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VEGETATION RECOVERY AND PLANT DIVERSITY DYNAMICS IN GRASSLAND COMMUNITIES FOLLOWING HERBICIDE TREATMENT

by

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The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Vegetation recovery and plant diversity dynamics in grassland communities following herbicide treatment** submitted by **Reginald Frank Newman** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Rangeland and Wildlife Resources**.

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DEDICATION

To my parents Leslie and Cecilia,

who brought their children to Canada

for the unlimited opportunities

ABSTRACT

The treatment of an area of rangeland with a herbicide for the control of a specific undesirable plant species results in the exposure of many non-target plant species to the herbicide. The potential damage that herbicides may cause to non-target plant species deserves careful consideration because of the implications for reduced forage values for livestock and wildlife, damage to wildlife habitat, the threat to rare and endangered plant species, and the threat to biological diversity. The objectives of this study were: to provide quantification of the response of non-target native plant species to the herbicide picloram; to examine methods of reducing the effects of picloram on non-target plant species; and, to examine a potential mechanism whereby extirpated plant species may re-enter the plant community following losses induced by herbicide treatment.

Picloram applied at 0.55 kg ai/ha (kg active ingredient/ha) reduced the cover of 14 plant species in addition to the target weed species, diffuse knapweed (*Centaurea diffusa*). Six of the affected non-target plant species, primarily perennials in the Asteraceae family, were eliminated from the plots. Plant species density declined by one-third following treatment with picloram at 0.55 kg ai/ha while species diversity was reduced by approximately 20%.

Reducing the application rate of picloram from 0.55 kg ai/ha to 0.15 or 0.05 kg ai/ha resulted in less damage to non-target plant species, and had less impact on species density and species diversity. The use of an alternative herbicide, clopyralid, applied at 0.15 kg ai/ha, resulted in less damage to some plant species, and had less impact on species density and species diversity, when compared to picloram applied at 0.55 kg ai/ha. The lower application rates and clopyralid treatments were equally as effective

as the full rate of picloram at suppressing densities of diffuse knapweed for at least 2 years.

Picloram-sensitive native plant species such as low pussytoes (*Antennaria dimorpha*) did not show increased seedling emergence in the gaps created by the removal of individual needle-and-thread (*Stipa comata*) plants. Needle-and-thread is the most likely plant to colonize gaps created by death of other needle-and-thread plants.

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CHAPTER 1. INTRODUCTION

1.0 Introduction

Herbicides have been used successfully to manage undesirable plant species on rangelands for almost 50 years. There is little doubt that the proper application of a herbicide results in substantial declines in undesirable plant species and often significant increases in desirable species. The use of herbicides to manage undesirable plants on rangelands can be more problematic than the use of herbicides on croplands. One aspect of this difference is that cropland is often used for the culture of a single plant species whereas rangelands typically are composed of many native plant species, numbering from about 20 to well over 100 (Daubenmire 1968). As a result, the treatment of an area of rangeland for the control of a specific undesirable plant species results in the exposure of many non-target plant species to the herbicide. Herbicides registered for use on rangelands are toxic to broad groups of plant species, therefore the potential exists for non-target plant species damage.

Management practices that reduce the diversity or alter the composition of plants in rangeland communities must be assumed to increase the potential for damage to ecological function. The potential damage that herbicides may cause to non-target plant species deserves careful consideration because of the implications for reduced forage values for livestock and wildlife, damage to wildlife habitat, the threat to rare and endangered plant species, and the threat to biological diversity.

1.1 Research Objectives

The objectives of this research are as follows:

1) to quantify the response of non-target plant species to the herbicide picloram (4-amino-3,5,6 trichloro-2-pyridinecarboxylic acid) in the bunchgrass grasslands

of southern British Columbia (B.C.);

2) to evaluate alternative herbicide practices aimed at mitigating damage to non-target plant species;

3) to determine the efficacy of these alternative methods on control of *Centaurea diffusa*; and,

4) to examine a mechanism for the recovery of non-target plant species following picloram damage.

2.0 Literature Review

2.1 Use of Herbicides on Rangelands

The release of 2,4-D (2,4-dichlorophenoxyacetic acid) in 1945 launched the era of chemical weed control on rangelands. This new chemical was the first to show distinct selective phytotoxity at relatively low rates of application. It was adopted very quickly by range managers. By the spring of 1946, control of *Artemisia filifolia* on Oklahoma rangeland had been demonstrated (McIlvain and Savage 1949). Extensive tests of aerial application of 2,4-D on this same vegetation followed soon after in 1947 and 1948 (McIlvain and Savage 1949). At the same time, Cornelius and Graham (1951) were conducting tests on California forest ranges using both 2,4-D and 2,4,5-T. The use of 2,4-D expanded rapidly from that point and by 1951 approximately 6100 ha of rangeland had been treated in Clark County, Idaho alone (Blaisdell and Mueggler 1956). Extensive use of 2,4-D on rangelands continues to date and many other herbicides such as picloram (4-amino-3,5,6 trichloro-2-pyridinecarboxylic acid), released in 1963, have been adopted for use on rangelands. Norris (1986) reported that 2,4-D, together with picloram, accounted for 75% of all herbicide use in 1982 in the U.S. National Forests.

2.2 Non-target Plant Damage Due to Herbicides

2.2.1 Forage

The negative effects of 2,4-D on non-target plant species were recognized soon after its first use on rangelands. Initially, concern centered on the potential loss of desirable forage plants to cattle and other kinds of livestock. McIlvain and Savage (1949) were perhaps the first to comment on the impact of herbicides on desirable native species and provided information on the susceptibility of many non-target species to 2,4-D. No recommendations were provided, however, on methods to reduce the impact on non-target species. As early as 1951, Cornelius and Graham cautioned that:

> "some desirable native legumes and browse plants are susceptible to the herbicides now in use. Where these desirable plants occur the range manager will need to decide whether control of the undesirable brush and weeds will be worth more than the loss of desirable plants."

Others considered forbs, the most generally susceptible plants, to be of minor importance as forage for cattle (Hurd 1955).

2.2.2 Wildlife Habitat

It was also soon recognized that the extensive use of 2,4-D could impact forage for big game species in certain habitats (Blaisdell and Mueggler 1956). Big game species such as mule deer (*Odocoileus hemionus*) and pronghorn antelope (*Antilocapra americana*) rely on shrubs and forbs that are traditionally considered unimportant as livestock forage (Yoakum 1986, Cooperrider and Bailey 1986). For example, Yoakum (1986) viewed the large scale chemical control of big sagebrush (*Artemisia tridentata*) as a direct threat to pronghorn antelope populations.

The concerns over the herbicide-induced loss of wildlife habitat were extended to include game birds such as sage grouse (Roberson 1986) and lesser prairie-chicken (Olawski and Smith 1991) and non-game species such as Brewer's sparrow (Schroeder and Sturges 1975).

2.2.3 Biodiversity

Increasing concern regarding the conservation of biological diversity (Dasmann 1984, Biodiversity Convention Office 1995) has resulted in renewed interest in non-target species damage. Ever increasing pressures from conservationists, ecologists and the concerned public regarding the conservation of biodiversity is motivating the managers of public rangelands to seriously address issues concerning potential losses of diversity.

One of the concerns is the indication that the loss of native plant species results in less stable communities. Tilman and Downing (1994) found that primary productivity in more diverse Minnesota grassland communities was more resistant to, and recovered more fully from, major drought than in less diverse grassland communities. If this relationship exists on other rangelands and for other site perturbations, then there is a potential for greater long-term forage production decreases associated with periodic extremes in factors such as drought, insect epidemics, rodent cycles or fire.

On a theoretical level, the continual loss of species will eventually result in an unstable community (MacArthur 1955). The response of a monoculture to perturbations such as drought, insect epidemic or fire illustrates this point. A variety of species in a community provides a range of responses to a particular perturbation. Catastrophic change from a single event is less likely in a diverse community because there is a greater probability that one or more species will show resistance to the perturbation. In contrast, a lack of vegetative complexity results in a more monotonic response, and therefore a greater potential for catastrophic change (Odum 1971).

The impact of species removals on ecosytems is largely unknown. The functioning of an ecosystem is at least risk when all species are preserved in their original composition. While certain species provide overlapping function with others (functional redundancy hypothesis; e.g., Johnson and Mayeux 1992), not enough is known about individual species characteristics to be able to sacrifice species or groups of species without some risk to ecological function. Therefore, management practices that reduce the diversity or composition of plants in rangeland communities have the potential to increase the risk for a change in ecological functioning.

2.3 Characteristics of the Herbicides Picloram and Clopyralid

Clopyralid and picloram belong to a group of auxin-type herbicides that are best regarded as synthetic auxins (Cobb 1992). Clopyralid has been grouped with picloram, fluroxypyr, and triclopyr to form a sub-group of the auxin-type herbicides based on their pyridine derivation. Clopyralid has also been classified together with picloram as a pyridinecarboxylic acid (Kirkwood 1991). Both picloram and clopyralid have low mammalian toxicities. The LD_{50} of picloram in rats ranges from 5,000 to 10,330 mg/kg body weight (Olson 1963, Chen 1982). The LD_{50} of clopyralid is stated to be >2000 mg/kg body weight (DowElanco Canada 1996). Picloram is widely used on rangeland with more limited use on cropland. Clopyralid was registered for use on rangeland in northern B.C. in 1996, but has been used on croplands for 15 years, primarily for the control of Canada thistle (*Cirsium arvense*).

Picloram and clopyralid cause responses similar to some of the reactions that plants have to auxins. In sensitive plants, these include the induction of severe epinasty (deformation by bending downward), hypertrophy (excessive growth such as swelling), fasciation (malformation of parts by enlargement or flattening) of the crown and leaf petioles, and premature abscission of leaves (Hall and Vanden Born 1988). Although chemically quite similar, clopyralid and picloram can produce quite dissimilar effects in some plant species. For example, the treatment of *Brassica napus* with 5000 g/ha of clopyralid resulted in no symptoms after 18 days, while the treatment of *Brassica napus* with 75 g/ha of picloram resulted in severe symptoms within 6 hours (Hall *et al.* 1985).

Clopyralid is a selective postemergence herbicide with activity on a relatively narrow spectrum of broadleaf plants. Members of the Asteraceae, Polygonaceae and

Fabaceae families are particularly sensitive to clopyralid, while members of the Brassicaceae family are generally resistant (Hall & Soni 1989, Cobb 1992). Most of the evidence in selectivity studies involving clopyralid and picloram points to differences in the inherent potency at the target site. There is little support for selectivity differences based on variation in absorption, retention or translocation. Picloram provides a wider spectrum of broadleaf weed control than clopyralid. For instance, unlike clopyralid, picloram is toxic to both conifers and deciduous woody species.

The Environmental Protection Agency of the United States has classified picloram as a restricted use pesticide because of its long persistence, its relatively high water solubility with potential for leaching in soil, and its high phytotoxicity to broadleaf plants. Picloram cannot be used near trees, surface water, or areas with sandy soil and a high water table because of its long soil residual and high leaching potential (Lym 1992, Lym and Messersmith 1988). In general, clopyralid causes fewer environmental concerns than picloram in persistence, mobility and phytotoxicity (Mullison 1985). The half life of picloram varies from 30 to 400 days (Lym 1992, Lym & Messersmith 1988, Smith *et al.* 1988) while the half life of clopyralid is only 30 to 90 days under aerobic conditions. The phytotoxic residues of picloram can persist for up to 5 years after application, depending on the dose and soil type (Lym & Messersmith 1988).

Both picloram and clopyralid are phloem-mobile, clopyralid being especially so (Orfanedes *et al.* 1993, Kirkwood 1992). Herbicides with phloem mobility are generally required for control of perennial species, especially deep-rooted plants. The efficacy of phloem-mobile herbicides is generally influenced by the phenological stage at application (Orfanedes *et al.* 1993). For example, Turnbull & Stephenson (1985) report that the maximum effectiveness of phenoxy and benzoic acid herbicides is achieved when applications are made at the early-bud to bloom stage of *Cirsium arvense*. This phenological stage is said to coincide with the period of lowest root reserves and maximum basipetal translocation of plant assimilates.

Picloram and clopyralid are readily taken up by both roots and leaves of many plant species. Picloram is highly active in the soil and residues as low as 0.25 µg/kg soil can be toxic to sensitive broadleaf crops (Jotcham *et al.* 1989). *Glycine max* can be affected for up to 5 years after application of 2.75 kg/ha of picloram and seedling growth may be affected by as low a rate as 38 g/ha (Jotcham *et al.* 1989). On rangelands, this soil activity can be a benefit for long-term control of broad-leaved weed species. However, seedlings of susceptible non-target plant species will be impacted as well.

2.4 Herbicide-Plant Interactions

The effects of a herbicide on weed control and subsequent plant community composition and ecological processes depend on a number of factors including:

1) the herbicide treatment, such as type of herbicide, application rate of herbicide,

and timing of herbicide treatment;

2) the composition of the plant community before treatment;

3) the weather patterns before and after treatment; and

4) the abiotic influences such as climate, soils, and landform type.

It is unlikely that the plant community resulting after a particular herbicide treatment will be immediately, completely stable. A certain amount of recovery of herbicide-sensitive vegetation is probable with time, depending on a number of circumstances discussed in the next section. It is also possible that the vegetation will never recover to the original pre-treatment state.

2.4.1 Vegetation Dynamics Following Herbicide Treatment

The selective destruction of vegetation caused by a herbicide, or by natural perturbation, results in the creation of gaps where space and resources become available for plant colonization. Colonization of these gaps may occur by vegetative proliferation, where the surviving plants fill the gap by increasing in size, or by reproductive means from newly dispersed seeds or soil seed banks (Harper 1977). The earliest plants to colonize gaps will be those that can expand vegetatively, or disperse seed widely and in great numbers, or have longevity in the soil seed bank. Noble and Slatyer (1980) suggest that the pattern of species that colonize after a disturbance results from the relative availability of a range of species and their life history characteristics.

Theoretically, the recovery of plant diversity after a herbicide treatment should not generally differ from recovery after a natural perturbation such as plant mortality due to disease. Insect herbivory, disease, and drought may all cause selective plant species losses with minimal physical disturbance of the substrate in a similar way to the action of selective herbicides (Grubb 1977, Heady 1973). Disturbances such as large mammal trampling, fire, or erosion, however, result in physical changes at the soil surface as well as plant species losses. These physical changes have a significant effect on vegetation recovery and are effects that are not found after herbicide treatment.

2.4.2 Recovery of Vegetation after Application of Non-persistent Herbicides

The persistence of a herbicide is a crucial property determining long-term plant response in the treated community. Non-persistent herbicides, such as 2,4-D and glyphosate (N-[phosphonomethyl]glycine), do not remain active in the soil after application. Conversely, persistent herbicides, such as picloram or clopyralid (3,6-

dichloro-2-pyridinecarboxylic acid), remain active in the soil and interfere with the regrowth and seedling establishment of sensitive plants over extended periods of time. Picloram is classified as a long residual herbicide because it interferes with vegetation for more than one growing season (Ross and Lembi 1985), while clopyralid, which is less long-lasting, is classified as persistent only (Lym and Messersmith 1992).

The recovery of plant communities treated with a non-persistent herbicide can occur by both tolerant and sensitive species starting soon after treatment. Tolerant species that survive a non-persistent herbicide treatment will have an advantage over sensitive species in colonizing the gaps because of well dispersed and proximate aerial seed sources, and availability of vegetative sources for expansion. Recovery from the soil seed bank, however, will be similar for sensitive and tolerant species because a non-persistent herbicide is likely to have minimal effects on seedling emergence from the permanent soil seed bank.

2.4.3 Recovery of Vegetation after Application of Persistent Herbicides

Persistent herbicides have continuing toxic effects on sensitive plant species for a number of years following application. Seedling germination in contaminated soil may be especially impacted. Initially, only species that are tolerant of the persistent herbicide are able to colonize the gaps left by sensitive species. Eventually, after dissipation of the herbicide in the soil, sensitive species will no longer be disadvantaged, and may also colonize. At that time, however, the gaps may be fully occupied by tolerant species, resulting in a different community from that which occurs after application of non-persistent herbicides (Tomkins and Grant 1977). There is a substantial difference in the potential impact of non-persistent herbicides compared to persistent herbicides. Information gathered from studies of non-persistent herbicides such as 2,4-D provides little indication of the impacts of persistent herbicides.

It is not known to what extent, and at what rate, the dominance attained by herbicide-tolerant species is lost over time. In fact, Connell and Slatyer (1977) provide compelling evidence that the first species to colonize a natural disturbance may often exclude or suppress subsequent colonist species for long periods (see also Egler 1954). Alternatively, many field-based studies report that recovery of the original plant species composition is rapid, occurring within 2-5 yrs after treatment with herbicide (Malone 1972, Murray *et al.* 1991, Rice *et al.* 1992).

2.4.4 Herbicides and the Creation of Stable Vegetation States

There is a possibility that certain plant species may never recover to pre-treatment levels due to the existence of stable post-treatment vegetation states. Theories on alternate stable states suggest that once a threshold has been passed in certain vegetation types, transition back to the original vegetation state is unlikely without certain major disturbances or human intervention (Law and Morton 1993, Tausch *et al.* 1993, Laycock 1991). Simply removing the cause of the initial change in vegetation, such as heavy grazing, does not necessarily mean a reversal to the pre-treatment vegetation state. For example, stable vegetation states have been demonstrated for grasslands converted to shrublands by excessive grazing. These vegetation states showed no recovery toward the original vegetation composition after decades of complete removal of grazing (Laycock 1991). The common factors in the formation of stable vegetation states seem to be: 1) a semi-arid or arid environment; 2) an aggressive dominant plant species; and 3) loss of seed source of recovering species (Laycock 1991). Many of these factors may pertain to vegetation states created after herbicide use in semi-arid rangelands of North America.

2.4.5 Mechanisms of Plant Diversity Recovery

Grubb (1977) hypothesized that, in natural systems at equilibrium, species richness is maintained by mechanisms such as:

1) variation in life-form;

2) phenological separation;

3) fluctuations in the environment (biotic and abiotic);

4) competitive balance;

5) variation in competitive ability with physiological age; and

6) differences among species regeneration requirements.

Some of these mechanisms may be applicable to the recovery of plant communities whose diversity has been lowered by treatment with herbicides. In particular, mechanisms 1 and 2 (above) may be important because not all of the resources (in time and space) of a low-diversity community are completely tied up. Thus, given time, there are opportunities for invasion by complementary species (Tilman 1993).

Mechanism 3 may be important because it has been suggested that, in semi-arid grasslands, natural succession does not progress in average years but requires an episodic event consisting of one or more growing seasons of above-average moisture to progress (Harris 1967). Thus, plant communities that may appear to be stable in the short-term, may be unstable over longer periods.

Mechanisms 5 and 6 are potentially important because it seems intuitive that plant diversity may be increased in the community through gaps created by senescence and death of dominant individuals. These gaps provide areas of increased resource availability that significantly enhance the chances of recruitment. Connell and Slatyer (1977) suggest that even though earlier species may continue to exclude or suppress later ones for long periods, the former eventually are damaged or killed and are then replaced. Others have proposed that the species richness of a community is maintained by a dynamic equilibrium between local extinction and local colonization.

2.5 Relationships Between Species Diversity and Ecosystem Function

There is an unresolved controversy over whether ecosystems with lower species diversity have reduced stability (Luckinbill 1979, Zaret 1982). There are some analyses supporting this hypothesis (e.g. MacArthur 1955, Zaret 1982, Tilman and Downing 1994). For example, Tilman and Downing (1994) found that the productivity of less diverse grassland communities did not recover as quickly, or as fully, from major drought compared to more diverse grassland communities. This relationship also exists on other rangelands and for other perturbations (McNaughton 1985), and may also pertain to treatment with herbicide. Thus, there is a potential for long-term forage production decreases associated with less diverse plant communities. Other analyses conclude that increased species diversity reduces stability (e.g. May 1973, Zaret 1982) or have no effect on stability (e.g. Johnson and Mayeux 1992). Even though the controversy remains unresolved, some concern is justified because it has been suggested that environmental sustainability is best attained by the "maintenance of natural capital" (Goodland 1995). That implies that management practices that result in alterations and losses within ecosystems are ecologically unsustainable to some degree.

There is some evidence that more diverse ecosystems are more productive (Baskin 1994). For example, Naeem *et al.* (1994) tested 14 artificially-constructed ecosystems and found that those with greater species richness were the most productive. Relationships between species diversity and other ecosystem functions, such as nutrient cycling and decomposition have also been suggested. However, conclusive evidence for these relationships is non-existent (Baskin 1994).

3.0 Need for Research

While knowledge of the response of many rangeland weed species to herbicides is generally adequate, less is known about the response of non-target rangeland plants and treated plant communities.

3.1 Studies of Herbicide Impacts on Weeds

Many herbicide studies on rangelands have focused on the measurement of a target weed species, providing no individual consideration of other plants in the community (e.g., Renney and Hughes 1969, Boyd *et al.* 1978, Mayeux *et al.* 1979). The degree of control of the target weed and changes in forage production following treatment are routinely reported. These studies provide crucial information on herbicide efficacy and the economic returns expected after control, but they do not provide all the information required for informed decisions regarding herbicide use in an ecological context.

Evans and Young (1985) illustrate this point well. They reported that *Juniperus occidentalis* could be controlled successfully with picloram, resulting in manyfold increases in forage yield. They also reported that most of the increase was due to undesirable annual grasses. For that particular rangeland ecosystem, information on the composition of desirable and undesirable plants in the community before treatment, as well as the probable impacts of picloram on existing desirable forb species, would have aided in the prediction of the results of *Juniperus occidentalis* control.

3.2 Studies of Herbicide Impacts on Weeds and Associated Species

Other studies have included floristic information beyond the target species (e.g., Hurd 1955, Arnold and Santelmann 1966, Martin and Morton 1980, Whisenant 1986, Murray *et al.* 1991). However, these rarely approached a quantitative consideration of the full flora of the site. This is due, in part, to the difficulty of conducting proper sampling and statistical treatment of nonabundant and patchily distributed plant species (Houston 1977, Scifres and Mutz 1978). In contrast, Blaisdell and Mueggler (1956) and Houston (1977) provided a comprehensive quantitative analysis on the sites they studied. In general, impacts on the most abundant non-target species are reported. All other things being equal, these species are the least threatened because of their greater numerical capacity to recover. Less abundant species, which are potentially the most threatened, are rarely considered.

For example, some authors report that herbicide-susceptible forbs recover quickly to pretreatment levels (Murray *et al.* 1991) or that forb damage is not substantial (Lacey *et al.* 1989). In these two studies, however, the conclusions were based on quantitative information on only seven and five forb species, respectively. As a result, the authors' conclusions regarding the impact of herbicides on all associated non-target plant species are incomplete, since each plant species is likely to respond to the herbicide and recover on the site in an individual manner.

3.3 Studies of Herbicide Impact on Plant Diversity

3.3.1 Picloram

Although the effects of 2,4-D and other auxin-type herbicides have been widely investigated (e.g. McIlvain and Savage 1949, Cornelius and Graham 1951, Whisenant 1986), few authors have conducted research on the impacts of picloram on plant diversity (N.R.C.C. 1984). Rice *et al.* (1992) examined the effects of picloram on plant species diversity for two seasons in Montana and reported small reductions after the first year. The authors suggest that the reductions in plant species diversity are probably transitory, based on a trend toward full recovery by the second year following treatment. No statistical analyses were performed on the data which reduces the reliability of the conclusions. An earlier report of a study in the same region found "few significant differences in forb density and diversity", but did not present data nor analyses on the effects on forb diversity (Lacey *et al.* 1989). Murray *et al.* (1991) examined the response of tall-forb communities in Montana to treatment with mixtures of picloram and 2,4-D. The authors concluded that native forbs in the tall-forb community have great resilience to these herbicides and that no long-term effects can be expected. Tomkins and Grant (1977) reported that picloram and picloram plus 2,4-D had dramatic effects on early seral and mature plant communities in the first year following treatment of right-of-ways in Ontario. In contrast to the other studies, the authors provided data suggesting relatively long-term (3 year) changes, especially in the early seral community. Nevertheless, they concluded that recovery of plant species diversity was rapid.

Tschirley (1969) was requested to provide an assessment of the ecological effects of the defoliation program in Vietnam. He reported that the herbicides used, including agent white (4:1 ratio of 2,4-D and picloram), would not have irreversible effects on the vegetation. He predicted that complete recovery of a treated mangrove forest could take 20 years. He also predicted that herbicide treatment of the semideciduous forest type would have no ecological effect unless the treatment was repeated.

3.3.2 Other Herbicides

Malone (1972), Tomkins and Grant (1977), and Viragh and Genencser (1988) examined the use of herbicides, other than picloram, on plant species diversity. Most of these authors reported initial declines in species diversity followed by rapid recovery (Malone 1972, Tomkins and Grant 1977). Tomkins and Grant (1977) also reported long-lasting and persistent reductions in species diversity, depending upon the herbicide type that was used. In contrast, Nolte (1995) reported no difference in plant species richness and diversity two years after treating mesquite and *Opuntia* spp. with a mix of picloram and triclopyr.

3.4 Theoretical Studies on Herbicide Impacts

Few field-based studies have investigated the interaction between plant and herbicide, especially in regard to non-target species damage. Herbicides have been employed to kill selected components of grassland vegetation in order to examine hypotheses regarding mutual interference (Sagar 1959, Foster 1964, Putwain and Harper 1970). However, the authors of these studies were not concerned with the effects of the herbicide itself but used the herbicide as a tool for selective removal of plants.

3.5 Conclusions

In contrast to the few complete studies on plant community response following herbicide treatment, the impact of range management practices such as grazing and fire on vegetation has received great attention from researchers. Results of hundreds of studies are available on the recovery of vegetation after grazing or fire, and many of these provide detailed floristics and information on plant diversity dynamics (e.g., Ellison 1960, Vogl 1974, Peek *et al.* 1978, Humphrey 1984).

Initially, much attention was directed to studying the environmental effects of the non-persistent herbicide 2,4-D on rangelands. Few authors have examined the impacts of picloram at the plant community level. The environmental effects of picloram are expected to differ from those of 2,4-D because picloram is a persistent herbicide capable of affecting susceptible plants long after the date of application.

The appropriate use of herbicides on rangelands is a valuable tool for range managers. However, more information is needed on the impact of herbicides on non-target species and on the recovery of affected species over time. A clear understanding of the impact of herbicides on rangeland vegetation cannot be achieved without new knowledge gained from field-based studies of the response of susceptible non-target plant species.

4.0 Research Rationale

The study was initiated in response to a concern regarding the impacts of picloram on native plant species diversity. This concern was well summarized in an National Research Council of Canada (1984) report:

> "Blanket-spraying with picloram, because of its broad spectrum of phytotoxicity, might push the community back to the initial stages of the rate-of-change curve. The result is a simple biotic community with a high rate-of-change potential, offering little inherent stability or competition to trees or weeds."

Little detailed information exists on this topic. Nevertheless, assumptions have been made that picloram does not affect plant species diversity. This situation is problematic when viewed from both the pesticide management and environmental points of view. The largely unsubstantiated position that picloram does not affect plant species diversity opens up the possibility of successful appeals of pesticide licences on the basis of lack of information. The potential loss of the use of picloram would have a serious impact on weed management efforts in certain areas of B.C. From the environmental point of view, the continued use of potentially damaging standard operational practices may result in plant species extirpations and loss of ecosystem function. An evaluation of the impacts of standard herbicide practices on native plant species diversity will provide a sound basis for the retention or alteration of these practices.

This research does not have the objective of improved weed control. It is recognized, however, that the maintenance of weed control on rangelands is a vitally important function. Therefore, the focus of the research will be on providing solutions that integrate weed management objectives with biodiversity objectives.

4.1 Quantification of the Problem.

Three components were used to address the information gap. The first component addresses the need to quantify the response of non-target native plant species to the herbicide picloram on the bunchgrass grasslands of southern B.C. This information will provide the basis with which to determine the severity of the problem.

A series of areas operationally treated with picloram in 1991 for the control of *Centaurea diffusa*, were used to collect this information. *Centaurea diffusa*-infested areas were used because *Centaurea diffusa* is the most widespread and problematic weed in southern B.C. (Sturko 1996, Noxious Weed Control Function Managers 1996). The treatment of these areas by contracted spray crews was well documented and the exact treated locations can still be determined due to highly visible discontinuities in the vegetation. Areas immediately beyond the vegetation discontinuities provided representative controls for the each of the treated locations.

4.2 Evaluation of Methods to Mitigate the Problem

The second component examines methods of reducing the effects of picloram on non-target plant species. Three previously untreated areas with a uniform and dense infestation of *Centaurea diffusa* were selected for these trials. Standard operational practices were compared to alternative practices as follows:

1) The use of reduced application rates of picloram from the standard application rates was examined for the potential to reduce non-target plant species damage.

2) The use of an alternative herbicide, clopyralid, was examined for the

potential to reduce non-target plant species damage.

3) The use of alternative application dates was examined for the potential to reduce non-target plant species damage.

The use of standard application rates and timing in experiments 2 and 3 above, also provides additional, and more controlled, information to address the first component (quantification of the problem).

