harvest to 30.6% of available seed four months after harvest. By seven months after harvest the percentage germinability had further increased to 51.5%. There was a marginal decline to 50.0% germinability of available seed at nine months after harvest with the final assessment eleven months after harvest showing germinability rate increasing to 53.0%. This also represented an overall significant increase (single factor ANOVA $p<0.00027$) in germinability of *T. triandra* seed over nine months of storage (Figure 22).

The significant increase in germination rates with storage time of *T. triandra* seed harvested from basalt plains grassland remnants in the St Albans region of Melbourne (progressive increase from 15.1% to 26.1% over five months in 1994 [single factor ANOVA $p<0.02$]; progressive increase from 29.5% to 57.1% over nine months in 1995 [single factor ANOVA $p<0.000004$]) are indicative of dormancy operating in *T. triandra* seed which is relieved with storage of the seed. Dormancy and relief of dormancy appears to be independent of whether the seed is stored within the hay, or separated and stored. Significant increases in germination rates were observed both in seed that had been stored within hay samples, 29.5% increasing to 57.1% germinability (single factor ANOVA $p<0.000004$) (Figure 21) and seed that had been stored after being separated from hay samples (awns removed), 29.5% increasing to 53.0% germinability (single factor ANOVA $p<0.00027$) (Figure 22). The apparent dormancy in *T. triandra* in southern Victorian populations of the species is contrary to the general findings of Hagon (1976) and suggests that seed dormancy might vary more widely between sites in the one geographic region and between years than previously believed. This is worthy of further investigation.

The discovery of dormancy indicates that storage of *T. triandra* seed for a period approximating at least eight months after harvest is likely to be advantageous to *T. triandra* revegetation projects (see section 3.5). However, what happens to seed dormancy in hay distributed on revegetation sites immediately after harvesting also requires further consideration.

Figure 20

1994 *T. triandra* percentage seed germination over time N=10

Seed in hay harvested summer (Jan), and stored in dark at temperatures ranging from 10º - 35º celsius. Samples drawn and seed separated at time of trials

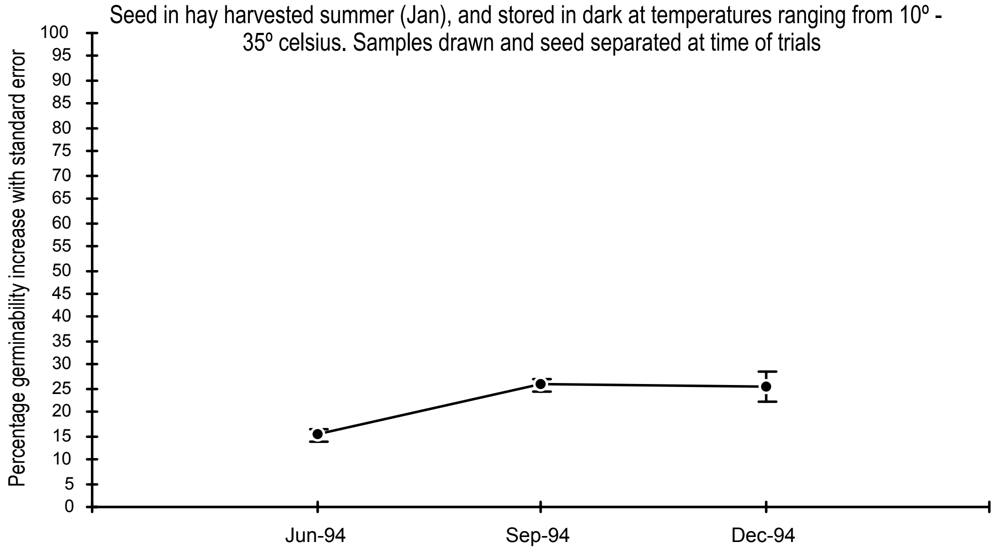
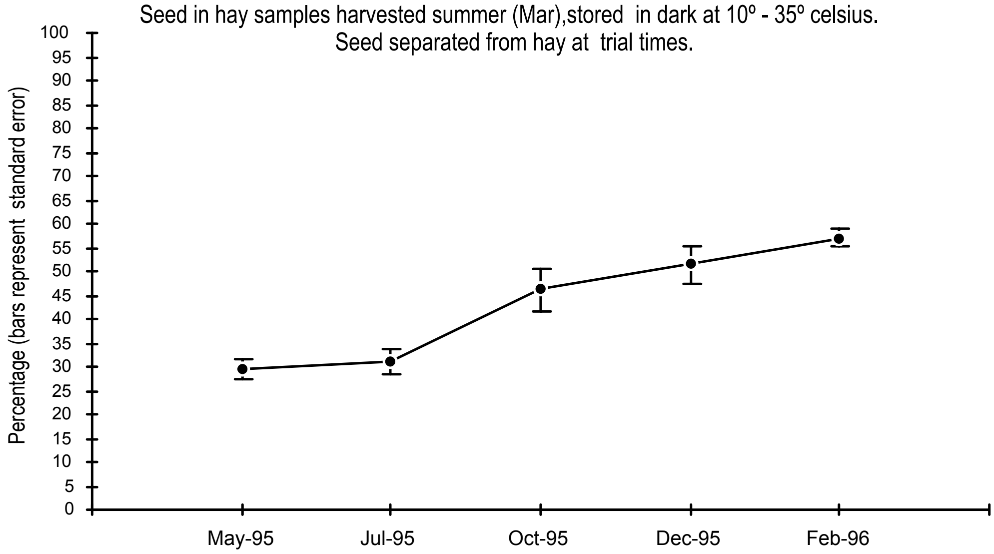
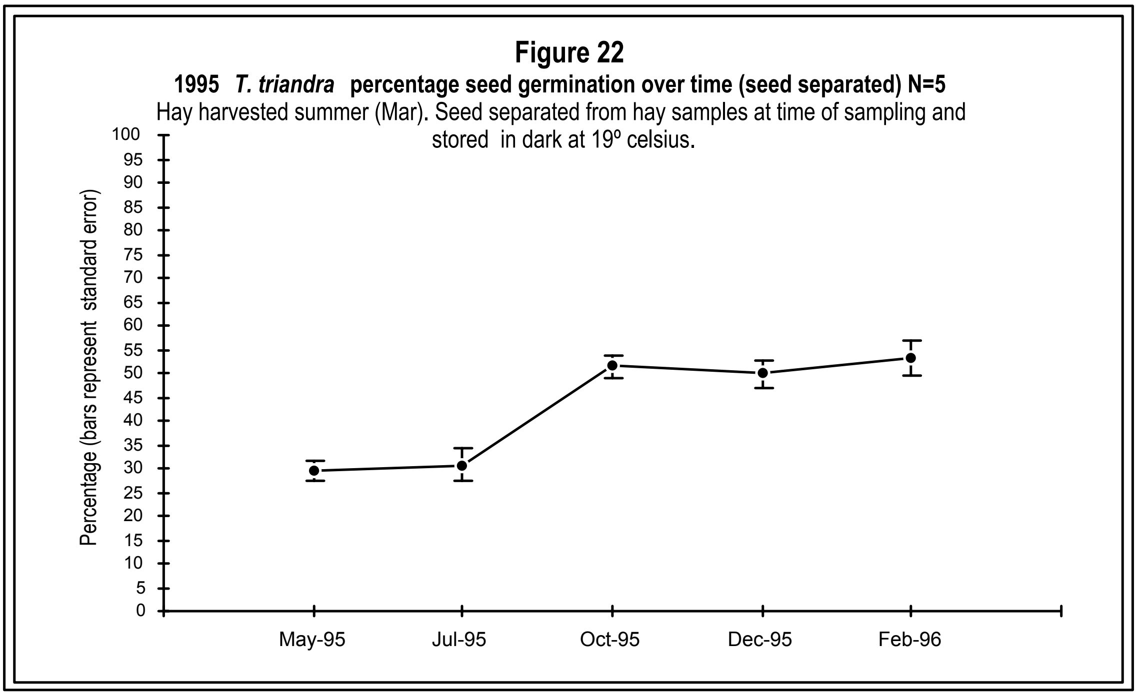


Figure 21

1995 *T. triandra* percentage seed germination over time (seed in hay) N=5

Seed in hay samples harvested summer (Mar),stored in dark at 10º - 35º celsius. Seed separated from hay at trial times.





3.5 Implications for Revegetation of Hay Seed Quality

Seed characteristics such as the amount of available seed and its germinability are likely to affect, in major ways, the success of programs to re-establish *T. triandra* using seed-bearing *T. triandra* hay, such as the 'spray and hay' method (Chapters 4 and 5).

Investigations showed that *T. triandra* seed germinability increased with increased storage time. The assessment of available seed from hay harvested in March 1995 showed the possibility for major and minor differences in seed production rates within and between sites in one season (Figure 18). Trials reported here also demonstrate that florets continue to dehisce available seed while hay is in storage after harvest.

Based on germination and seed drop data produced by the above trials it is likely that *T. triandra* restoration projects will be significantly advantaged if hay were to be stored for at least eleven months before thatching is carried out. Anecdotally, it has been the practice of people attempting this method to lay hay soon after harvest. Trials *in situ* (i.e. under field conditions) to determine variation in the amount of available seed over time in the field, as well as effects of predation on seed from thatch over time would be a valuable comparison to the laboratory studies reported here.

Results from these trials demonstrated how important it is to carry out a thorough assessment of seed production rates and germinability percentage as an early element in the 'spray and hay' methodology. Without accurate knowledge of the seed characteristics of the thatch used, *T. triandra* re-establishment projects will be destined for an uncertain outcome.

4. Replacing *Nassella trichotoma* with *Themeda triandra* using the 'spray and hay' method (1): Effect of herbicide type, seed quality and hay removal technique

4.1 Summary

A 'spray and hay' method was developed to introduce kangaroo grass (*Themeda triandra* Forrsk.) seedlings onto sites previously dominated by serrated tussock (*Nassella trichotoma* (Nees) Arech.). Serrated tussock was previously removed by herbicide application. Field trials over three years demonstrated that *T. triandra* swards can be established with predictable densities, with very low densities of *N. trichotoma* seedlings. The method utilised seed-bearing *T. triandra* hay harvested from adjacent grassland remnants which was laid down approximately three months after slashing and herbicide treatment of the revegetation site. Hay was subsequently removed, by either burning or physical removal, about three months after laying. Laboratory-based assessments of *T. triandra* seed characteristics and field studies of the effectiveness of herbicide type on *N. trichotoma* were used to refine the 'spray and hay' procedure. Field results suggest that improved seed availability and germinability occurs when seed-bearing *T. triandra* hay is stored for up to eight months after storage (harvest summer, store until the following spring). Both atrazine and glyphosate were found to be suitable herbicides in the revegetation process, although atrazine produced lower numbers of *N. trichotoma* seedlings and higher numbers of *T. triandra* seedlings across a range of specific treatments. Flupropanate was not a suitable herbicide to use at backpack rates due to apparent residual levels in the soil which was lethal to many of the emerging *T. triandra* seedlings. A comparison between *T. triandra* seed characteristics determined prior to revegetation and on-site seedling densities found that the conversion rate of germinable seed into seedlings in the field was around twenty percent.

4.2 Introduction

The herbicides glyphosate, atrazine and flupropanate have been successfully used to remove *Nassella trichotoma* in lowland indigenous grassland remnants and create revegetation opportunities for *Themeda triandra* (Chapter 2). Revegetation to establish effective cover of *T. triandra* seedlings using seed-bearing *T. triandra* hay has been shown to be possible on sites cleared of weeds (McDougall 1989) – this revegetation procedure will be referred to herein after as the 'spray and hay' method. As has been discussed in section 1.4, questions remained about the reproducibility of the method and specific conditions required for optimum effect and post-establishment control of weeds. In addition the seed content of *T. triandra* hay harvested from indigenous grassland remnants can vary widely within sites, between sites and between years of harvest (Chapter 3), with potentially major consequences for revegetation projects using the 'spray and hay' method. If the method is to be used successfully, there is a need to determine the optimum conditions for revegetation, and in particular, how the type of herbicide used for weed removal, the seed content of *T. triandra* hay, and the method for removal of hay (burning or physical removal) affect *T. triandra* seedling densities and associated weed invasion. The results of investigations of these parameters over three years are reported in this chapter. Additional investigations of the effects of season of application of the 'spray and hay' method are reported on in Chapter 5.

4.3 Materials and methods

4.3.1 Trial site and quadrat parameters

The trials were carried out in the Victoria University (St Albans) indigenous grassland conservation reserve which has *T. triandra* as the dominant indigenous tussock grass in surviving remnant areas, with *N. trichotoma* scattered across the site. *N. trichotoma* is also dominant in areas where soil disturbance (ploughing, sheep encampments) has occurred (Chapter 1).

Quadrats in all three years of trials were set up in areas with full *N. trichotoma* cover. Treatment quadrats measured 1.3 m x 1.5 m (1.95 m^{-2}), with the central 1.0 m x 1.0 m (1.0 m^2) area designated for data collection, as a way of minimising 'edge effects' (Chapter 1). These dimensions also allowed for maximum access for inspection from the sides of the plots while minimising disturbance from foot traffic, and provided a total treatment area approximating 2.0 m^2 which simplified calculations for applying herbicide, laying of hay rates and related operations.

4.3.2 Harvesting *T. triandra* hay

Seed-bearing *T. triandra* hay was harvested using a sickle-bar mower from nearby indigenous grassland remnants and packed loosely in wool bales. These bales were stored in either a control temperature room at around 27°C (year 1 and year 3) or in a garage lock-up (year 2) (Chapter 3).

4.3.3 Quadrat treatment

Slashing or burning as a first treatment

Initial *N. trichotoma* biomass reduction for 1993-1994 trial sites was by an incidental wildfire that burnt the conservation reserve in February 1993. Additional *N. trichotoma* biomass reduction on the 1994-1995 trial site was carried out by slashing with a domestic lawn mower in March 1994, producing a *N. trichotoma* stubble averaging 80 mm in height. A similar slash treatment was undertaken on the 1995-1996 trial site in January 1995.

Herbicide application

Herbicide control of weed regrowth was carried out in July of each of the trial years. Weed control herbicides were prepared at label recommended strengths for back pack application:

- atrazine (as Nufarm Nutrazine®) 130 ml : 15 L,
- glyphosate (as Monsanto Roundup®) 150 ml : 15 L,
- flupropanate (as ICI Frenock®) 30 ml : 15 L (used in year 1 trials only).

Each of the herbicides was applied using a manually operated pressure pump attached to a 15 litre backpack spray unit and calibrated for a standard delivery of a set volume to *N. trichotoma* regrowth following initial biomass reduction by slashing (Chapter 2). Each herbicide was applied at rates common for weed control in indigenous grassland remnants, which equated to approximately five times higher than for broad-acre agricultural treatments (Chapter 2).

Thatching with seed-bearing *T. triandra* hay

Seed-bearing *T. triandra* hay was laid down as thatch on trial plots in September. For consistency and comparability of data, all trials in all years used 770 grams of hay m^{-2} as a single volume thatch applied to each 2 m^2 quadrat. During the trials however, it became clear that, because the seed content of the hay was highly variable from year to year (Chapter 3) the actual amount of seed added with the hay was very different between years. In years 2 and 3, just prior to thatching in spring, the hay was removed from the wool bales into which it was packed at harvest, layed out indoors on a concrete floor, thoroughly mixed by hand, and returned to wool bales for transport to the trial site. Any loose seed was distributed evenly amongst the hay across wool bales. On site, thatch was held in place using Cyclone® anti-bird netting with 25 mm x 25 mm diamond mesh, anchored over each quadrat with six standard 175 mm tent pegs.

T. triandra thatch removal

Thatch removal was carried out in December of each trial year using either burning or manual removal. In the manual removal technique the hay was pulled by hand off each site and packed into bags for extraction from the trial site. Plots which had hay removed by hand were left with a covering of dead *N. trichotoma* stubble.

The burning removal of hay employed the use of a steel burn box to contain the fire precisely to the quadrat to be treated. The burn box was constructed using 20 gauge steel. Burn box dimensions were 1.3 m x 1.5 m in plan and 0.8 m in elevation. The box was hinged in each corner for ease of transport. Hay was ignited across one end of the boxed quadrat and allowed to burn unassisted to the opposite end. Fires were not extinguished at any point and burned until fuel (*T. triandra* hay and other dead organic matter) was exhausted. See Appendix 2 for illustrations of steel burn box.

Estimating germinable *T. triandra* seed levels in hay

The seed content and germinability of the seed-bearing *T. triandra* hay used in year 1 trials were not assessed. For other years, prior to thatching, random samples of hay were drawn from each of the wool bales used in the trials (ten samples per bale in year 2 and five samples per bale in year 3). Each sample weighed 37.5 g which represented five percent of the hay placed on each revegetation quadrat (Chapter 3). The hay in each sample was assessed for seed availability (Chapter 3). The separated seed from each hay sample was then assessed for germinability according to the methods outlined in Chapter 3, at the time of the spring thatching on the trial sites (September 1994 and October 1995).

4.3.4 Vegetation Assessment on Quadrats

In each year of trials vegetation was assessed in the centre 1.0 m^{-2} quadrat of each 1.95 m^{-2} treatment quadrat area, in late autumn (May) of the year following removal of hay (previous December). In year 1 pilot trials, vegetation was assessed as cover, using a 100 point intercept frame. In years 2 and 3, the number of individual seedlings of *T. triandra* and other plant species were counted. Apart from control (untreated) quadrats, no mature plants of any species survived the herbicide application, with the exception of *Romulea rosea* which resprouted from underground corms in winter in some quadrats. The mean and standard error for each parameter measured was calculated for data from the five replicates per treatment set, except for the year 1 pilot treatments, for which only two or three replicate quadrats were established . For standard comparisons of treatments one or two factor analysis of variance (ANOVA) was carried out after numerical data had been log transformed to minimise skewness, with significant difference being recognised at 0.05 probability of type I error.

4.3.5 Densities of mature *T. triandra* and *N. trichotoma* tussocks in an adjacent remnant grassland

Within the area of remnant indigenous grassland adjacent to the experimental revegetation site, replicated quadrats were established in areas dominated by swards of mature *T. triandra* alone and *N. trichotoma* alone. Plant densities of each species were assessed and used to estimate the appropriate *T. triandra* target densities on revegetation sites. Three discrete locations in each of the *T. triandra*-dominated areas and *N. trichotoma*-dominated areas were used for analysis. At each locations a 1.0 m^2 quadrat frame was placed at five random locations. All *T. triandra* or *N. trichotoma* plants were counted in each 1.0 m^2 quadrat regardless of maturity and size. The mean and standard error was then calculated for each sample set. All sites used for this investigation had been burnt by a wild fire eighteen months prior to the survey.

4.4 Results

4.4.1 *T. triandra* and *N. trichotoma* densities in a remnant grassland site

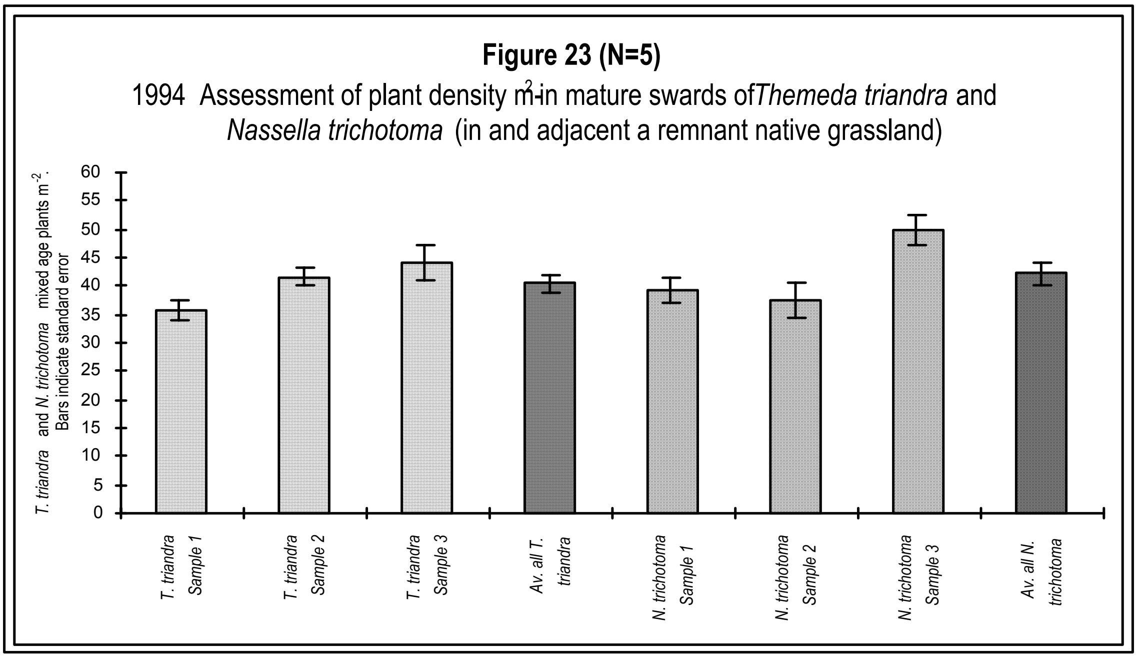
Average density of *Themeda triandra*

Previous assessment of mature *T. triandra* tussocks in indigenous grassland remnants populations have shown average densities at 38.0 *T. triandra* plants m^{-2} (McDougall 1989). The investigation of the remnant indigenous grassland adjacent to the experimental revegetation site reported in this thesis revealed an overall average density of just over 40 *T. triandra* plants m^{-2} of mixed age classes (Figure 23).

For years 2 and 3 of the revegetation trials outlined below the density of at least 40 *T. triandra* plants m^2 was proposed as an appropriate target density for assessing *T. triandra* for establishment success. The density requirement for a competitive sward of *T. triandra* seedlings able to exclude *N. trichotoma* and other weed seedlings led to the proposal that initial *T. triandra* seedling densities on revegetation sites should be well in excess of 40 plants m^{-2} for successful *T. triandra* re-establishment with low weediness.

Average density of *Nassella trichotoma*

Investigations into the density of *N. trichotoma*, the key weed species in this research, revealed an overall average of the three sample location averages to be a density of 42 plants m^{-2} (Figure 23). This result, along with the similar life histories and growth habits of *T. triandra* and *N. trichotoma*, added weight to the proposal, for revegetation trials in years 2 and 3, that in excess of 40 *T. triandra* plants m^{-2} was an appropriate initial target for revegetation densities when attempting to replace *N. trichotoma* with *T. triandra* .



4.4.2 Results of Revegetation in Year 1 Pilot Trials (1993 – 1994)

Atrazine and Glyphosate

With the exception of trials using glyphosate as the herbicide for the control of *N. trichotoma*, combined with the removal of thatch by burning (N=3), calculation of standard errors for year 1 trials was not possible. This was because there were only one or two plots for each pilot treatment. Even though three replicates was probably not sufficient to give an accurate estimate of standard error for the glyphosate – thatch burn treatment, standard errors were calculated for this data to provide some indication of the extent of variability of the results (Figure 26).

The seed quality parameters (availability and germinability) of the seed-bearing hay added to plots were not measured in pilot trials. However, the very high initial densities of *T. triandra* seedlings on most hay treatment plots (Figures 24 to 27) indicated that there had been very high numbers of germinable seed in the hay applied. The highest initial densities of *T. triandra* seedlings were established on plots pre-treated with atrazine or glyphosate to remove the *N. trichotoma*, along with either manual removal or burning to remove the thatch. The initial seedling density in every revegetation plot pre-treated with either atrazine or glyphosate was greater than 500 plants m^{-2} (Figures 24 to 27). Over the following seventeen months, across atrazine and glyphosate plots, *T. triandra* densities fell, but always remained higher than 60 plants m^{-2}. Atrazine plots showed lower rates of *N. trichotoma* invasion than glyphosate plots seventeen months after treatment (Figures 24 to 27). The only treatment not to exhibit *N. trichotoma* invasion at seventeen months after treatment was the atrazine treatment with manual removal of thatch (Figure 25). Seventeen months after treatment the lowest rates of *C. lanatus* invasion and *R. rosea* regrowth were recorded on plots pre-treated with atrazine and glyphosate and with manual removal of thatch (Figures 25 and 27).

Flupropanate

As for atrazine and glyphosate treatments in the year 1 pilot trials, calculation of standard error or the use of statistical comparisons was also not possible for results of the pilot fluproponate trials because there was only one or two plots for each treatment. However, the two replicate plots treated with fluproponate and with removal of thatch by burning resulted in densities of *T. triandra* seedlings in excess of 430 plants m^{-2} (Figure 28). Seventeen months later this figure had reduced to 45 plants m^{-2} for each of the plots from which thatch had been removed by burning (Figure 28) and 34 plants m^{-2} on the plot with thatch manually removed (Figure 29). Flupropanate plots with either burn or manual removal of thatch showed substantial *C. lanatus* invasion in the spring following revegetation (Figures 28 and 29). The flupropanate plot with burn removal of hay also had substantial growth of *R. rosea* around this time (Figure 29).

T. triandra seedlings remaining on flupropanate sites had a chlorotic (absence of chlorophyll) appearance suggesting a phytotoxic impact of the herbicide on the plants. It is possible that the persistence of high levels of flupropanate, a non-specific systemic herbicide, in the soil resulted in unfavourable impacts on the new *T. triandra* seedlings. Flupropanate was also observed to be moving laterally in the soil and to be killing mature tussocks of *T. triandra* adjacent to either the burn or remove plots (data not shown).

Figure 24 ***Themeda triandra*** **& other seedling densities 1993 (N=2)**
Quadrats burnt Feb 93, sprayed with atrazine June 93,
thatch Sept 93, burn thatch Dec 93

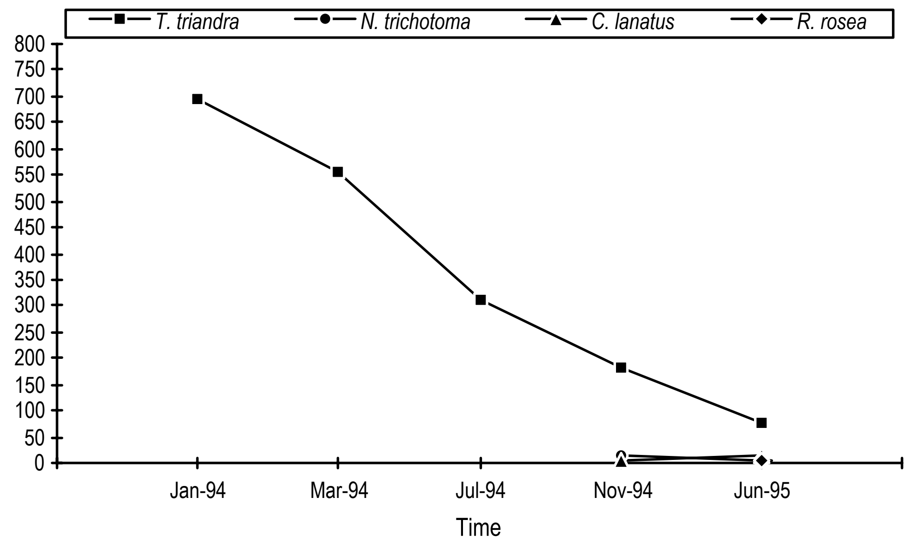


Figure 25 ***Themeda triandra*** **& other seedling densities 1993 (N=1)**
Quadrat burnt Feb 93, sprayed with atrazine June 93,
thatched Sept 93, thatch removed Dec 93

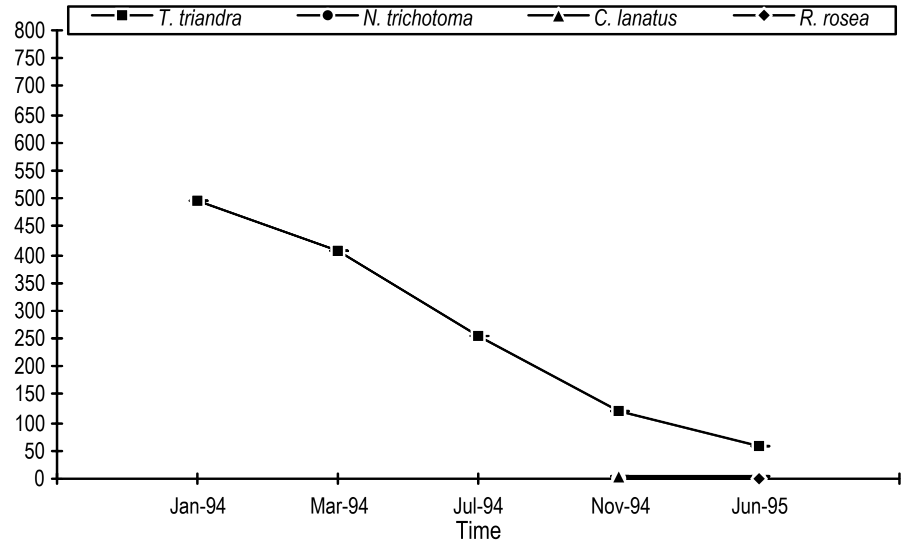


Figure 26 ***Themeda triandra*** **& other seedling densities 1993 (N=3)**
Quadrats burnt Feb 93, sprayed with glyphosate June 93,
thatched Sept 93, thatch burnt Dec 93

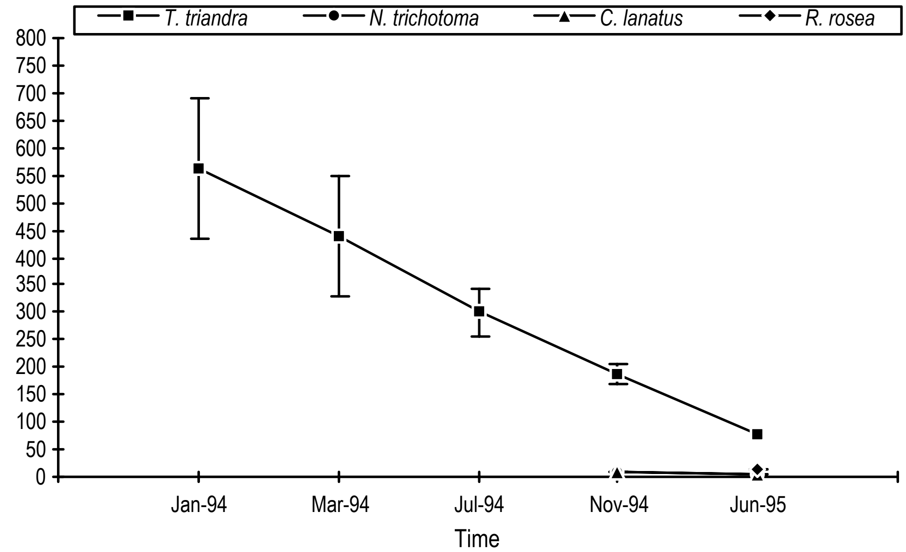


Figure 27 ***Themeda triandra*** **& other seedling densities 1993 (N=2)**
Quadrats burnt Feb 93, sprayed with glyphosate June 93,
thatched Sept 93, thatch removed Dec 93

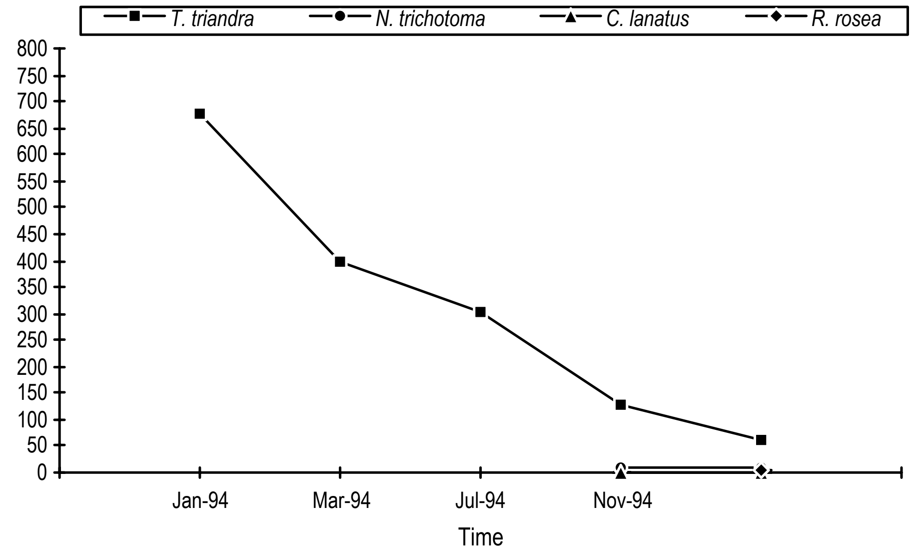


Figure 28 ***Themeda triandra*** **& other seedling densities 1993 (N=2)**
Quadrats burnt Feb 93, sprayed with flupropanate June 93,
thatched Sept 93, thatch burnt Dec 93

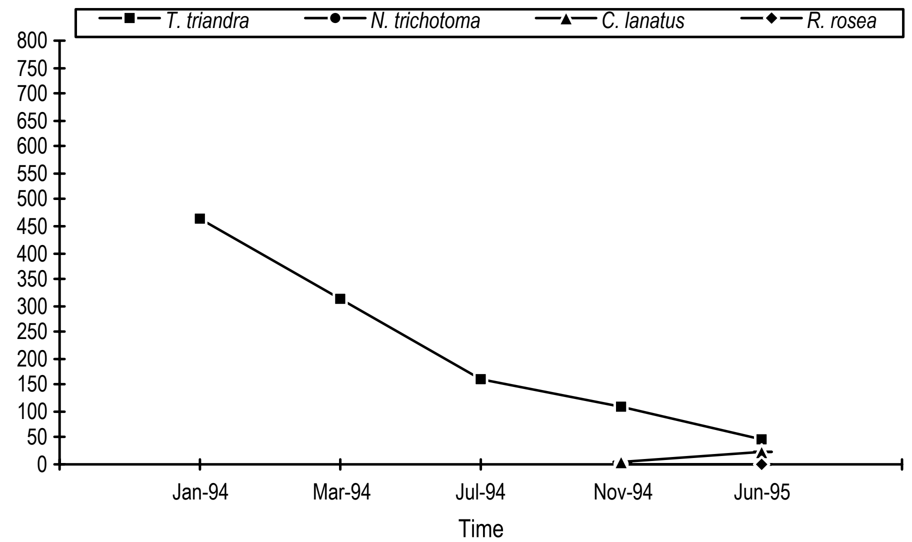
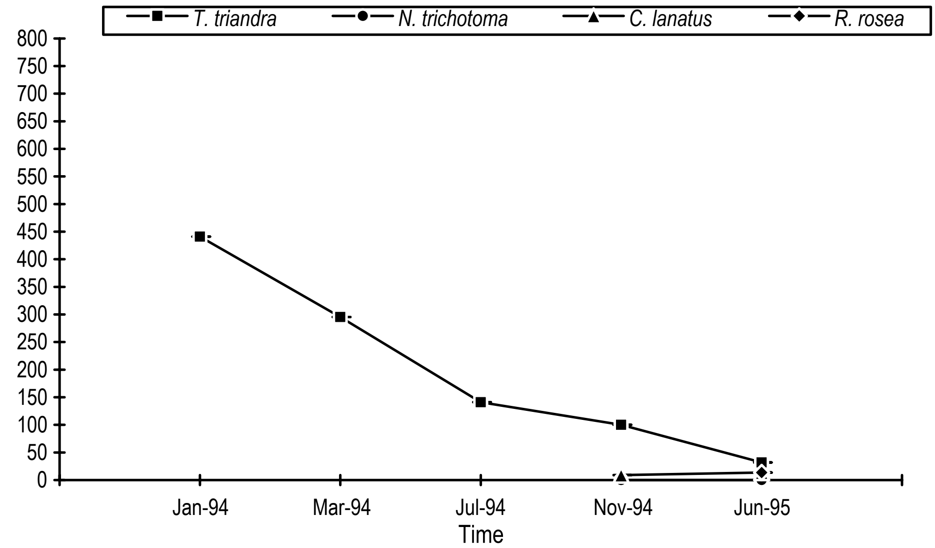


Figure 29 *Themeda triandra* & other seedling densities 1993 (N=1)
Quadrats burnt Feb 93, sprayed with flupropanate June 93,
thatched Sept 93, thatch removed Dec 93



4.4.3 Results of Revegetation in Year 2 (1994 – 1995)

An extensive range of treatments and accessions (i.e. seasons of treatment) were investigated in the year 2 trials for their effectiveness in removing *N. trichotoma* and replacing this with *T. triandra* (see Appendix 3 for a full list of treatments). The results of three of these trials and matching control data are reported on in this section. Due to the drought experienced in south-east Australia during the time of these trials, data collection was abandoned after two data accession times due to concerns that the uncommonly dry conditions were influencing outcomes. Sufficient data was collected from year 2 trials to form conclusions about the effects of seed quality and herbicide type on *T. triandra* establishment and *N. trichotoma* seedling invasion, and to allow planning for year 3 trials to focus on testing tightly defined hypotheses.

An examination of the densities of tussocks on untreated plots dominated by either mature *N. trichotoma* or *T. triandra* plants in remnant grassland adjacent to the study site indicated that revegetation treatments would require the establishment of at least 40 *T. triandra* plants m^{-2} after the removal of the mature *N. trichotoma* (Figure 23). To allow competitive exclusion by *T. triandra* seedlings of *N. trichotoma* seedlings, it was thought *T. triandra* seedling densities considerably higher than 40 plants m^{-2} would be needed in the initial stages of revegetation. The assessment of the seed qualities of the *T. triandra* hay used in year 2 trials showed that an average of around 88 germinable seeds m^{-2} were applied to trial plots in seed-bearing hay (Chapter 3). The highest *T. triandra* average densities of seedlings m^{-2} observed in the trials reported below equated with 25% conversion rate of germinable seed to seedlings in the field (data not shown). As such, the 40 plants m^{-2} minimum target was not achieved in any of the treatments in year 2 trials.

Note: The classification 'sundry' in graphs for year 2 trials refers to weed species which were observed sporadically on plots in numbers too low to graph as individual species. These plants included: *Picris echioides*, *Conyza* sp., *Anagallis arvensis*, *Myagrum perfoliatum*, *Vulpia bromoiodes*, *Bromus* sp., *Briza minor*, *Plantago lanceolata* and *Sonchus oleraceous*.

Burn thatch removal (glyphosate)

In January 1995, one month after treatments concluded with burning of hay, *T. triandra* seedlings were observed at an average of 14.3 plants m^{-2} (details of data not shown). At this data collection *C. lanatus* was present at an average of 7.7 plants m^{-2} with plots remaining clear of *N. trichotoma*. The February data revealed that *T. triandra* seedling densities had increased marginally to average 17.8 plants m^{-2} and *C. lanatus* had increased to 11.7 plants m^{-2} (details of data not shown)

The final data collected for this trial in April 1995, four months after treatment, showed an average density of *T. triandra* of 12.1 plants m^{-2} (Figure 30) or 60% of the initial densities in the previous February, while *C. lanatus* had increased substantially to 44.0 plants m^{-2}. At this point two additional weed species were also observed: *R. rosea* at 7.8 plants m^{-2} and *N. trichotoma* at 5.7 plants m^{-2}. Average *R. rosea* densities on these plots was similar to *R. rosea* at 8.6 plants m^{-2} on the control plots (Figure 30). *T. triandra* densities at this final data collection equated with a 13.7% conversion to seedlings of germinable seed in seed-bearing hay applied to plots.