4.3 Testing a Potential Mechanism of Recovery

The third component tests a hypothesis regarding the mechanism for the recovery of herbicide-impacted non-target plant species. The intent of this component is to increase our understanding of species recovery in an ecosystem treated with picloram.

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CHAPTER 2. HERBICIDE-INDUCED VEGETATION CHANGES AND PLANT REGENERATION ON A PICLORAM-TREATED GRASSLAND AFTER FIVE YEARS

1.0 Introduction

Grassland plant communities that have been treated with the herbicide picloram (4-amino-3-5,6-trichloro-2-pyridinecarboxylic acid) often appear visibly different from untreated communities by the next growing season following treatment. Much of the difference is due to an increase in the abundance of picloram-tolerant grass species and a decline in abundance of picloram-sensitive broad-leaved species. Few herbicide studies on rangelands have provided quantification of these plant species changes. Many have focused on the measurement of a target weed species and have provided little information regarding other plants in the community (e.g., Renney and Hughes 1969, Boyd *et al.* 1978, Mayeux *et al.* 1979). The degree of control of the target weed and changes in forage production following treatment are often reported. These studies have provided crucial information on herbicide efficacy and on the potential forage gains after herbicide treatment, but additional information is required for well-informed decisions regarding herbicide use.

An understanding of the total plant community response to herbicides is necessary to avoid unexpected outcomes. Evans and Young (1985) illustrated this point well. They reported that western juniper (*Juniperus occidentalis*) could be controlled successfully with picloram, resulting in large increases in above-ground annual biomass. They also reported that most of the increased biomass was due to undesirable annual grasses of low value to livestock. For that particular rangeland ecosystem, information on the composition of desirable plants in the community before treatment, as well as the probable impacts of picloram on existing desirable forage species, would have assisted in predicting the outcome of western juniper control.

A few authors have included floristic information beyond the target weed species (e.g., Hurd 1955, Arnold and Santelmann 1966, Martin and Morton 1980, Whisenant 1986, Murray *et al.* 1991). However, these studies did not quantify effects on the full flora of the plant community (for the exceptions, see Blaisdell and Mueggler 1956, Houston 1977). This is due, in part, to the difficulty of conducting adequate sampling and statistical treatment of non-abundant and patchily distributed plant species (Houston 1977, Scifres and Mutz 1978). In general, herbicide impacts on only the most abundant non-target species are reported. All other things being equal, these species are the least threatened because of their greater numerical capacity to recover. Tilman and Pacala (1991) provide evidence that the rarity of a species increases its probability of being lost during a drought event. This relationship is likely applicable to environmental stresses other than drought and is probably also pertinent to the stresses imposed by herbicides.

Plant regeneration dynamics, such as the response of soil seed banks and of seedling emergence to picloram treatment, are also poorly documented in the literature. Soil-persistent herbicides such as picloram have a continuing toxic effect on sensitive plant species for a number of years following application (Hamaker *et al.* 1967, Goring and Hamaker 1971). Initially, only species that are tolerant of the persistent herbicide are able to colonize the gaps left by loss of the sensitive species. Eventually, after degradation of the herbicide in the soil, sensitive species are no longer at a disadvantage and may also colonize. The rate of recovery of herbicide-sensitive species will depend on many factors. One of the primary factors is the availability of seeds, both newly dispersed seeds and those in the soil seed bank.

The objectives of this study were to describe the floristic changes that occurred in a grassland community 5 years after treatment with picloram and to quantify regeneration of picloram-sensitive plants on the site.

2.0 Study Areas

The study was conducted at three areas (Redhill, Racetrack and Stinklake) spaced about 1 km apart, within a low elevation grassland site (50° 43'/ 120° 23') in the southern interior of British Columbia, Canada. An adjacent area (Prudens exclosure), exclosed since 1959, was sampled to provide descriptive information on the ungrazed plant community. The grassland occurs in the Bunchgrass (Very Dry-Warm) biogeoclimatic subzone (Lloyd *et al.* 1990). The dominant plant species on the three areas in 1995 was *Stipa comata*, with *Poa secunda* often codominant. *Elymus spicatus* and *Koeleria macrantha* are codominant within the Prudens exclosure. Plant taxonomic nomenclature follows Douglas *et al.* (1989) . The weed *Centaurea diffusa* is common on portions of the experimental areas not treated with picloram, and also within the Prudens exclosure. The soils are Orthic Dark Brown Chernozems (Typic Borolls) of aeolian origin with fine sandy loam textures. The aspects are mostly southerly with slopes of 0 to 10%. Elevation is about 700 m at all locations.

Mean annual precipitation at Kamloops airport (200 m lower in elevation and 10 km southeast) is 256 mm with peaks in June-August and December-January. At Kamloops, the highest daily maximum temperatures occur in July (28.8° C) and lowest daily mean minimum temperatures occur in January (-9.8° C) (Environment Canada 1984).

The grasslands of this area have a grazing history dating back to 1842 when they were first used by Hudsons Bay Company horses. Continuous heavy grazing has resulted in a change in vegetation composition from *Elymus spicatus*-dominated to *Stipa comata*-dominated (Tisdale 1947). Range condition in the 1930's was described as poor, but more recent conservative management has led to significant recovery

(McLean 1982). Light grazing occurred on the Racetrack area in the fall of 1995. The other areas were undisturbed by grazing during the study.

3.0 Methods

3.1 Treatments

On July 22 and 23, 1991, *Centaurea diffusa* infestations along several rough four-wheel drive trails at the grassland site were treated with picloram by the Kamloops Forest District to prevent further spread of this weed. Spray crews treated the infestation in a 15-m swath along both sides of many trails using an ATV-mounted boom-sprayer to apply picloram at a rate of 0.54 kg ai/ha. Heavy infestations of *Centaurea diffusa* still occur beyond the 15-m treated swath at all experimental areas. Five years later, in 1996, the treated areas remain highly visible due to the vegetation changes that occurred following herbicide application. The Prudens exclosure received no herbicide treatment.

3.2 Site Selection and Plot Layout

Experimental locations were selected based on clear, visual evidence of a sharp transition from a grass-dominated community to a *Centaurea*-dominated community. Such areas were common along the trails that had been treated with picloram in 1991. Further selection criteria were based on homogeneity of slope and surface characteristics.

Four 18-m transects were established within the picloram-treated swath and an additional four transects were established in the adjacent untreated area, at each of the three locations. The two innermost transects within each cluster of four were used to measure plant recruitment, basal area, and plant density. For this purpose, ten 0.25-m^2 circular plots were systematically located about 3 m apart along the transects. Each plot was centered on a single *Stipa comata* plant so the final plot location varied slightly from a strict 3-m spacing.

3.3 Measures and Records

The taxonomic identification and dimensions of all seedlings and mature plants were recorded within the circular plots during July 1995 and June 1996. Additional sampling of seedlings occurred during September 1995 and during May 1996. Ephemeral annuals were also recorded at the May 1996 sampling date. The parameter measured for plant dimension varied according to plant growth type and age class as follows:

1) The basal areas of juvenile and mature caespitose grasses were estimated by taking two basal diameters at right angles.

2) The heights of grass and forb seedlings and of mature forbs with upright growth habit were recorded.

3) Reproductive culms of established *Stipa comata* plants were counted.

Soil seed bank samples were taken adjacent to each circular plot. The soil seed bank was sampled on 17 July 1995 by taking ten 5-cm-diameter soil cores to a 2-cm depth at each experimental location, both within the picloram-treated area and in the adjacent untreated area. Surface litter was included in the sample. Seeds of all species were extracted using direct count methods including sieving and flotation (Malone 1969). Extracted seeds were placed on moistened filter paper at 20° C, under a 16 hr light/8 hr dark regime for 3 weeks to determine germinability. Seeds were not pretreated in any manner. Seed viability of ungerminated seeds was not determined.

Plant canopy cover was estimated using 12 20 x 50 cm quadrats (0.1 m^2) systematically located along each of the four transects (Daubenmire 1959). Canopy cover was sampled in April 1996 while the ephemeral spring forbs were still detectable. Species density (Hurlbert 1971) was calculated by totaling the number of species identified in the 48 quadrats. The Shannon index of species diversity was calculated for each experimental unit using canopy cover estimates from the quadrats. The Shannon index was chosen from a number of possible diversity indices because it is widely used and has a moderate ability to discriminate species diversity differences (Magurran 1988). The Shannon index was calculated using the formula:

$$H' = -\Sigma p_i \ln p_i$$

The value of p_i was estimated as n_i/N (Pielou 1969) where n_i is the cover of a plant species and N is the total cover of all plant species considered in the calculation.

Soil samples from picloram-treated areas at the Redhill and Racetrack locations were bioassayed using picloram-sensitive *Helianthus annuus* to determine if active picloram residues remained (Leasure 1964). Triplicate soil samples to a 25 cm depth were taken for each bioassay and used to fill four 10-cm-diameter pots. Four *Helianthus annuus* seeds were sown per pot, at 2 cm depth, and germinated at room temperature. After four days the pots were transferred to a growth chamber set at a constant temperature of 25 °C with a 12/12 hr light regime. After 3 weeks, percentage germination, height growth, and survival were assessed and compared to plants grown on control soils.

3.4 Analysis

The experiment had a completely randomized block design with one factor (picloram treatment) and three replicates (locations). Plant species variates were analyzed separately using ANOVA to test for significant effects attributable to the July 1991 picloram treatment. The density of seedlings, by species, was tested separately for July 1995, September 1995, May 1996 and June 1996 sampling dates. All analyses were performed using the Statistical Analysis System (SAS Institute 1988). Arcsine transformations or square root transformations were used to adjust for non-normally distributed data where necessary.

4.0 Results and Discussion

4.1 Plant Cover and Plant Density

Centaurea diffusa cover was greatly reduced on picloram-treated areas. Five years after treatment, the cover of this species is still nine times lower than on untreated areas (Table 2.1). The results of a soil bioassay suggest that the current reduced abundance of *Centaurea diffusa* is not due to the continuing direct effects of herbicide activity. Bioassays of the top 25 cm of the soil (Leasure 1964) at the Redhill and Racetrack sites using picloram-sensitive *Helianthus annuus* indicated that picloram had degraded to the point that germination and growth of this plant was no longer affected. *Helianthus annuus* germination, 3-week height growth, and 3-week survival were similar on picloram-treated and control soils (Table 2.2). No evidence of picloram injury such as epinasty, hypertrophy, or fasciation of crown and leaf petioles was observed on any plant. The loss of picloram activity after 4 years on these grasslands is consistent with local knowledge and the literature. Picloram applied at 0.54 kg ai/ha is predicted to remain active in the soils of semi-arid areas of southern British Columbia for approximately 2 - 4 years (Goring and Hamaker 1971, Hamaker *et al.* 1967). The rate of picloram degradation is controlled principally by temperature and moisture. Cooler and drier climates result in the lowest degradation rates. Given that picloram is no longer active in these soils, factors such as the reduced availability of seed and the increased abundance of other competitive species are the most likely causes of reduced *Centaurea diffusa*.

The cover of *Stipa comata* was nearly three times greater on picloram-treated areas than on untreated areas (Table 2.1). *Stipa comata* density, however, was not significantly different from untreated areas. This suggests that *Stipa comata* plants responded by increasing in size following application of picloram. In fact, the average

Table 2.1 Density (plants/m^2) of perennial plant species in July 1995 and canopy cover (%) of vascular plants in May 1996 following July 1991 application of picloram at 0.54 kg ai/ha.

	Density[1]		Canopy cover[2]	
Species	Picloram	Untreated	Picloram	Untreated
	---------- plants/ m^2 ---		------------ (%) ---------	
Grasses				
Bromus tectorum	-	-	0.5	+
Elymus spicatus	+	+	-	-
Koeleria macrantha	0.8	1.5	1.5	2.3
Poa compressa	+	+	+	1.2
Poa pratensis	+	+	-	-
Poa secunda	26.9	32.2	15.2	11.3
Sporobolus cryptandrus	4.8	1.2	3.4	1.0
Stipa comata	52.5	38.5	42.3	15.1*
Vulpia octoflora	-	-	1.1	+
Grass Total	85.0	74.2	64.3	31.0
Forbs				
Achillea millefolium	-	-	+	+
Androsace occidentalis	-	-	+	+
Antennaria dimorpha	+	22.1(*)	0.5	10.8(*)
Antennaria microphylla	+	0.5	+	1.2
Arabis holboellii	+	+	1.0	1.4
Arenaria serpyllifolia	-	-	+	+
Astragalus collinus	-	-	+	+
Calochortus macrocarpus	-	-	+	+
Castilleja thompsonii	-	-	+	+
Centaurea diffusa	-	-	1.2	11.0(*)
Cirsium undulatum	-	-	+	+
Collinsia parviflora	-	-	+	+
Crepis atrabarba	-	-	+	+
Draba nemorosa	-	-	+	+
Draba verna	-	-	+	+
Erigeron compositus	-	-	+	+
Erigeron linearis	-	-	+	+
Erigeron pumilus	-	-	+	+
Fritillaria pudica	-	-	+	+
Kochia scoparia	-	-	+	+
Microsteris gracilis	-	-	+	2.1**
Myosotis stricta	-	-	+	0.6
Plantago patagonica	-	-	+	+
Tragopogon dubius	-	-	+	+
Forb Total	+	22.7	4.0	28.5
Shrubs				
Artemisia frigida	+	2.3	+	1.2
Artemisia tridentata	+	+	1.2	3.0
Chrysothamnus nauseosus	+	+	0.5	+
Shrub Total	+	2.7	2.0	4.5
Overall Total	85.5	99.6	70.3	63.9

(*), *, ** significantly different at $P \leq 0.10$, 0.05, and 0.01, respectively.

[1]n=10; [2]n=40; + Less than 0.5 plants/m^2 or less than 0.5 % cover; - not sampled.

Table 2.2 Germination (%), 3-week height growth (cm), and 3-week survival of *Helianthus annuus* grown in soil collected in April 1995, following July 1991 treatment with picloram at 0.54 kg ai/ha at the Redhill and Racetrack locations.

	Germination			Growth			Survival	
	Picloram	Control	Prob >t	Picloram	Control	Prob >t	Picloram	Control
	(%)			(cm)			(%)	
Redhill	58	83	0.2508	10.6	9.6	0.5518	100	100
Racetrack	67	58	0.8340	9.3	10.5	0.5555	100	100

basal area of *Stipa comata* plants was more than doubled on picloram-treated areas (30 cm^2 vs. 13 cm^2; $P \leq 0.05$). There is substantial evidence, therefore, that much of the increase in *Stipa comata* cover, on picloram-treated areas, can be accounted for by vegetative increase of individual plants. The cover and density of grasses other than *Stipa comata* were unaffected by the picloram treatment (Table 2.1). It is notable that *Poa secunda*, a codominant on some areas, had almost the same cover and density on treated and untreated areas. There is a possibility that *Poa secunda* is not as opportunistic as *Stipa comata* in capturing resources released by the removal of the herbicide-sensitive species.

Antennaria dimorpha was nearly eliminated on the picloram-treated sites, dropping from 11% to less than 0.5% cover (Table 2.1). Mature plants of this species were not recorded on picloram-treated plots but had a density of 22 plants/m^2 on untreated plots. Like other Asteraceae, *Antennaria dimorpha* appears to be highly sensitive to picloram. This species commonly forms low-growing mats in the interspaces of perennial grasses. The loss of *Antennaria dimorpha* may potentially result in changed ecosystem function because no other species with similar growth form was observed to replace this species in the bunchgrass interspaces. The growth form of *Antennaria dimorpha* suggests that it may be important for stabilizing the soil against erosion, although no published account of this exists. *Poa secunda*, the only picloram-resistant species with similar size and habit, did not increase in cover or density on the picloram-treated areas.

The cover of *Microsteris gracilis*, an ephemeral annual species in the Polemoniaceae family, dropped from 2.1% on untreated areas to 0.5% on picloram-treated areas (Table 2.1). This species was also over eight times less frequent (85.4 vs. 10.4; $P \leq 0.003$) on treated areas. It is apparent that *Microsteris gracilis* is sensitive to picloram and that recovery of this species is not occurring despite its high reproductive capacity. *Microsteris gracilis* forms about 2% of the cover at the sites, which is a relatively minor component of the plant community. It may, nonetheless, provide a unique but unknown ecological function. There are several other species with similar life strategy and growth form that were unaffected by picloram. *Collinsia parviflora*, *Draba nemorosa*, *Draba verna*, and *Myosotis stricta* appear to share a similar niche with *Microsteris gracilis*; all are annual forbs of low stature that complete their life cycles early in the spring before soil moisture becomes limiting. It is possible that much of the ecological role of *Microsteris gracilis* overlaps with the role of these other species (Johnson and Mayeux 1992).

Changes in species composition, especially grass/forb ratios, were evident on all picloram-treated areas. Grasses formed 91% of the total cover on picloram-treated areas compared to only 48% on untreated areas, with the majority of the response due to an increase in *Stipa comata* and a decline in *Centaurea diffusa*. *Stipa comata* had similar cover to *Poa secunda* on untreated areas but it dominated on picloram-treated areas (Table 2.1). Similar grass increases in response to treatment with picloram have been reported by others (e.g., Renney and Hughes 1969, Scotter 1975).

The use of picloram on weed-infested rangeland often leads to increased forage value for cattle because of the increased proportion of grasses after treatment and because of control of weed competition. Nevertheless, the practice of large-scale herbicide treatment of *Centaurea diffusa* infestations is generally regarded to be uneconomical when based on increased forage values alone (Cranston *et al.* 1983, Griffith and Lacey 1989). These authors determined that 20 - 30 years would be needed to repay the cost of picloram treatment, while only 7 years of control of *Centaurea diffusa* could be expected. Intangible losses such as environmental damage, decreased property values, deteriorations of wildlife habitat, and increased grazing management costs were not included in the calculations but were deemed to be important considerations (Cranston *et al.* 1983).

Plant diversity, calculated using the Shannon index, was consistently lower on picloram-treated areas (Table 2.3). There is little doubt that increased *Stipa comata* dominance combined with the reduced abundance of several forb species is the reason for the reduced Shannon index. It has not been resolved whether ecosystems with lower diversity have reduced stability (Luckinbill 1979). There are some analyses that support this hypothesis (e.g., MacArthur 1955, Zaret 1982, Tilman and Downing 1993), but other authors have concluded that increased species diversity reduces stability (e.g., May 1973, Zaret 1982). Even though the controversy remains unresolved, some concern is justified because it has been suggested that environmental sustainability is best attained by the "maintenance of natural capital" (Goodland 1995). That implies that management practices that result in alterations and losses within ecosystems are ecologically unsustainable to some degree.

Species density was not statistically different between treatments although on two of the three sites there was a trend toward fewer species on picloram-treated locations (Table 2.3).

4.2 *Stipa comata* Reproductive Culms

There was a small and statistically weak ($P \leq 0.10$) increase in the number of *Stipa comata* reproductive culms, from an average of 2.3 per plant on untreated areas to 2.8 per plant on picloram-treated areas, at the July 1995 sample date. At the June 1996 sampling date, however, reproductive culms were similar in number between treatments. This suggests that the reproductive capacity of *Stipa comata* plants is only slightly, if at all, greater in picloram-treated areas than in untreated areas.

4.3 Soil Seed Bank

Seeds of 26 species were extracted from the soil seed bank at the three locations, including eight grasses, 17 forbs and one shrub (Table 2.4). Three non-

Table 2.3 Species density (*N*) and plant diversity (*H'*) in April 1996 at the Redhill, Racetrack and Stinklake locations following July 1991 treatment with 0.54 kg ai/ha picloram.

	Species density			Shannon index			
Site	Picloram	Control	Prob. >F	Picloram	Control	Prob. >F	
	---------(*N*)---------			---------(*H'*)--------			
Redhill	17.0	15.0		0.70	1.30		
Racetrack	18.0	23.0		0.92	1.60		
Stinklake	14.0	22.0		0.84	1.32		
mean	16.3	20.0	0.3414	0.82	1.41	0.0097	**

** significantly different at $P \leq 0.01$

Table 2.4 Number of germinable seeds recovered from the upper 2 cm of soil in July 1995 following July 1991 application of picloram at 0.54 kg ai/ha.

Species	Seed density	
	Treated	Untreated
	------ seeds per core[1] ------	
Grasses	12.43	3.46
Bromus tectorum	0.37	0.00
Festuca campestris	0.43	0.00
Koeleria macrantha	1.00	0.23
Poa compressa	0.10	0.13
Poa secunda	0.27	1.40
Sporobolus cryptandrus	2.93	1.23
Stipa comata	0.40	0.30
Vulpia octoflora	6.93	0.17
Forbs	1.02	8.26
Antennaria dimorpha	0.00	1.20
Androsace occidentalis	0.06	0.03
Arabis holboellii	0.03	0.33**
Castilleja thompsonii	0.00	0.03
Centaurea diffusa	0.37	2.50**
Descurainia pinnata	0.00	0.10
Draba verna	0.00	0.40
Gaillardia aristata	0.07	0.00
Lapula echinata	0.13	0.03
Lactuca scariola	0.00	0.13
Lithophragma glabra	0.10	0.07
Microsteris gracilis	0.10	0.57
Montia linearis	0.00	0.07
Myosotis stricta	0.00	1.87
Plantago patagonica	0.03	0.00
Taraxacum officinale	0.00	0.03
Unknown	0.13	0.90
Shrubs		
Artemisia frigida	0.00	0.27
Total	13.45	11.99

** significantly different at $P \leq 0.01$; n=10

[1]Soil cores were 40 cm^3 (5 cm in diameter by 2 cm deep).

native weed species, *Lappula echinata*, *Lactuca scariola* and *Taraxacum officinale*, and three perennial native species, *Gaillardia aristata*, *Lithophragma glabra* and *Festuca campestris* were found in the soil seed bank but were not represented in the above-ground vegetation. In contrast, 20 species were sampled above-ground as seedlings or mature plants but were not found in the soil seed bank.

The most abundant germinable seed extracted was *Vulpia octoflora*, accounting for 52% of the total germinable seeds in the soil seed bank on picloram-treated areas. This species accounted for only 1% of the total above-ground cover (Tables 2.1 and 2.4). Similarly, *Myosotis stricta* formed 16% of the total germinable seeds extracted but only 1% of the total above-ground cover in the untreated areas.

The patchy distribution of most seeds in the soil seed bank combined with a relatively small sample size resulted in high variability among replicates and, consequently, low power to detect treatment differences. For example, there was 40 times more seed of *Vulpia octoflora* in picloram-treated soils than in untreated soils; however, this difference was not statistically significant (P>0.10). Similarly, the number of germinable forb seeds averaged eight times greater in untreated areas but no treatment differences were detected. Such high variability is prevalent in soil seed bank studies and can be overcome only by extracting larger volumes of soil (Major and Pyott 1966, Benoit *et al.* 1989). Few conclusions can be drawn regarding these non-significant differences because there is little confidence that true treatment effects could be detected (Peterman 1990). Valid inferences can still be made regarding significant effects (Nemec 1991).

The density of *Centaurea diffusa* seeds was seven times lower on picloram-treated areas than on untreated areas (Table 2.4). This is probably directly related to the reduced cover of mature *Centaurea diffusa* which was nine times lower on treated areas (Table 2.1), and supports the theory that low seed availability in picloram-treated areas contributes to low abundance of mature *Centaurea diffusa*. A reduction in mature, seed-producing *Centaurea diffusa* plants limits the seed production considerably since much of the seed falls close to the parent plant. Over time, and without further input, seed bank numbers will decline due to losses by germination, decay and predation. For example, Chichoine (1984) found that *Centaurea maculosa* seed reserves declined by 61 and 81% of original numbers when seed production was limited by herbicide application for 10 months.

The density of *Arabis holboelii* seeds was about 11 times lower in the treated areas (Table 2.4). Unlike *Centaurea diffusa*, this species was present in the above-ground vegetation. *Arabis holboellii* cover was virtually identical between treatments and no difference could be detected in the density of mature plants (Table 2.1). It is possible that the reproductive capacity of *Arabis holboellii* was reduced by competition from increased *Stipa comata* abundance on picloram-treated areas. *Arabis holboellii* plants on picloram-treated areas may not produce as many seeds. Direct measurement of the reproductive capacity of *Arabis holboellii* was not obtained; however, seed production can be estimated based on the abundance of bolted plants, since the rosette form does not produce seed. Plant heights above 10 cm were assumed to indicate that *Arabis holboellii* had bolted. Observations of the rosette form of this species indicated a range in height from about 1 to 7 cm. Only a single plant taller than 10 cm was recorded on the picloram-treated areas, compared to 5 plants on the untreated areas. This suggests that the reproductive stage of *Arabis holboellii* , a monocarpic perennial, was suppressed on picloram-treated areas.

4.4 Seedling Density

Centaurea diffusa seedlings were at least 70 times more dense on untreated areas than on picloram-treated areas, at every sampling date, except for June 1996 when *Centaurea diffusa* was omitted from sampling (Table 2.5). *Centaurea diffusa* seedling density was as high as 3.5/m^2 on picloram-treated soils but reached 251/m^2 on untreated soils in May 1996. About 13% of the seed available in the top 2 cm of the untreated soil seed bank in July 1995 germinated in the fall (Sept. 1995). In comparison, less than 1% of the seeds available in the picloram-treated areas germinated in the fall (Tables 2.4 and 2.5). Since *Centaurea diffusa* seeds mature in mid-August (Watson and Renney 1974), no new seed input had occurred since the 1994 seed crop was dispersed. *Centaurea diffusa* germinates best in full light (Nolan and Upadhyaya 1988); therefore, the lower rate of emergence from available seed on treated areas may be related to shading effects resulting from an increased cover of *Stipa comata*.

An additional factor may be that seeds on picloram-treated areas became less germinable over time due to losses by germination of non-dormant seed and by physiological aging. In fact, germination tests on extracted seeds revealed that 55% of the seeds recovered from untreated areas were readily germinable compared to only 22% from picloram-treated areas.

It is notable that *Stipa comata* seedling densities were similar at all sampling periods (Table 2.5). This is in contrast to cover of this species which was three times higher on picloram-treated areas. Apparently, picloram treatment is followed by minimal increases in *Stipa comata* seedling recruitment. This evidence corroborates findings that most of the increase in *Stipa comata* cover following picloram treatment is due to vegetative enlargement of individual plants and not to increases in density (section 4.1).

Antennaria dimorpha seedling density was lower on picloram-treated areas at the last three sampling dates (Table 2.5). These effects are consistent with similar decreases in *Antennaria dimorpha* cover and density on treated areas (Table 2.1). Like *Centaurea diffusa*, this species may be negatively affected by the increased cover of

Table 2.5 Seedling density (plants/m^2) of vascular plant species following July 1991 application of picloram at 0.54 kg ai/ha at the Redhill, Racetrack and Stinklake locations.

Species	July 1995		Sept 1995		May 1996		June 1996	
	Picloram	Control	Picloram	Control	Picloram	Control	Picloram	Control
	plants/ m^2							
Grasses								
Bromus tectorum	+	0.0	-	-	8.3	0.0	-	-
Koeleria macrantha	+	+	-	-	0.7	0.5	5.6	1.6
Poa compressa	-	-	-	-	0.0	+	-	-
Poa pratensis	0.0	3.8	-	-	+	0.0	-	-
Poa secunda	0.0	+	-	-	15.7	10.3	2.3	1.3
Sporobolus cryptandrus	2.2	0.5	+	0.5	+	0.0	1.0	1.3
Stipa comata	9.7	12.5	2.8	1.1	17.9	16.5	24.7	23.6
Vulpia octoflora	0.5	+	-	-	117.5	1.2	2.6	0.0
Grass Total	12.8	17.1	3.3	1.6	160.5	29.0	36.2	27.8
Forbs								
Achillea millefolium	0.0	+	-	-	-	-	-	-
Antennaria dimorpha	+	+	0.0	0.5(•)	1.9	4.5(•)	2.0	5.9(•)
Antennaria microphylla	-	-	+	0.0	-	-	-	-
Androsace occidentalis	-	-	-	-	0.8	0.7	-	-
Arabis holboellii	2.2	4.8	4.2	13.3(•)	8.4	16.9	10.5	24.2
Calochortus macrocarpus	-	-	-	-	+	+	0.0	+
Castilleja thompsonii	+	+	+	0.0	-	-	4.3	0.0
Centaurea diffusa	0.1	53.5•	1.5	162.4•••	3.5	251.5•	-	-
Cirsium undulatum	0.0	+	-	-	-	-	-	-
Collinsia parviflora	-	-	-	-	1.1	0.0	-	-
Descurainia pinnata	-	-	-	-	+	0.0	-	-
Draba nemorosa	-	-	-	-	+	0.5	-	-
Draba verna	-	-	-	-	0.0	2.2	-	-
Echium vulgare	-	-	-	-	-	-	+	0.0
Erigeron pumilus	+	0.7	+	0.8	0.5	1.9	0.7	2.0
Fritillaria pudica	-	-	-	-	+	+	-	-
Kochia scoparia	0.7	0.0	-	-	13.5	3.7	10.5	2.3
Lomatium macrocarpum	+	0.0	-	-	-	-	-	-
Microsteris gracilis	0.0	+	-	-	1.8	37.8	-	-
Myosotis stricta	+	0.0	0.5	0.0	0.7	116.1	-	-
Plantago patagonica	+	0.0	+	+	3.0	+	2.0	0.0
Polygonum douglasii	0.0	+	-	-	0.0	+	-	-
Sonchus arvensis	-	-	-	-	-	-	0.7	0.0
Tragopogon dubius	-	-	+	+	0.7	0.5	1.0	+
Forb Total	3.7	59.8	7.1	178.1	37.8	436.0	31.9	35.0
Shrubs								
Artemisia frigida	+	0.5	1.8	12.7	3.1	6.9	3.0	7.2
Artemisia tridentata	0.7	+	-	-	8.3	11.4	5.6	18.0
Chrysothamnus nauseosus	+	0.0	0.0	+	2.2	0.7	+	1.0
Shrub Total	1.1	0.7	1.8	12.9	13.5	19.0	8.9	26.2
Total	17.6	77.6	12.1	192.6	211.8	484.8	76.9	89.1

(•), •, ••• significantly different at $P \leq 0.10$, 0.05, and 0.001, respectively.
+ Less than 0.5 plants/m^2

Stipa comata on picloram-treated areas. Similarly, in September 1995, seedlings of *Arabis holboellii* were three times less dense on picloram-treated areas. This corresponds to an 11-fold difference in soil seed banks.