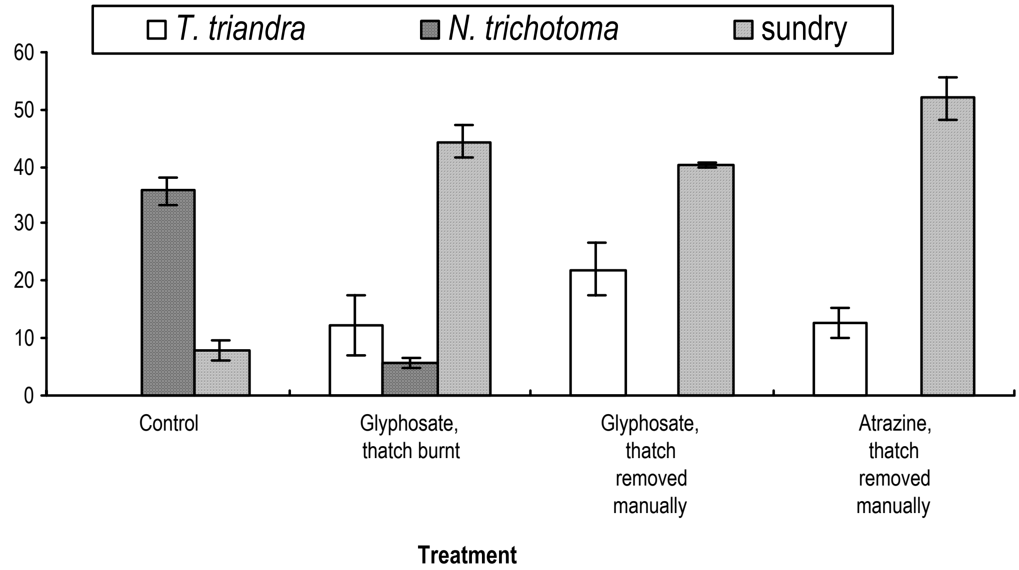
The application of seed-bearing *T. triandra* hay with approximately 88 germinable seeds m^{-2} resulted in the establishment of a low density, uncompetitive sward of *T. triandra* seedlings on all trial plots. The establishment of high rates of *C. lanatus* on all plots corresponded with the establishment of low *T. triandra* numbers.

Manual thatch removal (atrazine and glyphosate)

The first seedlings of any species were not observed on either glyphosate or atrazine treated plots with manual removal of hay during revegetation until two months after treatment. At this time (late January) only *T. triandra* seedlings were recorded, at an average of 5.0 seedlings m^{-2} on the atrazine treated plots and at 10.8 seedlings m^{-2} on glyphosate treated plots (data not shown). At this data collection these plots demonstrated a complete clearance of *N. trichotoma* mature tussocks and seedlings and other weed species.

The April 1995 data collection (four months after completing treatments) revealed *T. triandra* had increased to an average of 12.8 plants m^{-2} on the atrazine plots and 22.0 plants m^{-2} on glyphosate plots (Figure 30). Although the target weed species *N. trichotoma* was controlled, the thistle species *C. lanatus* was present in unacceptably high densities at 52.0 plants m^{-2} (atrazine plots) and 40.0 plants m^{-2} (glyphosate plots) (Figure 30). *T. triandra* densities at this final data collection equated with a 14.5% (atrazine) and 25% (glyphosate) conversion to seedlings of germinable seed from seed-bearing hay applied to plots.

Figure 30 *Themeda triandra* & other seedling densities 1995 (N=5)
April 1995 data collection



4.4.4 Results of Revegetation in Year 3 (1995 – 1996)

The 1995-1996 trials focussed on further developing and refining the most favourable outcomes from the 1994-95 trials and the significance to optimising revegetation success and minimising weediness of adding high densities of germinable *T. triandra* seed. A range of treatments and accessions were examined in year 3 trials (Appendix 3). The outcomes of four trials examining the effects on revegetation success of the type of herbicide used and the type of removal of thatch (manual removal or burning) are reported on in this chapter. The outcomes of other experiments in year 3 trials are reported in Chapter 5.

The seed-bearing *T. triandra* hay used in year 3 trials delivered an average of 457 germinable seeds m^{-2} into revegetation sites (Chapter 3). The highest average *T. triandra* seedling densities observed in the trials reported here equated with a 12.5% conversion rate of germinable seed to seedlings in the field (data not shown). As discussed in the previous section, examination of *N. trichotoma* and *T. triandra* tussock densities in remnant grassland areas adjacent to the experimental site suggested that, to be successful, treatments would need to achieve densities of *T. triandra* seedlings approximating 40 plants m^{-2} (Figure 23).

Burn thatch removal (glyphosate)

T. triandra was recorded at 61.6 *T. triandra* seedlings m^{-2} one month after the thatch was removed, with no weed species observed (details of data not shown), and after a further month at 60.8 seedlings m^{-2} with *C. lanatus* at 2.4 seedlings m^{-2} (details of data not shown). The final data set, collected five months after treatment showed that there had been a decline in average *T. triandra* seedling density to 17.8 plants m^{-2} (Figure 31). High densities of some weed species were also observed at this time: *N. trichotoma* at 6.8 plants m^{-2}, *C. lanatus* at 11.6 plants m^{-2} and *R. rosea* 8.2 plants m^{-2} (summarized as 'sundry' in Figure 31 - further details in Chapter 5). The average density of *R. rosea* on treated plots was similar to the average *R. rosea* density of 8.8 plants m^{-2} on the control plots.

The average *T. triandra* density of 17.8 plants m^{-2} at the final data collection in May, six months after the hay had been removed by burning, represented a 3.9% conversion of the 457 germinable seeds m^{-2} applied to the site.

Manual thatch removal (glyphosate)

One month after thatch was removed (January 1996), the manual hay removal plots showed a poor rate of *T. triandra* establishment at 19.4 plants m^{-2} with no weed species recorded (details of data not shown). One month later *T. triandra* had increased to 35.2 plants m^{-2} with sundry weed species present at 6.4 plants m^{-2}. The average *T. triandra* density at this time compared unfavourably with *T. triandra* at 60.8 plants m^{-2} for same data accession on the burn thatch remove plots (data not shown).

Five months after treatment, the final data collection showed *T. triandra* to be present at 21.2 plants m^{-2}, a higher establishment rate than for the same period on the burn remove plots (Figure 31). Cool season weed species were also present, but at lower rates than on comparable glyphosate treated plots from which the hay had been removed by burning (Figure 31). The average density of *N. trichotoma* at 0.8 plants m^{-2} was significantly (single factor ANOVA $p<0.0006$) lower than for the comparable burn thatch treatment at 6.8 plants m^{-2}

(Figure 31) as was the average density of the other main weed species *C. lanatus* at 1.0 plants m^{-2} (included under 'sundry' - details in Chapter 5). The average density of *R. rosea* at 3.6 plants m^{-2}, although low, was not significantly lower (single factor ANOVA p>0.13) than the comparable burn thatch removal plots at 8.2 plants m^{-2} (Figure 31).

The average density of *T. triandra* of 21.2 plants m^{-2} at the final data collection represented a 4.6% conversion of the 457 germinable seeds m^{-2} applied to the site.

Burn thatch removal (atrazine)

T. triandra had established at 83.2 seedlings m^{-2} with no weed species present one month after *T. triandra* hay was removed (details of data not shown). The February 1996 assessment one month later showed that plots had remained clear of weed species with *T. triandra* declining marginally to 78.0 plants m^{-2}.

The May 1996 data collection five months after treatment showed *T. triandra* to be present at 31.6 plants m^{-2} (Figure 31). At this final data collection time the only weed species recorded was the cool season geophyte *R. rosea* emerging from its dormant phase at 11.4 plants m^{-2}, an average density which was comparable to *R. rosea* on control plots at 8.8 plants m^{-2}.

The average density of 31.6 *T. triandra* plants m^{-2} at the final data collection represented a 6.9% conversion of the 457 germinable seeds m^{-2} applied to the site.

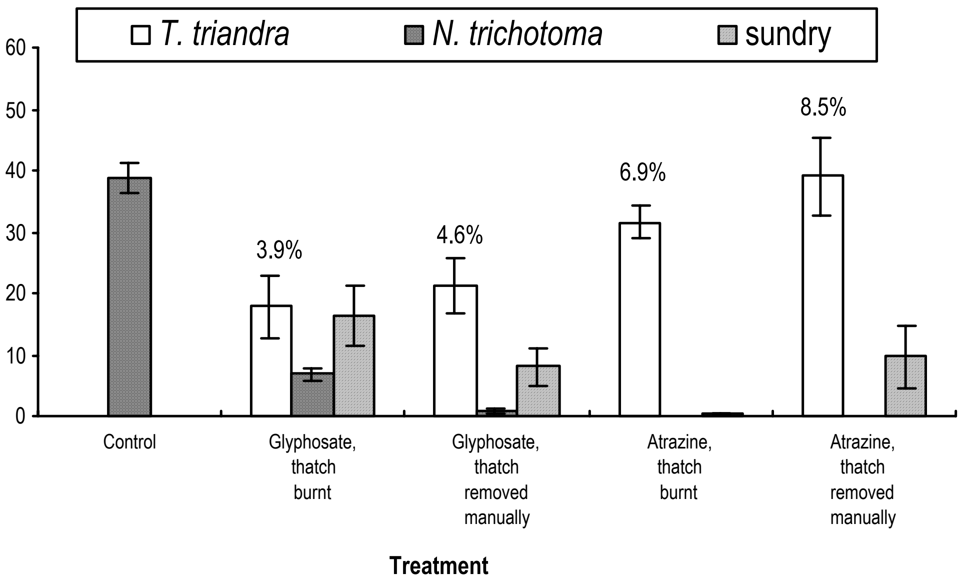
Manual thatch removal (atrazine)

One month after treatments were complete an assessment of plots showed *T. triandra* to be present at 20.0 seedlings m^{-2} with no weed species present (data not shown). This was less than a quarter of the *T. triandra* plants present at the same time on the burn remove plots (see above). Two months after treatment *T. triandra* had increased to 47.4 seedlings m^{-2} (data not shown). It was highly likely that the atrazine used in this treatment continued to suppress weed regrowth and no weed species were recorded the second data collection time.

Five months after treatment the average density of *T. triandra* was 39.0 plants m^{-2}, the highest (two factor ANOVA $p<0.046$) *T. triandra* density of any of the four treatments (Figure 31). The geophytic weed species *R. rosea* was present at 6.2 plants m^{-2} which was 50% of the *R. rosea* average density recorded at the same time on equivalent burn remove plots (above) and 75% of the *R. rosea* population on control plots.

In contrast to the low average densities of *C. lanatus* and other weeds on burn thatch removal plots, on manual removal of thatch treatments *C. lanatus* was recorded at an average 2.6 plants m^{-2} and sundry weed category 7.0 plants m^{-2}. No plants in either of these weed categories were recorded for the atrazine burn thatch remove plots (Figure 31). The density of *T. triandra* at 39.0 plants m^{-2} in the final data collection represented an 8.5% conversion of the 457 germinable seeds m^{-2} applied to the site (detailed data not shown).

Figure 31 *Themeda triandra* & other seedling densities 1996 (N=5
May 1996 data collection



4.5 Discussion

The outcomes of this research demonstrated that the initial average *N. trichotoma* density on revegetation sites of around 42 plants m^{-2} was generally able to be replaced successfully by approximately equivalent densities of *T. triandra*, and the regrowth or invasion of either *N. trichotoma* seedlings or other weed species was impeded when *T. triandra* was re-established at 30 plants m^{-2} or above. These outcomes were shown to be reproducible across years. Treatments to achieve these *T. triandra* densities included initial weed biomass reduction (by fire or slashing), followed by removal of mature *N. trichotoma* tussocks using herbicide, and later by thatching with seed-bearing *T. triandra* hay, and subsequent removal of hay by either burning or physical removal several months after thatching. Although *T. triandra* establishment rates varied across treatment sets, and across years, results suggested that the following key factors interact to determine the success of re-establishing *T. triandra*:

- the seed content of seed-bearing *T. triandra* hay and the germinability rates of the seed,
- the type of herbicide used for follow-up weed removal after an initial slash or burn treatment, and prior to thatching with seed-bearing *T. triandra* hay,
- the type of technique used to remove thatch (whether thatch was burnt or manually removed).

High densities of *T. triandra* seedlings and low densities of *N. trichotoma* seedlings are possible during initial revegetation

The outcomes of year 1 pilot revegetation trials demonstrated that it was possible to establish very high densities of *T. triandra* seedlings on the research site, in excess of 500 seedlings m^{-2}, regardless of the type of herbicide pre-treatment or type of thatch removal (Figures 24 to 29). Seedlings of *T. triandra* always reached their maximum densities within two to three months of removal of the thatch in December (see section 4.4.1 and Chapter 5) and in all years where the initial maximum seedling density was above 30 seedlings m^{-2} there was a subsequent downward trend in *T. triandra* seedling density (see for examples Figures 24 to 29). By comparison, after herbicide removal of mature *N. trichotoma* plants from revegetation sites, the establishment of seedlings of *N.*

trichotoma from the seedbank was slow, and high densities did not appear until the spring of year following revegetation, although the extent of *N. trichotoma* seedling establishment during this second spring period was affected by other revegetation treatment parameters (see discussion below). Recent investigations at Victoria University of the germination characteristics of *N. trichotoma* seeds (Puhar 1996 and investigations of soil seedbank conducted as part of the revegetation trials, but not reported in this thesis due to mixed results) have shown that there is around 90% dormancy which is progressively relieved under optimum germination conditions, at least for seed collected in the vicinity of the study area used for revegetation trials. Extrapolating these results to the field, *N. trichotoma* seedlings would be expected to establish slowly and progressively from the seedbank over several growing seasons.

It is likely that the spray and hay method for replacement of *N. trichotoma* by *T. triandra* is successful if the timing of actions in the revegetation process is set (in this instance; summer slash, winter herbicide, spring thatch, late spring thatch removal). This is probably because the *T. triandra* seedlings are able to establish in large numbers quickly on the revegetation site (provided sufficient viable seeds are present) whereas *N. trichotoma* seedlings are only able to re-establish slowly from the seedbank, beginning in the spring of the year following winter herbicide clearance, and clearance of subsequent cover of the site by removing hay.

Effects of Seed Content of Hay and Germinability on *T. triandra* Seedling Densities

There would appear to be a clear and important relationship between the number of germinable *T. triandra* seeds broadcast onto a site during 'spray and hay' revegetation and the density of *T. triandra* seedlings which subsequently become established. Measured conversion rates of germinable seed to seedlings (for the highest average density of seedlings in each treatment across the monitoring period) varied between 25% for some treatments in year 2 (section 4.4.3) down to 12.5% in some treatments for year 3 trials (section 4.4.4). As pointed out above, the highest density of *T. triandra* seedlings with each treatment also appeared to have an effect on the overall weediness of the site during revegetation. The density of germinable seeds being applied to a revegetation site (germinable seed content of seed-bearing hay being applied), therefore appears to be a key consideration for successful revegetation with *T. triandra* when using the 'spray and hay' method. The wide variability in the content of *T. triandra* seed-bearing hay (Chapter 3) may have been one of the key parameters affecting the outcomes of previous attempts to use the 'spray and hay' method for revegetation with *T. triandra* of sites previously occupied by weeds (for example: McDougall 1989, Carr and Muir 1993, Carr and Todd 1991). None of these revegetation programs assessed the germinable seed content of the hay prior to revegetation, or took into account the variability in germinable seed content of the hay from batches taken from the same day from a harvest site (Chapter 3).

Effects of Type of Herbicide on *T. triandra* Seedling Densities

Flupropionate (as ICI Frenock®), the recommended herbicide for removal of *N. trichotoma*, was found to be unsuitable at label strength and backpack rates for clearance of *N. trichotoma* from sites destined for revegetation by *T. triandra*. There was extensive on-site evidence that flupropionate remained residual in the soil, as reported elsewhere (Campbell 1998), well into the time period when *T. triandra* seedlings were establishing on 'spray and hay' revegetation sites (section 4.4.2) and was associated with the chlorotic appearance of *T. triandra* seedlings. The high residuality of flupropionate was also probably responsible for the high progressive rates of death of *T. triandra* seedlings on revegetation sites, compared with sites treated with glyphosate, which is minimally residual, or atrazine, which is residual but selective for C_3 species and appears to leave *T. triandra* seedlings relatively unaffected (figures 24-29). In addition to its negative effects on *T. triandra* seedling survival, flupropionate also promoted the growth of a number of weeds noted as problem species within lowland indigenous grassland remnants (figures 28 and 29). Recently there has been a brief report that flupropionate, applied at low rates approximating one fifth of agricultural rates (agricultural label strength) is selective for removal of *N. trichotoma* seedlings from amongst some indigenous grasses, including *T. triandra* (Campbell 1998). Trials are currently under way at the revegetation site at Victoria University to determine whether this applies in the local situation (C. Hocking 1999, pers. comm.).

Both glyphosate and atrazine were successful if used as part of the 'spray and hay' method for replacing *N. trichotoma* with *T. triandra* in each of the three years tested (sections 4.4.2, 4.4.3 and 4.4.4). In year 3 of trials, plots treated with glyphosate tended to have significantly higher levels of weed establishment in the autumn following treatment than atrazine (single factor ANOVA $p<0.01$) (Figure 31). This outcome was most likely achieved because atrazine is a root systemic herbicide and resides temporarily in the soil as an active control agent, while glyphosate is a foliar absorbed herbicide with no described soil-borne residual capacity for uptake through root systems (Parsons 1992). Plots treated with atrazine in the year 3 trials (section 4.4.4) resulted in significantly higher *T. triandra* densities than plots treated with glyphosate, (single factor ANOVA $p<0.004$) (Figure 31). In year 3, plots treated with atrazine with manual or burn hay removal resulted in final *T. triandra* densities significantly higher than on matched plots treated with glyphosate (two factor ANOVA $p<0.046$). The C_3 selective function of atrazine is likely have advantaged the establishing C_4 *T. triandra* seedlings over the C_3 *N. trichotoma* seedlings leading to higher *T. triandra* density on atrazine plots.

Effects of Burning and Physical Removal of Thatch on *T. triandra* Seedling Densities

In glyphosate treated plots in year 2 trials (section 4.4.3), and glyphosate and atrazine treated plots in year 3 trials (section 4.4.4) manual removal of hay resulted in slightly higher final *T. triandra* densities than on plots where hay had been removed by burning (Figures 30 and 31). However, the differences were not significantly different when tested alone in:

- year 2 for glyphosate (single factor ANOVA $p>0.1$)
- year 3 for glyphosate (single factor ANOVA $p>0.6$)
- year 3 for atrazine (single factor ANOVA $p>0.3$)

or in combination with herbicide type in:

- year 3 (two factor ANOVA, $p>0.3$).

Although not measured, it is possible the heat from the fire killed a proportion of surface-located germinable seeds while manually removing hay left surface-located germinable seeds undamaged. Overall, there would not appear to be a distinct advantage in either burning thatch or manually removing it.

Interactions between Type of Herbicide and Removal of Thatch

As outlined previously, the highest average *T. triandra* densities recorded in year 3 trials (section 4.4.4) were on plots treated with atrazine with hay either removed manually or burnt. Burn removal of thatch on atrazine plots also resulted in higher densities of *T. triandra* than on either the burn or manual hay remove glyphosate plots (Figure 31). In statistical comparisons of the effects of herbicide type and method of hay removal for year 3 *T. triandra* densities, there was no detectable interaction between the two factors. It would appear that, in assessments of the *T. triandra* densities established on 'spray and hay' trial plots for up to five months after removal of hay, the type of herbicide used is the key factor for optimizing outcomes, or at least for the July spray - September thatch - December remove hay series.

4.5.1 Optimum conditions for revegetation

The specific 'spray and hay' treatment applied to revegetation plots where atrazine was used for removal of mature *N. trichotoma* tussocks, with either burning or manual removal of *T. triandra* thatch, produced substantial *T. triandra* numbers, few other weed species and no *N. trichotoma* invasion for many months. Of the options tested for the July-September-December seasonal accession the most favourable treatment emerged as: January slash, July herbicide, September thatch, December removal of thatch. However, as will be discussed in Chapter 5, the seasons of treatment using the 'spray and hay' method also affects the outcomes.

Specifically, the most successful outcomes of methods for the July-September-December accession were as follows:

In the one month period immediately following thatch removal in year 3 trials, the highest average density of *T. triandra* seedling establishment was assessed at 83.2 plants m^{-2} (data detailed in Chapter 5) on plots with the following treatment regime:

- *N. trichotoma* sward slashed in January,
- *N. trichotoma* regrowth sprayed with label strength atrazine at knapsack rates in July,
- plots thatched with seed-bearing *T. triandra* hay in September,
- thatch removed by burning in December.

The highest final average *T. triandra* establishment density recorded five months after thatch was removed in year 3 trials was 39.0 plants m^{-2} on plots with the following treatment regime:

- *N. trichotoma* sward slashed in January,
- *N. trichotoma* regrowth sprayed with label strength atrazine at knapsack rates in July,
- plots thatched with seed-bearing *T. triandra* hay in October,
- thatch removed manually in December.

5. Replacing *Nassella trichotoma* with *Themeda triandra* using the 'spray and hay' method (2): Effect of treatment timing

5.1 Summary

The 'spray and hay' method for replacing serrated tussock (*Nassella trichotoma* (Nees) Arech.) with kangaroo grass (*Themeda triandra* Forrsk.) developed in previous trials (Chapter 4) was investigated in detail to determine the effects on *T. triandra* establishment densities and levels of infestation by *N. trichotoma* and other weed seedlings. The seasonal timing of the method, the type of herbicide used, the method for removing *T. triandra* hay, and possible interactions between these parameters was also investigated. The most favourable *T. triandra* establishment rates resulted from slashing weed biomass in summer (January), controlling weed regrowth with herbicides in winter (July), thatching with seed bearing *T. triandra* hay in early spring (September) and removing hay in late spring (December). Across all seasonal accessions, treatments in which atrazine was the herbicide used resulted in higher rates of *T. triandra* seedlings, higher final *T. triandra* densities and lower densities of *N. trichotoma* and other weeds than treatments which tested the herbicide glyphosate. In treatments using glyphosate, the highest initial and final densities of *T. triandra* seedlings, with low final densities of *N. trichotoma* and other weeds resulted from the May – October accession in which the *T. triandra* hay was removed by burning. Glyphosate with July – December accessions also produced acceptable initial and final densities of *T. triandra*, although when the hay was burnt in this treatment accession, higher final densities of *N. trichotoma* resulted than for the equivalent treatment in which the hay was manually removed. Glyphosate with February – July accessions resulted in very high densities of both *N. trichotoma* seedlings and other weeds, even though *T. triandra* seedling densities were also high. Rates of conversion of *T. triandra* germinable seed in the hay to seedlings on revegetation trial plots varied from 8% to 26%. Treatments where hay was removed by burning produced higher initial seedling rates than equivalent manual hay removal treatments.

5.2 Introduction

The successful removal of *Nassella trichotoma* in lowland indigenous grassland remnants using the herbicides glyphosate and atrazine has been described in Chapter 2 and techniques for the successful revegetation of these cleared sites with *Themeda triandra* , in a process referred to as the 'spray and hay' method has been outlined in some detail in Chapter 4 (see Chapter 1 for a broader discussion of the principles underlying this method). The effects on the success of the 'spray and hay' method of herbicide selection and timing of application (Chapter 2) and of seed content and germinability in harvested seed-bearing *T. triandra* hay (Chapter 3) have been investigated. In Chapter 4 the final average densities of *T. triandra* and weed establishment on revegetation plots four to five months after treatment resulting from the treatment accession: July herbicide – September thatch - December remove thatch over two years was presented. Anecdotal evidence from other revegetation programs attempting to use the spray and hay method for replacement of weeds, including *N. trichotoma* with *T. triandra* suggested that the seasonal timing of the steps in the 'spray and hay' method might influence the outcomes, in terms of *T. triandra* densities and overall weed establishment on revegetation sites (C. Hocking 1995, pers. comm.). As a consequence, in year 3 trials (1995-96), in parallel with the studies of the effects of herbicide type and type of method for removal of hay, the effects of altering the accession times of treatments were also investigated. The results of these investigations, including detailed time sequences for establishment of *T. triandra* seedlings and weed seedlings on revegetation plots, are reported in Chapter 5.

5.3 Materials and methods

5.3.1 Quadrat location and size, and pretreatment for biomass reduction

The trials reported here were carried out in the Victoria University (St Albans) indigenous grassland conservation reserve, as detailed in Chapter 2. Quadrats in all trials measured 1.3 m x 1.5 m (1.95 m^{-2}), with the central 1.0 m x 1.0 m (1.0 m^{-2}) used for data collection.

Initial weed biomass reduction on the trial site, across all quadrat areas to be investigated, was carried out by slashing *N. trichotoma* with a domestic lawn mower in January 1995. Slashed *N. trichotoma* was raked and removed by hand from the site, as detailed in Chapter 4. Slashing produced a stubble averaging 80 mm in height across the site.

5.3.2 Accession Times and Treatments for 'Spray and Hay' Method

Accession Times and Treatments Applied

The following combinations of treatments were applied to replicated, randomized quadrats within a total area of 20 m x 50 m:

glyphosate or atrazine	x	burn hay or remove hay	x	July – December accession or February – July accession

Integrated with these treatments was another set of treatments with the accession May – October, for which the only herbicide used was glyphosate, but which did incorporate each of the two hay removal treatments (i.e. burn or manual remove). It was not possible to incorporate atrazine into these trials because of the limited availability of quality seed-bearing *T. triandra* hay.

There were five randomised replicate quadrats for each treatment, making up a total of 50 quadrats (see Appendix 3 for full list of treatments).

Herbicide Treatment and Thatching with seed-bearing *T. triandra* hay

Herbicides were prepared at label recommended strengths and applied by knapsack at standard rates for weed management in conservation areas, but higher than agricultural recommendations (see Chapter 2 for details).

Seed-bearing *T. triandra* hay was laid down as thatch on trial plots in May, July or October and held in place using anti-bird netting (Chapter 4). All trials used 770.0 grams of hay m^{-2} as a single volume thatch (Chapter 4).

Germinable *T. triandra* seed applied to quadrats

Seed-bearing *T. triandra* hay was harvested from adjacent remnant indigenous grassland sites in January 1995 (see Chapter 3 for details). All hay used in the trials was laid out on a concrete floor and thoroughly mixed, before being packed into wool bales and stored in a cool, mouse-proof room until needed for laying on quadrats. Assessments of the seed qualities of the hay was carried out according to the methods reported in Chapter 3 immediately prior to laying out on the site. When required, wool bales containing hay were selected randomly from amongst the available stock. While all of the hay began with the same seed content and quality, with storage time both the availability of the seed and the germinability of the seed increased (see Chapter 3 for details). Assessments of seed characteristics of the hay at the times of laying produced the following characteristics at each of the three different accessions (available seed in 770g of hay applied to each m^2 of quadrat):

- February: 749.3 available seed m^{-2} averaging 221.1 germinable seeds m^{-2},
- July: 928.0 available seed m^{-2} averaging 289.7 germinable seeds m^{-2},
- October: 992.0 available seed m^{-2} averaging 457.5 germinable seeds m^{-2}.

T. triandra thatch removal

Thatch removal was carried out in July, October or December using either burning or manual removal, as detailed in Chapter 4.

5.3.3 Quadrat Assessment

T. triandra and other species were counted in the centre 1.0 m^{-2} quadrat of the larger treated quadrat area, to determine seedling densities, as detailed in Chapter 4. Data collection on all trial plots commenced at the first observed *T. triandra* germination. The period between the end of treatments (i.e. the final treatment in the 'spray and hay' accession, which was removal of *T. triandra* thatch either manually or by burning) and first observed *T. triandra* germination varied considerably between treatment sets. The mean and standard error was calculated and where comparisons between treatments was made, an analysis of variance (ANOVA) was carried out, after numerical data had been log transformed (Chapter 4).

5.4 Results

Note: the classification 'sundry weeds' refers to an aggregate of weed species which at times (but not always) included *Picris echioides*, *Conyza* sp., *Anagallis arvensis*, *Myagrum perfoliatum*, *Vulpia bromoiodes*, *Bromus* sp., *Briza minor*, *Plantago lanceolata* and *Sonchus oleraceous*. These plants were commonly observed in numbers too low to graph as individual species.

5.4.1 July – December accession with atrazine or glyphosate

Trial sites slashed in January, weed regrowth treated with atrazine or glyphosate in July, seed-bearing *T. triandra* hay laid as thatch in October, thatch removed manually or by burning in December.

End-point results for these trials, collected in May 1996, six months after the removal of hay, were reported on and discussed in Chapter 4. Briefly these results showed that either glyphosate or atrazine were suitable for use in 'spray and hay' revegetation, but that atrazine treated plots resulted in significantly higher densities of *T. triandra* seedlings and lower densities of *N. trichotoma* seedlings than glyphosate treated plots (figure 31). Burning or manual removal of hay did not make a significant difference to densities of *T. triandra* for either glyphosate or atrazine treated plots, but densities of *N. trichotoma* seedlings were significantly lower on glyphosate treated plots where the hay had been manually removed than on equivalent plots where the hay had been burnt.

The time sequence for establishment of *T. triandra* seedlings was broadly similar for both atrazine and glyphosate treated plots, but varied within each of these treatments according to whether hay was removed manually or by burning (figures 32 – 35). In both atrazine and glyphosate treatments where hay was burnt, *T. triandra* seedlings germinated rapidly, and by the first assessment in the January following hay removal in December, were averaging 83.2 seedlings m^{-2} on atrazine treated plots (Figure 32) and 61.6 seedlings m^{-2} on glyphosate treated plots (Figure 34). At the next assessment in February, *T. triandra* seedling densities remained high for both treatments, and then fell markedly by the final assessment in May (figures 32 and 34).

By comparison, in both atrazine and glyphosate treatments where hay was manually removed, *T. triandra* seedling densities were still low at the first assessment in January, one month after hay removal, with an average of 20.0 seedlings m^{-2} on atrazine treated plots (Figure 33) and 19.4 seedlings m^{-2} glyphosate treated plots (Figure 35). At the next assessment in February, *T. triandra* seedling densities had increased markedly for each of the herbicide treatments, and then fell again by the final assessment in May (Figures 32 and 34).

An interesting feature of the results is that although the hay removal process differed, by May both sets of atrazine treatments (burn and remove), had similar densities of *T. triandra* seedlings, as did both sets of glyphosate treatments (burn and remove) (see Figure 31 in Chapter 4).

Another feature of the data in Figures 32 – 35 is that the *T. triandra* seedling densities observed on atrazine plots were always higher than comparable glyphosate plots. The densities of weeds (*N. trichotoma* and sundry) only became high by the May assessments – these were always higher on glyphosate plots than comparable atrazine plots, as discussed in Chapter 4.

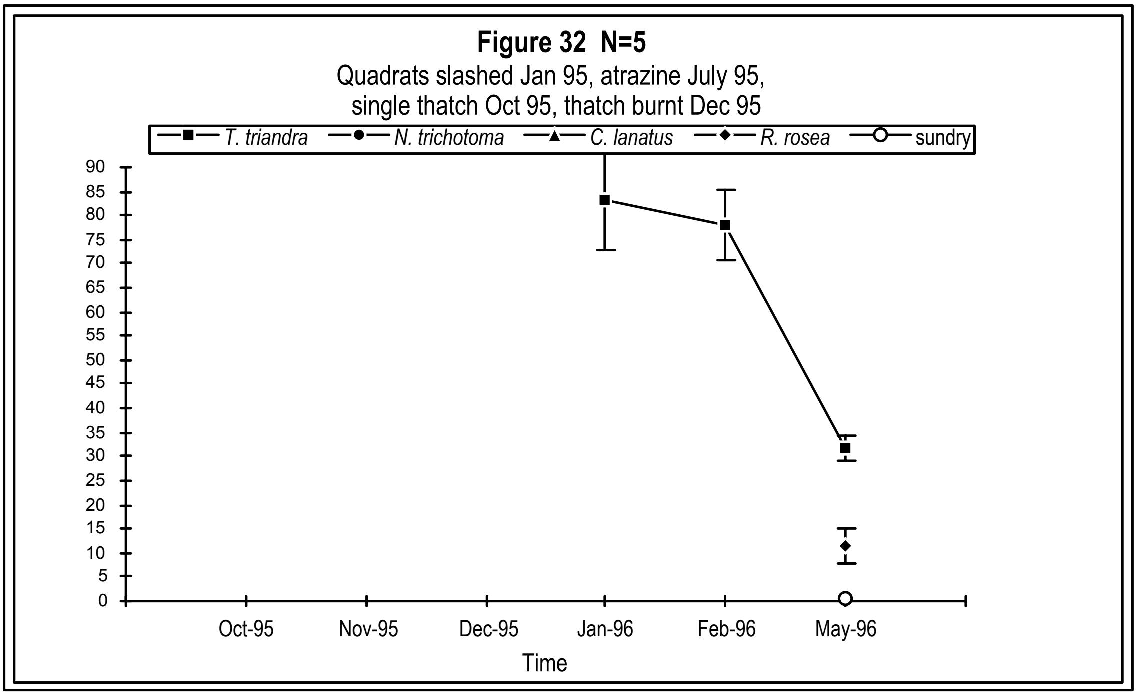


Figure 32 N=5
Quadrats slashed Jan 95, atrazine July 95,
single thatch Oct 95, thatch burnt Dec 95
T. triandra
N. trichotoma
C. lanatus
R. rosea
sundry
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
Oct-95
Nov-95
Dec-95
Jan-96
Feb-96
May-96
Time

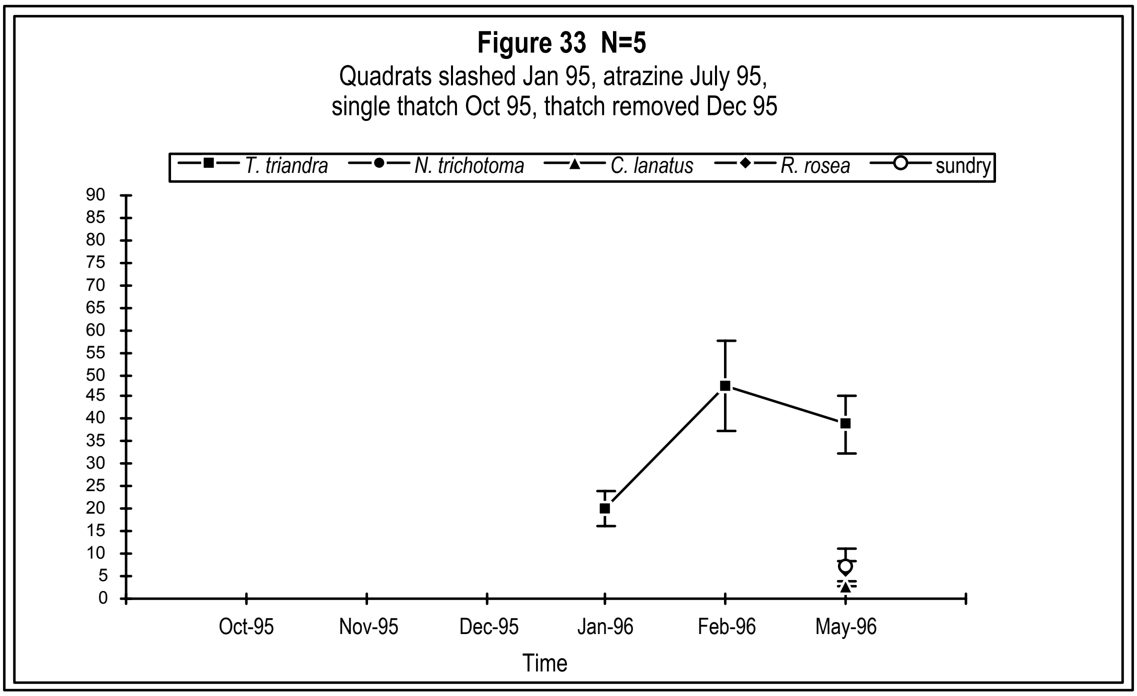


Figure 33 N=5
Quadrats slashed Jan 95, atrazine July 95,
single thatch Oct 95, thatch removed Dec 95
T. triandra
N. trichotoma
C. lanatus
R. rosea
sundry
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
Oct-95
Nov-95
Dec-95
Jan-96
Feb-96
May-96
Time

Figure 34 N=5

Quadrats slashed Jan 95, glyphosate July 95, single thatch Oct 95, thatch burnt Dec 95

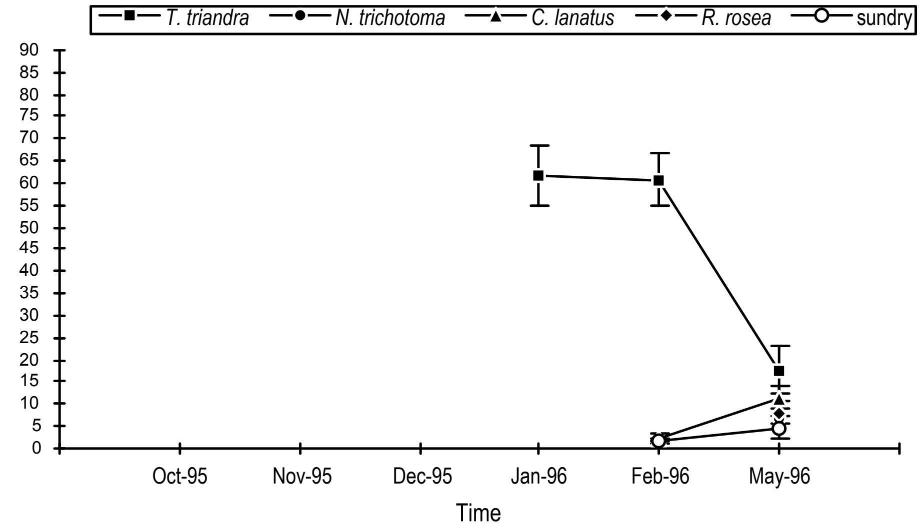
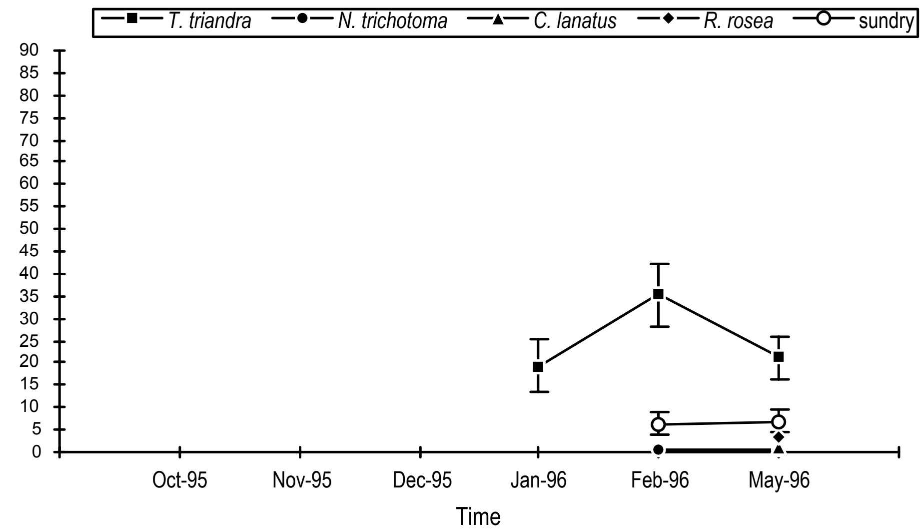


Figure 35 N=5

Quadrats slashed Jan 95, glyphosate July 95, single thatch Oct 95, thatch removed Dec 95



5.4.2 February – July accession with atrazine or glyphosate

Trial sites slashed in January, weed regrowth treated with atrazine or glyphosate in February, seed-bearing *T. triandra* hay laid as thatch in May, thatch removed manually or by burning in July.