5.0 Conclusions

Quantification of vegetation changes was provided for a low elevation grassland site following application of picloram at 0.54 kg ai/ha. Plant diversity was reduced on picloram-treated areas. This is a potential concern if plant diversity is a valid index of reduced ecosystem stability. Only one species, *Antennaria dimorpha*, was shown to be threatened by extirpation on herbicide-treated areas. The loss of *Antennaria dimorpha* may affect ecosystem function if it proves to provide a unique role in the plant community.

Centaurea diffusa remained suppressed after 5 years despite the fact that residual picloram had dissipated to non-toxic levels. The cover of the dominant grass, *Stipa comata*, increased by vegetative expansion and accounted for 82% of the total grass response. It is likely that the increase in *Stipa comata* resulted in the continued suppression of *Centaurea diffusa*. Another factor may be the decrease of *Centaurea diffusa* seed in the soil seed bank. Nevertheless, 188 seeds/m^2 still remained in the soil seed bank, which is more than enough to quickly re-establish this weed, even at relatively low germination rates.

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CHAPTER 3. RESPONSE OF NON-TARGET GRASSLAND PLANT SPECIES TO MODIFIED PICLORAM AND CLOPYRALID TREATMENTS

1.0 Introduction

The herbicide picloram (4-amino-3,5,6 trichloro-2-pyridinecarboxylic acid) is widely used for weed control and containment on grassland range in North America (Norris 1986). Picloram is effective against many serious grassland weeds but many non-target plant species are also susceptible to this herbicide (Scotter 1975, Murray *et al.* 1991). Increasing concern regarding the conservation of biological diversity (Dasmann 1984, Biodiversity Convention Office 1995) has resulted in a focus on a variety of rangeland values in addition to the forage resource. Range managers are now often required to address concerns regarding endangered species, wildlife habitat and clean water values (Heady 1994). The effects of picloram on non-target plant species deserve consideration because of the potential damage to forage values for livestock and wildlife, the threat to rare and endangered plant species, and the threat to biological diversity.

The forage value of weed-infested areas is often improved following treatment with picloram, due to substantial increases in the production of grasses (e.g., Renney and Hughes 1969). However, information regarding the susceptibility of non-target species, as well as the composition of the pre-treatment plant community, is required for well-informed use of herbicides on rangelands. Evans and Young (1985) illustrated this point well. They reported that western juniper (*Juniperus occidentalis)* could be controlled successfully with picloram, resulting in large increases in herbage yield. They also reported that, overall, the herbicide treatment was a failure because most of the herbage increase was due to undesirable annual grasses of poor forage value. For that particular rangeland ecosystem, information on the proportion of desirable plants in the community before treatment, as well as the probable impacts of picloram on existing desirable forb species, would have aided in the prediction of the results of western juniper control. McIlvain and Savage (1949) were perhaps the first to comment on the negative impact of herbicides on desirable native forage plant species and provided information on the susceptibility of many non-target species to 2,4-D. As early as 1951, Cornelius and Graham (1951) cautioned that some desirable native forage legumes and browse plants were susceptible to rangeland herbicides. They noted that the loss of desirable forage plants may sometimes outweigh the benefits of weed control.

Extensive use of herbicides can also impact forage values and habitat for wildlife in certain situations (Blaisdell and Mueggler 1956). Herbicide-susceptible shrubs and forbs that are considered unimportant as livestock forage may be important to wildlife species (Yoakum 1986, Cooperrider and Bailey 1986). For example, Yoakum (1986) viewed the large-scale chemical control of *Artemisia tridentata* as a direct threat to pronghorn antelope populations. The concerns over the herbicide-induced loss of wildlife habitat are also pertinent to game birds such as sage grouse (Roberson 1986) and lesser prairie-chicken (Olawski and Smith 1991), and to non-game species such as Brewer's sparrow (Schroeder and Sturges 1975).

The protection of rare plant species should be a concern wherever herbicides are used because of the potential threat of plant species extirpation. Tilman and Pacala (1991) provided evidence that the probability of a species being lost due to drought increases with its rarity. This relationship is likely applicable to environmental stresses other than drought and is perhaps also pertinent to the stresses imposed by herbicides. The impact of current herbicide practices on rare native plant species is generally unknown. It is sometimes assumed, based on recovery of the more abundant plant species, that most plant species recover rapidly after treatment with herbicide (e.g., Murray *et al.* 1991, Rice *et al.* 1992). However, the most abundant plant species are generally the least threatened because of their greater numerical capacity to recover. This is because the chances of successful germination and establishment are increased with increasingly dense seed rain (Harper 1977). Less abundant species, that are potentially the most threatened, are rarely considered in the literature. This is due, in part, to the difficulty of conducting adequate sampling and statistical treatment of non-abundant and patchily distributed plant species (Houston 1977, Scifres and Mutz 1978).

The objectives of this study were to determine the extent of non-target plant species damage in a plant community treated with the herbicide picloram. A number of strategies aimed at reducing non-target plant species damage were examined and compared to the standard practice. The use of the herbicide clopyralid (3,6-dichloro-2-pyridinecarboxylic acid) was tested as a less-damaging alternative to picloram. Reduced rates and application during different seasons were also examined as potential methods to reduce non-target plant species damage by picloram. The susceptibilities of non-target plant species treated with picloram were determined on *Centaurea diffusa*-infested grassland sites in British Columbia (B.C.).

The herbicide clopyralid is closely related to picloram but possesses some important differences. Picloram is a wide-spectrum herbicide that is toxic to many herbaceous broad-leaved plant species, while clopyralid is more selective (Kelpsas and White 1986, Whisenant 1986, Hall and Vanden Born 1988, Cobb 1992, Orfanedes *et al.* 1993). For example, plant species in the Rosaceae family as well as coniferous species are resistant to clopyralid, but generally susceptible to picloram. Canola (*Brassica napus*) treated with 5000 g/ha of clopyralid showed no symptoms after 18 days, while treatment with 75 g/ha of picloram resulted in severe symptoms within 6 hr (Hall *et al.* 1985). Clopyralid is less persistent than picloram and so is expected to provide shorter-term control of *Centaurea diffusa* (Hogue 1982, Jotcham *et al.* 1989). For these reasons, the use of clopyralid was examined as a potential method to reduce damage to non-target plant species.

The current recommended rate for control of *Centaurea diffusa* on grassland in B.C. using picloram is 0.54 kg ai/ha (kg active ingredient/ha) (DowElanco Canada Inc. 1993). This rate was based on the achievement of good control with little damage to forage grasses (Renney and Hughes 1969, Cranston 1977). Rates as low as 0.1 - 0.14 kg ai/ha, however, have resulted in adequate control of *Centaurea diffusa* for at least 16 months (Renney and Hughes 1969, Hogue 1982). Theoretically, reducing the rate of picloram may reduce losses of non-target broad-leaved species that are less susceptible to picloram than *Centaurea diffusa*.

Spring application of picloram for the control of *Centaurea diffusa* is a common operational practice in B.C. Previous research has provided evidence of adequate *Centaurea diffusa* control regardless of the application date of picloram (Renney and Hughes 1969, Hogue 1982, Cranston 1983a, Cranston 1983b). The use of summer and fall herbicide application was therefore examined as a strategy to reduce non-target plant damage. By summer, a wide variety of rangeland plant species enter a state of drought-induced dormancy (Pitt and Wikeem 1990). In the fall, the majority of plant species have completed their seasonal growth. Some species, such as *Centaurea diffusa* and many grasses, regrow or germinate in the fall in years when adequate moisture is available (Shirman 1981). Under those conditions, *Centaurea diffusa* may be relatively more susceptible to picloram than broad-leaved plants that are entering winter dormancy.

The efficacy of phloem-mobile herbicides (such as clopyralid and picloram) is generally influenced by the phenological stage at application (Orfanedes *et al.* 1993). For example, Turnbull & Stephenson (1985) report that the maximum effectiveness of phenoxy and benzoic acid herbicides is achieved when applications are made at the early-bud to bloom stage of Canada thistle (*Cirsium arvense*). This phenological stage is said to coincide with the period of lowest root reserves and maximum basipetal translocation of plant assimilates. There is some evidence of differential susceptibility to clopyralid at varying phenological stages (O'Sullivan *et al.* 1985, Zollinger *et al.* 1992). However, Donald (1988) found that clopyralid provided equal control of Canada thistle at the vegetative, bud, and flowering stages. Penetration of clopyralid into leaf tissue is generally greater in younger than older leaves (Donald 1988).

Some evidence of differential susceptibility to picloram at varying phenological stages also exists (Gorrell *et al.* 1988). Leafy spurge (*Euphorbia esula*) was less susceptible to picloram in the vegetative growth stage than in the flowering or seed-filling stages (Hickman *et al.* 1989, Lym and Messersmith 1990). Similarly, Lym and Moxness (1989) found that picloram translocation to leafy spurge roots was greatest during the flowering and late seed-set growth stages. This was postulated to be due to differences in plant size which results in the more mature plants receiving the highest rate of herbicide (Hickman *et al.* 1989). Treatment of leafy spurge during fall regrowth (late August until a killing frost) was effective, but the rate of picloram had to be doubled compared to treatment during the true-flower stage (Lym and Messersmith 1985, Lym and Messersmith 1990).

The specific objectives of this study were:

1) to explore lower rates of picloram than recommended, as a method to reduce non-target plant species damage;

2) to explore an alternative herbicide, clopyralid, as a method to reduce non-target plant species damage; and

3) to explore alternative application dates than currently used, as a method to reduce non-target plant species damage.

2.0 Study Areas

The study was conducted on two grassland sites in the southern interior of B.C. that were heavily infested with the weed *Centaurea diffusa*. The two sites, referred to as the Pritchard and Prudens sites, occur in the Bunchgrass (Very-Dry Warm) biogeoclimatic subzone (Lloyd *et al.* 1990). The Pritchard site was divided into two blocks, Block-A and Block-B, to provide for two treatment application years. The dominant vegetation at both sites includes *Stipa comata*, *Centaurea diffusa*, and *Koeleria macrantha*. *Elymus spicatus* is expected to dominate these sites at their potential natural community (Tisdale 1947) but is currently present at less than 1% and 6% canopy cover at the Pritchard and Prudens sites, respectively. Plant taxonomic nomenclature follows Douglas *et al.* (1989).

The soils are Dark Brown Chernozems (Typic Borolls) of aeolian origin, with generally fine sandy loam textures. The aspect at the Pritchard site (50° 45' N, 119° 55' W) is 5% southeast with an elevation of 450 m. The aspect at the Prudens Site (50° 43' N, 120° 23' W) is 2% north with an elevation of 700 m. Mean annual precipitation at the Kamloops airport, which is within 350 m elevation and 35 km from both sites, is 256 mm, with peaks in June-August and December-January. At Kamloops, the highest daily maximum temperatures occur in July (28.8° C) and lowest daily mean minimum temperatures occur in January (-9.8° C) (Environment Canada 1984).

3.0 Methods

Herbicides were individually mixed for each plot in 1.1-L portions and were applied on 2 X 11-m plots using a portable pressurized tank sprayer with a hand-held boom. Water volume was 400 L/ha, pressure was 240 kPa, and Teejet 8004 nozzles were used. The temperatures at the time of treatments varied between 13° C and 21° C and relative humidities averaged from 55% to 63%. Total precipitation in the 1993 and

1994 treatment years was within 10% of the 45-year normal at Kamloops Airport (Fig. 3.1). Total precipitation in the 1995 treatment year exceeded the 45-year normal by 38%. Five replications were established at each location. Five untreated control plots were also established at each location.

A single 11-m transect was located along the longitudinal centerline of each plot for vegetation sampling purposes. The canopy cover of all plant species was sampled on 10 20 X 50-cm (0.1 m^2) quadrats that were permanently established at a 1-m spacing along the transects (Daubenmire 1959). The plots were sampled in 1995 and 1996 during May, while the ephemeral plant species were still recognizable.

Data for each plant species were analyzed separately. Significant differences were declared at $P \leq 0.05$ for all tests. In cases of non-significant effects, post-hoc power tests were used to determine whether a 100% change in the control means could have been detected. If statistical power was less than 80% for these tests then the analysis for that plant species was not discussed because little confidence can be placed on non-significant tests with statistical power less than 80% (Peterman 1990).

3.1 Reduced Rates of Picloram

Single applications of picloram at 0.05, 0.15 and 0.55 kg ai/ha were made on 5 May 1994 at the Pritchard Block-A location to test the effect of reduced rates of picloram from the currently recommended application rate. The same treatments were applied on 9 May 1995 at Pritchard Block-B, and on 12 May 1995 at the Prudens location. Precipitation in May 1994 was within 10% of the 45-year normal while precipitation in May 1995 was only 29% of normal (Fig. 3.2). Rainfall events were of low intensity, less than 5 mm/day, during the 2-week period following herbicide treatment for all treatment dates. Canopy cover was sampled 1 year after treatment in May 1995 or May 1996. The cover data from each site were analyzed separately using analysis of variance (SAS 1988) in a completely randomized design. Linear and

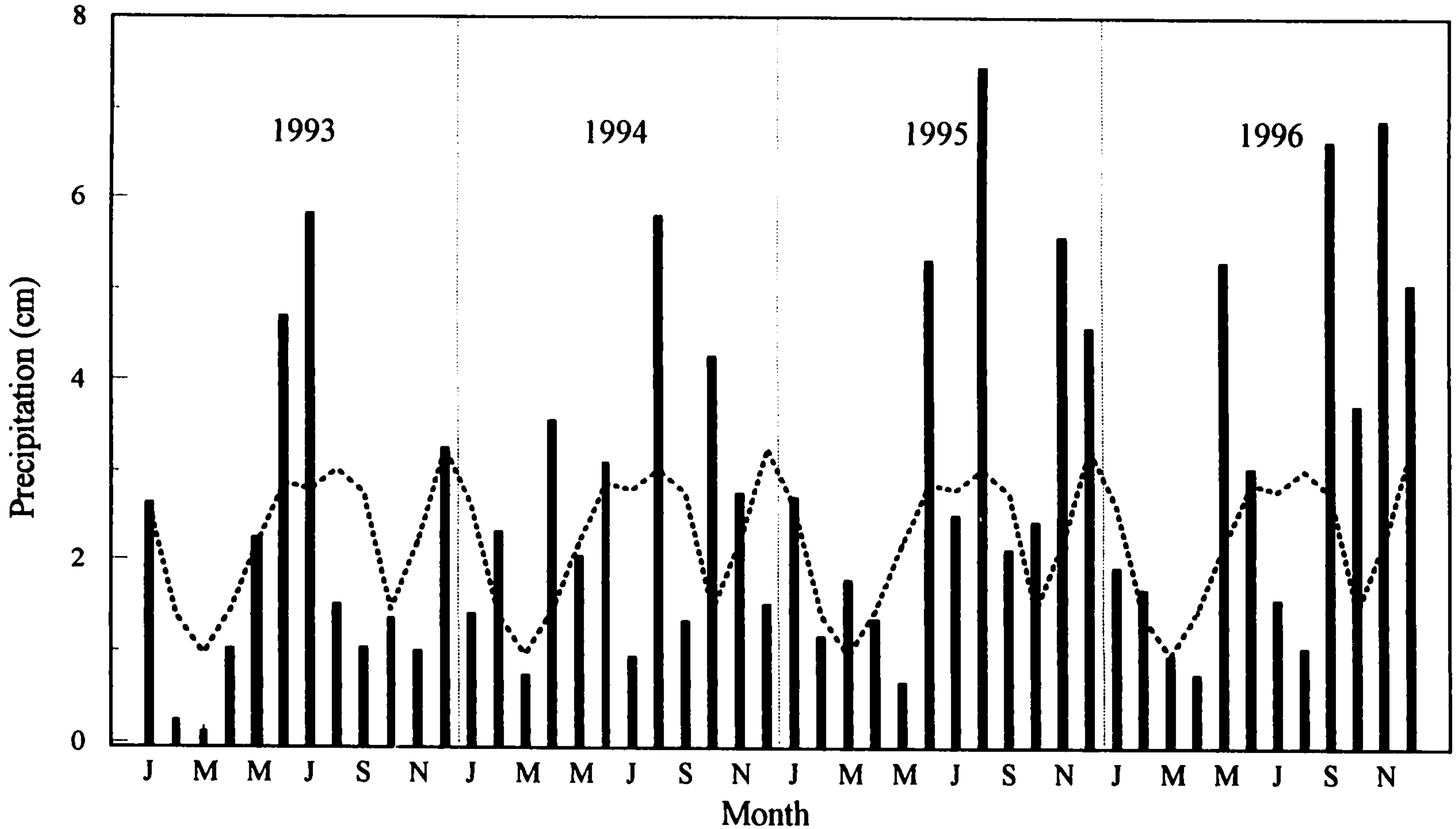

Fig. 3.1 Monthly precipitation totals for 1993 through 1996 (bars) compared to the 45-year normal (dashed line) at Kamloops, British Columbia.

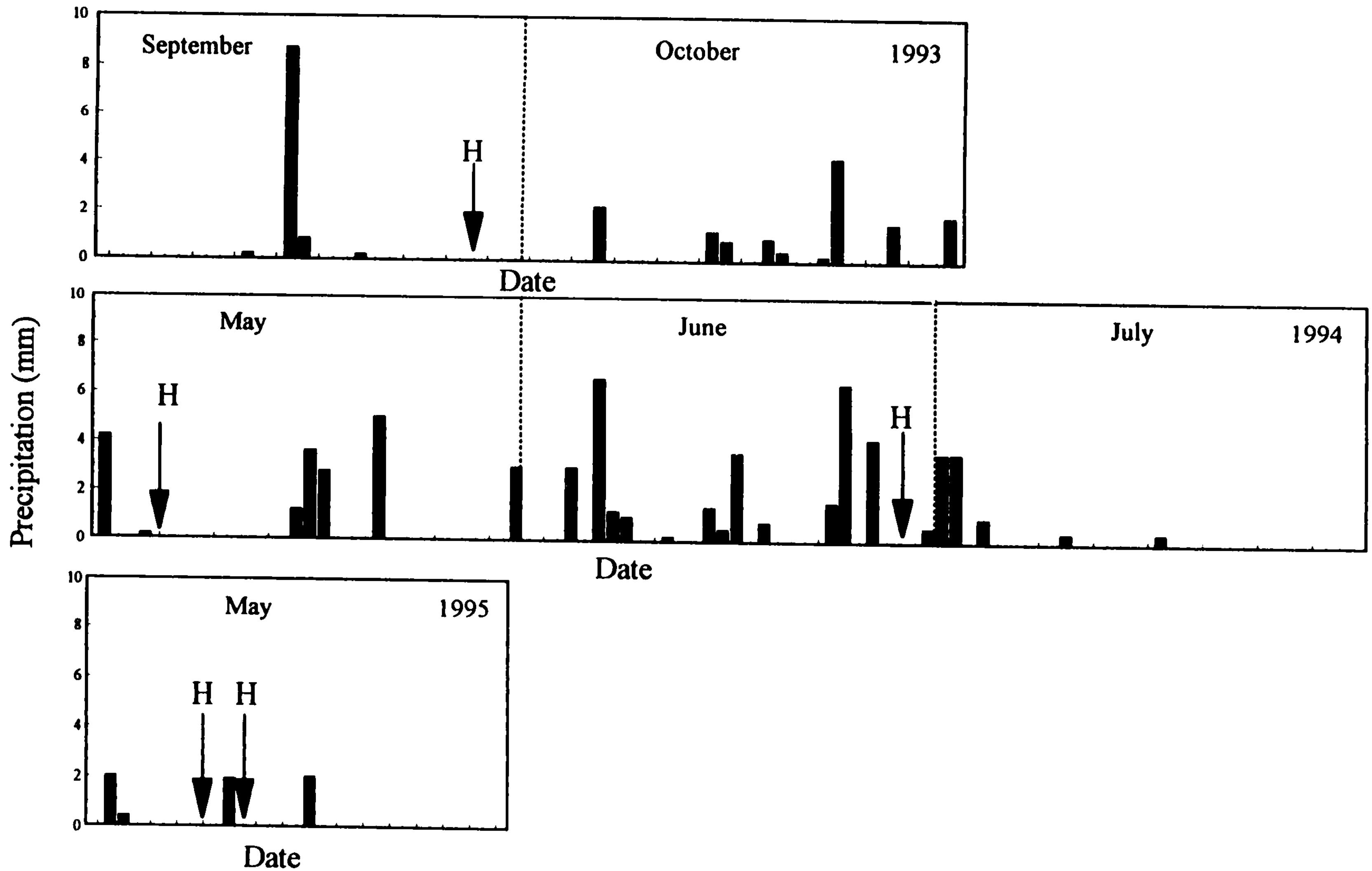

Fig. 3.2 Daily precipitation (mm) at Kamloops, B.C. during and after the herbicide treatment dates (H) in 1993, 1994 and 1995.

quadratic polynomial contrasts were used to test the nature of the response to the rate effect.

3.2 Alternative Dates of Application and Clopyralid as an Alternative Herbicide to Picloram

In a separate experiment, picloram and clopyralid were applied at three dates to examine the effects on non-target plant species damage. Single applications of picloram at 0.55 kg ai/ha and clopyralid at 0.15 kg ai/ha were made in the fall (27 September 1993), spring (5 May 1994), and summer (28 June 1994) at the Pritchard Block-A location. Precipitation for the month following herbicide application was within 10% of the 45-year normal in October 1993 and May 1994 (Fig. 3.2). Precipitation was only 32% of normal during July 1994. Rainfall events were of low intensity, less than 5 mm/day, during the 2-week period following herbicide treatment for all treatment dates. Canopy cover was sampled in May 1996. The cover data were analyzed using analysis of variance in a 2 X 3 (type X date) factorial arrangement of treatments in a completely randomized design. Orthogonal contrasts were used to test for differences between the spring and summer dates, and between fall compared to spring and summer combined.

4.0 Results and Discussion

A total of 55 plant species (Table 3.1) were recorded in the plots at the three study sites (Pritchard Block-A 38; Pritchard Block-B 42; Prudens 28). Meaningful analyses were possible for only 24 of the species (Fig. 3.3) because sparse distribution often resulted in high variability among replicates, and consequently low statistical power.

4.1 Non-Target Plant Species Susceptibility to Picloram

Picloram applied at 0.55 kg ai/ha reduced the cover of *Centaurea diffusa* by

Table 3.1 Canopy cover (%) of vascular plants 1 year after application of picloram at 0.05, 0.15 and 0.55 kg ai/ha at Pritchard Block-A (May 1994), Pritchard Block-B (May 1995) and Prudens (May 1995).

	Pritchard Block-A					Pritchard Block-B					Prudens				
	0	0.05	0.15	0.55	Signif.	0	0.05	0.15	0.55	Signif.	0	0.05	0.15	0.55	Signif
	------- cover (%) --------					--------- cover (%) ------					--------- cover (%) ------				
Grasses															
Bromus japonicus	-	-	-	-		0.8	3.1	0.0	4.5		-	-	-	-	
Bromus tectorum	5.9	3.0	4.5	3.3		+	0.5	0.7	0.6		-	-	-	-	
Elymus spicatus	-	-	-	-		0.0	0.0	0.0	+		6.2	3.7	7.60	5.4	
Koeleria macrantha	0.7	3.4	2.7	6.2		6.7	8.2	8.5	12.4		17.5	12.4	17.90	24.3	l
Poa compressa	-	-	-	-		0.0	0.8	+	0.0		-	-	-	-	
Poa pratensis	4.1	7.5	4.4	12.8		4.3	6.8	10.6	6.2		-	-	-	-	
Poa secunda	7.4	7.2	9.1	8.7		7.0	6.7	7.0	11.9		9.2	8.7	10.30	10.4	
Sporobolus cryptandrus	+	+	+	+		-	-	-	-		-	-	-	-	
Stipa comata	23.6	39.2	29.1	33.9		32.5	29.5	33.7	35.4		8.6	14.3	10.45	9.7	
Vulpia octoflora	0.05	0.10	0.10	0.10		0.30	0.05	0.05	0.00		-	-	-	-	
Forbs															
Achillea millefolium	2.3	4.3	+	0.0	R l q	1.9	1.4	0.6	0.0	R l	3.2	0.5	0.15	0.0	R l q
Androsace occidentalis	1.6	1.4	1.9	2.0		0.8	0.9	1.1	1.4		0.0	0.6	0.10	0.0	
Antennaria dimorpha	-	-	-	-		-	-	-	-		2.0	0.6	0.35	0.0	R l q
Antennaria microphylla	1.1	1.1	+	0.0		3.6	+	1.4	0.0		4.0	3.7	0.95	0.0	R l
Arabis holboellii	+	0.9	+	0.0		+	+	+	0.0	R l	1.5	1.5	0.40	+	R l q
Arenaria serpyllifolia	2.4	2.3	1.8	0.3	R l	1.9	1.9	2.0	1.5		-	-	-	-	
Artemisia dracunculus	0.0	0.0	0.0	0.3		-	-	-	-		-	-	-	-	
Artemisia frigida	-	-	-	-		-	-	-	-		+	+	0.00	0.0	
Astragalus miser	9.7	3.6	1.9	0.5	R l q	2.4	2.8	1.5	+		-	-	-	-	
Balsamorhiza sagittata	0.8	0.2	+	0.0		+	1.4	+	0.0		-	-	-	-	
Calochortus macrocarpus	0.0	0.0	+	0.0		-	-	-	-		0.6	0.5	0.45	0.0	

Table 3.1 (continued)

	Pritchard Block-A					Pritchard Block-B					Prudens				
	0	0.05	0.15	0.55	Signif.	0	0.05	0.15	0.55	Signif.	0	0.05	0.15	0.55	Signif.
	--------- cover (%) ------					--------- cover (%) ------					--------- cover (%) ------				
Forbs (continued)															
Castilleja thompsonii	0.0	0.0	+	0.0		-	-	-	-		+	0.5	0.30	+	
Centaurea diffusa	6.9	2.5	2.1	+	R l q	8.1	3.7	1.9	+	R l q	9.3	2.4	0.05	+	R l q
Chrysopsis villosa	-	-	-	-		0.0	0.0	0.0	+		-	-	-	-	
Cirsium undulatum	-	-	-	-		+	0.0	0.0	0.0		-	-	-	-	
Collinsia parviflora	2.5	2.6	3.0	2.9		2.0	3.0	2.2	3.6	l	0.0	+	0.05	0.0	
Crepis atrabarba	-	-	-	-		-	-	-	-		0.8	+	0.30	0.0	
Delphinium nuttallianum	+	0.5	0.5	+		0.7	0.9	0.7	+	l	0.0	+	0.00	0.0	
Dodecatheon cusickii	-	-	-	-		-	-	-	-		0.0	0.0	0.05	0.0	
Draba nemorosa	-	-	-	-		-	-	-	-		0.0	+	0.05	0.0	
Draba verna	2.2	2.0	2.2	1.9		2.0	1.8	2.0	2.5		-	-	-	-	
Erigeron flagellaris	-	-	-	-		+	+	0.0	0.0		-	-	-	-	
Erigeron pumilus	-	-	-	-		-	-	-	-		0.6	+	0.00	0.0	R l q
Fritillaria pudica	0.5	0.7	0.7	+		1.1	0.8	0.8	0.6		+	0.9	0.85	+	R l q
Gaillardia aristata	+	0.8	0.0	+	R	+	0.6	+	+		-	-	-	-	
Lesquerella douglasii	+	0.0	0.0	0.0		0.0	+	0.0	0.0		-	-	-	-	
Lithophragma glabra	0.0	+	+	+		0.9	0.6	1.0	+		-	-	-	-	
Lithospermum ruderale	+	1.1	+	2.8		1.3	1.1	1.1	0.6		0.0	0.0	0.05	0.0	
Lomatium ambiguum	0.0	1.3	0.0	0.0		+	+	+	+		-	-	-	-	
Lomatium macrocarpum	1.2	2.3	1.8	0.9		1.3	0.8	0.8	0.5		+	+	0.30	+	
Microsteris gracilis	-	-	-	-		+	+	0.0	0.0		+	+	0.15	0.0	
Montia linearis	1.3	1.2	2.1	1.2		1.6	0.9	1.5	0.8		-	-	-	-	
Myosotis stricta	2.5	2.3	1.9	+	R l	2.4	2.5	2.3	+	R l q	7.9	6.5	0.30	0.0	R l
Oxytropis campestris	0.0	0.1	0.0	0.0		3.3	6.6	1.7	0.4		-	-	-	-	

Table 3.1 (continued)

	Pritchard Block-A					Pritchard Block-B					Prudens				
	0	0.05	0.15	0.55	Signif.	0	0.05	0.15	0.55	Signif	0	0.05	0.15	0.55	Signif.
	--------- cover (%) ------					--------- cover (%) ------					--------- cover (%) ------				
Forbs (continued)															
Phacelia linearis	+	+	0.0	0.0		+	0.7	+	0.4		-	-	-	-	
Plantago patagonica	-	-	-	-		+	+	+	0.0		-	-	-	-	
Polemonium micranthum	0.6	+	+	0.0		+	+	+	+		-	-	-	-	
Polygonum douglasii	+	0.7	+	+		1.1	0.5	+	+	R l q	-	-	-	-	
Ranunculus glaberrimus	+	+	0.0	0.0	q	0.8	+	+	0.0	l	+	0.0	0.00	0.0	
Rosa acicularis	0.0	+	0.0	0.0		-	-	-	-		-	-	-	-	
Stellaria nitens	1.8	1.9	1.7	1.1		1.5	1.8	1.3	1.1		-	-	-	-	
Taraxacum officinale	-	-	-	-		-	-	-	-		+	+	0.00	0.0	
Tragopogon dubius	-	-	-	-		1.8	0.5	0.0	0.0	R l q	+	+	0.05	0.0	
Shrubs															
Artemisia tridentata	-	-	-	-		-	-	-	-		8.7	0.8	2.4	4.7	
Pinus ponderosa	0.0	+	+	0.0		+	0.0	0.0	0.0		-	-	-	-	

R=significant main effect for rate of picloram ($P \leq 0.05$).

l=significant linear polynomial contrast across rates ($P \leq 0.05$).

q=significant quadratic polynomial contrast across rates ($P \leq 0.05$).