Unlike the results of the July – December accession, the pattern of *T. triandra* seedling establishment in the February – July accession was broadly similar on both burn and remove plots, across atrazine and glyphosate treatments (Figures 36 – 39). By October some *T. triandra* seedlings had already begun to establish, and this increased two- to three- fold in all treatments by December. Two factor analysis of variance revealed that initial seedling establishment (assessed in October) was significantly higher ($p<0.05$) in burnt hay plots compared with physical hay removal plots, and that densities on plots treated with atrazine were significantly higher than on plots treated with glyphosate, but for burnt plots only. The highest overall density of seedlings recorded was on the atrazine burn hay treatment in December (57 plants m^{-2}). The next highest was on glyphosate burn hay removal (47 plants m^{-2}) in November, followed by atrazine with manual hay removal (40.2 plants m^{-2}) in December and lastly glyphosate with manual hay removal (40 plants m^{-2}) in December

After December, the densities of *T. triandra* seedlings remained relatively constant over the summer (Figures 36 - 39), except on atrazine burn plots, where they began to decrease soon after the very high relative seed densities had been achieved. For all treatments there was a downward trend in seedling densities in the autumn of 1996 (February – May). The final *T. triandra* seedling densities recorded on plots, in May 1996, were very similar for all treatments, at around 30 seedlings m^{-2} (Figures 36 - 39). Two factor analysis of variance revealed that there were no significant differences in *T. triandra* seedling densities between treatments, or interactions between treatments ($p>0.5$).

There were marked differences between treatments in the number of weed seedlings establishing on February-July accession revegetation plots. On atrazine treated plots, with either burn or manual removal of hay treatments, no weed seedlings were recorded until May 1996, ten months after removal of hay

(Figures 36 and 37). At the final assessment in May, very low densities of *N. trichotoma* and other weeds were recorded. In comparison, high densities of weeds were recorded on glyphosate treated plots at the first assessment in October 1995, for both burn and manual removal of hay treatments (Figures 38 and 39). Weed densities remained high over the summer and autumn assessment periods, with sundry weeds at around 10 to 15 plants m^{-2}.

No *N. trichotoma* seedlings were recorded at the first assessment in October 1995 for either burn or manual remove glyphosate treatments (Figures 38 and 39). However, *N. trichotoma* was present at around an average of 10 plants m^{-2} at the November 1995 assessment for both types of glyphosate treatment, and these average densities increased to about 15 plants m^{-2} for each of the two types of glyphosate treatments at some point in the summer-autumn assessment period (Figures 38 and 39). The densities of *N. trichotoma* on glyphosate burn and glyphosate physical removal treatments appeared to peak in December 1995 at 14 and 10 plants m^{-2} respectively and then remain at constant densities over the summer and into autumn.

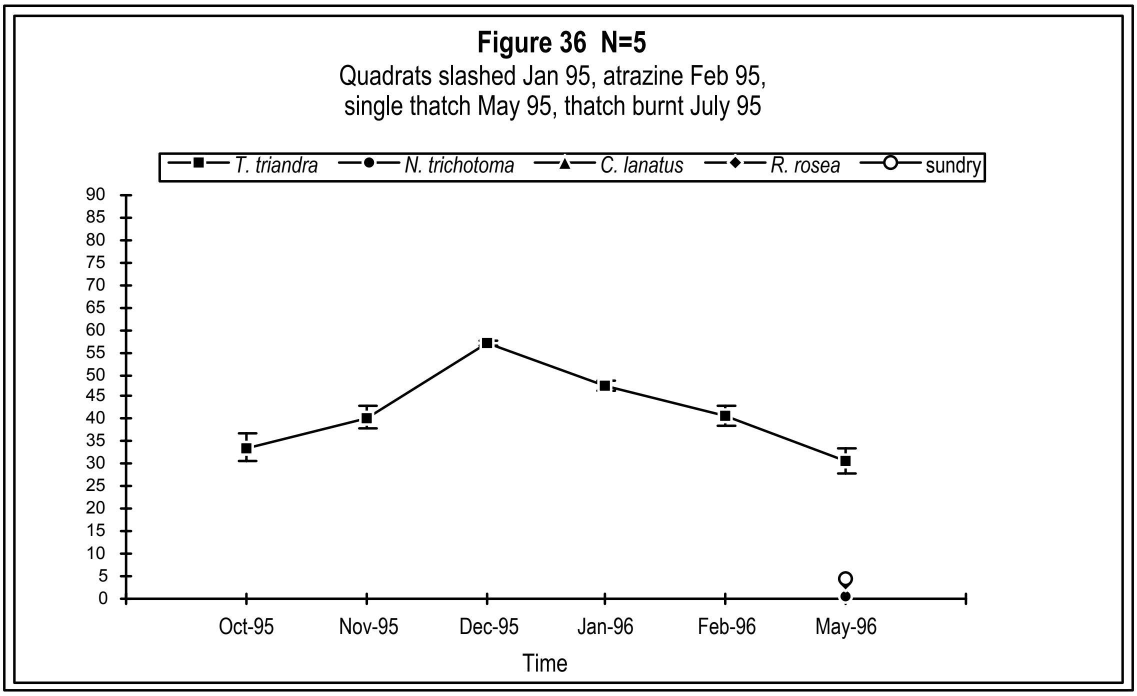


Figure 36 N=5
Quadrats slashed Jan 95, atrazine Feb 95,
single thatch May 95, thatch burnt July 95
T. triandra
N. trichotoma
C. lanatus
R. rosea
sundry
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
Oct-95
Nov-95
Dec-95
Jan-96
Feb-96
May-96
Time

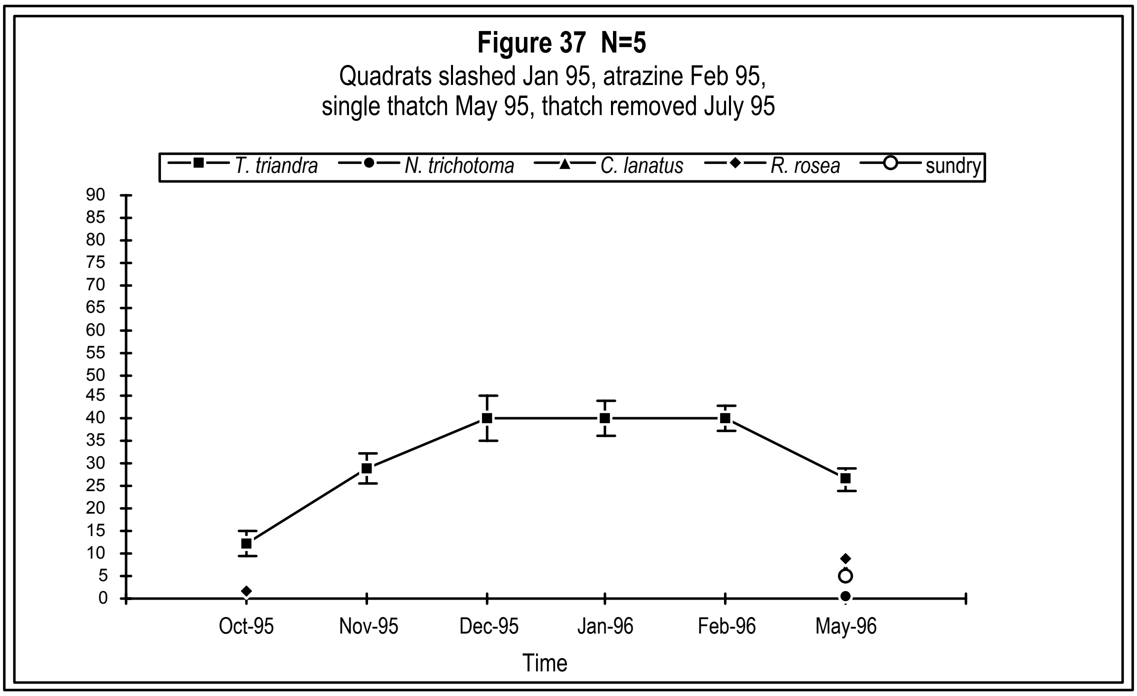


Figure 37 N=5
Quadrats slashed Jan 95, atrazine Feb 95,
single thatch May 95, thatch removed July 95
T. triandra
N. trichotoma
C. lanatus
R. rosea
sundry
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
Oct-95
Nov-95
Dec-95
Jan-96
Feb-96
May-96
Time

Figure 38 N=5
Quadrats slashed Jan 95, glyphosate Feb 95, single thatch May 95, thatch burnt July 95

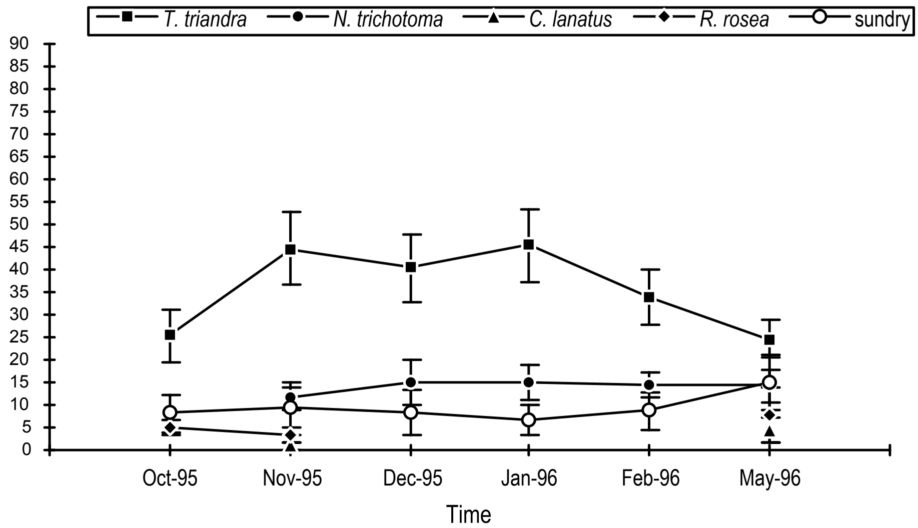
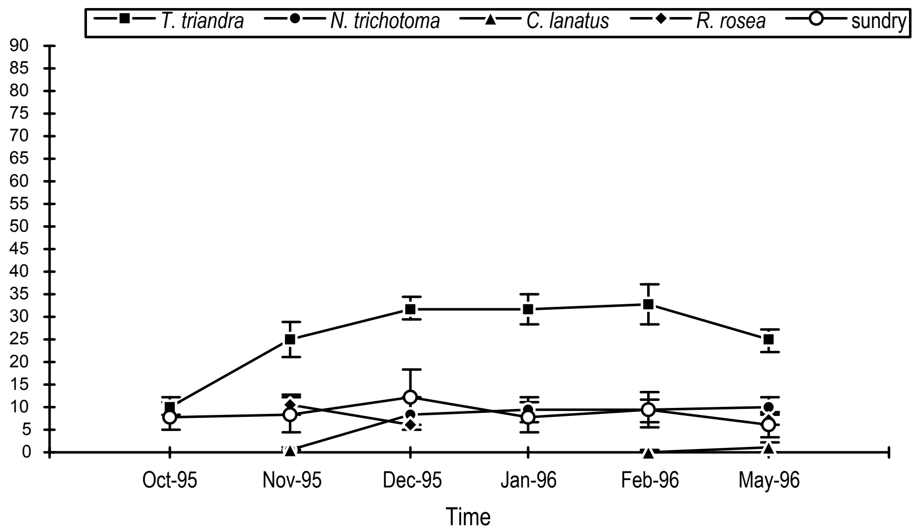


Figure 39 N=5
Quadrats slashed Jan 95, glyphosate Feb 95, single thatch May 95, thatch removed July 95



5.4.3 May – October accession with glyphosate only

Trial site slashed in January, weed regrowth treated with glyphosate (no matched atrazine treatment) in May, seed-bearing *T. triandra* hay laid as thatch in July, thatch removed manually or by burning or manually in October.

At the first assessment in November 1995, one month after removal of hay, average *T. triandra* seedling densities were much higher in burn hay treatments (64 plants m^{-2}) than in manual hay removal treatments (8.8 plants m^{-2}) (Figures 40 and 41). By December 1995 both treatments were at maximum *T. triandra* seedling densities, and over the summer – autumn period seedling densities declined progressively. At the final assessment in May of 1996 average seedling densities on burn hay treatments (35.6 plants m^{-2}) were significantly higher (single factor ANOVA $p<0.0008$) than on hay manual removal treatments (14.8 plants m^{-2}).

The pattern of establishment of weed seedlings on May – October accession (glyphosate treatment) revegetation plots was also different between burn hay and manual removal of hay treatments. Few weed seedlings had established on burn hay treatments by November 1995, one month after treatment (Figure 40). Weed seedling densities continued to increase slowly over the summer and autumn period for this treatment, to reach a density for sundry weeds of 5 plants m^{-2} by the final assessment in May 1996. By comparison, average sundry weed densities in manual hay removal plots had reached 14 plants m^{-2} by December 1995, two months after hay removal (Figure 41), and by May 1996, at the final assessment, this had further increased to 16 plants m^{-2}.

The average densities of *N. trichotoma* seedlings remained low on both burn hay and manual removal of hay treatments, across the assessment period. At the final assessment in May of 1996, average *N. trichotoma* densities were around 3 to 4 plants m^{-2} on both burn hay and manual removal of hay treatments (Figures 40 and 41).

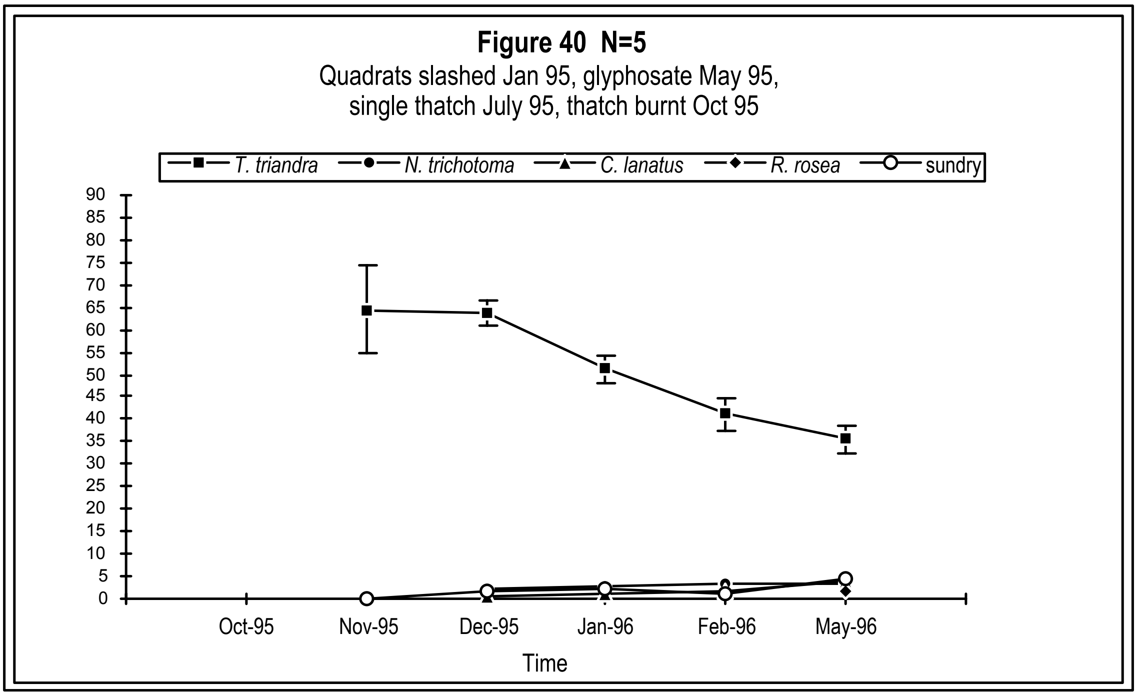


Figure 40 N=5
Quadrats slashed Jan 95, glyphosate May 95,
single thatch July 95, thatch burnt Oct 95
T. triandra
N. trichotoma
C. lanatus
R. rosea
sundry
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
Oct-95
Nov-95
Dec-95
Jan-96
Feb-96
May-96
Time

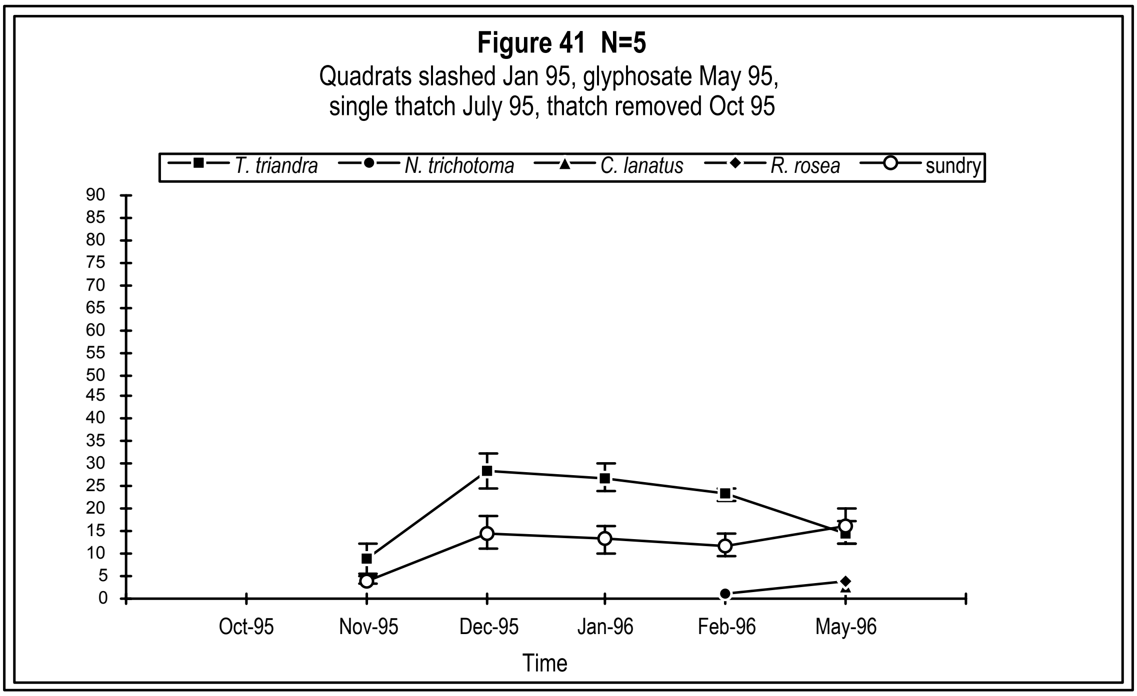


Figure 41 N=5
Quadrats slashed Jan 95, glyphosate May 95,
single thatch July 95, thatch removed Oct 95
T. triandra
N. trichotoma
C. lanatus
R. rosea
sundry
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
Oct-95
Nov-95
Dec-95
Jan-96
Feb-96
May-96
Time

5.4.4 Other treatments investigated (data not shown)

January slash sward, no further treatment (no treatment control sets)

Two randomised control sets of plots (each with five replicates) were established in this trial randomly scattered amongst revegetation treatments, to form foundation data as a comparative measure for all treated plots. These sets were used as a comparison for herbicide trial plots (see Chapter 2) and *T. triandra* establishment trial plots. Control plots were slashed in January 1995 at the same time as all other plots with no further treatment applied.

The average density of *N. trichotoma* on these no treatment control plots was consistently around 40 plants m^{-2} across quadrats (data not shown). Key weed species observed across all quadrats in low numbers were the cool season *C. lanatus* and *R. rosea* (May to October) and the warm season *A. arvensis* (November to February). The observation of *C. lanatus* and *R. rosea* at these times accorded with their appearance at the same time on revegetation plots. *A. arvensis* was either not present, or present at minimal densities on treated plots.

Seed-bearing thatch plus seedless thatch – glyphosate only, burn or physical removal of hay, July – December accession

This treatment was designed to test whether extra hay added to the revegetation process made any difference to the final revegetation outcomes. The treatments resulted in essentially the same seasonal pattern of densities of *T. triandra* seedlings, *N. trichotoma* seedlings and other weed seedlings as for the July – December glyphosate burn or physical removal of hay treatments. It would appear that the amount of hay in the thatch, and its effect on fire intensity during burning removal, does not make a great deal of difference to the final outcomes.

Initial biomass reduction prior to application of herbicide by burning not slashing– glyphosate only, burn or physical removal of hay, July – December accession

The burn hay treatment on plots burnt prior to herbicide application, rather than slashed treatment, resulted in around 30% lower average *T. triandra* seedling densities than the equivalent treatment where the *N. trichotoma* had been slashed prior to application of herbicide. The overall seasonal pattern of establishment of *T. triandra* seedlings was similar to the equivalent control (i.e. slash prior to herbicide application) treatment, but at lower densities of *T. triandra* (details of data not shown). The average *T. triandra* densities on burn pre-treatment plots at the final assessment, in May 1996, was also about 30% lower than on the equivalent control plots (details of data not shown). Patterns of *N. trichotoma* and other weed seedling establishment across the seasons were similar to equivalent control plots with burn removal of hay treatments.

By comparison, the manual removal of hay treatments on plots burnt prior to herbicide application resulted in around 30% higher average *T. triandra* seedling densities than the equivalent control treatment where the *N. trichotoma* had been slashed prior to application of herbicide (details of data not shown). These higher average densities of *T. triandra* seedlings persisted across the seasons, although the overall pattern of seedling establishment was similar to that for equivalent control plots, which had been slashed prior to herbicide application. The average *T. triandra* densities on burn pre-treatment plots at the final assessment, in May 1996, was about 20% higher than on the equivalent control plots. Patterns of *N. trichotoma* and other weed seedling establishment across the seasons were similar to equivalent control plots with manual removal of hay treatments.

5.4.5 Field germination rates

The highest *T. triandra* seedling densities recorded for each treatment set, across the seasons that each treatment was assessed, were used to calculate percentage conversion to seedlings in the field from germinable seeds applied to plots. This data has been summarized in Table 2. Two general trends are apparent from the data: (1) in almost all cases, where treatments involved manual removal of thatch the percentage conversion into seedlings was a lot lower than for the equivalent treatment in which thatch was removed by burning; and (2) glyphosate used as part of treatment resulted in somewhat lower maximum densities of *T. triandra* than for equivalent treatments where atrazine was used, although this second generalization is only based on a comparison of four treatments within the February – July and July – December accessions.

Table 2

Field conversion to seedling of germinable seeds applied to trial plots

Treatments (N=5)				Percentage conversion to seedling
January slash	July atrazine	October thatch	December thatch burnt	18.2%
January slash	July glyphosate	October thatch	December thatch burnt	13.5%
January slash	July atrazine	October thatch	December thatch removed manually	10.4%
January slash	July glyphosate	October thatch	December thatch removed manually	7.7%
*January slash	July glyphosate	October thatch plus thatch seedless	December thatch burnt	14.4%
*January slash	July glyphosate	October thatch plus thatch seedless	December thatch removed manually	10.1%
January slash	February atrazine	May thatch	July thatch burnt	25.8%
January slash	February glyphosate	May thatch	July thatch burnt	20.5%
January slash	February atrazine	May thatch	July thatch removed manually	18.1%
January slash	February glyphosate	May thatch	July thatch removed manually	14.8%
January slash	May glyphosate	July thatch	October thatch burnt	22.3%
January slash	May glyphosate	July thatch	October thatch removed manually	9.3%
*March burnt	July glyphosate	October thatch	December thatch burnt	9.7%
*March burnt	July glyphosate	October thatch	December thatch removed manually	12.2%
* Not discussed in detail in text				

5.5 Discussion

5.5.1 Establishment of *T. triandra* follows predictable patterns

The overall pattern for *T. triandra* establishment is similar for atrazine and glyphosate treatments, but different for burn hay and manual removal of hay treatments.

For each of the 'spray and hay' accessions incorporating burning of hay as part of the treatment, seedlings of *T. triandra* established quickly at high densities, then showed further increase in some cases over early to mid summer, before declining over late summer and autumn (Figures 32, 34, 36 and 38). This pattern appeared to be unaffected by whether the herbicide treatment was atrazine or glyphosate. By comparison, for all treatments incorporating manual removal of hay as part of the treatment, initial *T. triandra* seedling densities were always low, regardless of other aspects of treatment, and progressively increased to reach a maximum in late spring or early to mid summer (Figures 35, 37, 39 and 41).

A likely explanation for why burn hay treatments had higher initial *T. triandra* seedling densities than physical removal of hay treatments is that, in physical hay removal plots, the dead *N. trichotoma* stubble left behind after spraying and subsequent manual hay removal, produces shading and depressed temperatures which slows down *T. triandra* seed germination. By comparison, on burn hay treatments, all of the dead *N. trichotoma* stubble is removed, which would result in low levels of shading, and higher ground temperatures than on physical hay removal treatments. Sindel *et. al.* (1993) found that germination of *T. triandra* is markedly increased with increasing day time temperatures, up to 40° C, and Puhar (1996) has found that *T. triandra* seedlings do not germinate in the dark, so presumably partial shading may reduce percentage germination on physical hay removal plots, at least until the exposed, dead *N. trichotoma* stubble begins to break down.

The pattern of *T. triandra* establishment is affected by time of accession (i.e. season of the sequence: herbicide application – laying hay – removal of hay)

For treatments in which hay was burnt, any further increases *T. triandra* seedling densities after the initial assessment following burning, appeared to depend on the time at which the hay was burnt. When hay was burnt in mid spring (Figure 40) or late spring (Figures 32 and 34) the highest *T. triandra* seedling densities were achieved at the first assessment (i.e. late spring and early summer respectively). By comparison, when hay was burnt in mid winter (Figures 36 and 38), high densities of *T. triandra* seedlings were recorded at the first assessment in mid spring (October) but densities continued to increase over the following few months, to peak in late spring or early summer. It would appear from these results that some *T. triandra* seedlings are able to establish in early spring, but that the majority of seedlings become established in the period November to January (see below for detailed discussion on this point).

In a similar way, for treatments involving physical removal of hay, the extent to which *T. triandra* seedling densities increased after removal of hay and the time taken to reach maximum seedling densities, depended on the time at which the hay was removed. For accessions involving hay removal in mid spring (figure 41) or late spring (Figures 33 and 35), there was a rapid increase in *T. triandra* densities after the initial assessment, reaching a peak in late spring or early summer respectively. For treatments in which the hay had been physically removed in mid winter (Figures 37 and 39), while there was some seedling establishment by mid spring (October), there was also a progressive increase in seedling densities on sites over spring, until maximum densities were reached in late spring (December). As for the burn hay treatments, the results of physical hay removal treatments suggest a specific window of time which is optimum for *T. triandra* seedling establishment

Highest densities of *T. triandra* seedlings occur in November to December; after summer, seedling densities fall to levels approaching those of mature *T. triandra* tussock densities

The results across all of the accession treatments suggest that while some *T. triandra* seedlings are able to establish as early as September to October, the optimum time for seeding establishment is late spring (December) to early or mid summer. These results accord with the findings of Sindel *et. al.* (1993) that the optimum conditions for *T. triandra* seed germination are high temperatures, up to 40^{o} C and high moisture. More recent trials at Victoria University investigating the success of the ‘spray and hay’ method under drought conditions (Dare 1996, C Hocking 1997, pers. comm.) have demonstrated that *T. triandra* seedlings will not establish over summer if water levels are low, but if water is supplied artificially then *T. triandra* seedling establishment is improved.

The decline in *T. triandra* seedling densities following maximum densities achieved in late summer, is consistent with the proposal put forward in the discussion of Chapter 4 that, when *T. triandra* seedling densities are in excess of around 30 plants m^{-2}, the density at which mature *T. triandra* tussocks are found in adjacent indigenous grassland remnants, competition between plants is such that some seedlings die. When *T. triandra* seedling densities in the autumn

(May) following revegetation are compared across treatments (Table 3) it is clear that, for most treatments, seedling densities had reduced to around 25 to 35 plants m^{-2}. Many factors would have affected the final densities of *T. triandra* seedlings. One such factor would be the densities of *N. trichotoma* which, being a tussock grass with similar requirements for growth to that of *T. triandra*, would probably compete in a similar way to *T. triandra* for resources for growth. If the combined densities of *T. triandra* and *N. trichotoma* plants in autumn are compared across treatments (Table 3), most densities are found to lie between 30 and 40 plants m^{-2}, a value very similar to the densities of mature *T. triandra* and *N. trichotoma* plants found in grassland remnants adjacent to the revegetation site (see Chapter 4). Another factor which would have influenced the final densities of *T. triandra* and *N. trichotoma* establishing on site would have been the large scale growth of other weeds. The three lowest combined densities of *T. triandra* and *N. trichotoma* plants across the treatments were on plots with high persistent numbers (and cover) of the exotic thistle *C. lanatus* (Table 3). The treatment with the lowest overall average density of *T. triandra* and *N. trichotoma* combined (glyphosate, May-October accession, physical hay removal) was also had the lowest maximum *T. triandra* seedling density recorded of any of the treatments (Table 3), indicating that this treatment probably produced the poorest conditions for establishment of tussock grasses of any type.

Table 3 A comparison of the average densities of *T. triandra* and *N. trichotoma*, individually and combined, in various 1995 treatments in the 1996 autumn following revegetation, with the highest overall average densities of *T. triandra* seedlings recorded on each treatment during revegetation.				
	Average Seedling Densities (plants m^{-2})			
Revegetation Treatments	*T. triandra* in Autumn	*N. trichotoma* in Autumn	*T. triandra* + *N. trichotoma*	Highest *T. triandra*
Atrazine Burn (Feb-July)	30.8	0.5	31.3	57.0
Atrazine Remove (Feb-July)	26.8	0.5	27.3	40.2
Glyphosate Burn (Feb-July)	24.6	14.2	38.8	45.4
Glyphosate Remove Feb-July	24.8	10.2	35.0	31.6
Glyphosate Burn (May-Oct)	35.6	3.6	39.2	64.4
Glyphosate Remove (May-Oct)	14.8*	3.8	18.6*	27.0
Atrazine Burn July-Dec	31.6	0.0	31.6	83.2
Atrazine Remove (July-Dec)	39.0	0.0	39.0	47.4
Glyphosate Burn (July-Dec)	17.8*	6.8	24.6*	61.6
Glyphosate Remove (July-Dec)	21.2*	0.8	22.0*	35.2
* Consistently high *C. lanatus* (in excess of 10 plants m^{-2}) over the seedling establishment phase of *T. triandra* and *N. trichotoma*.				

5.5.2 Densities of *N. trichotoma* seedlings are affected by herbicide type and time of accession

Final densities of *N. trichotoma* were lower on atrazine treatment plots than on equivalent glyphosate treatment plots.

Seedlings of *N. trichotoma* were either absent or at average densities below a single plant m^{-2} on all revegetation plots treated with atrazine at all stages during the revegetation process, regardless of the time at which the herbicide was administered, or how hay was removed from sites (Figures 32, 33, 36, 37). The success of atrazine as a general treatment for preventing *N. trichotoma* seedling establishment during 'spray and hay' revegetation with *T. triandra* is clearly illustrated by comparing the densities of *N. trichotoma* in the autumn following revegetation across treatments (Table 3). *N. trichotoma* is essentially absent from any plots treated with atrazine, but in varying densities, up to 14 plants m^{-2} on plots treated with glyphosate. The effectiveness of atrazine in preventing establishment of *N. trichotoma* is almost certainly due to the residuality of atrazine in the basaltic clay soils of the revegetation site, and the highly selective action of atrazine in removing *N. trichotoma* seedlings while leaving *T. triandra* seedlings unaffected. Preliminary measurements of atrazine residuality in the soil at the revegetation site over several years suggest that around half of the original levels of atrazine applied to revegetation sites are still present at eight months after application (levels probably depend on extent of rainfall following herbicide application, which in turn is affected by seasonality – C. Hocking 1997, pers. comm.). Other revegetation trials at the St Albans site suggest that even very high levels of atrazine in the soil causes minimal or no mortality of *T. triandra* seedlings (C. Hocking 1999, pers. comm.). By comparison, glyphosate is reported to break down almost immediately on contact with the soil (Parsons 1992).

Seedlings of *N. trichotoma* in glyphosate treatments established in higher densities on February – July accessions than on July – December accessions

The pattern of *N. trichotoma* seedling establishment and the final (May) seedling densities were very different for the February – July (Figures 38 and 39) and July – December (Figures 34 and 35) accessions. For the February – July accession, high densities of *N. trichotoma* had established by the November assessment in each of the burn hay (Figure 38) and physical hay removal (Figure 39) treatments. These densities increased further in December and were then maintained without further increase into the following autumn. The results are consistent with a lack of residual herbicide protection from weed establishment, as expected for the non-residual glyphosate, and with the early to mid spring germination times previously observed for *N. trichotoma* (Campbell 1998), compared with the late spring to summer germination times for *T. triandra* (see discussion above). The final (May) *N. trichotoma* densities on burn hay plots (February-July accession), averaging 14.2 plants m^{-2} were not significantly higher (single factor ANOVA $p>0.3$) than for equivalent physical hay removal plots, which averaged 10.2 plants m^{-2}. These results, and possible reasons for the differences are discussed later in this section.

For the July - December accessions with glyphosate, no *N. trichotoma* seedlings were detected at the first assessment after hay removal, either for hay burn or physical hay removal treatments. By February, a few seedlings had been able to establish on burn treatments but not on physical removal treatments. By May, the final assessment, significant differences in *N. trichotoma* seedling densities had appeared between burn hay (average 6.8 plants m^{-2}) and physical hay removal (average 0.8 plants m^{-2}) treatments (single factor ANOVA $p<0.0005$). It would appear that the retention of hay on revegetation sites until December protects the sites from *N. trichotoma* seedling establishment over early summer, but that, for burn hay treatments at least, some *N. trichotoma* seeds are able to germinate and establish seedlings in the autumn following revegetation. This outcome is consistent with other reports of the capability of *N. trichotoma* seedlings to germinate and establish in autumn as well as spring (Campbell 1998). Possible reasons for the differences in final *N. trichotoma* seedling densities between hay burn and physical hay removal treatments are discussed later in this section.

The pattern of *N. trichotoma* seedling establishment on glyphosate treated May – October accessions displayed features of each of the equivalent February – May and July – December accessions

For the burn hay treatments in the May-October accession, *N. trichotoma* seedlings had established at low levels (average 2.2 plants m^{-2}) by one month (November) following hay removal (Figure 40). Densities of *N. trichotoma* continued to increase slowly over the spring and level out in summer, to reach a final average density of 3.6 plants m^{-2} by the final assessment in May 1996. By comparison, no *N. trichotoma* seedlings were able to establish on physical hay removal treatment plots over the spring or early summer following hay removal (Figure 41). By February 1996, a few *N. trichotoma* seedlings were recorded, and there appeared to be an autumn burst of establishment, leading to a final (May) density of 3.8 seedlings m^{-2}. In summary, while there was a clear difference in the pattern of establishment of *N. trichotoma* seedlings between hay burn and physical hay removal treatments, the final densities of *N. trichotoma* seedlings in the autumn following revegetation were not significantly different (single factor ANOVA $p>0.2$). It is possible that the burn hay treatments would have lead to higher *N. trichotoma* seedling numbers on May – October accessions, in a similar response to the burn hay treatments for February – July and July – December accessions (see discussion below), but that *N. trichotoma* seedling establishment on May – October accessions was dampened by the high persistent densities of *T. triandra* seedlings on these treatments over the key spring and autumn establishment times for *N. trichotoma* seedlings (Figure 40).

Final densities of *N. trichotoma* in glyphosate treatments were affected by the type of treatment used to remove hay (burning versus physical removal) in a variety of ways across different accessions.

For both February – July and July – December accessions with glyphosate treatments, the final (May 1996) *N. trichotoma* seedling densities were significantly higher on burn hay treatments than physical hay removal treatments (two factor ANOVA $p<0.003$) (Figures 34, 35, 38 and 39). Densities of *N. trichotoma* on burn hay treatments were sufficiently high (average 14.2 plants m^{-2} on February – July accessions and average 6.8 plants m^{-2} on July – December accessions) to present significant difficulties for ongoing management of sites with this type of revegetation outcome. A possible explanation for the higher *N. trichotoma* seedling densities on burn hay treatments compared with physical hay removal treatments is that the stubble of dead *N. trichotoma* remaining after *T. triandra* hay removal on physical removal treatments provides sufficient cover to depress the rate of germination of *N. trichotoma* seeds. A similar proposal was put forward in section 5.5.1 to explain the significantly lower densities of *T. triandra* seedlings, and the more gradual pattern of establishment of *T. triandra* seedlings on physical hay removal treatments compared with burn hay treatments. Recently Puhar (1996) has found that *N. trichotoma* seed germination is inhibited in the dark. On plots in which the *T. triandra* hay was burnt, shading effects would have been minimal, as all above-ground dead *N. trichotoma* plant material was removed during burning. The resulting higher light levels, possibly combined with higher soil temperatures in spring and autumn, which potentially promoted the germination of *N. trichotoma* seeds and growth of seedlings.

5.5.3 Densities of weeds post-establishment are affected by herbicide type and time of accession

Weed seedling establishment was negligible in all atrazine treatments, across all other combinations of treatments

As for densities of *N. trichotoma*, the establishment on atrazine treated plots of seedlings of other weeds commonly occurring on revegetation plots was negligible (Figures 32, 33, 37 and 38). The effect of atrazine, in preventing the establishment of a range of weeds, was consistent across the two times of accession investigated (February – July and July – December), as well as across the two types of hay removal (burning and physical removal). The reasons for this positive effect of atrazine are most likely similar to its effect in suppressing *N. trichotoma* seedling establishment (discussed above); that is, atrazine is a residual herbicide, selective for a wide range of monocotyledon and dicotyledon plants with C_3 photosynthesis (Parsons 1992).

Weed seedling establishment for glyphosate treatments was much higher in early accessions than later accessions.

The densities of sundry weeds (thistles and annual grasses combined) on plots treated with glyphosate in February, with hay removal in July (Figures 38 and 39) was much higher than on comparable plots treated with glyphosate in July, with hay removal in December (Figures 34 and 35). This difference is most likely due to the shading effect of the hay, in the July-December accessions, preventing weed seedling establishment over the spring. For this accession, by the time that conditions were again suitable for weed seedling establishment on plots, in the following autumn, it is probable that there were sufficient *T. triandra* seedlings established to minimize seedling establishment, although the densities of weeds in autumn did rise to some extent (Figures 34 and 35).

The February – July accession glyphosate treatment results (Figures 38 and 39) clearly show that weeds were able to establish in high densities on plots by the third month (October) after hay removal. Densities of weeds were similar for both burn hay and physical hay removal treatments. High densities of weeds persisted on both hay removal treatments over the spring and summer. In autumn, sundry weed densities declined marginally on physical hay removal treatments and increased on hay burn treatments, probably reflecting the individual responses of autumn germinating weeds in the sundry category (thistles, other broadleaf weeds and annual grasses) to the different soil temperature, moisture and shade levels produced by the two treatments. As a generality, the densities of these types of weeds on plots at the levels observed for the glyphosate February – July accessions would present ongoing problems for management if such results translated into similar outcomes on a broad scale during revegetation. Most of the observed weed species are persistent in the seedbank and easily dispersed and would continue at sufficient densities to maintain high numbers in the seedbank at the trial site.

Weed seedling establishment for glyphosate treatments in May – October accessions was different for burning versus physical removal of hay, but not for other accessions.

Unlike the two other accessions, for which sundry weed densities were similar for burn hay and physical hay removal treatments, for the May – October treatment the densities of sundry weeds was consistently much higher for the physical removal treatment (Figure 41) than for the burn hay treatment (Figure 40). The most likely explanation for this outcome is that, in the May – October burn hay treatment, the fire was applied at a time that the sundry weeds were most sensitive to mortality by fire. The results of the physical removal of hay treatments for the February – July accession (Figure 39) show that high densities of sundry weeds can become established on revegetation plots by October if the cover afforded by the *T. triandra* hay is removed. This suggests that the seeds of these weeds are responding to the spring conditions. It is likely that the fire applied in October to glyphosate, burn hay May – October accession treatment plots caused significant mortality to seedlings beginning to germinate and grow up through the *T. triandra* hay at this time. It is also apparent that the burning of the hay in October does not fully remove all weeds from the spring growth period, because by November, some weeds were recorded on the plots (Figure 40). By December sundry weed densities had increased further, with still more increases by the following May (1996).