\+ Less than 0.5 %

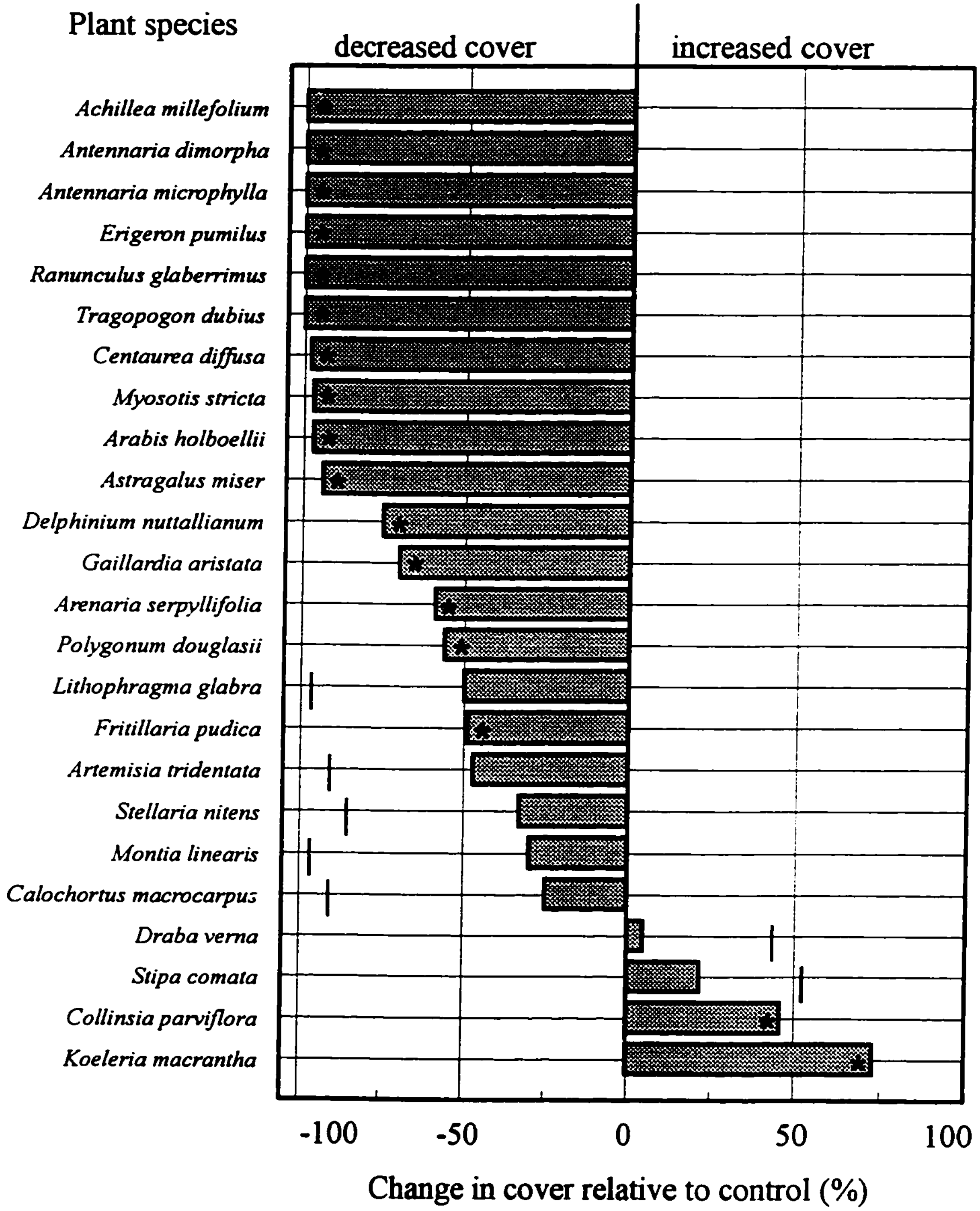

Fig. 3.3 Change in canopy cover (%) relative to control 1 year after May 1994 or May 1995 application of picloram at 0.55 kg ai/ha at the Pritchard Block-A, Pritchard Block-B and Prudens sites. Values are the average of sites at which species occur. * significant at $P \leq 0.05$; | minimum detectable change at 80% power.

98% compared to control (Table 3.1, Fig. 3.3). Fourteen other plant species were also susceptible to picloram at this rate. Six of these species were more susceptible than *Centaurea diffusa* and were completely eliminated from the plots. *Ranunculus glaberrimus* (Ranunculaceae) was the only species eliminated that was not in the Asteraceae family. Seven species, representing 88% of the Asteraceae on the sites, were reduced by picloram. This result is consistent with other reports of the sensitivity of the Asteraceae family to picloram. A single species from the Boraginaceae, Brassicaceae, Fabaceae, Ranunculaceae, Caryophyllaceae, Polygonaceae, and Liliaceae plant families also showed susceptibility to picloram at 0.55 kg ai/ha. In general, these species were not as greatly reduced as species in the Asteraceae family (Fig 3.3).

It is notable that five of the six species eliminated (83%) were perennial even though perennial species comprise only 54% of the total species. It appears that perennial species may be more susceptible to short-term extirpation than annuals or monocarpic perennials. A potential mechanism for this observed behaviour is that annual and monocarpic perennial species recover faster because of greater reproductive capacity. Annual and monocarpic perennial species are generally prolific seed producers adapted for rapid germination and establishment while perennial species are generally more conservative seed producers with more restrictive conditions for germination and establishment (Grime 1979, Grime *et al.* 1981).

Koeleria macrantha and *Collinsia parviflora* increased in cover after treatment with picloram, while *Stipa comata* and *Draba verna* showed a tendency toward increased cover (Table 3.1, Fig. 3.3). These four plant species fall into two broad categories, mid-sized perennial grasses and ephemeral annuals. Like most members of the Poaceae family, *Koeleria macrantha* and *Stipa comata* are resistant to picloram. The removal of sensitive forb species by picloram probably resulted in greater soil moisture availability for the remaining resistant plants such as the grasses. Soil moisture is the overriding factor controlling plant distribution in the bunchgrass grasslands of B.C. (Tisdale 1947). The increased cover of *Collinisia parviflora* and *Draba verna* is likely a combination of herbicide resistance and high reproductive capacity. These ephemeral spring annuals usually compete with as many as 10 other annual species for soil moisture and other resources in the interspaces of perennial grasses. Although herbicide assays were not conducted, picloram applied at 0.55 kg ai/ha is likely to remain active in the soil for 2 to 4 years in the environmental conditions at these sites (Hamaker *et al.* 1967, Goring and Hamaker 1971, Smith *et al.* 1988). Herbicide resistance allows *Collinisa parviflora* and *Draba verna* to establish in the absence of usual herbicide-sensitive competitors resulting in greater resource capture than normal.

4.2 Reduced Rates

Picloram applied at rates of 0.05 and 0.15 kg ai/ha resulted in less reduction in cover of most species than the 0.55 kg ai/ha rate (Table 3.1, Figs. 3.4a,b,c). Unlike the 0.55 kg ai/ha treatment, no species were eliminated when treated with the two lower herbicide rates. Plant species response to picloram rate can be broken into two major groups based on the shape of the response curve. Group 1 species are those that are sensitive to the lowest rates of picloram (0.05 and 0.15 kg ai/ha) and have a curved-shaped response to increasing rates. These species display a relatively sharp reduction in cover at low rates, with proportionately less reduction in cover between the 0.15 kg ai/ha rate and the 0.55 kg ai/ha rate. Statistical verification of this curved-shaped response is provided by a significant quadratic (q) polynomial contrast (Table 3.1). The species in this group include *Achillea millefolium*, *Antennaria dimorpha*, *Arabis holboellii*, *Astragalus miser*, *Centaurea diffusa*, *Erigeron pumilus*, *Myosotis stricta*, *Polygonum douglasii*, *Ranunculus glaberrimus*, and *Tragopogon dubius*. Group 1 species can be further categorized based on the sensitivity to the two lowest

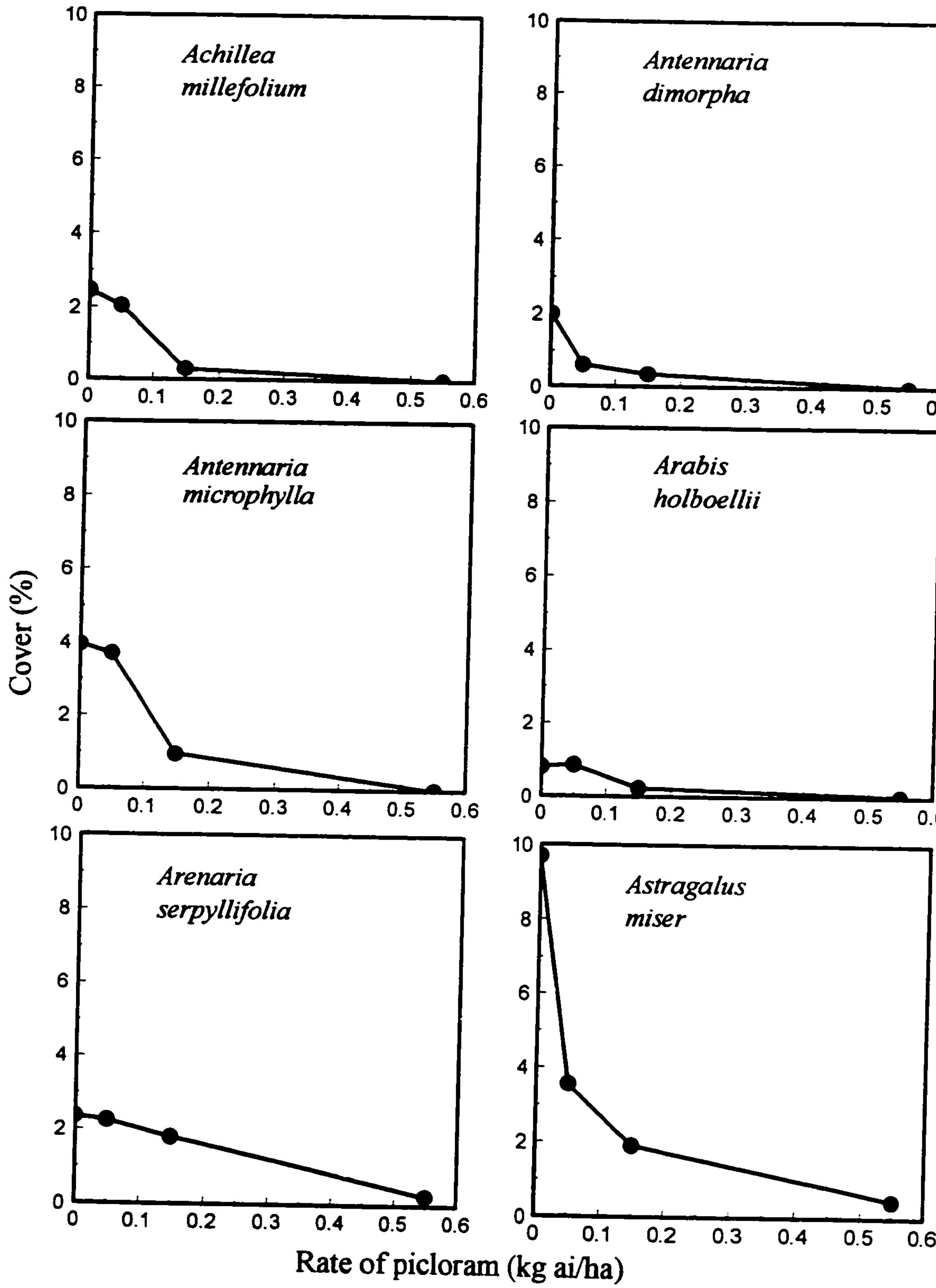

Fig. 3.4a Canopy cover (%) of vascular plants 1 year after May 1994 or May 1995 application of picloram at 0.05, 0.15 or 0.55 kg ai/ha at the Pritchard Block-A, Pritchard Block-B and Prudens sites. Values are the average of sites showing significant effects.

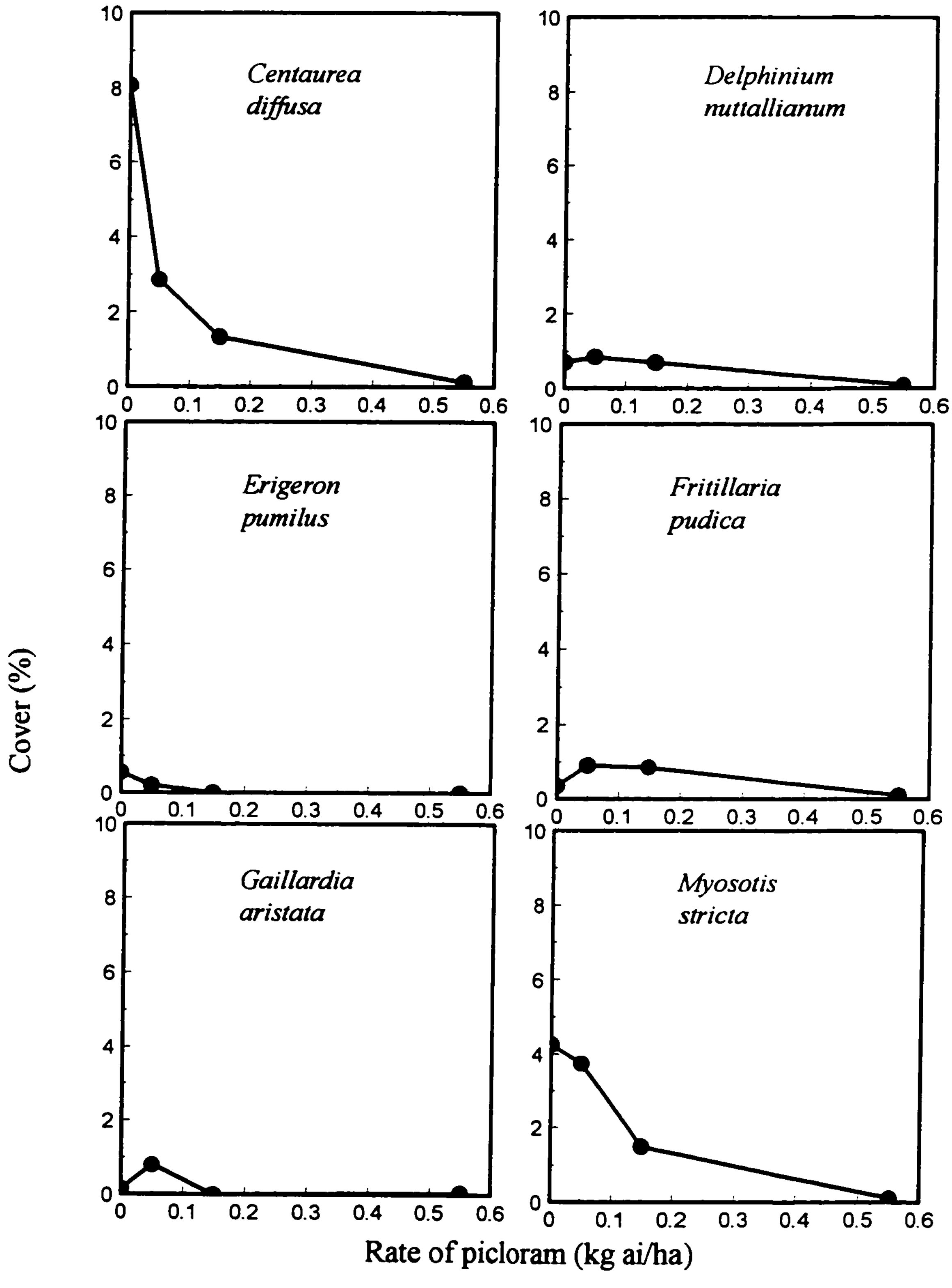

Fig. 3.4b Canopy cover (%) of vascular plants 1 year after May 1994 or May 1995 application of picloram at 0.05, 0.15 or 0.55 kg ai/ha at the Pritchard Block-A, Pritchard Block-B and Prudens sites. Values are the average of sites showing significant effects.

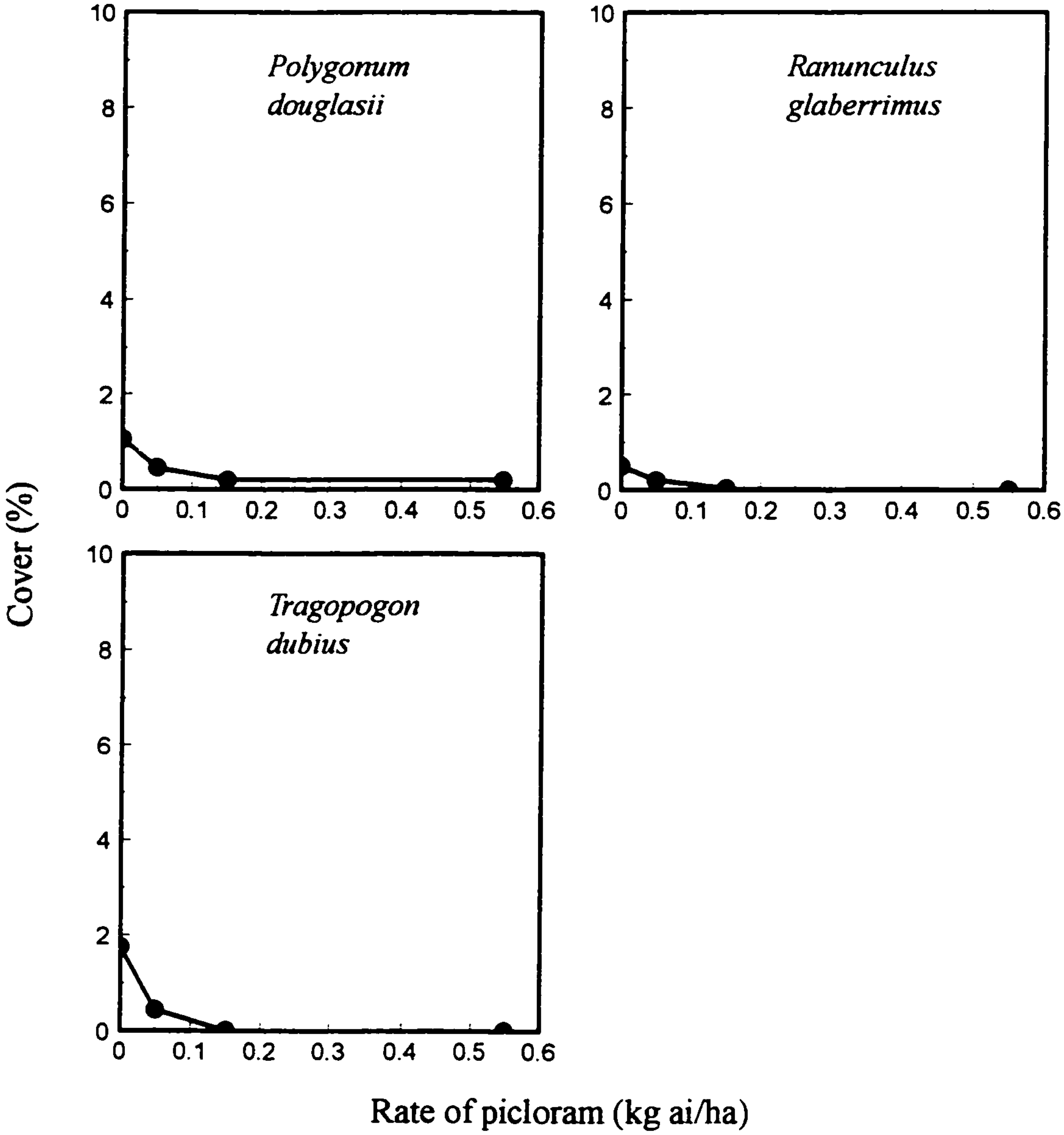

Fig. 3.4c Canopy cover (%) of vascular plants 1 year after May 1994 or May 1995 application of picloram at 0.05, 0.15 and 0.55 kg ai/ha at the Pritchard Block-A, Pritchard Block-B and Prudens sites. Values are the average of sites showing significant effects.

rates:

1a) Sensitive to the lowest rate (0.05 kg ai/ha). These species include *Antennaria dimorpha*, *Astragalus miser*, *Centaurea diffusa*, *Erigeron pumilus*, *Polygonum douglasii*, *Ranunculus glaberrimus*, and *Tragopogon dubius*.

1b) Sensitive to the intermediate rate (0.15 kg ai/ha) but not the lowest rate. These species include *Achillea millefolium*, *Arabis holboellii*, and *Myosotis stricta*.

Group 2 species are those that are not sensitive to the lowest rates of picloram and have a linear response to increasing rates. These species display little response to the 0.05 kg ai/ha rate (and sometimes to the 0.15 kg ai/ha rate) with proportionately more reduction in cover between the 0.15 kg ai/ha rate and the 0.55 kg ai/ha rate. Statistical verification of the linear response is provided by a significant linear (l) polynomial contrast and a non-significant quadratic polynomial contrast (Table 3.1). The species in this group include *Antennaria microphylla*, *Arenaria serpyllifolia*, and *Delphinium nuttallianum*.

Fritillaria pudica and *Gaillardia aristata* are not easily categorized into the two groups because their untreated control cover values are lower than the herbicide-treated cover values at 0.05 and 0.15 kg ai/ha. Visual examination of the cover response for these two species suggests that *Fritillaria pudica* fits best in group 2 while *Gaillardia aristata* fits best in group 1b.

Reduced herbicide rates appear most useful for minimizing damage to plant species in groups 1b and 2, although group 1a species may also benefit slightly. It is obvious that the lowest rate is the best option for protection of non-target plant species. *Centaurea diffusa* is reduced by 65% of the control value at the lowest rate, which is likley to be insufficient for weed management purposes. However, only seven non-target plant species are highly sensitive at this rate. Eight other non-target plant species, sensitive to picloram at 0.55 kg ai/ha, are relatively undamaged by the lowest rate.

The intermediate rate (0.15 kg ai/ha) appears to be a good compromise between *Centaurea diffusa* control and reduced damage to non-target plant species. The intermediate rate provides 84% control of *Centaurea diffusa*, which is only 14% less control than from the full label rate. Twelve non-target plant species are highly sensitive at rates of 0.15 kg ai/ha. Three other non-target plant species, sensitive to picloram at 0.55 kg ai/ha, are relatively undamaged at the intermediate rate.

It is notable that no plant species were eliminated at the two lower rates while treatment with 0.55 kg ai/ha picloram resulted in the complete loss of six species (Table 3.1, Figs. 3.4a,b,c). This may be the most important benefit to using lower rates of picloram. Recovery of a herbicide-sensitive species within a treated plant community is likely to occur more rapidly if scattered residual plants remain. These plants can provide dispersed sources of vegetative and reproductive material. In the absence of these plants, recovery will be slower because plant propagules must then be dispersed from the untreated periphery. The presence of residual plants will be critical to the recovery of plant species that have limited abilities to disperse or whose main method of increase is by vegetative reproduction. The importance of residual plants to the recovery of plant species increases as the size of the herbicide-treated area increases due to the greater dispersal distances involved.

4.3 Alternative Dates of Herbicide Application

Six plant species were sensitive to the herbicide application date (Table 3.2, Figs. 3.5a,b,c). These species were affected differently by herbicide, depending on the date of treatment.

4.3.1 Spring vs. Summer

Summer herbicide treatment with clopyralid or picloram resulted in less impact

Table 3.2 Canopy cover (%) of vascular plants in May 1996 after application of picloram at 0.55 kg ai/ha or clopyralid at 0.15 kg ai/ha at Pritchard Block-A in fall (Sept 1993), spring (May 1994) or summer (June 1994).

Plant species	Control	Clopyralid			Picloram			Tests of significance						
		Spring	Summer	Fall	Spring	Summer	Fall	a	b	c	d	e	f	g
	Cover (%)													
Grasses														
Bromus tectorum	4.3	0.50	2.2	+	7.1	4.2	0.9	-	-	-	-	-	-	-
Koeleria macrantha	1.3	8.5	3.5	1.0	5.0	+	2.5	-	-	-	-	-	-	-
Poa pratensis	5.4	11.8	16.5	14.9	15.9	20.3	7.2	-	-	-	-	-	-	-
Poa secunda	7.2	4.4	4.7	6.8	9.1	7.3	6.7	-	-	-	-	-	-	-
Stipa comata	33.2	34.5	31.9	34.5	44.2	32.5	44.0	-	-	-	-	-	-	-
Forbs														
Achillea millefolium	3.5	0.6	1.3	0.7	+	0.0	+	-	-	-	*	-	-	-
Antennaria microphylla	1.1	+	1.0	1.4	0.0	0.0	0.0	-	-	-	*	-	-	-
Androsace occidentalis	+	+	0.8	1.0	1.0	1.1	1.4	*	-	*	*	-	-	-
Arabis holboellii	+	+	0.2	+	0.0	+	+	-	-	-	-	-	-	-
Arenaria serpyllifolia	2.3	1.8	3.1	1.8	1.9	2.4	1.9	*	*	-	-	-	-	-
Astragalus miser	4.4	+	4.5	1.2	0.9	+	0.5	*	*	-	*	*	-	-
Centaurea diffusa	11.5	2.9	2.5	6.2	1.4	1.1	2.0	*	-	*	*	-	-	*
Collinsia parviflora	2.4	2.4	2.4	2.8	2.7	2.6	5.6	-	-	*	-	-	-	-
Delphinium nuttallianum	0.5	0.8	0.8	1.3	+	+	+	-	-	-	*	-	-	-
Draba verna	2.2	1.6	2.2	2.0	2.3	1.7	2.2	-	-	-	-	-	-	-
Fritillaria pudica	1.1	0.6	0.9	1.1	+	+	+	-	-	-	*	-	-	-
Gaillardia aristata	+	+	0.8	1.1	+	+	+	-	-	-	-	-	-	-
Lithophragma glabra	1.3	1.0	1.2	0.8	0.5	+	0.0	-	-	-	*	-	-	-
Lithospermum ruderale	+	1.2	1.1	1.1	1.4	1.1	0.5	-	-	-	-	-	-	-
Lomatium macrocarpum	0.9	1.3	1.9	0.9	0.6	+	1.0	-	-	-	-	-	-	-
Montia linearis	2.0	1.5	1.4	1.1	1.8	1.4	2.1	-	-	-	-	-	-	-
Myosotis stricta	2.4	2.4	2.2	2.5	2.3	2.1	2.4	*	*	*	-	-	-	-
Phacelia linearis	+	+	+	+	+	+	+	-	-	-	-	-	-	-
Plantago patagonica	0.0	+	+	+	+	+	+	-	-	-	-	-	-	-
Polygonum douglasii	1.1	1.2	1.1	1.0	+	+	0.9	-	-	-	*	-	-	*
Polemonium micranthum	+	+	+	+	0.0	+	+	-	-	-	*	-	-	-
Ranunculus glaberrimus	0.8	+	1.9	1.2	0.0	0.0	0.0	-	-	-	*	-	-	-
Stellaria nitens	1.1	1.7	1.3	1.6	1.2	0.9	1.9	-	-	-	-	-	-	-
Tragopogon dubius	0.5	+	+	+	+	0.0	0.4	-	-	-	-	-	-	-

a=Date main effect; d=Type of herbicide main effect; e= Date X Type interaction.

Orthogonal contrasts: b=Spring vs. Summer; c=Fall vs. other; f=Type X Spring vs. Summer; g=Type X Fall vs. Other.

* significant at $P \leq 0.05$. + Less than 0.5 %

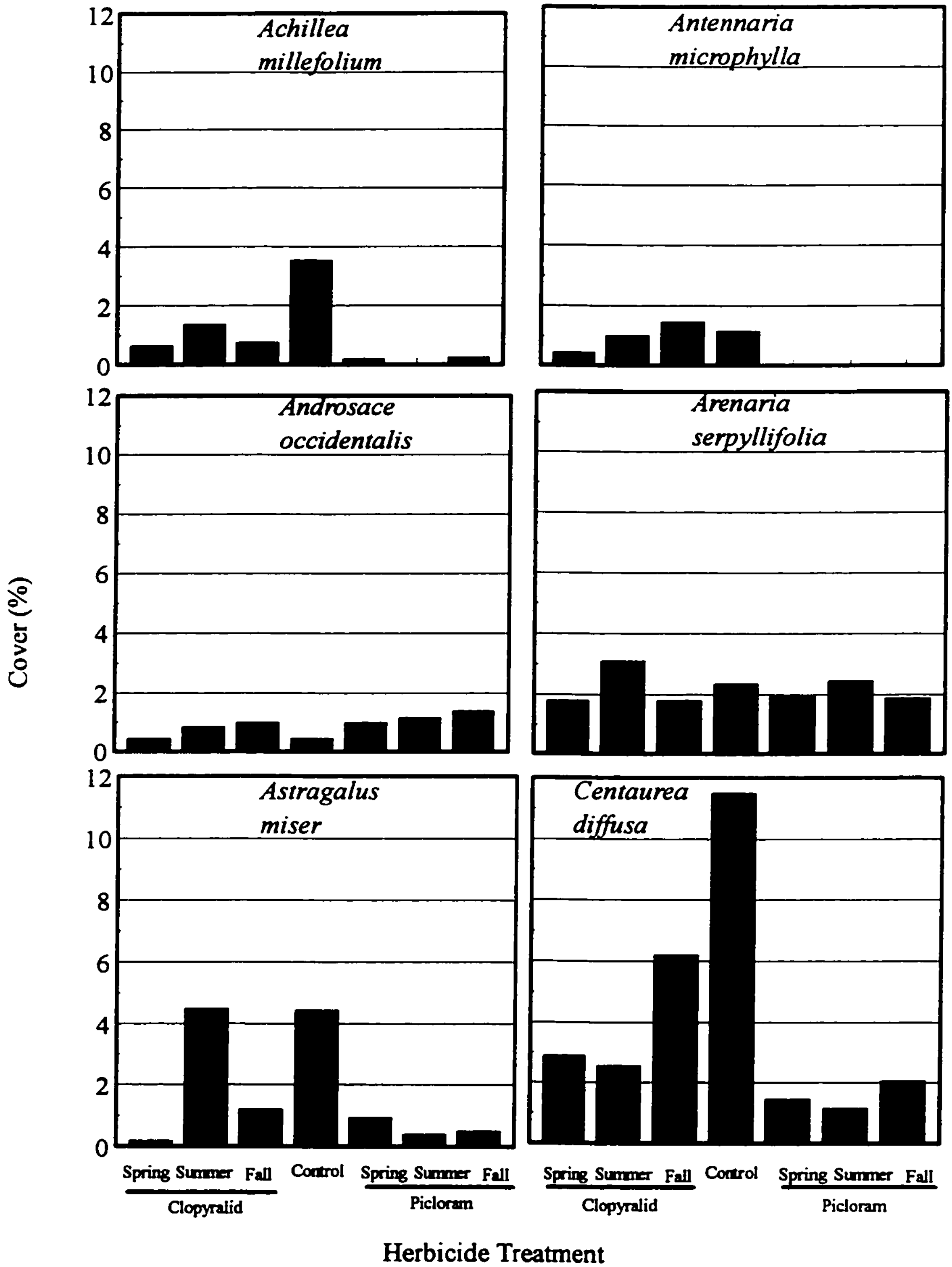

Fig. 3.5a Canopy cover (%) of vascular plant species 2 growing seasons after treatment with clopyralid at 0.15 kg ai/ha or picloram at 0.55 kg ai/ha in spring, summer or fall at the Pritchard Block-A site.

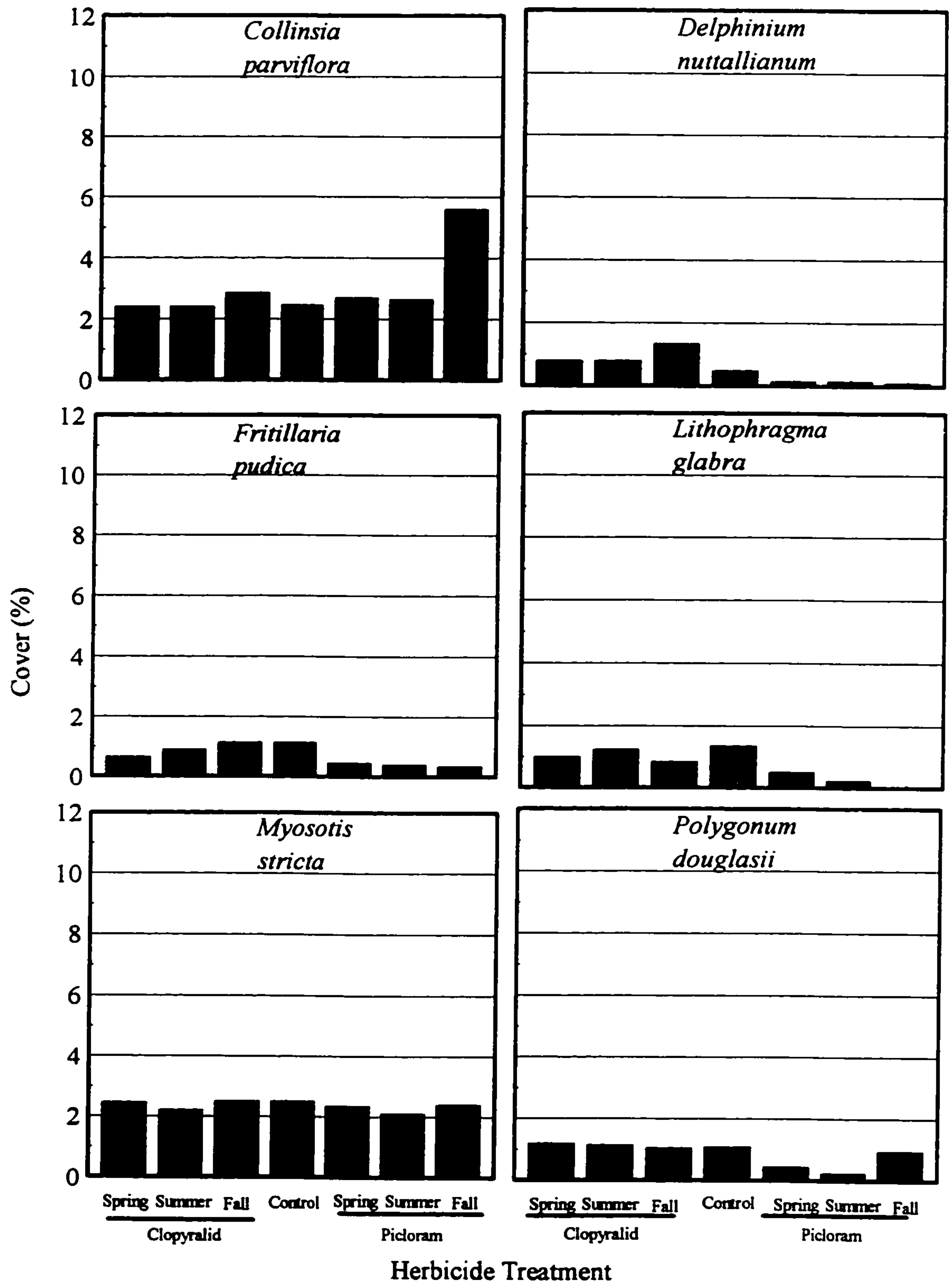

Fig. 3.5b Canopy cover (%) of vascular plant species 2 growing seasons after treatment with clopyralid at 0.15 kg ai/ha or picloram at 0.55 kg ai/ha in spring, summer or fall at the Pritchard Block-A site.

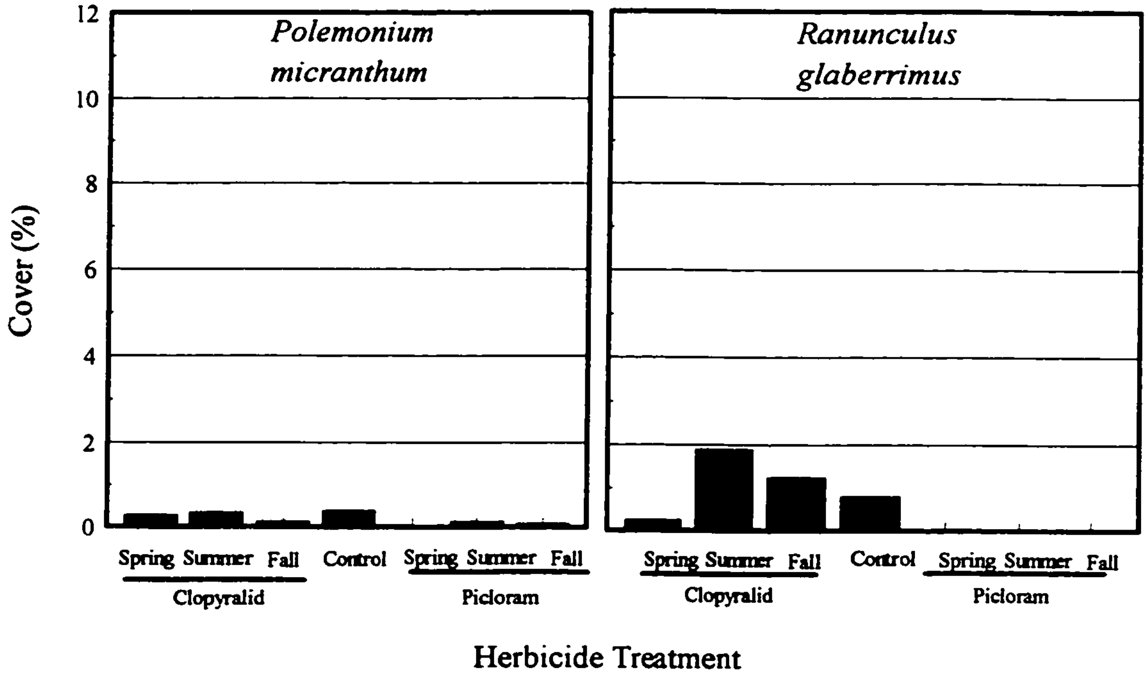

Fig. 3.5c Canopy cover (%) of vascular plant species 2 growing seasons after treatment with clopyralid at 0.15 kg ai/ha or picloram at 0.55 kg ai/ha in spring, summer or fall at the Pritchard Block-A site.

on the cover of *Arenaria serpyllifolia* than the spring herbicide treatment (Table 3.2, Fig. 3.5a). In fact, the cover values of *Arenaria serpyllifolia* on summer-treated areas were as high, or higher than on the untreated control. *Astragalus miser* responded in a similar manner when treated with clopyralid in the summer, but not when treated with picloram. When clopyralid was applied, the cover of *Astragalus miser* was virtually identical on summer-treated and untreated areas. *Myosotis stricta* responded in the opposite manner, although the differences were only slight (Table 3.2, Fig. 3.5b). The cover of *Myosotis stricta* was reduced more when treated in summer than when treated in the spring.

Two possible explanations exist for the effects due to date of application. One possibility is that plant species may show different susceptibilities to herbicides at different phenological stages during the year. This is a strong possibility since the efficacy of clopyralid and picloram is generally influenced by the phenological stage at the time of application. The differences in cover of *Arenaria serpyllifolia* may reflect the different phenological stage of this species at the two dates. On 2 May 1994, three days before the spring herbicide treatment, *Arenaria serpyllifolia* was still in a vegetative stage and was most likely killed at this stage. By 24 June 1994, four days before the summer herbicide treatment, this species was at the seed development stage and it may have produced viable seeds after treatment with herbicide. It is possible that the loss of the entire season's seed production after spring herbicide treatment may have resulted in reduced populations of *Arenaria serpyllifolia* in subsequent years. It is also possible that summer-treated *Arenaria serpyllifolia* did not absorb or translocate sufficient herbicide to kill the plant because of the advanced phenological stage. Variations on this kind of explanation can also be postulated to account for the different effects due to the date of treatment on *Astragalus miser* and *Myosotis stricta*.

A second explanation for the effects due to date of application is that environmental conditions before, during and after herbicide treatment may have differed at the two application dates. Temperature, soil moisture, and relative humidity are the most important environmental conditions affecting the uptake and translocation of phloem-mobile herbicides such as clopyralid and picloram. Absorption of picloram generally increases with increasing temperature. For example, for each 1° C increase in temperature the day before treatment, there was a 1% increase of picloram absorption into *Euphorbia esula* (Lym and Messersmith 1990). The absorption of herbicides into the leaf tissue of plants grown under water stress can be reduced because of a thickened and dehydrated leaf cuticle. For example, Kloppenburg and Hall (1990) found that the toxicities of clopyralid and picloram were reduced when applied to water-stressed *Fagopyron tataricum* plants. Relative humidity, before and after herbicide application, can affect herbicide absorption because of the effect on the level of cuticle hydration and the amount and composition of epicuticular waxes. Higher relative humidity is conducive to greater herbicide absorption.

Temperatures during application of the spring treatments varied from 19 to 24° C, while temperatures during application of summer treatments varied from 26 to 29° C. These small temperature differences were probably not the primary factor responsible for herbicide efficacy differences among application dates. Relative humidity was within a range of 55 to 63% at all application dates, and therefore not a differentiating factor either. Although precipitation for May 1994, the month following spring herbicide application, was within 10% of normal, precipitation for July, the month following summer herbicide application, was only 32% of normal (Fig. 3.2). This difference may account for some of the differential herbicide effects between the spring 1994 and summer 1994 application dates.

The varied responses among the affected plant species to the summer application date illustrate the difficulty of managing rangeland plant communities using herbicides. Each plant species possesses unique characteristics, including phenological development. The phenological stages of the 40-50 plant species at the sites are unlikely to be completely synchronous yet the plant community is often treated with a single herbicide prescription. It can be expected that mixed results will often occur among plant species in diverse plant communities managed in this way.

4.3.2 Fall vs. Spring and Summer

Fall herbicide treatment application with clopyralid and picloram resulted in less damage than the average effect of spring and summer herbicide treatment for *Androsace occidentalis*, *Collinsia parviflora*, and *Myosotis stricta* (Table 3.2, Figs. 3.5a,b). *Centaurea diffusa* responded in a similar manner when treated with clopyralid but not when treated with picloram. When *Centaurea diffusa* was treated with clopyralid in the fall, cover was reduced by less than half of the amount compared to spring and summer treatment. It is possible that different phenological stages and differences in environmental conditions among the three application dates may have contributed to these effects. However, the reduced damage exhibited by fall-treated plant species may simply be due to the 7-month difference between the fall and spring/summer treatment dates. Fall treatments were applied on 27 September 1993, compared to 5 May 1994 and 28 June 1994 for the spring and summer treatments, respectively. Herbicides applied in the fall had 7 months longer to degrade. Three of these months occur when temperature and soil moisture is adequate for microbial degradation of the herbicide (Goring and Hamaker 1971, Hamaker *et al.* 1967). Plants on the fall-treated plots also had 7 months longer to recover, compared to spring- and summer-treated plots. It is notable that the four plant species that showed greater cover after fall treatment were annuals or monocarpic perennials with high reproductive capacity. These types of plant species are expected to recover rapidly as soon as residual herbicide in the soil degrades to non-toxic levels.

4.3.3 Conclusions

Varying the date of application resulted in a few, mixed responses by six plant species. The benefits of alternative dates appear to be minimal, especially considering that late growing season (summer and fall) treatments may allow *Centaurea diffusa* to complete seed maturation after herbicide treatment.

4.4 Clopyralid as an Alternative to Picloram

Picloram was more damaging than clopyralid to ten of the eleven affected species that showed a response to herbicide type (Table 3.2, Figs. 3.5a,b,c). *Achillea millefolium*, *Antennaria microphylla*, *Astragalus miser*, *Centaurea diffusa*, *Delphinium nuttallianum*, *Fritillaria pudica*, *Lithophragma glabra*, *Polygonum douglasii*, *Polemonium micranthum*, and *Ranunculus glaberrimus* all had lower cover after treatment with picloram compared to treatment with clopyralid and to the untreated control.

Clopyralid appears to be less toxic than picloram to plant species in the Ranunculaceae family. In fact, *Ranunculus glaberrimus* and *Delphinium nuttallianum*, the only Ranunculaceae on the site, appeared to be resistant to clopyralid (Figs. 5a,b,c). The cover of these species on untreated control plots was lower than on clopyralid-treated plots in most cases. Clopyralid also appears to be less toxic to the Asteraceae. Three of 5 species examined in this family showed greater sensitivity to picloram (Table 3.2).

Clopyralid was more effective than picloram in reducing the cover of only one species, *Androsace occidentalis*. It is notable that another species of the genus *Androsace* has been reported to be resistant to picloram. Scotter (1975) reported that *Androsace chamaejasme* was one of only two forb species that showed resistance to picloram treatment at a grassland site in Alberta, Canada.

The differences between the impacts of clopyralid and picloram on non-target plant species damage are sufficient to warrant further investigation. It should be noted, however, that picloram-resistant species such as *Androsace occidentalis* may be damaged to a greater extent by clopyralid.

4.5 Response of Rare Plants to Herbicides

Six rare plant species of the dry interior of B.C. are found at the Pritchard site. These are: *Androsace occidentalis, Erigeron flagellaris, Lithospermum incisum, Monarda fistulosa, Polemonium micranthum*, and *Tetradymia* (Munro 1993). Only one of these, *Androsace occidentalis*, also occurs at the Prudens site. Three of these species, *Lithospermum, Monarda* and *Tetradymia canescens* were not abundant enough on the sites to provide meaningful analyses. For example, only four plants of *Lithospermum incisum* and five plants of *Monarda fistulosa* were sampled at the site and, therefore, no analysis was possible for these species.

At the Pritchard-Block A site, clopyralid reduced the cover of *Androsace occidentalis* by 37% more than picloram (Table 3.2). In contrast, picloram reduced the cover of *Polemonium micranthum* by 79% more than clopyralid at this site. Spring treatment with picloram eliminated *Polemonium micranthum* completely, while fall treatment reduced its cover by 86% compared to the untreated control (Table 3.2). *Erigeron flagellaris* was statistically unaffected by the herbicide date or type treatments, but examination of the individual plot means suggests that *Erigeron flagellaris* may be sensitive to picloram. This is not unexpected, since many Asteraceae are sensitive to picloram.

Very little quantitative information was derived on the response of rare plants to picloram and clopyralid. The proper examination of rare plant species will require studies designed to focus on individual species. For example, at the Pritchard site there are sufficient *Lithospermum incisum* and *Monarda fistulosa* plants to provide a well replicated study, if these plants were to be located, tagged, and individually treated with herbicide.

Although only one rare Asteraceae species occurred at the study sites, there is a need for concern regarding the use of picloram in areas where these species occur because of the high sensitivity of this family (Sect. 4.1). Ten Asteraceae are listed as rare in the dry interior of B.C.; two of these in the most rare category (R1) (Munro 1993). Avoidance of damage to rare plant species can be accomplished in two ways:

1) development of pre-treatment plant species lists. This would enable weed managers to determine if highly sensitive, rare plant species existed on the area to be treated;

2) mapping the distribution of highly sensitive, rare plant species. These maps could be provided to weed managers who would take special precaution in areas identified as high risk for rare plant species damage.

5.0 Summary

Picloram reduced the cover of 14 plant species in addition to the target weed species, *Centaurea diffusa*. Six of the affected non-target plant species, primarily in the Asteraceae family, were completely eliminated. Special care is needed where rare perennial species in the Asteraceae family exist on the area to be treated with picloram. It appears that perennial species may be the most threatened in natural systems due to their reduced reproductive capacity and slower dispersal. Reduced rates from the currently recommended application rate have good potential to ameliorate non-target plant species damage.

Altering the date of herbicide application proved quite ineffective as a method to ameliorate herbicide impacts on non-target plant species because only six species responded to this treatment and no treatment date appeared to be a clear winner. Picloram was more phytotoxic than clopyralid to 10 plant species. Although picloram will probably provide longer-term control of *Centaurea diffusa*, it also has greater impact on a number of non-target plant species.

A complete understanding of the impacts of a particular herbicide on non-target plant species requires:

1) a pre-treatment plant species list;

2) information on the herbicide sensitivity for each plant species.

In areas where conservation of diversity is a priority, the use of reduced rates or alternative herbicides, such as clopyralid, will minimize non-target plant species damage.

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CHAPTER 4. CHANGES IN GRASSLAND PLANT DIVERSITY AFTER TREATMENT WITH PICLORAM AND CLOPYRALID

1.0 Introduction

Grassland plant communities treated for weed control with the herbicide picloram (4-amino-3-5,6-trichloro-2-pyridinecarboxylic acid) often appear visibly different from untreated communities. Much of the difference is due to changes in plant species composition consisting of increases of picloram-tolerant plant species and the decline of picloram-sensitive plant species. These changes may impact plant community attributes such as species richness and species diversity.

Increasing concern regarding the conservation of biological diversity (Dasmann 1984; Biodiversity Convention Office 1995) has resulted in renewed interest in the impacts of herbicides on native plant communities. Pressures from conservationists, ecologists, and the concerned public, along with legislation regarding the conservation of biodiversity are motivating the managers of public rangelands to address issues concerning potential losses of diversity (Lewis *et al.* 1988, West 1993, Fleischner 1994).

There is an unresolved controversy over whether ecosystems with lower species diversity have reduced stability (Luckinbill 1979, Zaret 1982). A few studies support this hypothesis (e.g., MacArthur 1955, Zaret 1982, Tilman and Downing 1994). Tilman and Downing (1994) found that less diverse grassland communities did not recover as quickly, nor as completely, from major drought as more diverse grassland communities. This relationship exists on other rangelands and for other perturbations (McNaughton 1985), and may also pertain to treatment with herbicide. Thus, there is a potential for long-term forage production decreases associated with less diverse plant communities. Other authors have concluded that lower species diversity increases stability (e.g., May 1973, Zaret 1982) or has no effect on stability (e.g., Johnson and Mayeux 1992). Even though the controversy remains unresolved, some concern is justified because it is possible that the conclusions from none of these studies are incorrect. The differing results of these studies may be due to differences in the ecosystems examined.

There is also evidence that less diverse ecosystems are less productive (Baskin 1994). For example, Naeem *et al.* (1994) tested 14 artificially-constructed ecosystems and found that those with greater species richness were the most productive. Relationships between species diversity and other ecosystem functions, such as nutrient cycling and decomposition, also have been suggested. However, conclusive evidence for these relationships was not found.

Goodland (1995) defined environmental sustainability as "the maintenance of natural capital". The definition implies that management practices that result in permanent alterations or losses within ecosystems are ecologically unsustainable to some degree because the "natural capital" is not being maintained. The functioning of an ecosystem is likely to be at least risk when all species are preserved in the composition that has evolved over thousands, or even millions of years (Ritchie 1976). Grasslands in North America have persisted through changes in climate, fire, drought and have re-established after periodic glaciation (Ritchie 1976, Ryder 1982, Bailey 1997). The surviving plant species are not necessarily highly coevolved and interdependent, such as in tropical forests (Johnson and Mayeux 1992), however, they represent the suite of species best able to respond to natural perturbation.

While certain species provide overlapping function with others (functional redundancy hypothesis; Johnson and Mayeux 1992, Baskin 1994), not enough is known about individual species' characteristics to be able to sacrifice species or groups of species without some risk of ecosystem change. For example, keystone species are species whose importance cannot be judged by their abundance alone. These species provide critical roles in ecosystem function. The loss of a single keystone species may be catastrophic in certain ecosystems (Tanner *et al.* 1994). Management practices that permanently reduce the diversity or alter the composition of plants in rangeland communities have a high risk of changing ecological functioning. Management practices that minimize reductions in plant diversity or alterations of plant composition provide less risk of change to ecological functioning.

Although the effects of 2,4-D (2,4-dichlorophenoxyacetic acid) and other auxin-type herbicides on plant species diversity have been widely investigated (e.g., McIlvain and Savage 1949, Cornelius and Graham 1951, Whisenant 1986), few authors have conducted research on the impacts of picloram on plant species diversity and composition. Rice *et al.* (1992) examined the effects of picloram on plant species diversity for two seasons in Montana and reported small reductions after the first year. The authors suggest that the reductions in plant species diversity are probably transitory, based on a trend toward full recovery by the second year following treatment. No statistical analyses were performed on the data, which reduces the reliability of the conclusions. An earlier report of a study in the same region found "few significant differences in forb density and diversity", but did not present data or analyses on the effects on forb diversity (Lacey *et al.* 1989). Murray *et al.* (1991) examined the response of tall-forb communities in Montana to treatment with mixtures of picloram and 2,4-D. The authors conclude that native forbs in the community studied have great resilience to these herbicides and that no long-term effects can be expected. Tomkins and Grant (1977) report that picloram, and picloram plus 2,4-D, had dramatic effects on early seral and mature plant communities in the first year following treatment of right-of-ways in Ontario. In contrast to the other studies, the authors provide data suggesting slightly longer-term (3 year) changes, especially in the early seral community. Nevertheless, they conclude that recovery of plant species diversity was rapid.

One objective of this study was to determine the changes in plant species richness and diversity that occur in plant communities treated with the herbicide picloram. A number of strategies aimed at reducing changes in species density and species diversity were also examined and compared to the standard practice. The use of the herbicide clopyralid (3,6-dichloro-2-pyridinecarboxylic acid) was tested as an alternative to picloram to determine if it had different effects on non-target species. Reduced herbicide rates, below the currently recommended rate, and different times of application from the standard practice, were also examined as potential methods to reduce changes in species density and species diversity after treatment with picloram.

The herbicide clopyralid is closely related to picloram but possesses some important differences. Picloram is a wide-spectrum herbicide that is toxic to many herbaceous broad-leaved plant species, while clopyralid is more selective (Kelpsas and White 1986, Whisenant 1986, Hall and Vanden Born 1988, Cobb 1992, Orfanedes *et al.* 1993). For example, plant species in the Rosaceae family as well as coniferous species are resistant to clopyralid, but generally susceptible to picloram. Canola (*Brassica napus*) treated with 5000 g/ha of clopyralid showed no symptoms after 18 days, while canola treated with 75 g/ha of picloram showed severe symptoms within 6 hr (Hall *et al.* 1985). Clopyralid is less persistent than picloram and so is expected to provide shorter-term control of *Centaurea diffusa* (Hogue 1982, Jotcham *et al.* 1989). For these reasons, the use of clopyralid was examined as a potential method to reduce effects on non-target plant species.

The currently recommended rate of picloram for control of *Centaurea diffusa* on grassland in B.C. is 0.54 kg ai/ha (kg active ingredient/ha) (DowElanco Canada Inc. 1993). The recommended rate for use of picloram on *Centaurea diffusa* was based on the achievement of good control with little damage to forage grasses (Renney and Hughes 1969, Cranston 1977). Rates as low as 0.1 - 0.14 kg ai/ha, however, have resulted in adequate control of *Centaurea diffusa* for at least 16 months (Renney and Hughes 1969, Hogue 1982). Theoretically, reducing the rate of picloram may reduce losses of non-target broad-leaved species that are less susceptible to picloram than *Centaurea diffusa*.

Spring application of picloram for the control of *Centaurea diffusa* is a common practice in B.C. Previous research has provided evidence of adequate *Centaurea diffusa* control regardless of the application date of picloram (Renney and Hughes 1969, Hogue 1982, Cranston 1983a, Cranston 1983b,). The use of summer and fall herbicide application was therefore examined as a strategy to reduce effects on non-target plants. By summer, a wide variety of rangeland plant species enter a state of drought-induced dormancy (Pitt and Wikeem 1990). In the fall, the majority of plant species have completed their seasonal growth. Some species, such as *Centaurea diffusa* and many grasses, regrow in the fall in years when adequate moisture is available (Shirman 1981). Under those conditions, *Centaurea diffusa* may be relatively more susceptible to picloram than broad-leaved plants that are entering winter dormancy.