5.5.4 Predictability of *T. triandra* seedling establishment

Overall, the rates of conversion of germinable seed into seedlings during revegetation ranged between 8% and 26% (Table 2). This is the range that might be expected in most revegetation trials, because the trials in this investigation varied widely across a number of key determining parameters for 'spray and hay' revegetation. Other revegetation projects by Victoria University using the 'spray and hay' method have found a similar range in the percentage conversion of germinable seeds to seedlings. The reasons for the maximum conversion rate being approximately 25% are not known, but are likely to include scavenging of germinable seed by ants, bird and other animals, inappropriate germination sites for some germinable seed and mortality of young seedlings due to herbivory by invertebrates, rabbits and other animals.

As noted in the results section, the maximum percentage conversion of germinable *T. triandra* seeds into seedlings was higher for hay burn treatments than for physical hay removal treatments (Table 2), probably because burning removes the dead *N. trichotoma* stubble in addition to the *T. triandra* hay, and exposes the revegetation sites to higher light and temperature conditions at ground level than for sites where the dead *N. trichotoma* stubble is intact. The generally higher rates of *T. triandra* seed conversion into seedlings on atrazine treatments compared to glyphosate treatments was probably because the residual atrazine was more effective at preventing the establishment of *N. trichotoma* and other weeds, whereas these species establishing on glyphosate treated plots would have taken up a proportion of the light, water and nutrient resources otherwise available to *T. triandra* seedlings, resulting in lower rates of germination and growth of *T. triandra* seedlings.

5.5.5 General Discussion: A possible model for the effects of different treatments on outcomes

The investigations reported in this thesis were intended to determine what the most effective treatments were for utilizing the 'spray and hay' method for replacing *N. trichotoma* with *T. triandra* during revegetation of Western (basalt) plains grassland. The reasons why some treatments involving herbicides, type of removal of *T. triandra* hay and timing of accession of treatments were more successful than others was not a key focus for study. However, the outcomes of the investigations do lead to some reflections as to why particular treatments may have been more successful than others, which may form a basis to guide future investigations aimed at further developing and refining the 'spray and hay' method. In brief, these reflections are as follows:

Seedlings of both *T. triandra* and *N. trichotoma* can begin to grow on revegetation sites cleared of vegetation (above ground and below ground) in October. However, the pattern of seedling establishment of the two species is different. Germination and establishment of *T. triandra* seedlings can take place over late spring (December) and even into January and February, depending on how much water is available in the soil over summer, whereas germination and establishment of *N. trichotoma* seedlings is mostly complete by November. A small percentage of *N. trichotoma* seedlings are also able to establish in the autumn following revegetation.

Other weeds which are capable of establishing on revegetation plots (thistles, other broad leaf weeds and annual grasses) begin germination and establishment in the early spring and are mostly finished establishing by November or December. There is an additional short period of germination and establishment for some of these species in the autumn following revegetation.

The application of atrazine during 'spray and hay' revegetation, at label strength and backpack rates, in any of the possible accession times, ensures that there is sufficient residual atrazine in the soil to prevent *N. trichotoma* seedling establishment during the optimum period for this species (October – December). Also there is sufficient atrazine residuality in the autumn following revegetation to minimize *N. trichotoma* seedling establishment during February – May. Residual levels of atrazine are also effective in preventing the establishment of other weed species in spring, and in minimizing establishment of these species in the autumn following revegetation.

Whether hay is removed from revegetation plots by burning or physical removal affects the pattern of *T. triandra* seedling establishment, but not the final outcome. Burning of the *T. triandra* hay also removes dead *N. trichotoma* stubble and results in rapid establishment of *T. triandra* seedlings, whereas physical removal of hay leaves some dead *N. trichotoma* stubble in place, and hence partial shading of the ground. The effect of this is a slower, more progressive germination and establishment of *T. triandra* seedlings over late spring and summer, and may leave *T. triandra* seedling establishment more susceptible to the effects of summer drought.

Application of glyphosate as part of 'spray and hay' revegetation kills the mature *N. trichotoma* on site, but does not provide residual protection. The time at which the 'spay and hay' revegetation steps take place have an important bearing on the outcomes. If the *T. triandra* hay (and hence protective cover) is removed from revegetation sites during early or mid-spring, the opportunity arises for *N. trichotoma* and other weed seedlings to establish at high densities. If protective hay cover is retained until October, then establishment of *N. trichotoma* and other weeds is lessened. However, the lower level establishment of weeds in autumn is not avoided.

Burning of *T. triandra* hay in July on glyphosate treated plots does not have a major effect on the establishment of annual grasses and broadleaf weeds, because the main time for seedling establishment of these weeds is after this time. However, burning hay in October or December reduces the level of spring establishment of annual grasses and broadleaf weeds, presumably because by this stage some seedlings are beginning to germinate under the thatch, and burning kills these seedlings.

However, when hay is burnt on glyphosate treated plots in July or December, the establishment of *N. trichotoma* seedlings is promoted more than on physical hay removal treatments, presumably because the fire removes the dead *N. trichotoma* stubble on the revegetation sites, and exposes the seeds to light and higher soil temperatures, both of which would promote seed germination and seedling establishment.

It is also apparent that there is a more complex set of interactions between plant species growing on revegetation sites after 'spray and hay' treatments (especially for glyphosate treatments), which affect the outcomes of revegetation. Two examples of this are: (1) *N. trichotoma* seedling establishment on glyphosate, burn, May – October succession treatments is probably kept low because the treatment also results in high persistent *T. triandra* seedling densities over the spring and autumn periods of optimum establishment for *N. trichotoma*. The high densities of *T. triandra* seedling would most likely offer vigourous competition for resources which would limit *N. trichotoma* seedling establishment; and (2) the removal of the dead *N. trichotoma* stubble by burning can also advantage the establishment of high densities of *T. triandra* seedlings, but only if the burning takes place immediately prior to the optimum time for *T. triandra* seedling establishment (late spring to mid summer). Burning at other times (e.g. July) can advantage the establishment of *N. trichotoma* and other weed seedlings over *T. triandra* seedling establishment. If significant weed numbers are on revegetation sites at the optimum time for *T. triandra* seedling establishment, the cover of these weeds and their utilization of underground resources (water, nutrients, etc.) will probably have an inhibitory effect on *T. triandra* seedling establishment.

6. General conclusions and recommendations for future research

6.1 Conclusions

Research reported in this thesis involving removal of *N. trichotoma* and its replacement by *T. triandra* has resulted in the following recommended techniques:

- Harvest seed-bearing *T. triandra* hay and store in a weather and vermin-proof location in a container such as a wool bale which allows air circulation,
- Reduce *N. trichotoma* weed biomass by slashing the weed sward to approximately 80 mm,
- Spray *N. trichotoma* weed regrowth with recommended label strength atrazine or glyphosate at back pack rates (wet to run-off point) – preferably in late autumn,
- Thatch site with seed-bearing *T. triandra* hay in winter or early spring,
- Remove hay through controlled burning or manual removal in late spring.

6.1.1 Critical elements

Harvest and storage of *T. triandra* hay

Summer harvest of seed-bearing *T. triandra* hay over the three years of this research revealed the variability that can occur in optimum harvest time. Hay harvest for the three years were carried out on the following dates:

1993 – 94 trials	8 January 1993,
1994 – 95 trials	14 January 1994,
1995 – 96 trials	7 March 1995.

***N. trichotoma* weed biomass removal**

N. trichotoma biomass was reduced on trial plots in early summer (January), prior to thatching by slashing the sward to 80 mm length. This was followed by herbicide control of regrowth using glyphosate or atrazine at label recommended dilutions, applied at 770 ml/m^{-2} in autumn or winter. Note: Subsequent monitoring in 1997 of the year 3 trial plots established in 1995-96 suggest that the late autumn (May) herbicide application and associated treatment accession dates (thatching in July and thatch removal in October) make up the optimum treatment process (C. Hocking 1997, pers. comm.).

Seed content and germinability

Laboratory-based assessment of seed content and germinability revealed a dormancy operating on *T. triandra* seed germination. The assessments showed an increase over time of both available (free) seed, and germination rates. Data from laboratory-based assessment of seed was used to determine rates of germinability occurring in the field. It was revealed that conversion into seedlings in the field were averaging a rate of 15% of the available germinable seed being applied to plots. Data from these trials also showed variation of seed availability and germinability from hay within, and between sites.

Optimum *T. Triandra* establishment and thatch rates

Both the 1994 - 95 and 1995 - 96 trials which showed establishment rates of 30 to 40 *T. triandra* plants m^{-2} provided the optimum seedling densities. In order to achieve 30.0 plants m^{-2}, and using a modifier of 15% conversion into seedlings in the field of germinable seed (see above) a minimum of 200 available germinable seeds m^{-2} should be applied in seed-bearing thatch.

Thatching and thatch removal

The seed content of hay was used to select which wool bales of hay were used on the 1995 - 96 trials. Homogenising the hay used was critical in equalising seed content across all thatch treatments. This is proposed as necessary in any *T. triandra* establishment using the ‘spray and hay’ technique to avoid patchiness in seedling establishment (too many seedlings in some areas and not enough in others). Either burn or manual removal of hay in late spring or early summer produced satisfactory (>30 plants m^{-2}) rates of *T. triandra* establishment.

6.2 Transferability of results

The work reported in this thesis has resulted in techniques for removing *N. trichotoma* from *T. triandra*-dominated grassland remnants and replacing this with *T. triandra* at St Albans, Victoria. The consistency of results over the three years of research suggest that the methods are reliable in the context of the site and conditions of this research. Caution is urged in the appropriation of the findings of this research to other sites and other target weed species. It is proposed that the methodology produced by this research be assessed and modified as required before use on other sites and other target species.

6.3 Setting up trials as a first step to management

This research has produced an effective method for replacing *N. trichotoma* with *T. triandra* on western basalt soils on a site sixteen kilometres north-west of Melbourne. It is proposed this methodology could be used as a basis for further development of methods for:

1. The management of *N. trichotoma*, a noxious weed of south-east Australia on non-ploughable or marginal farmland, roadsides, rail corridors, firebreaks and other related land,
2. The conservation management of remnant *T. triandra*- dominated western basalt plains grassland.

However, before this management tool can be applied with any confidence in the predictability of results further site specific trialing of the methodology should be carried out. It is recommended the following four treatment regimes be trialed before selecting methods for broad-scale site treatment (Tables 4, 5, 6, and 7).

Table 4
Treatment 1

TIME	ACTION

• January	Slash target *N. trichotoma* weed sward
• At the optimum time prior to full seed dehiscence (generally mid - late summer)	Harvest and securely store seed-bearing *T. triandra* hay
• February	Treat *N. trichotoma* regrowth with atrazine
• March - April	Assess seed availability/germinability in *T. triandra* hay (see 3. Harvesting and assessing *Themeda triandra* seed-bearing hay for revegetation)
• May	Apply seed-bearing *T. triandra* thatch with appropriate volume to achieve = 30 plants/m^{-2}. Adjust thatch volume to provide highest possibility of achieving =30.0 *T. triandra* plants/m^{-2} (see *T. triandra* germination rates in the field, *in* 5.4.5 Field germination rates)
• July	Burn removal of thatch

Table 5
Treatment 2

TIME	ACTION
• January	Slash target *N. trichotoma* weed sward
• At the optimum time prior to full seed dehiscence (generally mid - late summer)	Harvest and securely store seed-bearing *T. triandra* hay
• July	Treat *N. trichotoma* regrowth with atrazine
• August - September	Assess seed availability/germinability in *T. triandra* hay (see 3. Harvesting and assessing *Themeda triandra* seed-bearing hay for revegetation)
• October	Apply seed-bearing *T. triandra* thatch at appropriate volume to achieve =30 plants m^{-2}. Adjust thatch volume to provide highest possibility of achieving =30.0 *T. triandra* plants m^{-2} (see *T. triandra* germination rates in the field, *in* 5.4.5 Field germination rates)
• December	Manually remove thatch

Table 6
Treatment 3

TIME	ACTION
• January	Slash target *N. trichotoma* weed sward
• At the optimum time prior to full seed dehiscence (generally mid - late summer)	Harvest and securely store seed-bearing *T. triandra* hay
• July	Treat *N. trichotoma* regrowth with atrazine
• August - September	Assess seed availability/germinability in *T. triandra* hay (see 3. Harvesting and assessing *Themeda triandra* seed-bearing hay for revegetation)
• October	Apply seed-bearing *T. triandra* thatch with appropriate volume to achieve =30 plants m^{-2}. Adjust thatch volume to provide highest possibility of achieving =30.0 *T. triandra* plants m^{-2} (see *T. triandra* germination rates in the field, *in* 5.4.5 Field germination rates)
• December	Burn removal of thatch

Table 7 Treatment 4	
TIME	ACTION
• January	Slash target *N. trichotoma* weed sward
• At the optimum time prior to full seed dehiscence (generally mid - late summer)	Harvest and securely store seed-bearing *T. triandra* hay
• May	Treat *N. trichotoma* regrowth with glyphosate
• May - June	Assess seed availability/germinability in *T. triandra* hay (see 3. Harvesting and assessing *Themeda triandra* seed-bearing hay for revegetation)
• July	Apply seed-bearing *T. triandra* thatch with appropriate volume to achieve =30 plants m^{-2}. Adjust thatch volume to provide highest possibility of achieving =30.0 *T. triandra* plants m^{-2} (see *T. triandra* germination rates in the field, *in* 5.4.5 Field germination rates)
• October	Burn removal of thatch

Trial structure

The following procedures are recommended when establishing field trials:

- A block area is recommended to be established at the trial site with randomised sets of quadrats established within the block,
- Quadrats should be a minimum area of 1.0 m^{-2} with a perimeter buffer zone external to each quadrat to minimise 'edge effect' biases,
- Each treatment plus control should have a minimum of six replicates,
- If possible data should be collected at monthly accessions, or at a minimum of two-monthly accessions for at least twelve months after final treatments,
- Data collected is recommended to be simply a recording of the species and their number present at each accession. Plants should not be removed, and should be recounted if still present at each assessment time. This should provide data regarding fluctuations in species present and their numbers over time.

References

Aberdeen, I. (1995). 'A strategy for the management of Serrated Tussock in Victoria' (Inland Agriculture Pty Ltd, Melbourne).

Adamson, D. A. & Fox, M. D. (1982). Change in Australasian vegetation since European settlement, *In* 'A History of Australasian Vegetation', ed. J.M.B. Smith, (McGraw-Hill, Sydney) pp 109 - 146.

Alard, D., Bance, J-F. and Frileux, P-N. (1994). Grassland Vegetation as an Indicator of the Main Agro-Ecological Factors in a Rural Landscape: Consequences for Biodiversity and Wildlife Conservation in Central Normandy (France), *Journal of Environmental Management* (42), (Academic Press), pp 91-109

Anderson, P. (1995). Ecological restoration and creation: a review. *Biological Journal of the Linnean Society* (Linnean Society of London), 56 (Supplement), pp 187 - 211.

Barr, N. and Cary, L. (1992). 'Greening a Brown Land: An Australian Search for Sustainable Land Use', (Macmillan Education Australia, Melbourne).

Best, E. P. H., van der Schaaf, S. and Oomes, M. J. M. (1995). Responses of restored grassland ditch vegetation to hydrological changes, 1989 – 1992. *Vegetatio* (116), (Kluwer Academic Publishers, Belgium), pp 107 - 122.

Bradley, J. (1986). 'Bringing Back the Bush', (Lansdowne Press, Sydney).

Buchanan, R. A. (1989). 'Bush Regeneration', (Department of Technical and Further Education [NSW], Sydney).

Cairns, J. (Jr) (1995). Restoration Ecology: Protecting Our National and Global Life Support Systems. *In* 'Rehabilitating Damaged Ecosystems', ed. J. Cairns Jr (second edition), (CRC Press, USA) pp 1-12.

Campbell, M. H. (1958). The problem of serrated tussock in the central tablelands of NSW. proceedings of Australian Agristology Conference, (University of New England), paper 73.

Campbell, M. H. (1982). The Biology of Australian Weeds 9: *Nassella trichotoma, Journal of Australian Institute of Agricultural Science*, (48), pp 76-84

Campbell, M. H. (1985). Serrated tussock identification and life history, AgFact P7.6.29, (Department of Agriculture, Orange, NSW).

Campbell, M. H. (1990a). Serrated tussock control, AgFact pamphlet, (NSW Agriculture and Fisheries, Orange, NSW).

Campbell, M. H. (1990b). Serrated tussock control, supplement to AgFact P7.6.30, (NSW Agriculture and Fisheries, Orange, NSW).

Campbell, M. H. (1995). Methods of Controlling Serrated Tussock, proceedings of Serrated Tussock Control Seminar, ed. L. Miller, (Grassland Society of Victoria), pp 8–10.

Campbell, M. H. (1998). Biological and ecological impact of serrated tussock *Nassella trichotoma* ([Nees] Arech.) on pastures in Australia, *Plant Protection Quarterly*, 13 (2) pp 80–86

Carr, G. W. and Muir, A. M. (1993). 'A Review of Revegetation at Organ Pipes National Park, Sydenham, Victoria. Ecology Australia' (unpublished report to the Department of Conservation & Natural Resources).

Carr, G. W. and Todd, J. A. (1991). 'Vegetation of the Brens Drive Site and an Evaluation of Native Grassland Establishment Techniques, Royal Park, City of Melbourne, Victoria', (Ecological Horticulture [unpublished report to Melbourne City Council]).

Carr, G. W., Robinson, K. E. and Yugovic, J. V. (1992). 'Environmental weed invasions in Victoria: Conservation and management implications', (Department of Conservation & Environment, Melbourne).

Coetzee, J. P., Bredenkamp, G. J. and van Rooyen, N. (1995a). Plant Communities of the Sub-humid Cool Temperate Mountain Bushveld of the Pretoria - Witbank - Heidelberg are, South Africa. *South African Journal of Botany*, 61 (3), pp 114 - 122.

Coetzee, J. P., Bredenkamp, G. J. and van Rooyen, N. (1995a). The phytosociology of the grasslands of the Ba and Ib land types in the Pretoria - Witbank - Heidelberg area. *South African Journal of Botany*, 61 (3), pp 123 - 133.

Conway, W. (1988). Can Technology Aid Species Preservation?, *In* 'Biodiversity', ed. E.O. Wilson, (National Academy Press, Washington), pp 263-268.

Craigie, V. and Hocking, C. eds. (1999). Down to Grass Roots. proceedings of a Conference on management of grassy ecosystems, 9 and 10 July 1998, Victoria University, St Albans, (Department of Natural Resources & Resource Environment, Melbourne).

Craigie, V. and Ross, J. (1995). 'Grassy Ecosystem Implementation Schedule', (Grassy Ecosystem Reference Group, Melbourne).

Craigie, V. and Stuwe, J. (1992). 'Derrimut Grassland Reserve Draft Management Plan', Department of Conservation & Environment, Melbourne (unpublished).

Dare, R. (1997). 'Control of Chilean Needle Grass and associated weeds using selected herbicides and replacement by Kangaroo Grass using the spray and hay method', unpublished Honours Thesis, (Department of Biology, Victoria University).

Delpratt, J. (in progress). 'Seed Production and Cultivation of Indigenous Wildflowers in Victoria'. MSc thesis, (University of Melbourne [Burnley]).

Department of Conservation & Environment (1990). 'Remnant Native Grasslands and Grassy Woodlands of the Melbourne Area: An Action Plan for Conservation Based on Biological Values', (Department of Conservation & Environment, Melbourne).

Department of Conservation & Environment (1991). 'Schedule 2 (1991), Flora and Fauna Guarantee Act, (1988)', (Department of Conservation & Environment, Melbourne).

Department of Conservation & Environment (1992). 'Draft conservation Program for Native Grasslands and Grassy Woodlands in Victoria', (Department of Conservation & Environment, Melbourne) (unpublished).

Department of Conservation & Environment, Australian Heritage Commission and Victorian National Parks Association (1992). 'Native Grassland Management in the Melbourne Area', (Department of Conservation & Environment, Melbourne).

Department of Conservation & Natural Resources (1994). 'Flora and Fauna Gaurantee Act (1988) Action Statement Nº 53 Western (Basalt) Plains Grassland', (Department of Conservation & Natural Resources, Melbourne).

Douglas, J. G. (1993). Geology and Geomorphology of Victoria. *In* 'The Flora of Victoria,' eds. D. B. Foreman, and N. G. Walsh, Vol 1, pp 24 – 46 (Royal Botanic Gardens Melbourne, Inkata Press, Melbourne).

Dutoit, T. and Alard, D. (1995). 'Permanent seed banks in chalk grassland under various management regimes: their role in the restoration of species-rich plant communities', *Biodiversity and Conservation* 4 (9), pp 939–950.

Estes, J. R., Tyrl, R. J. and Brunken, J. N. (1989). 'Grasses and Grasslands: Systematics and Ecology', (University of Oklahoma Press, Norman).

Evans, L. T. and Knox, R. B. (1969). Environmental Control of Reproduction in *Themeda australis* , *Australian Journal of Botany* (17), pp 375–389.

Gardener, M.R. and Sindel, B.M. (1998). The biology of *Nassella* and *Achnatherum* species naturalized in Australia and the implications for management of conservation lands. *Plant Protection Quarterly*, 13 (2) pp 76–80

Gibbons, F. and Rowan, J. (1993). Soils in Relation to Vegetation in Victoria, *In* 'The Flora of Victoria,' eds. D. B. Foreman, and N. G. Walsh, Vol 1, pp 159 - 194 (Royal Botanic Gardens Melbourne, Inkata Press, Melbourne).

Gott, B. and Zola, N. (1992). 'Koorie Plants Koorie People', (Koorie Heritage Trust, Globe Press, Melbourne).

Gott, B. (1993). Use of Victorian plants by Koories, *In* 'The Flora of Victoria,' eds. D. B. Foreman, and N. G. Walsh, Vol 1, pp 195–211 (Royal Botanic Gardens Melbourne, Inkata Press, Melbourne).

Govanstone, A., Ross J. and Robertson, M. (1992). 'Native Grassland Management in the Melbourne Area', (Department of Conservation & Environment, Melbourne).

Groves, R. H., Hagon, M. W., Ramakrishnan, P. S. (1982). Dormancy and Germination of Seed in Eight Populations of *Themeda australis*. *Australian Journal of Botany*, (30), pp 373–386.

Groves, R. (1965). Growth of *Themeda australis* Tussock Grassland at St Albans, Victoria. *Australian Journal of Botany*, (13), pp 291–302.

Groves, R. (1975). Growth and Development of Five Populations of *Themeda australis* in Response to Fire. *Australian Journal of Botany*, (23), pp 951–963.

Gugerli, F. (1993). Seed Bank as a Resource for Restoring of Extensively Managed, Species Rich Grassland. *Botanica Helvetica* 103 (2), pp 177–191.

Hagon, M. W. (1976). Germination and Dormancy of *Themeda australis*, *Danthonia* spp., *Stipa bigeniculata* and *Bothriacloa macra*. *Australian Journal of Botany*, (24), pp 319 - 327.

Hagon, M. W. (1977). Effects of Competition, Herbicides and Activated Carbon on Establishment of Australian Grasses. *Weed Research*, Vol 17 (5) pp 297-301

Hagon, M. W. and Chan, C. W. (1977). The Effects of Moisture Stress on the Germination of Some Australian Native Grass Seeds. *Australian Journal of Experimental Agriculture and Animal Husbandry*, 17 (84), pp 86-89

Hagon, M. W. and Groves, R. H. (1977). Some Factors Affecting the Establishment of Four Native Grasses. *Australian Journal of Experimental Agriculture and Animal Husbandry*, 17 (84), pp 90 - 96

Hagon, M. W. (1976). Germination and Dormancy of *Themeda australis*, *Danthonia* spp., *Stipa bigeniculata* and *Bothriacloa macra*. *Australian Journal of Botany*, (24), pp 319–327.

Hitchmough, J. D. (1994). The management of semi-natural and natural vegetation, *In* 'Urban Landcape Management' ed. J.D. Hitchmough pp 392–422. (Inkata Press, Sydney),

Hitchmough, J. D., Kilgour, R. A., Morgan, J. W., Shears, I. G. (1994). Efficacy of some grass specific herbicides in controlling exotic grasses in native grassy vegetation. *Plant Protection Quarterly*, 9 (1), pp 28–34.

Hitchmough, J., Berkley S. and Cross R. (1989). Flowering Grasslands in the Australian Landscape. *Landscape Australia* 4/89, pp 394 - 403

Hocking, C. (1998). Land management of *Nassella* areas – implications for conservation. *Plant Protection Quarterly*, 13 (2) pp 86–91

Jacobson, E. T., Wark D. B., Arnott R. G., Haas R. J. and Tober D. A. (1994). 'Sculptured Seeding: An Ecological Approach to Revegetation'. *Restoration and Management Notes* 12:1 pp 46 - 50

Jordan, W. R. (1995). Restoration Ecology: A Synthetic Approach to Ecological Research, *In* 'Rehabilitating Damaged Ecosystems', ed. J. Cairns Jr, 2nd edition, pp 373-384 (CRC Press, USA).

Katz, E. (1991). The Ethical Significance of Human Intervention in Nature. *Restoration and Management Notes* 9 (2), pp 90-96.

Kilby, M., Groves R., Woodruff B. and Green K. (1993). Factors Affecting Establishment of Native Grassland Forbs. *In* 'Technical Services Unit City Parks, Annual Report', p 117, (ACT Parks and Conservation Service).

Knapp, E. E. and Rice, K. J. (1994). 'Starting from Seed: Genetic Issues in Using Native Grasses for Restoration'. *Restoration and Management Notes* 12 (1), pp 40 - 45

Lamp, C. A., Forbes, S. J. and Cade, J. W. (1990). 'Grasses of Temperate Australia: A Field Guide', (Inkata Press, Melbourne).

Lamp, C. and Collet, F. (1989). 'Field Guide to Weeds in Australia', (Inkata Press, Melbourne).

Lunt, I. and Morgan, J. (1998). Lessons from grassland management of Laverton North and Derrimut Grassland Reserves: what can we learn for future grassland management? Down to Grass Roots. proceedings of a Conference on management of grassy ecosystems, 9 and 10 July 1998, Victoria University, St

Albans, pp 69 - 74 (Department of Natural Resources & Resource Environment, Melbourne).

Lunt, I. (1990). Impact of an autumn fire on a long-grazed *Themeda triandra* (Kangaroo Grass) grassland: implications for management of invaded, remnant populations. *The Victorian Naturalist* 107 (2), pp 45–51.

Lunt, I. (1991). Management of remnant lowland grasslands and grassy woodlands for nature conservation: a review. *The Victorian Naturalist* 108 (3), pp 56–66.

Lunt, I. (1992). Rare or threatened plants of lowland grasslands and grassy woodlands in Victoria. *Advances in Nature Conservation*, 1 (1) pp 7–12.

McDougall, K. L. (1989). 'The Re-establishment of *Themeda triandra* (Kangaroo Grass): Implications for the Restoration of Grassland', Technical Report Series Nº 89, (Department of Conservation, Forests and Lands, Melbourne).

McDougall, K. L. (1994). Grassland Flora and Significant Species. *In* 'Conservation of Lowland Native Grasslands in South-Eastern Australia', eds. McDougall K. L. and Kirkpatrick J.B. pp 4–7 (World Wide Fund For Nature, Australia).

McDougall, K. L. and Kirkpatrick, J.B. (1994). Synthesis and Recommendations. *In* 'Conservation of Lowland Native Grasslands in South-Eastern Australia', eds. McDougall K. L. and Kirkpatrick J.B. pp 158–167 (World Wide Fund For Nature, Australia).

McIntyre, S. (1993). Disturbance: Does it have a role in the maintenance of rare grassland plants?, Management of Relict Lowland Grasslands. proceedings of a workshop and public seminar, Conservation Series Nº 8, pp 38–41 (ACT Parks and Conservation Service).

McLaren, D.A., Stajsic, V. and Gardener, M.R. (1998). The distribution and impact of South/North American stipoid grasses (Poaceae: Stipeae) in Australia, *Plant Protection Quarterly*, 13 (2) pp 62–70.

Milberg, P. and Persson, T. S. (1994). Soil seed bank and species recruitment in road verge grassland vegetation. *Annales Botanici Fennici* 31 (3), pp 155–162.

Miller, G. T. (Jnr) (1992). 'Living in the Environment', Seventh Edition, p. 125 (Wadsworth Publishing, California).

Miller, L. (1998). Management of serrated tussock in farming areas, *Plant Protection Quarterly*, 13 (2) pp 91–93.

Mitchell, M. L. and Johnston, W. H. (1990). 'The Introduction and Evaluation of Native Perennial Grasses for Alleviating/Avoiding Salinity or Waterlogging', unpublished paper, (NSW Soil Conservation Service, Wagga Wagga, NSW).

Morgan, J. W. (1995a). Ecological Studies of the Endangered *Rutidosis leptorrhynchoides*. 1 Seed Production, Soil Seed Bank Dynamics, Population Density and their Effects on Recruitment. *Australian Journal of Botany* (43), pp 1–11.

Morgan, J. W. (1995b). Ecological Studies of the Endangered *Rutidosis leptorrhynchoides*. 2 Patterns of Seedling Emergence and Survival in a Native Grassland. *Australian Journal of Botany* (43), pp 13–24.

Muir, A. M. and Carr, G. W. (1994). 'Evaluation of Native Grassland Re-establishment – Air Liquide Australia Pipeline Easement, Altona North, Victoria'. Ecology Australia unpublished report (Air Liquide Australia P/L).

Parsons, J. M. ed. (1992). 'Australian Weed Control Handbook' Ninth Edition, (Inkata Press, Melbourne).

Phillips, A. (1994). 'Report N° 5 Flora Survey: Victoria University of Technology (St Albans)', unpublished report to Projects and Property Department, (Victoria University).

Puhar, P. (1996). 'The effects of herbicides on *in vitro* seed germination of native kangaroo grass (*Themeda triandra*) and introduced weed species serrated tussock (*Nassella trichotoma*) and Chilean needle grass (*Stipa neesiana*)', unpublished Honours Thesis, (Department of Biology, Victoria University).

Raven, P.H. and Johnson, G.B. (1989). 'Biology', Second Edition, pp 520–521 (Times Mirror/Mosby College Publishing, St Louis, USA).

Raven, P.H., Evert, R.F. and Curtis, H. (1981). 'Biology of Plants', Third Edition, pp 604–605 (Worth Publishers, New York).

Riser, P. G. (1988). Diversity in and Among Grasslands. *In* 'Biodiversity', ed. Wilson E.O. pp 176–180 (National Academy Press, Washington).

Robertson, D. (1986). 'Interrelationships between kangaroos, fire and vegetation dynamics at Gellibrand Hill Park, Victoria'. unpublished PhD thesis, (Botany Department, University of Melbourne).

Ryser, P., Langenauer, R. and Gigon, A. (1995). Species Richness and Vegetation Structure in a Limestone Grassland after 15 Years Management with Six Biomass Removal Regimes. *Folia Geobotanica and Phytotaxonomica* 30 (2), pp 157–167.

Scarlett, N. H., Wallbrink, S. J. and McDougall, K. (1992). 'Field Guide to Victoria's Native Grasslands', (National Trust, Melbourne, Victoria).

Scougall, S. A., Majer J. D. and Hobbs R. J. (1993). Edge effects in grazed and ungrazed Western Australian wheatbelt remnants in relation to ecosystem reconstruction. *In* 'Reconstruction of Fragmented Ecosystems' eds. Saunders D. A., Hobbs R. J. and Ehrlich P. R. pp 163–178, (Surrey Beatty and Sons, Chipping Norton, NSW).

Shears, I. G. (1998). 'Establishment of Native Forbs in Natural and Contrived Grasslands'. unpublished thesis for M. Appl. Sci. (Hort), (Melbourne University, Burnley).

Shepherd, R. C. H. and Richardson, R. G (1998). Outcomes from the *Nassella* workshop. *Plant Protection Quarterly*, 13 (2) p 103.

Sindel, B. M. (1993). Restoration Issues in Lowland Native Grassland. Management of Relict Lowland Grasslands. proceedings of a workshop and public seminar, Conservation Series N° 8, pp 31–38 (ACT Parks and Conservation Service).

Sindel, B. M. and Groves, R. H. (1991). Seed Production and Potential Domestication of Kangaroo Grass (*Themeda triandra*). Native Grass Workshop Proceedings, pp 171–172. (Australian Wool Corporation, Melbourne),

Sindel, B. M., Davidson, S. J., Kilby, M. J. and Groves, R. H. (1993). Germination and Establishment of *Themeda triandra* (Kangaroo Grass) as Affected by Soil and Seed Characteristics. *Australian Journal of Botany*, (41), pp 105–117.

Stafford, J. L. (1990). Revegetation with native grasses in conservation/ amenity areas. Proceedings of the ninth Australian Weeds Conference, Adelaide 1990, pp 140–143.

Stafford, J. L. (1991). Techniques for the Establishment of Kangaroo Grass in South Australian Conservation Reserves. *Plant Protection Quarterly* 6 (3), pp 120–122

Stuwe, J. (1986). (reprint 1989) 'An Assessment of the Conservation Status of Native Grasslands on the Western Plains, Victoria and Sites of Botanical Significance', Technical Report Series No. 48, (Department of Conservation, Forests and Lands, Melbourne).

Stuwe, J. and Parsons, R. F. (1977). *Themeda australis* grasslands on the Basalt Plains, Victoria: floristics and management effects. *Australian Journal of Ecology* (2), pp 467–476.

Trèmont, R. M. and McIntyre, S. (1994). Natural Grassy Vegetation and Native Forbs in Temperate Australia: Structure, Dynamics and Life Histories. *Australian Journal of Botany* (42), pp 641–658.

Wedin, D.A. (1992). 1 Grasslands: A Common Challenge. *Restoration & Management Notes* (10:2) pp 137-143.

Wells, T. C. E. (1989). The Recreation of Grassland Habitats. *The Entomologist* 108 (1 & 2) pp 97 - 108.

Wells, T. C. E., Bayfield, N. G., Cox, R. and McGowan, G. (1993). 'The Wildflower Handbook', (The Department of Transport, UK).

Wijesuriya, S. and Hocking, C. (1999). Why weeds grow when you dig up native grasslands. Down to Grass Roots. proceedings of a Conference on management of grassy ecosystems, 9 and 10 July 1998, Victoria University, St Albans, pp 102–109, (Department of Natural Resources & Resource Environment, Melbourne).

Willems, J. H. (1995). Soil Seed Bank, Seedling Recruitment and Actual Species Composition in an Old and Isolated Chalk Grassland Site. *Folia Geobotanica and Phytotaxonomica* 30 (2), pp 141–156.

Willis, J. H. (1992). Victoria's Grassland Heritage. *In* 'Field Guide to Victoria's Native Grasslands', eds. N. H. Scarlett S. J. Wallbrink and K. McDougall pp 2–4, (National Trust, Melbourne).

Willis, J.H. (1984). Plant Life of the Western Plains. *In* 'The Western Plains – A Natural and Social History', eds. D. Conley and C. Dennis, pp 27–38, (Australian Institute of Agricultural Science, Melbourne).

Zacharek, A. R. (1994). 'Restoration of Native Woodland in the Midlands, Tasmania: Weeding Techniques and Transplanting of Native Forbs and Grasses', (Tasmanian Conservation Trust & Department of Geography and Environmental Studies, University of Tasmania).

Zajicek, J. M., Sutton, R. K. and Sotero, S. S. (1986). Direct Seeding of Selected Forbs into an Established Grassland. *HortScience* 21 (1) pp 90 - 91

Personal Communications

Ms Judy Bush, Manager Conservation Programs, Merri Creek Management Committee, 2 Lee Street, East Brunswick, Victoria 3057.

Ms Vanessa Craigie, Grassland Coordinator, Flora & Fauna Program, Department of Natural Resources and Environment, 4th floor 250 Victoria Parade, East Melbourne VIC 3002

Dr Colin Hocking, Senior Lecturer Ecology, School of Life Sciences and Technology, Victoria University, PO Box 14428 Melbourne MC 8001.

Mr Keith McDougall, NSW National Parks & Wildlife Service, Southern Zone, PO Box 2115, Queanbeyan NSW 2620

Mr James Ross, Environmental Consultant, 7 Rushall Street, Fairfield, Victoria 3078 (former Grassland Officer, Victorian National Parks Association).

Appendix 1: Plant Protection Quarterly writing protocols

Appendix 2: Steel burn box illustrations

The steel burn box was constructed using 20 gauge steel and measured 1.3 m x 1.5 m in plan and 0.8 m in elevation. The weight of the box was sufficient to form a fire-resistant seal on the perimeter of all quadrats at the point of contact between the ground and the base of the box.

Appendix 3: Treatment listing for 1994–1995 and 1995–1996 trials

(Treatments for 1993–1994 trials not listed)

1994 - 1996 *Themeda triandra* revegetation trials list

(Thatch [x1] = 770 g seed-bearing hay, quadrats = 1.95 m^{-2} treated with centre 1.0 m^{-2} assessed)

Initial weed (*N. trichotoma*) biomass reduction	**Follow-up herbicide weed control**	**Thatch: seed-bearing *T. triandra* hay**	**Thatch removal method**	**Quadrats**
Control	no treatment	no treatment	no treatment	five random replicates
March slash	Control	no treatment	no treatment	five random replicates
March slash	March glyphosate	April thatch [x1]	July burn	five random replicates
March slash	March glyphosate	July thatch [x1]	October burn	five random replicates
March slash	July glyphosate	September thatch [x1]	December burn	five random replicates
March slash	July atrazine	September thatch [x1]	December remove	five random replicates
March slash	July glyphosate	September thatch [x1]	December remove	ten random replicates
March slash	July glyphosate	September thatch [x1]	December burn	ten random replicates
March slash	July glyphosate	September thatch [x1]	December part remove	five random replicates
March slash	July glyphosate	September thatch [x1]	December remove	five random replicates
March slash	July glyphosate	September thatch [x1]	December part remove	five random replicates
March slash	July glyphosate	September thatch [x1] + thatch seedless hay [x1]	December remove	ten random replicates
March slash	July glyphosate	September thatch [x1]	D`ecember burn	ten random replicates
March slash	July glyphosate	September thatch [x1]	December remove	five random replicates

1995 - 1996 *Themeda triandra* revegetation trials list

(Thatch [x1] = 770 g seed-bearing hay, quadrats = 1.95 m^{-2} treated with centre 1.0 m^{-2} assessed)

Initial weed (*N. trichotoma*) biomass reduction	**Follow-up herbicide weed control**	**Thatch: seed-bearing *T. triandra* hay**	**Thatch removal method**	**Quadrats**
January slash	February glyphosate	May thatch [x1]	July burn	five random replicates
January slash	February glyphosate	May thatch [x1]	July remove	five random replicates
January slash	February atrazine	May thatch [x1]	July burn	five random replicates
January slash	February atrazine	May thatch [x1]	July remove	five random replicates
January slash	May glyphosate	July thatch [x1]	October burn	five random replicates
January slash	May glyphosate	July thatch [x1]	October remove	five random replicates
January slash	July glyphosate	October thatch [x1]	December burn	five random replicates
January slash	July glyphosate	October thatch [x1]	December remove	five random replicates
January slash	July glyphosate	October thatch [x1] + blank [x1]	December burn	five random replicates
January slash	July glyphosate	October thatch [x1] + blank [x1]	December remove	five random replicates
March pre-burn	July glyphosate	October thatch [x1]	December burn	five random replicates
March burn	July glyphosate	October thatch [x1]	December remove	five random replicates
January slash	July atrazine	October thatch [x1]	December burn	five random replicates
January slash	July atrazine	October thatch [x1]	December remove	five random replicates
January slash	controls (as for Herbicide trials)			five random replicates
January slash	controls (as for Herbicide trials)			five random replicates