The efficacy of phloem-mobile herbicides (such as clopyralid and picloram) is generally influenced by the phenological stage at application (Orfanedes *et al.* 1993). For example, Turnbull & Stephenson (1985) reported that the maximum effectiveness of phenoxy and benzoic acid herbicides is achieved when applications are made at the early-bud to bloom stage of Canada thistle (*Cirsium arvense*). This phenological stage is said to coincide with the period of lowest root reserves and maximum basipetal translocation of plant assimilates. There is some evidence of differential susceptibility to clopyralid at varying phenological stages (O'Sullivan *et al.* 1985, Zollinger *et al.* 1992). However, Donald (1988) found that clopyralid provided equal control of Canada thistle at the vegetative, bud, and flowering stages. Penetration of clopyralid into leaf tissue is generally greater in younger than in older leaves (Donald 1988).

Some evidence of differential susceptibility to picloram at different phenological stages also exists (Gorrell *et al.* 1988). Leafy spurge (*Euphorbia esula*) was less susceptible to picloram in the vegetative growth stage than in the flowering or seed-filling stages (Hickman *et al.* 1989, Lym and Messersmith 1990a). Similarly, Lym and Moxness (1989) found that picloram translocation to leafy spurge roots was greatest during the flowering and late seed-set growth stages. This was postulated to be due to differences in plant sizes which result in the more mature plants receiving the highest rate of herbicide (Hickman *et al.* 1989). Treatment of leafy spurge during fall regrowth (late August until a killing frost) is effective, but the rate of picloram must be doubled compared to treatment during the true-flower stage (Lym and Messersmith 1985, Lym and Messersmith 1990a).

2.0 Study Area

The study was conducted on two grassland sites in the southern interior of B.C., that were heavily infested with the weed *Centaurea diffusa*. The two sites, referred to as the Pritchard and Prudens sites, occur in the Bunchgrass (Very-Dry Warm) biogeoclimatic subzone (Lloyd *et al.* 1990). The Pritchard site was divided into two blocks, Block-A and Block-B, to provide for two treatment application years. The dominant vegetation at both sites includes *Stipa comata*, *Centaurea diffusa*, and *Koeleria macrantha*. *Elymus spicatus* is expected to dominate these sites at their potential natural community (Tisdale 1947) but is currently present at less than 1% and 6% canopy cover at the Pritchard and Prudens sites, respectively. Plant taxonomic nomenclature follows Douglas *et al.* (1989).

The soils are Dark Brown Chernozems (Typic Borolls) of aeolian origin, with generally fine sandy loam textures. The aspect at the Pritchard site (50° 45' N, 119° 55' W) is 5% southeast with an elevation of 450 m. The aspect at the Prudens Site (50° 43' N, 120° 23' W) is 2% north with an elevation of 700 m. Mean annual precipitation at Kamloops airport, which is within 350 m elevation and 35 km from both sites, is 256 mm, with peaks in June-August and December-January. At Kamloops, the highest daily maximum temperatures occur in July (28.8° C) and lowest daily mean minimum temperatures occur in January (-9.8° C) (Environment Canada 1984).

3.0 Methods

Herbicides were individually mixed for each plot in 1.1-L portions and applied on 2 X 11-m plots using a portable pressurized tank sprayer with a hand-held boom. Water volume was 400 L/ha, pressure was 240 kPa, and Teejet 8004 nozzles were used. The temperatures at the time of treatments varied between 13° C and 21° C and relative humidities averaged from 55% to 63%. Total precipitation in the 1993 and 1994 treatment years was within 10% of the 45-year normal at Kamloops Airport (Fig. 3.1). Total precipitation in the 1995 treatment year exceeded the 45-year normal by 38%. Five replications were established at each location. Untreated control plots were also established for each replication.

A single 11-m transect was located along the longitudinal centerline of each plot for vegetation sampling purposes. The canopy cover of all plant species was sampled on ten 20 X 50-cm (0.1 m^2) quadrats that were permanently established at a 1-m spacing along the transects (Daubenmire 1959). The plots were sampled in 1995 and 1996 during May, while the ephemeral plant species were still recognizable.

Species density (Hurlbert 1971) was calculated for each main plot by totaling the number of unique species identified in the 10 quadrats. Two species diversity indices were calculated for each plot using canopy cover estimates from the 10 quadrats. The Shannon and Simpson's indices were chosen because they are widely used, have moderate ability to discriminate species diversity differences (Magurran 1988), and because they represent both a Type 1 and Type 2 heterogeneity measure (Peet 1974). Heterogeneity measures were defined by Peet (1974) as those indices combining measures of species richness and species evenness. The Shannon index (Type 1) is most affected by species richness while Simpson's index (Type 2) is most affected by abundance of the most common species. The Shannon index was calculated using the formula:

$$H' = -\Sigma p_i \ln p_i$$

The value of p_i was estimated as n_i/N (Pielou 1969) where n_i is the cover of a plant species and N is the total cover of all plant species considered in the calculation. Simpson's index was calculated using the formula:

$$D = \Sigma[n_i(n_i - 1)/N(N-1)]$$

using the same definitions of n_i and N. The reciprocal of the Simpson's index ($1/D$) was used for analysis for ease of presentation. Rank abundance plots (Magurran 1988) were constructed using cover, expressed as percentages, as the abundance measure. Significant differences were declared at $P \leq 0.05$ for all experiments. Analyses were conducted separately using analysis of variance (SAS Institute 1988).

3.1 Reduced Rates of Picloram

In this component of the study, the effects of reduced rates of picloram, below currently recommended rates, on plant species density and diversity were examined at the Prudens and Pritchard sites. Single applications of picloram at 0.05, 0.15 and 0.55 kg ai/ha were made on 5 May 1994 at the Pritchard Block-A location . The same treatments were applied on 9 May 1995 at Pritchard Block-B, and 12 May 1995 at the Prudens location. Precipitation in May 1994 was within 10% of the 45-year normal while precipitation in May 1995 was only 29% of normal (Fig. 3.2). Rainfall events were of low intensity, less than 5 mm/day, during the two-week period following herbicide treatment for all treatment dates. Canopy cover was sampled 1 year after treatment in May 1995 or May 1996. Species density and diversity at each site were analyzed separately using analysis of variance (SAS 1988) in a completely randomized, one-factor design.

Changes in plant species density and diversity over 2 years, after treatment with picloram at 0.05, 0.15, or 0.55 kg ai/ha, were assessed at the Pritchard Block-A site. Plots were sampled annually in May, from 1994 to 1996. Repeated measures analysis of variance was used for this analysis (Rowell and Walters 1976, Littel 1987).

3.2 Alternative Dates of Application and Clopyralid as an Alternative Herbicide to Picloram

In a separate experiment, picloram and clopyralid were applied at three dates to examine the effects on changes in species density and species diversity. Single applications of picloram at 0.55 kg ai/ha, and clopyralid at 0.15 kg ai/ha, were made in the fall (27 September 1993), spring (5 May 1994), and summer (28 June 1994) at the Pritchard Block-A location. Precipitation for the month following herbicide application was within 10% of the 45 year normal in October 1993 and May 1994 (Fig. 3.2). Precipitation was only 32% of normal during July 1994. Rainfall events were of low intensity, less than 5 mm/day, during the two-week period following herbicide treatment for all treatment dates. Canopy cover was sampled in May 1996. The cover data were analyzed using analysis of variance in a 2 X 3 (type X date) factorial arrangement of treatments in a completely randomized design. Orthogonal contrasts were used to test for differences between the spring and summer dates and for difference between fall dates and spring and summer dates combined.

4.0 Results and Discussion

4.1 Reduced Rates of Picloram

4.1.1 Species Density

Picloram applied at 0.55 kg ai/ha reduced species density by 32% compared to untreated plots, when averaged across the three sites (Table 4.1). These plots had an average of eight fewer species than untreated plots. Lower rates of picloram resulted in fewer species losses. The intermediate rate (0.15 kg ai/ha) resulted in an 11% decrease in species density, averaged across all sites. There were three fewer species on plots treated at the intermediate rate than on untreated plots. The low rate (0.05 kg ai/ha) had no effect on species density.

Species density generally declined in proportion to increasing picloram rate (Fig. 4.1); except for the lowest rate which appeared to have little or no effect. The strong rate effect reflects the differing susceptibilities of plant species to picloram. For example, control of the rangeland weed *Euphorbia esula* requires two to four times the

Table 4.1 Species density (no./plot), Shannon index (*H'*), and Simpson's index (1/*D*) 1 year after application of picloram at 0.05, 0.15 or 0.55 kg ai/ha at Pritchard Block-A (May 1994), Pritchard Block-B (May 1995), and Prudens (May 1995).

	Picloram rate (kg ai/ha)				
	0.00	0.05	0.15	0.55	Rate effect
	---species density (no./plot)----				Prob > F
Pritchard Block-A					
Species density	25.4	27.2	24.2	19.4	**
Shannon index	2.3	2.3	2.1	2.0	**
Simpson's index[1]	6.8	7.2	5.3	5.3	NS
Pritchard Block-B					
Species density	30.4	29.2	25.8	21.4	***
Shannon index	2.3	2.3	2.1	2.0	***
Simpson's index	6.1	6.4	5.5	5.4	*
Prudens					
Species density	18.4	18.8	15.8	9.4	***
Shannon index	2.1	1.9	1.7	1.6	***
Simpson's index	6.1	4.5	3.8	3.9	***

[1]Reciprocal of Simpson's index

*, **, *** significant at $P \leq 0.05$, 0.01, and 0.001, respectively

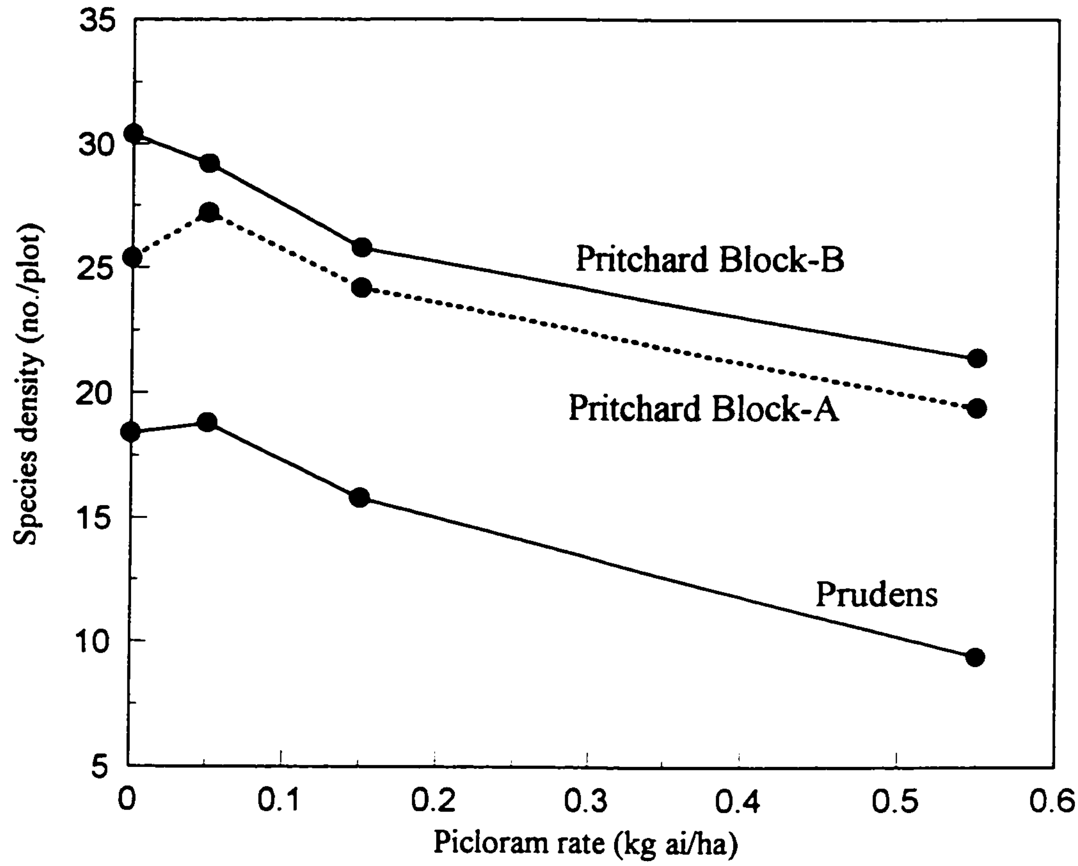

Fig. 4.1 Species density 1 year after application of picloram at 0.05, 0.15, or 0.55 kg ai/ha at the Pritchard Block-A, Pritchard Block-B, and Prudens sites.

rate of picloram required for control of *Centaurea diffusa* (Lym and Messersmith 1990b). In general, the selectivity of herbicides is reported to be due to variation in absorption, retention, translocation and the inherent potency at the target site (Kirkwood 1991).

Many plant species can be classified as to degree of susceptibility to picloram. The higher the rate of picloram, the greater the chance that species with greater tolerance will become affected. For example, at very high rates (>1 kg ai/ha), normally tolerant grass species are affected by picloram (Renney and Hughes 1969). Thus, more plant species are eliminated as the application rate of picloram is increased (Fig. 4.1).

Centaurea diffusa was highly sensitive to picloram since even the lowest experimental rate (0.05 kg ai/ha) reduced this weed by 64% at 1 year following treatment (Table 3.1). The highly sensitive nature of *Centuarea diffusa* provides an opportunity to control this weed while minimizing the effects on other less sensitive plant species.

4.1.2 Species Diversity

Picloram applied at 0.55 kg ai/ha reduced the Shannon index of species diversity by 17% compared to the untreated plots, when averaged across the three sites (Table 4.1, Fig. 4.2). Reducing the rate of picloram to 0.15 kg ai/ha resulted in only slightly less impact on species diversity. This rate reduced species diversity by 13% compared to the untreated plots. The Shannon index was unaffected by picloram applied at 0.05 kg ai/ha at the two Pritchard sites. Species diversity at the Prudens site responded differently to the low rate (Table 4.1). At this site, the low rate reduced the Shannon index by 12%, almost half of the decline that resulted from the highest rate (Fig. 4.2).

Picloram applied at 0.55 kg ai/ha reduced Simpson's index of species diversity

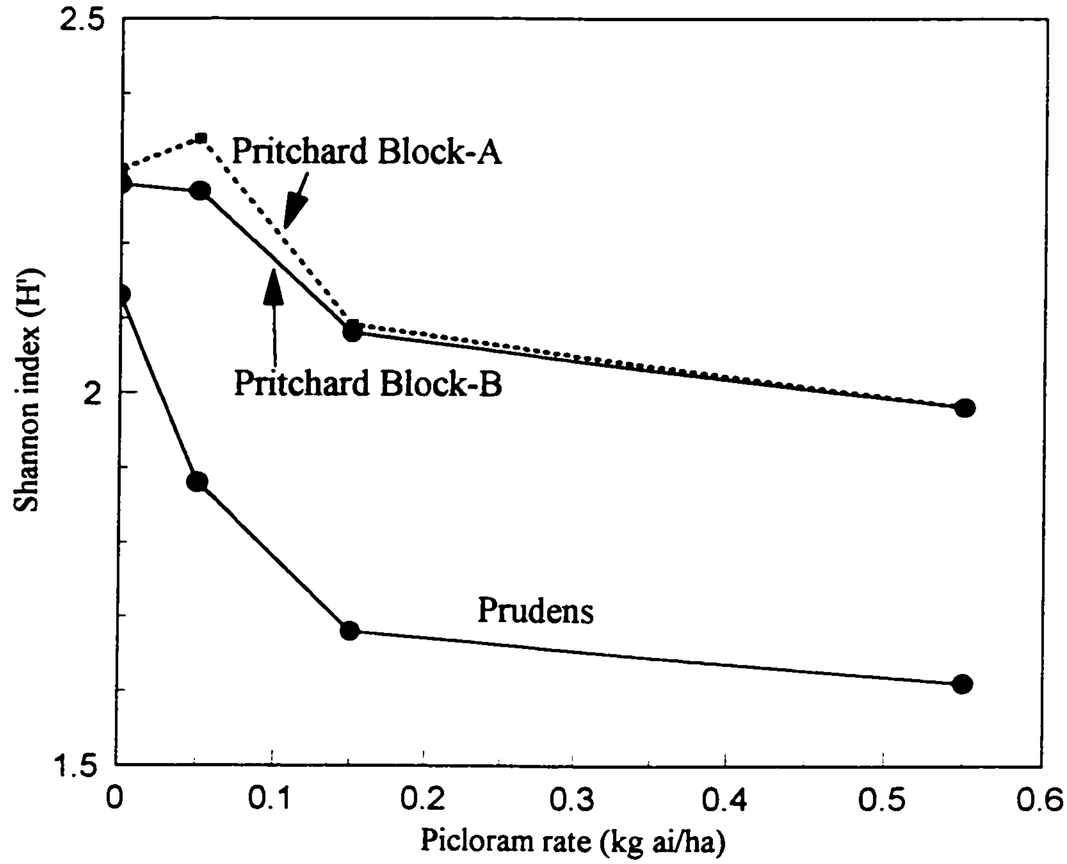

Fig. 4.2 Shannon index of species diversity 1 year after application of picloram at 0.05, 0.15, or 0.55 kg ai/ha at the Pritchard Block-A, Pritchard Block-B, and Prudens sites.

by 23% more than untreated plots, when averaged across the three sites (Table 4.1, Fig. 4.3). Picloram applied at 0.15 kg ai/ha reduced Simpson's index by almost exactly the same amount. Similar to the response of the Shannon index, the Simpson's index was unaffected by picloram applied at 0.05 kg ai/ha at the two Pritchard sites. The low rate reduced the Simpson's index by 27% at the Prudens site.

The difference in plant diversity response (Shannon and Simpson's index) to the low rates at the Prudens is likely due to a greater composition of picloram-sensitive species at this site. Species in the Asteraceae family are among the most sensitive to picloram (Fig. 3.3, Chapter 3). This family formed 56% of the species composition at Prudens, but only 19% and 27% at the Pritchard-Block A and Pritchard Block-B sites respectively. It is apparent that pre-treatment species composition is an important factor in determining the effect of picloram on plant diversity. Picloram can be expected to have increasing impact on plant diversity with increasing pre-treatment composition of picloram-sensitive plant species. In the extreme, a plant community entirely composed of picloram-sensitive plant species would be severely affected (perhaps eliminated) by treatment with picloram. Alternatively, a plant community with no picloram-sensitive species, other than the target weed, would be largely unaffected by treatment with picloram.

This observation has important implications for rangeland weed management using picloram because it provides a basis for ranking the sensitivity of entire plant communities to treatment with picloram. Plant communities with a large component of picloram-sensitive species have the greatest potential for ecosystem simplification and major shifts in species composition. The treatment of these types of plant communities with picloram should be carefully considered. Plant communities with a minor component of picloram-sensitive species will be relatively unaffected by treatment with

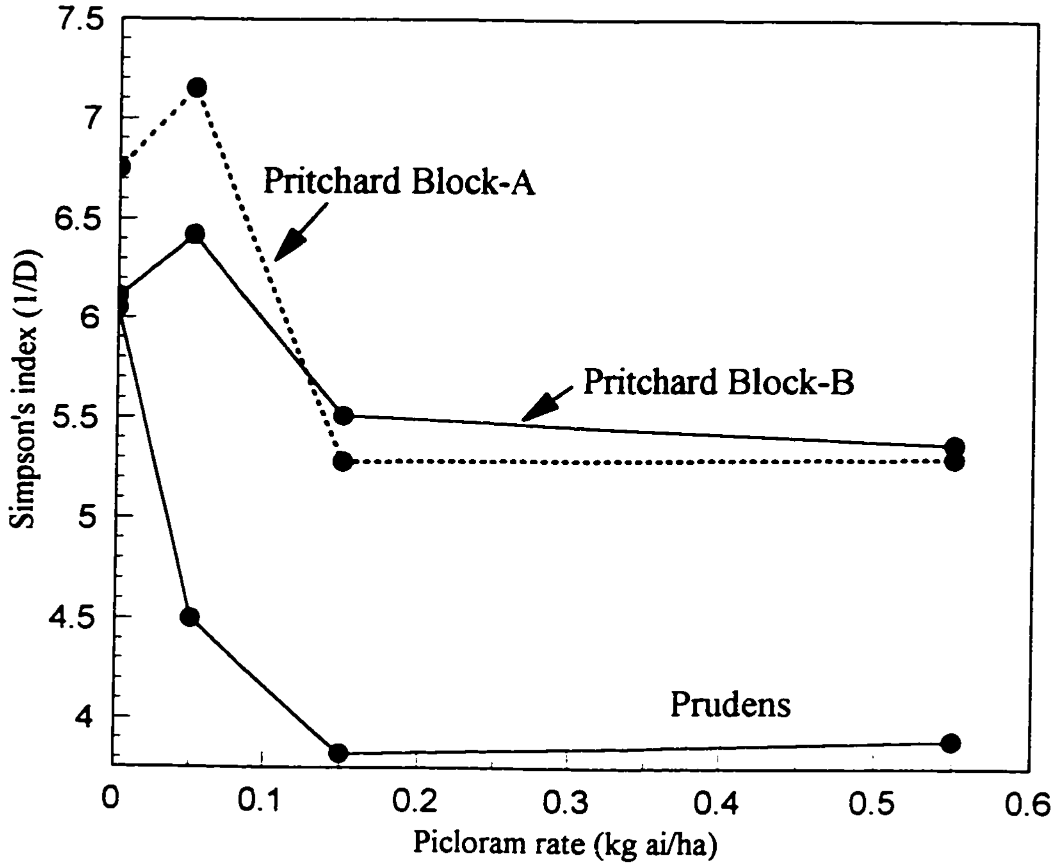

Fig. 4.3 Simpson's index of species diversity 1 year after application of picloram at 0.05, 0.15, and 0.55 kg ai/ha at the Pritchard Block-A, Pritchard Block-B, and Prudens sites.

picloram.

It is apparent that most of the reduction of species diversity occurs at the low (0.05 kg ai/ha) or intermediate (0.15 kg ai/ha) rates of picloram. This response is most evident for the Simpson's index (Fig. 4.3), but is also evident for the Shannon index (Fig. 4.2). For example, practically all of the drop in the Simpson's index occurred at the intermediate rate. Increasing the rate to 0.55 kg ai/ha did not result in an appreciable decrease in this index (Table 4.1). Similarly, 73% of the total decrease in the Shannon index occurred at the intermediate rate. This response indicates that plant diversity in the plant communities tested is quite sensitive to picloram, even at this reduced rate. There are minimal benefits to plant diversity from reducing the rate of picloram to 0.15 kg ai/ha. Greater potential exists for benefit using the low rate, especially at the two Pritchard blocks. However, the low rate may not provide sufficient *Centaurea diffusa* suppression to be useful for the weed control objectives of weed managers (Chapter 3).

4.1.3 Recovery of Species Density and Species Diversity.

Changes in species density in the 2 years following treatment with the low and intermediate rates of picloram did not differ from changes in control plots (Table 4.2, Fig. 4.4). In contrast, application at the high rate resulted in a sharp decline in species density at 12 months post-treatment; with no indication of recovery by 24 months post-treatment. Note that the apparent increase in species density from 12 to 24 months for the 0.55 kg ai/ha rate is not due to the treatment because the same increase occurs on control plots (Fig. 4.4).

Changes in the Shannon index of species diversity in the 2 years following treatment with the low rate of picloram did not differ from changes in control plots (Table 4.3, Fig. 4.5). Treatment with the intermediate and high application rates of

Table 4.2 Species density (no./plot) 0, 12 and 24 months after May 1994 treatment with picloram at 0.05, 0.15, and 0.55 kg ai/ha at the Pritchard Block-A location.

Months post-treatment	Picloram treatment control	0.05	0.15	0.55	Univariate ANOVA within year effects Rate	l	q
	---------- species density (no./plot) ----------				--Prob. > F ---		
0	23.2	25.8	23.6	25.6	NS	NS	NS
12	25.4	27.2	24.2	19.4	**	***	NS
24	28.2	29.2	27	22.2	***	***	NS
RM-ANOVA							
Year	***						
Year X Rate	***						

, * significant at $P \leq 0.01$, and 0.001, respectively

RM-ANOVA=Repeated measures analysis of variance

l=linear polynomial contrast among rates within a single year; q=quadratic polynomial contrast among rates within a single year

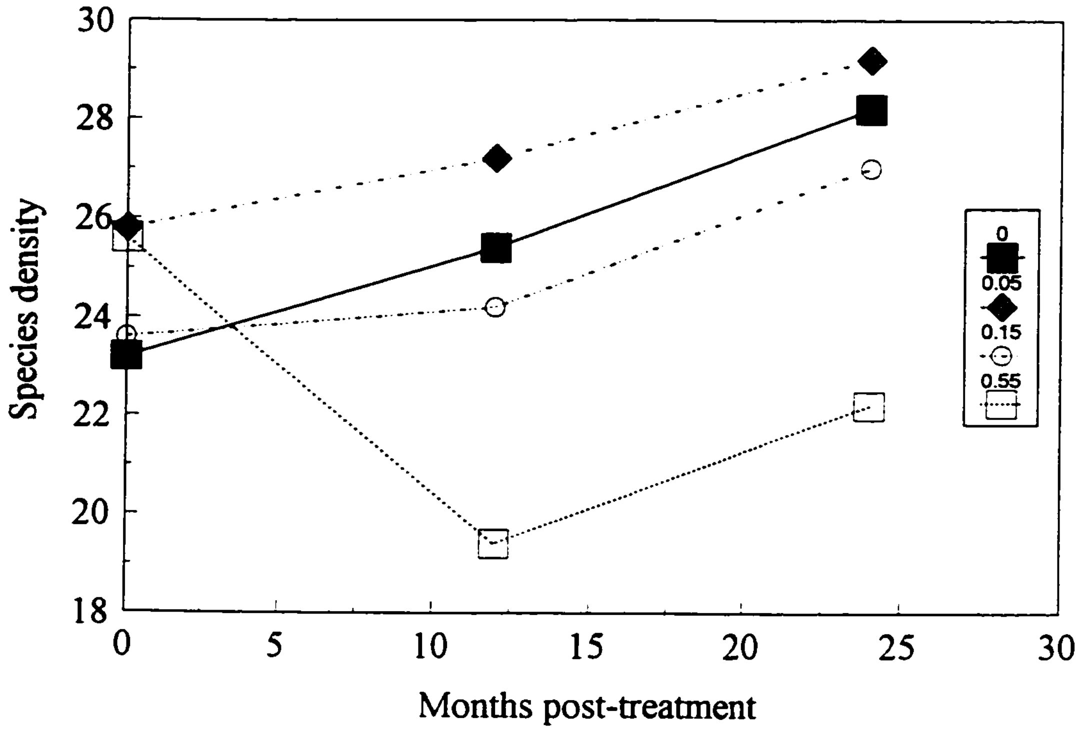

Fig. 4.4 Species density after treatment with 0.05, 0.15, or 0.55 kg ai/ha picloram on 5 May 1994 at the Pritch Block-A site.

Table 4.3 Shannon index of species diversity 0, 12 and 24 months after May 1994 treatment with picloram at 0.05, 0.15, and 0.55 kg ai/ha at the Pritchard Block-A location.

Months post-treatment	Picloram treatment				Univariate ANOVA within year effects		
	control	0.05	0.15	0.55	Rate	l	q
	Shannon index				Prob. >F		
0	2.432	2.424	2.332	2.408	NS	NS	NS
12	2.304	2.342	2.094	1.984	*	**	NS
24	2.288	2.188	2.102	1.948	***	***	NS
RM-ANOVA							
Year	***						
Year X Rate	NS						

, * significant at $P \leq 0.01$, and 0.001, respectively

RM-ANOVA=Repeated measures analysis of variance

l=linear polynomial contrast; q=quadratic polynomial contrast

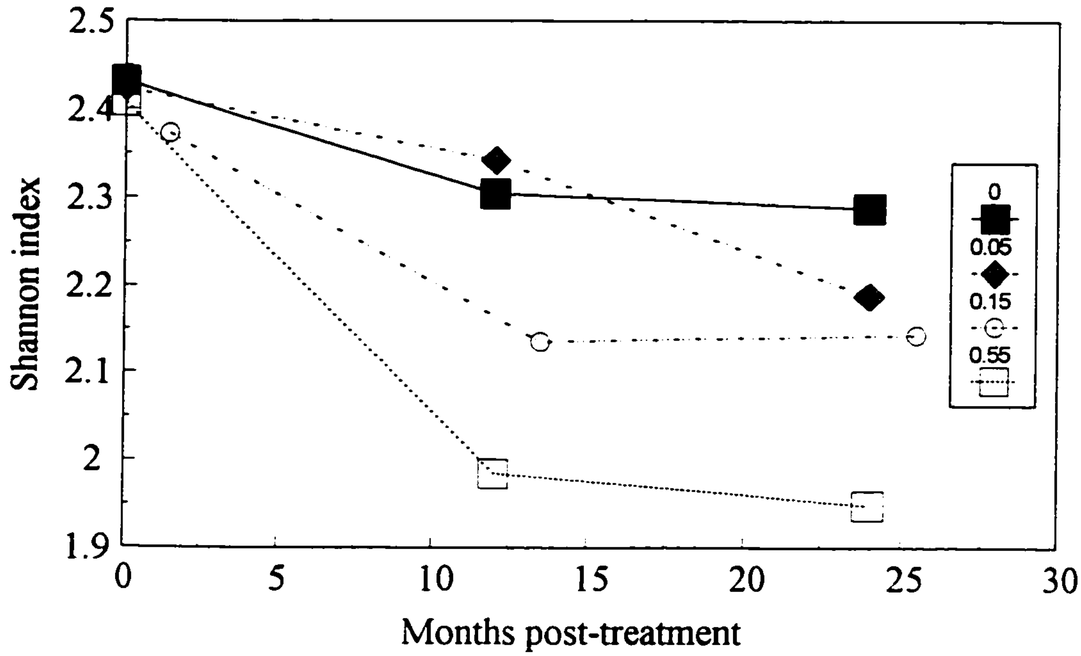

Fig. 4.5 Species diversity (Shannon index) after treatment with 0.05, 0.15, or 0.55 kg ai/ha picloram on 5 May 1994 at the Pritch Block-A site.

picloram resulted in declines in the Shannon index, with no recovery apparent by 24 months post-treatment. The Simpson's index showed little response (Table 4.4).

It is evident that changes in species density persist for at least 2 years on this site, when treated with the currently recommended application rate (0.55 kg ai/ha) of picloram. Similarly species diversity, measured as the Shannon index, was reduced for at least 2 years on this site, after treatment with the intermediate and high application rates of picloram.

The longevity of the effects of picloram on species richness and species diversity is an important question. If these community attributes recover relatively quickly, then there is no need for concern regarding herbicide practices on rangelands. Several studies on the effects of picloram on plant diversity report recovery within 3 years or less (Tompkins and Grant 1977, Lacey 1989, Murrary 1991, Rice *et al.* 1992). However, these studies encompass a wide range of vegetation types and climates, and their conclusions should be extrapolated with caution. For example, it is known that precipitation and over-winter temperature are major factors determining the degradation rate of picloram in soils (Hamaker *et al.* 1967). Picloram can remain active for 2 to 4 years in areas with very dry climates and cold winters. Recovery of picloram-sensitive species will be prevented until the amount of soil active picloram is reduced below toxic levels. Therefore, species diversity should be restored faster in climates with high precipitation and mild winters. Competitive interactions among plant species will alter this basic relationship. For example, Connell and Slatyer (1977) provide evidence that the first species to colonize a naturally disturbed site may often exclude or suppress subsequent colonist species for long periods (see also Egler 1954). Therefore, conclusions regarding the longevity of the effects of picloram on plant diversity should be framed, at a minimum, within broad climate and vegetation types.

Table 4.4 Simpson's index of species diversity 0, 12 and 24 months after May 1994 treatment with picloram at 0.05, 0.15, and 0.55 kg ai/ha at the Pritchard Block-A location.

Months post-treatment	Picloram treatment control	0.05	0.15	0.55	Univariate ANOVA within year effects Rate	l	q
	----Simpson's index----				-- Prob. > F --		
0	8.4098	8.0324	7.0854	7.633	NS	NS	NS
12	6.7476	7.1542	5.2756	5.3018	NS	NS	NS
24	6.5152	5.764	5.5172	5.2464	NS	*	NS
RM-ANOVA							
Year	***						
Year X Rate	NS						

*, *** significant at $P \leq 0.05$, and 0.001, respectively

RM-ANOVA=Repeated measures analysis of variance

l=linear polynomial contrast; q=quadratic polynomial contrast

Another concern regarding reports of rapid recovery of plant diversity is that individual plant species are not accounted for. For example, Beasom and Scifres (1977) reported rapid recovery of forb species density 27 months after treatment with 2,4-D in south Texas. However, not all of the original species returned to the treated plots. It is likely that picloram-sensitive plant species, such as those in the Asteraceae family, may be lost and replaced by picloram-tolerant species. Plant species with limited dispersal abilities or low reproductive capacities may also be replaced by plant species with opportunistic characteristics. These kinds of replacements would not be detected when using measures of community level plant diversity. Measures of plant diversity do not appear useful in describing the full impacts of herbicides on plant communities because of their site-specific nature and lack of species-specific information.

4.2 Alternative Dates of Herbicide Application

The application date of picloram or clopyralid had no effect on species density or species diversity (Table 4.5), even though the cover of nine plant species was affected by the application date (Chapter 3, Table 3.2). This may be due to compensating effects at the different dates. It is possible that the same numbers, but different plant species, may be affected by herbicides in the spring, summer and fall. When these species are examined as a group, using plant diversity indices, the species specific effects become masked.

The residual characteristic of both picloram and clopyralid may also be an important factor in the non-effect of application date. Herbicides that remain active in the soil for one or more growing seasons reduce the influence of initial date of application by distributing the herbicidal activity over the entire growing season. Plant species that are tolerant of the herbicide at a particular date of application, but sensitive

Table 4.5 Species density (*N*), Shannon index (*H'*), and Simpson's index (1/*D*) in May 1996 after application of picloram at 0.55 kg ai/ha or clopyralid at 0.15 kg ai/ha at Pritchard Block-A in fall (27 Sept 1993), spring (5 May 1994) or summer (28 June 1994).

		Clopyralid			Picloram			Tests of significance						
	Control	Spring	Summer	Fall	Spring	Summer	Fall	Date	Type	D X T	c1	c2	c3	c4
Species density	28.20	28.2	28.2	28.4	22.2	21.8	23.8	NS	*	NS	NS	NS	NS	NS
Shannon index	2.29	2.05	2.12	2.13	1.95	1.88	1.99	NS	*	NS	NS	NS	NS	NS
Simpson's index[1]	6.52	5.14	5.39	5.60	5.25	4.87	4.98	NS	NS	NS	NS	NS	NS	NS

D X T = Date X Type interaction term.

Contrast tests: c1=Spring vs. summer; c2= Fall vs. other; c3= Type X Spring vs. summer; c4=Type X Fall vs. other.

[1]Reciprocal of Simpson's index.

NS=Not significant at $P>0.05$;

*significant at $P\leq0.05$

Control values not included in the analysis but provided for reference only.

at other dates of application, remain exposed to the herbicide through root uptake for at least one growing season. Herbicidal effects due to differences in phenological stage at the time of herbicide application are therefore masked by the soil activity of the herbicide.

The possibility still exists for selectivity based on phenology if it is based on the difference in rooting depth of plant species. Theoretically, shallow-rooted species may be protected if they are treated when foliar uptake is not occurring, and the herbicide moves into the soil profile beyond the rooting depth of the plant before root uptake commences. In theory, there is little other basis for expecting plant species selectivity of sensitive species by alteration of the application date of a herbicide that persists at toxic levels for more than one growing season. Consequently, altering the date of herbicide application from spring is not a useful technique for avoiding plant diversity reductions.

4.3 Clopyralid as an Alternative Herbicide to Picloram

Treatment with clopyralid at 0.15 kg ai/ha resulted in less reduction in species density and species diversity (Shannon index) than treatment with picloram at 0.55 kg ai/ha (Table 4.5, Fig. 4.6) . In fact, clopyralid had no effect on species density while picloram reduced species density by 20% compared to control plots. Clopyralid and picloram reduced the Shannon index of species diversity by 9% and 15%, respectively. There were no differences between the effects of clopyralid or picloram on the Simpson's index (Table 4.3).

Clopyralid showed promise as a less damaging alternative to picloram, with respect to the impact on species density. This assessment can only be made, based on the currently recommended application rates of 0.15 kg ai/ha for clopyralid and 0.55 kg ai/ha for picloram. Most likely, the observed relationships will change as the application

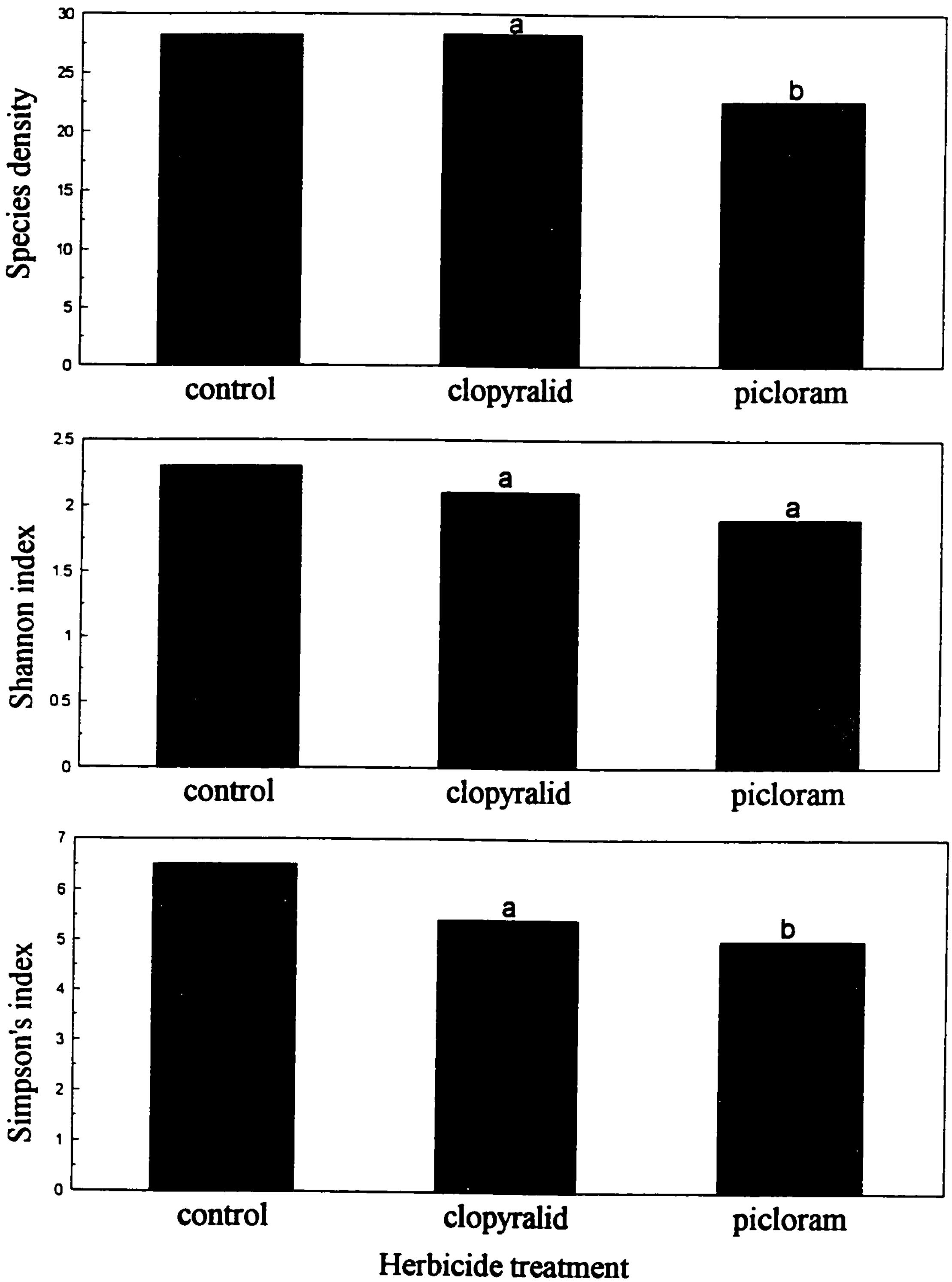

Fig. 4.6 Species density, Shannon index, and Simpson's index in May 1996, after application of clopyralid at 0.15 kg ai/ha or picloram at 0.55 kg ai/ha at the Pritchard Block-A site. Average for fall (27 Sept 1993), spring (5 May 1994), or summer (28 June 1995) treatment dates. Different letters above bars indicate significant differences at $P \leq 0.05$. Control values provided for reference only.

rates of both herbicides are varied. It can also be expected that the differences between the effects of these herbicides will change based on pre-treatment proportion of herbicide-sensitive plant species. Not all plant species are equally sensitive to clopyralid and picloram. For example, a plant community dominated by Rosaceae species, which are generally tolerant of clopyralid, would show relatively less reduction when treated with clopyralid than with picloram. Conversely, a plant community dominated by clopyralid-sensitive species would be affected in the opposite manner. Thus, any recommendation as to herbicide type would have to take into account the pre-treatment species composition of the plant community.

5.0 Summary

If management objectives on grassland sites place a high priority on plant species diversity then the use of picloram at a low (0.05 kg ai/ha) or intermediate rate (0.15 kg ai/ha) is preferable to the currently recommended rate (0.55 kg ai/ha). At currently recommended rates, clopyralid at 0.15 kg ai/ha should be considered as an alternative because of slightly less impact on species density and species diversity than picloram at 0.55 kg ai/ha. Varying the date of application of picloram is not a useful strategy to reduce the impacts on plant diversity.

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CHAPTER 5. EFFICACY OF CLOPYRALID AND OF LOW APPLICATION RATES OF PICLORAM FOR *Centaurea diffusa* CONTROL

1.0 Introduction

The weed *Centaurea diffusa* (diffuse knapweed) occurs on at least 80,000 ha of semi-arid rangeland in British Columbia (B.C.), Canada and has the potential to spread to 1 million ha of grassland and open forest (Muir 1986). Picloram (4-amino-3-5,6-trichloro-2-pyridinecarboxylic acid) is the most common herbicide used to contain *Centaurea diffusa* infestations in B.C. Although a biological control program was initiated in the 1970s, *Centaurea diffusa* is currently not controlled, despite the fact that eleven biocontrol agents have been released (Powell *et al.* 1994). Biological control has the potential to provide long-term control of *Centaurea diffusa* with minimal management input after the initial field release phase is complete (Cranston *et al.* 1983, Johnson and Wilson 1995). The operational application of biological control on a weed is normally a very slow process. An average of 20 years is needed for successful biological control of a weed (Harris 1984). Even then, there is no guarantee that an effective agent will be found, or that sufficient control of the weed will be attained (Messersmith and Adkins 1995). Of 30 agents released in Canada against 14 weed species, only one third inflicted major damage to the weed (Harris 1984, Myers 1984).

Other methods such as manual and mechanical control are not practical on rangelands because of the large areas involved and the high labour costs (Renney and Hughes 1969). For example, a study in south-central B.C. determined the cost of hand-pulling *Centaurea diffusa* to be $8600 per ha over 5 years compared to $110 per ha using picloram (Woods *et al.* 1996). Manual methods may be useful for controlling small infestations and where herbicide use is restricted.

Until biological control of *Centaurea diffusa* becomes effective, herbicides are the most effective tools for rapid control of small localized infestations and the containment of the spread to uninfested areas. Herbicides also play a valuable role in the immediate eradication of new weed species, before soil seed banks and population densities build up (Zamora *et al.* 1989). Herbicides are best suited for this purpose because of the rapidity and reliability of their action.

Operational practices involving herbicides for management of *Centaurea diffusa* in B.C. were developed in the late 1960's (Renney and Hughes 1969) and were not designed with consideration for the conservation of biological diversity. The use of picloram at the current recommended rate of 0.54 kg ai/ha (kg active ingredient/ha) (DowElanco 1993) has been demonstrated to reduce plant species density, species diversity, and abundance of picloram-sensitive native plant species for at least 2 years (Chapters 2, 3 and 4).

The objective of this study was to determine whether *Centaurea diffusa* can be controlled satisfactorily using two alternative methods that were shown to be effective in minimizing impacts on plant species density and diversity, and on the abundance of certain picloram-sensitive native plant species (Chapters 2,3 and 4). The use of an alternative herbicide to picloram, clopyralid (3,6-dichloro-2-pyridinecarboxylic acid) and the use of application rates below currently recommended rates of picloram, were examined for efficacy on *Centaurea diffusa*.

The herbicide clopyralid is closely related to picloram but possesses some important differences. Picloram is a wide-spectrum herbicide that is toxic to many herbaceous broad-leaved plant species, while clopyralid is more selective (Kelpsas and White 1986, Whisenant 1986, Hall and Vanden Born 1988, Cobb 1992, Orfanedes *et al.* 1993). Clopyralid is less persistent than picloram and so is expected to provide shorter-term control of *Centaurea diffusa* (Jotcham *et al.* 1989, Hogue 1982).

The currently recommended application rate of picloram on *Centaurea diffusa* was based on the achievement of good control with little damage to forage grasses (Renney and Hughes 1969, Cranston 1977). Rates as low as 0.1 - 0.14 kg ai/ha, however, have resulted in adequate control of *Centaurea diffusa* for at least 16 months (Renney and Hughes 1969, Hogue 1982). Reduced rates have good potential to provide adequate control of *Centaurea diffusa*.

2.0 Study Area

The study was conducted on two grassland sites in the southern interior of B.C., that were heavily infested with *Centaurea diffusa*. The two sites, referred to as the Pritchard and Prudens sites, occur in the Bunchgrass (Very-Dry Warm) biogeoclimatic subzone (Lloyd *et al.* 1990). The Pritchard site was divided into two blocks, Pritchard Block-A and Pritchard Block-B, to provide for two treatment application years. The dominant vegetation at both sites includes *Stipa comata*, *Centaurea diffusa*, and *Koeleria macrantha*. *Elymus spicatus* is expected to dominate these sites at climax (Tisdale 1947) but is currently present at less than 1% and 6% canopy cover at the Pritchard and Prudens sites, respectively. Plant taxonomic nomenclature follows Douglas *et al.* (1989).

The soils are Dark Brown Chernozems (Typic Borolls) of aeolian origin, with generally fine sandy loam textures. The aspect at the Pritchard site (50° 45' N, 119° 55' W) is 5% southeast with an elevation of 450 m. The aspect at the Prudens Site (50° 43' N, 120° 23' W) is 2% north with an elevation of 700 m. Mean annual precipitation at Kamloops airport, which is within 350 m elevation and 35 km from the sites, is 256 mm, with peaks in June-August and December-January. At Kamloops, the highest daily maximum temperatures occur in July (28.8° C) and lowest daily mean minimum temperatures occur in January (-9.8° C) (Environment Canada 1984).

3.0 Methods

Herbicides were individually mixed for each plot in 1.1-L portions and were applied on 2 X 11-m plots using a portable pressurized tank sprayer with a hand-held boom. Water volume was 400 L/ha, pressure was 240 kPa, and Teejet 8004 nozzles were used. The temperatures at the time of treatments varied between 13° C and 21° C and relative humidities averaged from 55% to 63%. Total annual precipitation in the 1994 treatment year was within 10% of the 45-year normal at Kamloops Airport (Fig. 1, Chapter 3). Total precipitation in the 1995 treatment year exceeded the 45-year normal by 38%. Rainfall events were of low intensity, less than 5 mm/day, during the 2-week period following herbicide treatment for all treatment dates. Monthly precipitation in May 1994 was within 10% of the 45-year normal while precipitation in May 1995 was only 29% of normal (Fig. 2, Chapter 3). Five replications were established at each location. Untreated control plots were also established for each replication.

A single 11-m transect was located along the longitudinal center line of each plot for vegetation sampling purposes. The density of *Centaurea diffusa* seedlings and established stages (rosette, bolted, and mature) was sampled on ten 20 X 50-cm (0.1 m^2) quadrats that were permanently established at a 1-m spacing along the transects.

3.1 Reduced Rates of Picloram

The study was comprised of two experiments. The first examined the effects of lower application rates of picloram than currently recommended rates on *Centaurea diffusa* at the Prudens and two Pritchard sites. The level of *Centaurea diffusa* control was determined by the reduction in density compared to untreated plots. Single applications of picloram at 0.05, 0.15 and 0.55 kg ai/ha were made on 5 May 1994 at the Pritchard Block-A location . The same treatments were applied on 9 May 1995 at

Pritchard Block-B, and 12 May 1995 at the Prudens location. *Centaurea diffusa* density was sampled in June 1995 at Pritchard Block-A, and in June 1996 at Pritchard Block-B and Prudens. The density data were analyzed using univariate analysis of variance in a completely randomized, one factor design.

A second component of the first experiment examined the changes in density of *Centaurea diffusa* over 2 years, after applications of picloram, at the Pritchard Block-A location. Repeated measures analysis of variance was used for this analysis (Rowell and Walters 1976, Littel 1987). *Centaurea diffusa* was sampled in June1994 (1 month after treatment), June 1995 (13 months after treatment) and June 1996 (25 months after treatment) to determine density.

3.2 Clopyralid as an Alternative Herbicide to Picloram

In a separate experiment, single applications of picloram at 0.55 kg ai/ha or clopyralid at 0.15 kg ai/ha were made on 5 May 1994 at the Pritchard Block-A location. *Centaurea diffusa* density data collected in June 1995 were analyzed using univariate analysis of variance. The experiment was laid out as a completely randomized, one factor design.

A second component of this experiment examined the recovery of *Centaurea diffusa* over 2 years after single applications of picloram or clopyralid. Repeated measures analysis of variance was used for this analysis. *Centaurea diffusa* was sampled in June1994 (1 month after treatment), June 1995 (13 months after treatment) and June 1996 (25 months after treatment) to determine density.

4.0 Results and Discussion

4.1 Reduced Rates of Picloram

Picloram applied at 0.55 kg ai/ha eliminated the established stages of *Centaurea diffusa* at all locations in both treatment years (Table 5.1, Fig. 5.1). *Centaurea diffusa* seedlings were also eliminated, except at the Pritchard Block-B location where a few seedlings remained (Table 5.1, Fig. 5.2). Picloram applied at rates of 0.05 and 0.15 kg ai/ha resulted in less control than the high rate; nevertheless, both lower rates were moderately effective at reducing *Centaurea diffusa* density. The intermediate picloram rate (0.15 kg ai/ha) reduced the established stages of *Centaurea diffusa* by 100, 94, and 100% compared to control at the Pritchard Block-A, Pritchard Block-B and Prudens locations, respectively (Table 5.1). The low rate (0.05 kg ai/ha), which is 11 times lower than the high rate, reduced the established stages of *Centaurea diffusa* by 94, 80, and 78% compared to control at the Pritchard Block-A, Pritchard Block-B and Prudens locations, respectively. *Centaurea diffusa* seedlings exposed to the two lower rates were similarly reduced, except at Pritchard Block-B where seedlings were reduced by only 39% (Table 5.1).

Examination of Figs. 5.1 and 5.2 shows that, proportionally, the majority of the reduction in *Centaurea diffusa* density occurs at the low rate of picloram. The intermediate and high rates do appear to provide additional control, but this was proportionally less than that provided by the low rate. The response of seedlings at Pritchard Block-B is the only nonconformity to this observation.

These results provide evidence that application rates of picloram lower than the recommended label rate are effective for control of *Centaurea diffusa* at these sites, under the climatic conditions that prevailed over both years of the study. The same results were attained in two different treatment years. Annual precipitation in these treatment years was relatively representative of the normal climate in this area (Fig. 3.1,

Table 5.1 Density of *Centaurea diffusa* (plants/m^2) 1 yr after treatment with picloram at 0.05, 0.15, or 0.55 kg ai/ha at Pritchard Block-A (May 1994), Pritchard Block-B (May 1995), and Prudens (May 1995).

Site	Growth stage	Picloram rate (kg ai/ha) Control	0.05	0.15	0.55	Signif.
		--------- (plants/m^2) -------------				
Pritchard Block-A	Established	19.4	1.2	0.0	0.0	*
(1994)	Seedlings	12.4	1.0	0.2	0.0	*
Pritchard Block-B	Established	20.6	4.2	1.2	0.0	*
(1995)	Seedlings	192.4	118.0	32.4	3.4	*
Prudens	Established	32.2	7.2	0.0	0.0	*
(1995)	Seedlings	36.2	4.2	0.2	0.0	*

* significant at $P \leq 0.05$

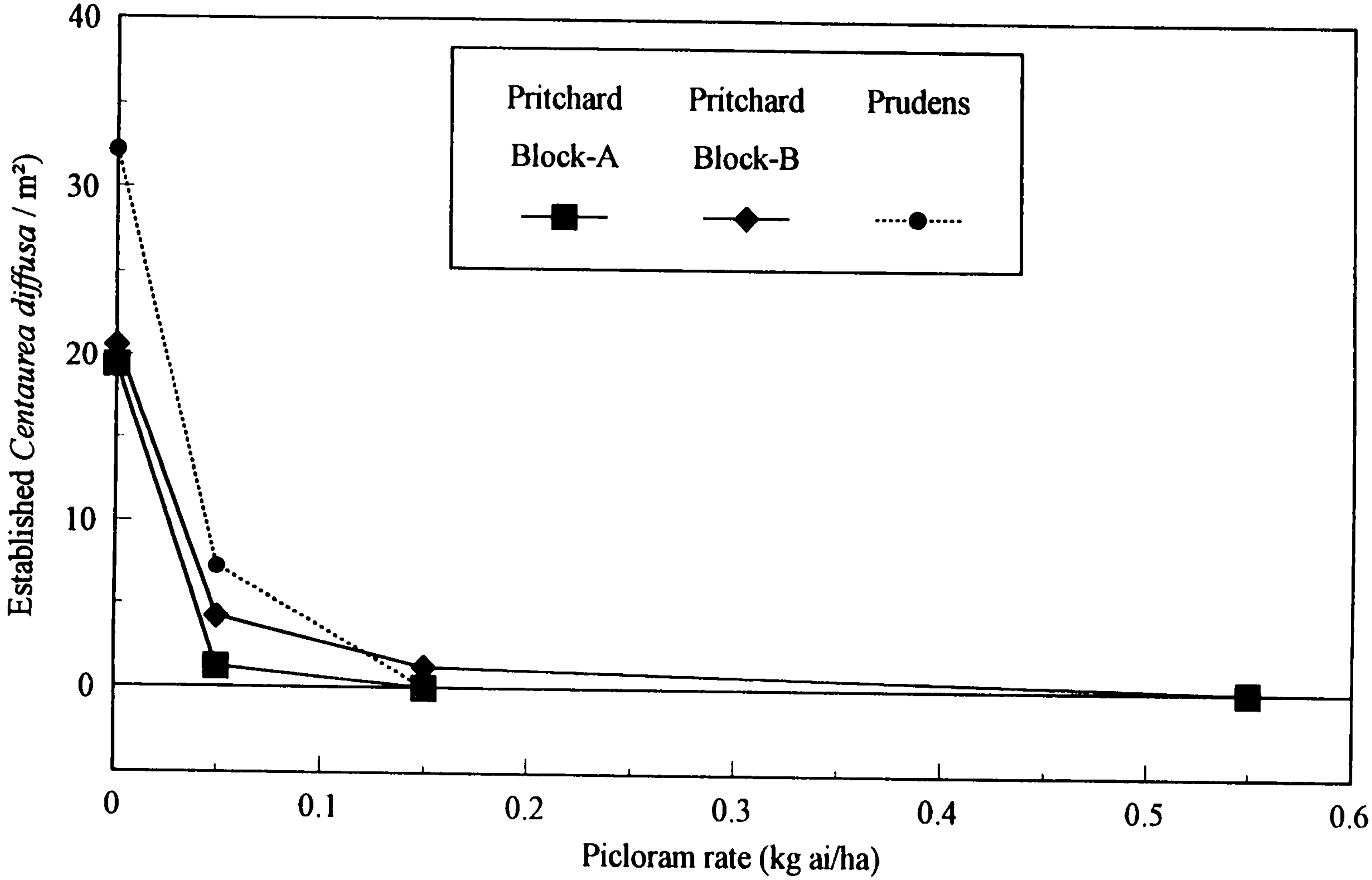

Fig. 5.1 Density of established *Centaurea diffusa* (plants/m^2) 1 yr after treatment with picloram at 0.05, 0.15, or 0.55 kg ai/ha at Pritchard Block-A (May 1994), Pritchard Block-B (May 1995), and Prudens (May 1995).

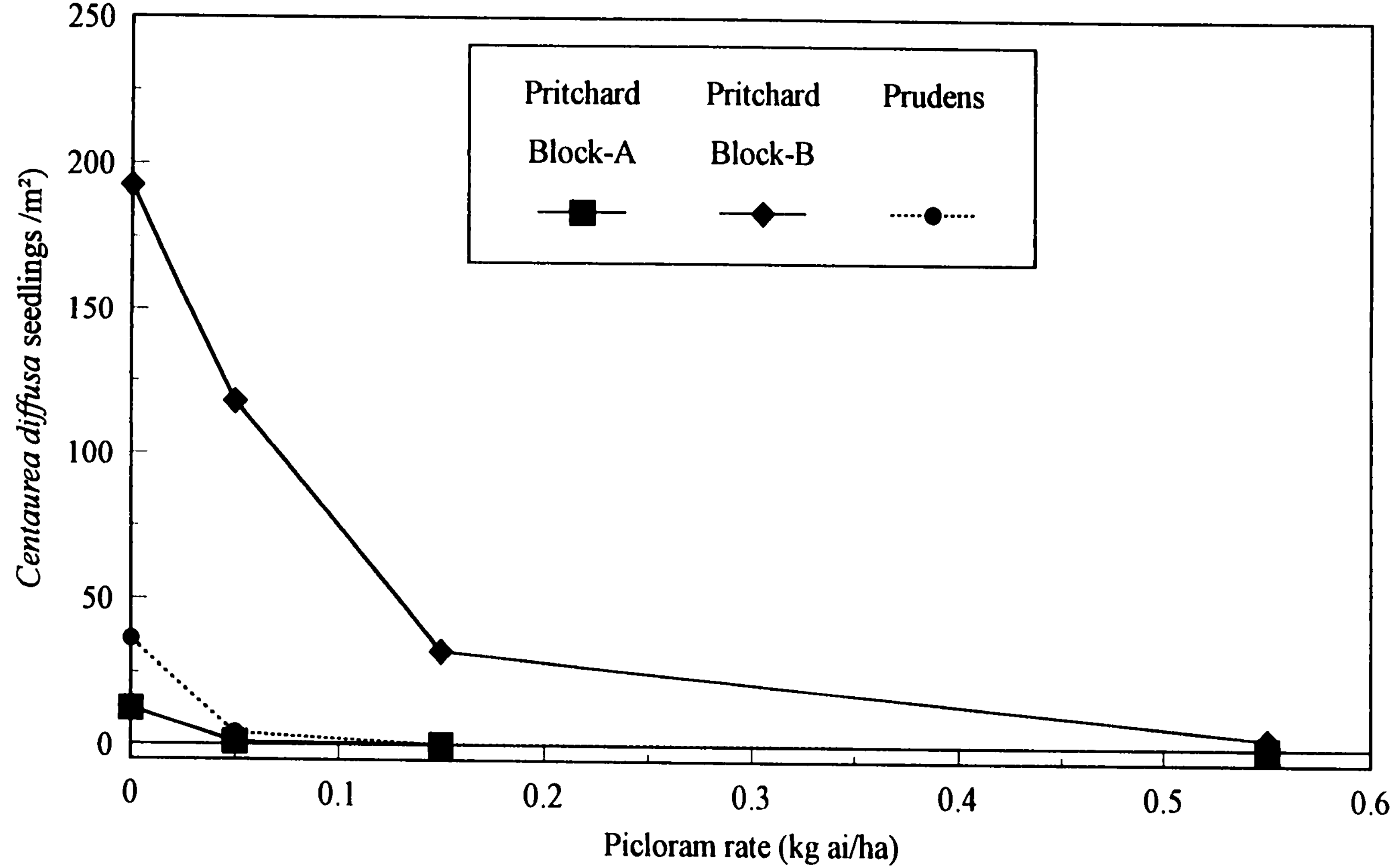

Fig. 5.2 Density of *Centaurea diffusa* seedlings (plants/m^2) 1 yr after treatment with picloram at 0.05, 0.15, or 0.55 kg ai/ha at Pritchard Block-A (May 1994), Pritchard Block-B (May 1995), and Prudens (May 1995).

Chapter 3). Precipitation during the treatment months in both years was also relatively representative (Fig. 3.2, Chapter 3). This reduces the possibility that the response was due to unusual weather conditions. The same results were attained at two geographically separate sites, at three locations, reducing the possibility that the results were due to anomalous site conditions.

Additional evidence for the efficacy of the intermediate application rate is provided by Woods *et al.* (1996) for a 5-year study near the Pritchard-A location. In this study, picloram at 0.14 kg ai/ha provided 4 years of control (>80%) of *Centaurea diffusa* after a single application in the spring of 1992. A similar treatment in the fall of 1992 provided 3 years of control (>80%). The response to the intermediate rate in the present study is somewhat better than that of Renney and Hughes (1969) who reported 60% control of *Centaurea diffusa* 16 months after it was treated with 0.14 kg ai/ha picloram. These authors recommended an application rate between 0.28 and 0.54 kg ai/ha. Several reasons were given for this recommendation:

1) Only slight damage occurred to the forage grasses at these application rates. (Rates up to 1.1 kg ai/ha were tested; these reduced subsequent forage grass production. There was no mention of the sensitivity of other non-target plant species.)

2) Good control (>88%) of *Centaurea diffusa* was achieved.

3) At least two years of control was achieved. The residual characteristic of picloram at these rates was deemed to make the practice economical.

Hogue (1982) reported a low level of control of *Centaurea diffusa* at an application rate of 0.10 kg ai/ha. The present study provides the only known evidence of control of *Centaurea diffusa* using 0.05 kg ai/ha of picloram and therefore requires verification by others.

4.1.1 Post-treatment Responses of Established *Centaurea diffusa*

The low and intermediate application rates of picloram continued to suppress established *Centaurea diffusa* below control plot densities after 25 months (Table 5.2, Fig. 5.3). Slight recovery of *Centaurea diffusa* occurred at 25 months on plots treated with the low and intermediate rates; nevertheless, the level of weed suppression was still 86 and 88%, respectively. These rates resulted in identical changes in the density of *Centaurea diffusa* (Fig. 5.3). At the high rate, *Centaurea diffusa* was eliminated after 13 months with no recovery after 25 months.

The density of established *Centaurea diffusa* in control plots varied annually (Table 5.2, Fig. 5.3) but this was expected since *Centaurea diffusa* is a biennial. Biennial species rely on annual germination to maintain populations of seedlings, rosettes and mature plants. Annual weather patterns strongly affect germination success (Maguire and Overland 1959, Watson 1972, Harper 1977, Nolan 1989), with consequent fluctuations in population densities over the years. Seedling densities in this study tended to vary with May precipitation; however, there are too few points to suggest causation (Fig. 3.1 (Chapter 3), Table 5.3). Other factors, such as variation in predation or disease, may also cause fluctuations in populations of seedlings and established growth stages. The result is a natural fluctuation in the density over years.

4.1.2 Post-treatment Responses of *Centaurea diffusa* Seedlings

Centaurea diffusa seedling numbers fluctuated to a greater degree, from year to year, than the numbers of established plants, and they appeared to recover faster (Fig. 5.4). As with the established stages, the low and intermediate application rates of picloram continued to suppress seedlings to densities below control plot levels after 25 months (Table 5.3, Fig. 5.4). However, the recovery of numbers of *Centaurea diffusa* seedlings at 25 months was greater than the recovery of numbers of established plants. Treatment with the low and intermediate rates suppressed seedlings by 55 and 58% of

Table 5.2 Density of established *Centaurea diffusa* (plants/m^2) 1, 13, and 25 months after May 1994 treatment with picloram at 0.05, 0.15, and 0.55 kg ai/ha at the Pritchard Block-A location.

Herbicide rate	Time post-treatment 1 mo	13 mo	25 mo	RM-ANOVA Year	Year X Rate
---- kg ai/ha ----	---------(plants./m^2) ---------			--- significance -----	
Control	32	19	29		
0.05	20	1	4		
0.15	21	0	4		
0.55	11	0	0	*	*
Univariate ANOVA of within year effects					
Rate	*	*	*		
linear	*	*	*		
quadratic	NS	*	*		

RM-ANOVA = Repeated measures analysis of variance.

* significant at $P \leq 0.05$; NS= not significant

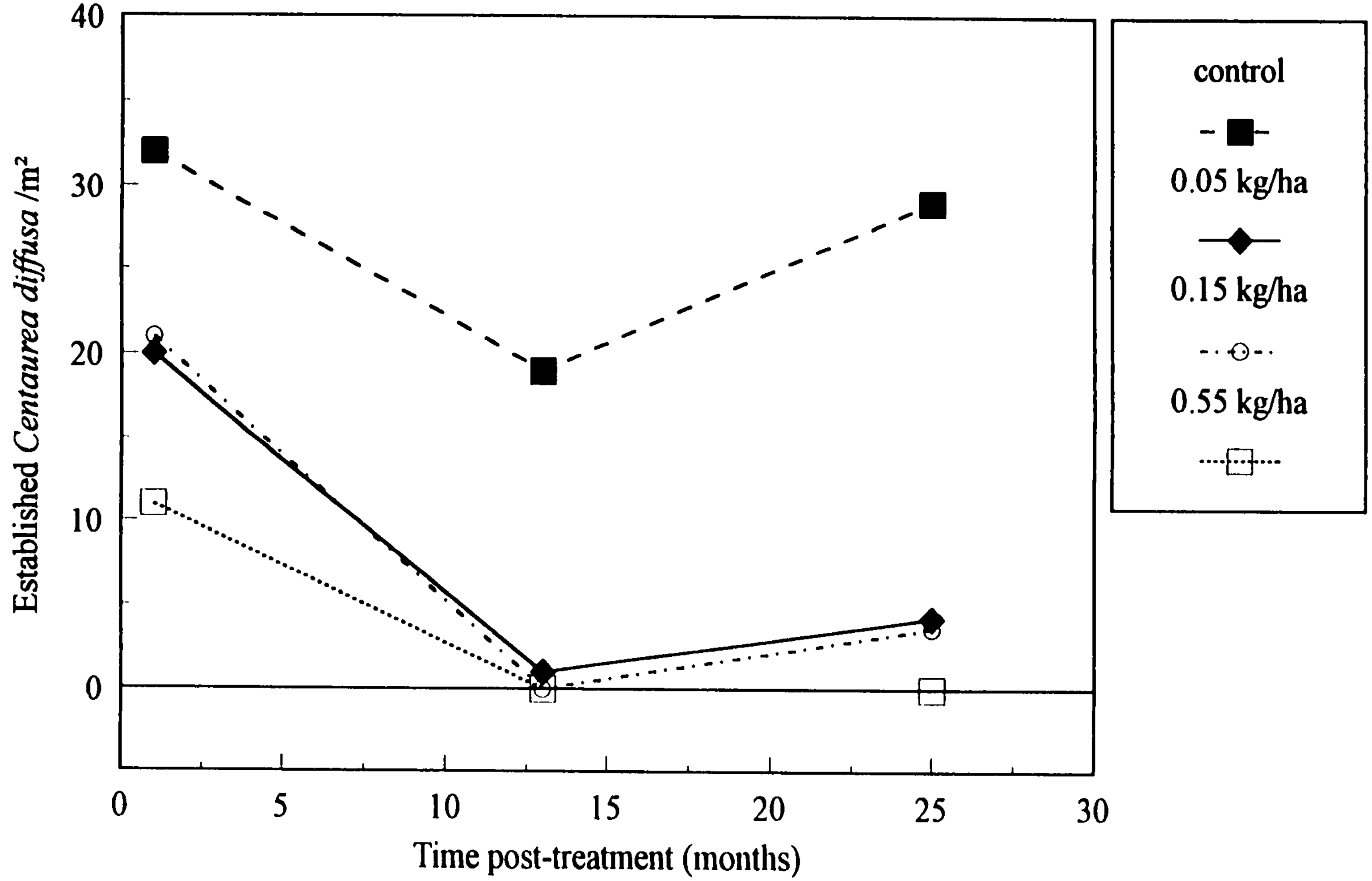

Fig. 5.3 Density of established *Centaurea diffusa* (plants/m^2) 1, 13, and 25 mo after May 1994 treatment with picloram at 0.05, 0.15, or 0.55 kg ai/ha at the Pritchard Block-A location.

Table 5.3 Density of *Centaurea diffusa* seedlings (plants/m^2) 1, 13, and 25 months after May 1994 treatment with picloram at 0.05, 0.15, and 0.55 kg ai/ha at the Pritchard Block-A location.

Herbicide rate	Time post-treatment 1 mo	13 mo	25 mo	RM-ANOVA Year	Year X Rate
---- kg ai/ha ----	--------(plants./m^2) ---------			--- significance -----	
Control	18	12	127		
0.05	9	1	57		
0.15	7	0	53		
0.55	1	0	22	*	*
Univariate ANOVA of within year effects					
Rate	*	*	*		
linear	*	*	*		
quadratic	NS	*	*		

RM-ANOVA = Repeated measures analysis of variance.

* significant at $P \leq 0.05$; NS= not significant

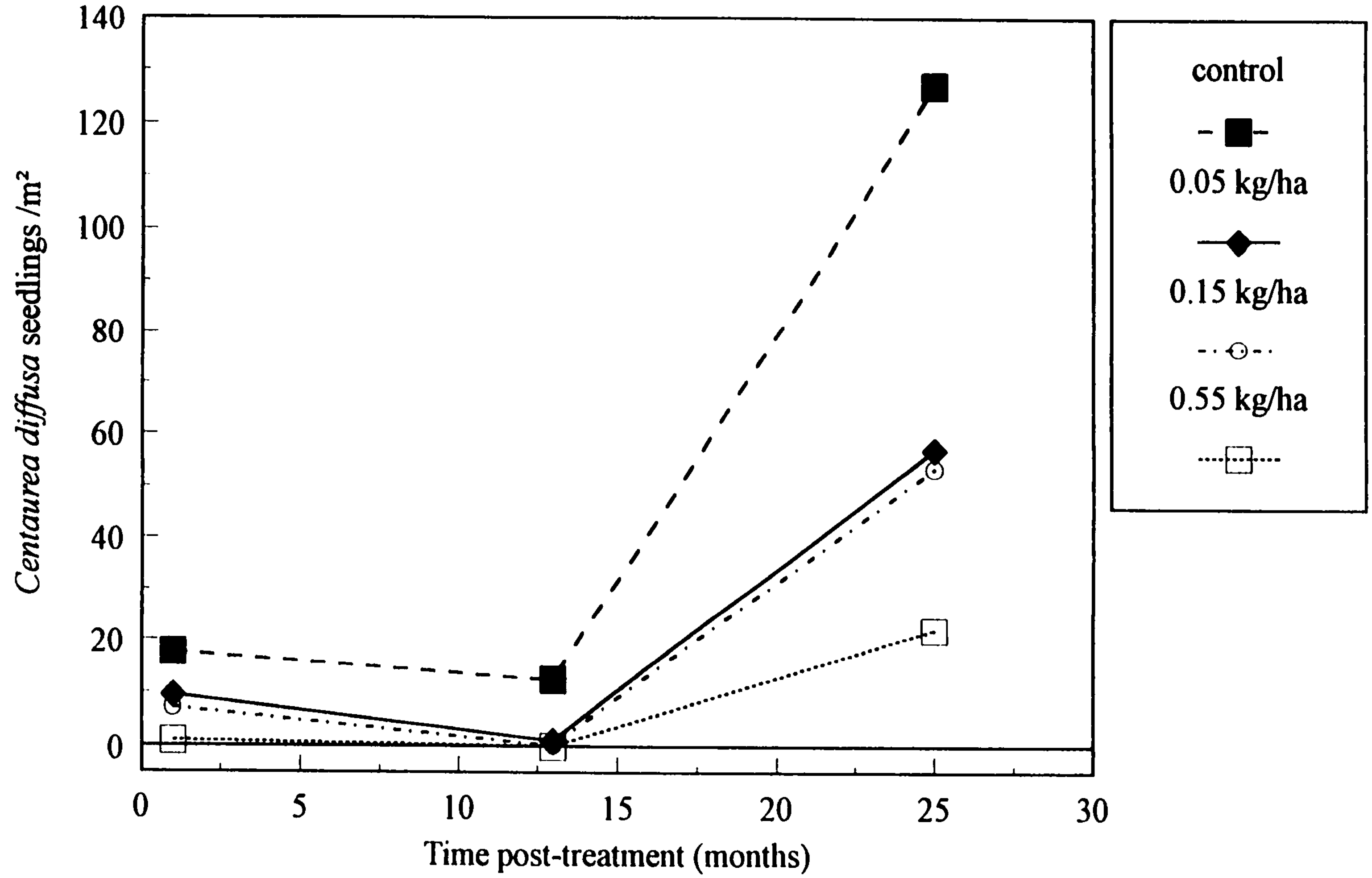

Fig. 5.4 Density of *Centaurea diffusa* seedlings (plants/m^2) 1, 13, and 25 mo after May 1994 treatment with picloram at 0.05, 0.15, or 0.55 kg ai/ha at the Pritchard Block-A location.

control densities, respectively, after 25 months. As with the established stages, the two lower rates of picloram resulted in identical changes in the density of *Centaurea diffusa* seedlings (Fig. 5.4). The high rate resulted in complete control of *Centaurea diffusa* after 13 months, but weed control was only 83% after 25 months.

The low and intermediate rates provided virtually the same control of *Centaurea diffusa* over a 25-month period at the Pritchard Block-A location. The large increase in *Centaurea diffusa* seedlings after 25 months indicates that densities of this species will likely approach control levels by the next growing season.

The demonstrated efficacy of the two lower rates of picloram provides lower-cost options for short-term *Centaurea diffusa* control. The cost of picloram is reduced by 3- and 11-fold for the 0.15 kg ai/ha and 0.05 kg ai/ha rates, respectively, compared to the currently recommended rate. Moreover, low rates of picloram have been shown to cause significantly less damage to non-target plant species (Chapters 3 and 4). These lower rates may, therefore, provide viable alternatives to the standard operational practice.

4.2 Clopyralid as an Alternative Herbicide to Picloram

Clopyralid and picloram were equally effective in controlling both established plants and seedlings of *Centaurea diffusa* at 1, 13 and 25 months after treatment (Tables 5.4 and 5.5, Figs. 5.5 and 5.6). Both herbicides were consistently more effective than no treatment, reducing established plants of *Centaurea diffusa* by 61%, 100% and 85% after 1, 13 and 25 months, respectively. Additionally, they were also consistently effective at reducing *Centaurea diffusa* seedling density (Table 5.5). Seedling density was reduced by 90%, 88% and 70%, after 1, 13 and 25 months, respectively.

Figure 5.5 shows that the density of established *Centaurea diffusa* on plots treated with picloram did not increase at the 25 month post-treatment period, whereas

Table 5.4 Density of established *Centaurea diffusa* (plants/m^2) 1, 13, and 25 months after May 1994 treatment with picloram at 0.55 kg ai/ha or clopyralid at 0.15 kg ai/ha at the Pritchard Block-A location.

	Time post-treatment			RM-ANOVA	
Herbicide type	1 mo	13 mo	25 mo	Year	Year X Type
	---------(plants/m^2) -----------			------significance-------	
Control	31.8	19.4	29.0		
Picloram	11.0	0.0	0.0		
Clopyralid	14.0	0.0	9.0	*	NS
Univariate ANOVA of within year effects					
Type	*	*	*		
Control vs. others	*	*	*		
Picloram vs. clopyralid	NS	NS	NS		

RM-ANOVA = Repeated measures analysis of variance.

* significant at $P \leq 0.05$; NS= not significant

Table 5.5 Density of *Centaurea diffusa* seedlings (plants/m^2) 1, 13, and 25 months after May 1994 treatment with picloram at 0.55 kg ai/ha or clopyralid at 0.15 kg ai/ha at the Pritchard Block-A location.

Herbicide type	Time post-treatment 1 mo	13 mo	25 mo	RM-ANOVA Year	Year X Type
	----------(plants/m^2) ----------			------significance-------	
Control	17.6	12.4	127		
Picloram	0.6	0.0	21.8		
Clopyralid	3.0	3.0	53.0	*	*
Univariate ANOVA of within year effects					
Type	*	*	*		
Control vs. others	*	*	*		
Picloram vs. clopyralid	NS	NS	NS		

RM-ANOVA = Repeated measures analysis of variance.

* significant at $P \leq 0.05$; NS= not significant

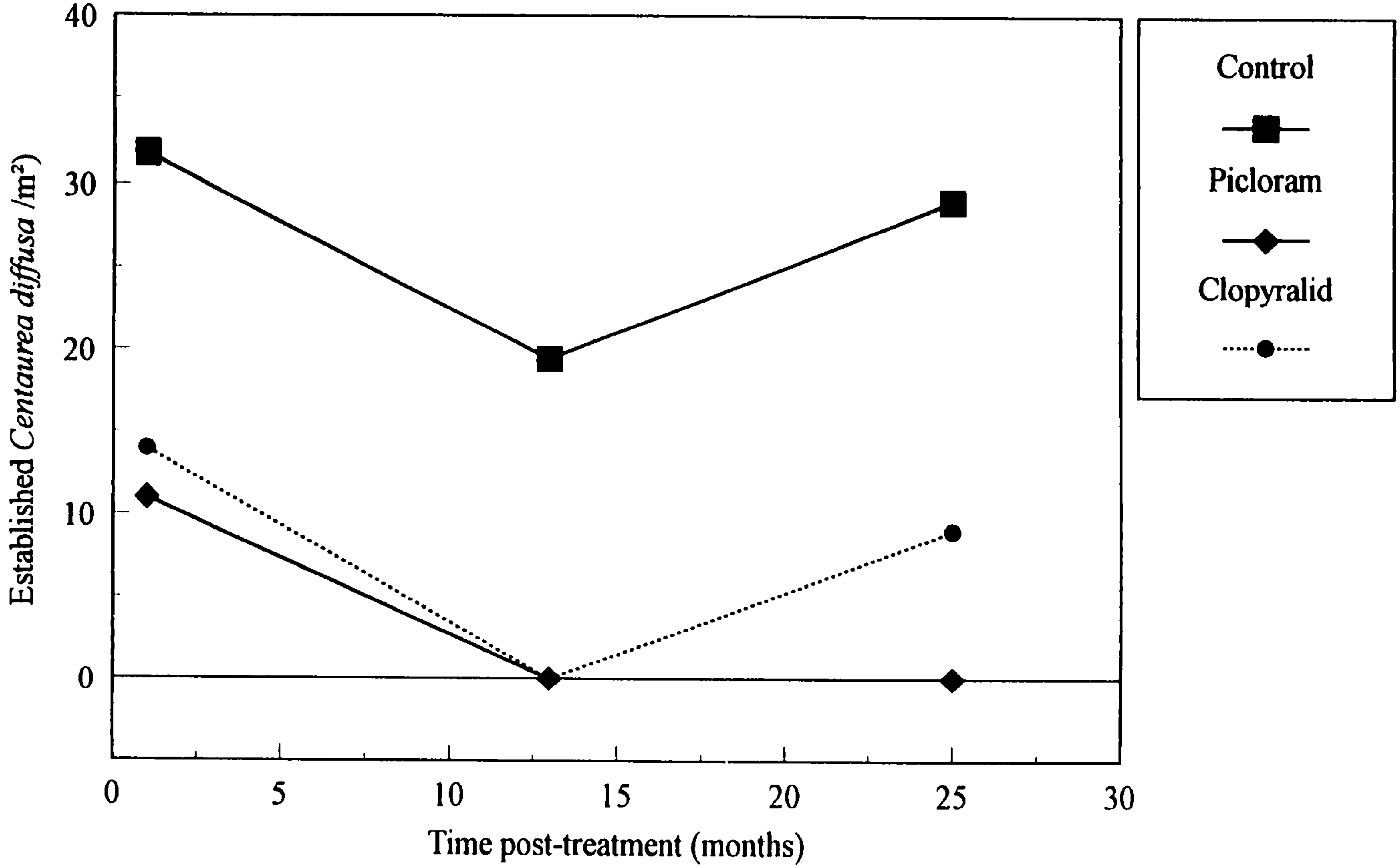

Fig. 5.5 Density of established *Centaurea diffusa* (plants/m^2) 1, 13, and 25 mo after May 1994 treatment with picloram at 0.55 kg ai/ha or clopyralid at 0.15 kg ai/ha at the Pritchard Block-A location.

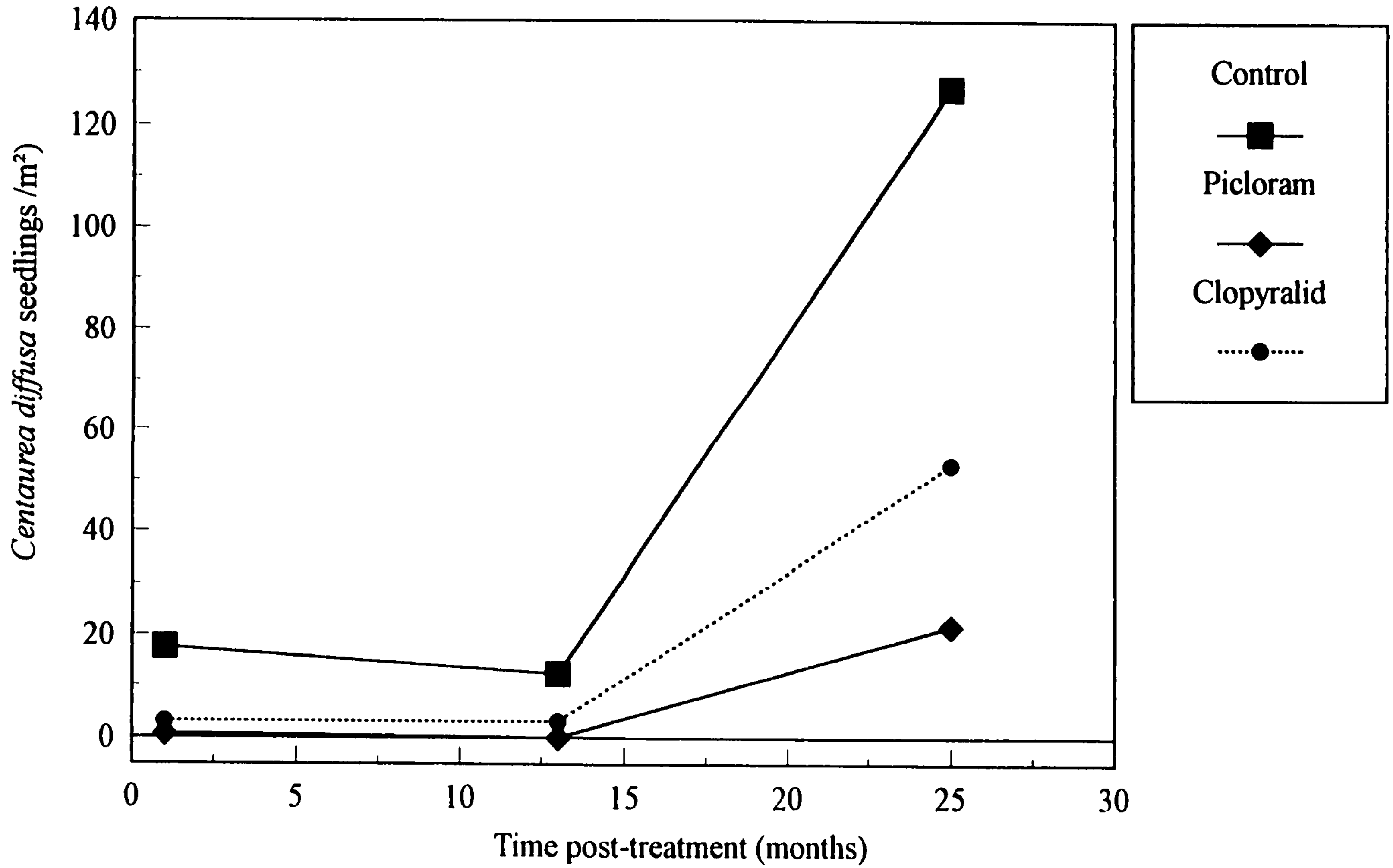

Fig. 5.6 Density of *Centaurea diffusa* seedlings (plants/m²) 1, 13, and 25 mo after May 1994 treatment with picloram at 0.55 kg ai/ha or clopyralid at 0.15 kg ai/ha at the Pritchard Block-A location.

those on clopyralid-treated plots did increase. This behaviour is consistent with the greater soil persistence of picloram. However, these apparent differences were not statistically significant ($P > 0.05$) when tested with univariate ANOVA (non-significant Picloram vs. Clopyralid contrast; Table 5.4). Therefore, *Centaurea diffusa* density at 25 months is the same for picloram and clopyralid.

The increase in control plot seedling density between the 13 month and 25 month sampling dates also occurred on treated plots, but not to the same extent (Fig. 5.6). It appears that natural fluctuations in *Centaurea diffusa* seedling density due to environmental conditions are restricted by the two herbicides.

Two possible explanations exist for this response:

1) Soil seed banks may be reduced on herbicide-treated areas, reducing the magnitude of seedling germination when conditions become favourable. Soil seed banks become depleted if seed "withdrawals" such as germination and predation exceed seed inputs. Seed input is largely controlled by the abundance and size of seed-bearing plants within 1 or 2 m of the area of soil under consideration (Shirman 1981). On herbicide-treated plots, seed input is likely to be negligible. Seed withdrawals occur by germination, predation, death due to aging, and death due to decay (Leck 1995).

2) A second possibility is that active herbicide still remains in the soil 25 months after treatment. This residual herbicide would be taken up by emergent seedlings and may result in toxic effects, depending on the level. Soil bioassays for picloram were not conducted at the Pritchard sites; however, it is known that picloram applied at 0.54 kg ai/ha remained active in the soils of semi-arid areas of southern British Columbia for approximately 2 - 4 years (Goring and Hamaker 1971, Hamaker *et al.* 1967).

4.2.1 Conclusions

These results provide evidence that clopyralid can provide the same level of control of *Centaurea diffusa* (85 to 100%) as picloram, for at least the first 2 years after treatment, under the prevailing conditions.

5.0 Summary

There is evidence that picloram application rates of 0.05 and 0.15 kg ai/ha are effective at controlling *Centaurea diffusa* densities for 2 years. There is also evidence that clopyralid at 0.15 kg ai/ha provides the same level of *Centaurea diffusa* control as picloram applied at 0.55 kg ai/ha, for at least the first 2 years after treatment.

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CHAPTER 6. LONG-TERM EFFECTS OF PICLORAM ON GRASSLAND COMMUNITIES

1.0 Introduction

The initial effects of picloram (4-amino-3-5,6-trichloro-2-pyridinecarboxylic acid) on sensitive grassland plant species are drastic and immediate, however, less is known about the recovery of these species in the years following treatment. Persistent herbicides such as picloram may have continuing toxic effects on the emergence and establishment of seedlings of sensitive plant species due to residual soil activity (Ross and Lembi 1985, Scifres 1977). In contrast, tolerant plant species are immediately able to utilize the resources released by the death of herbicide-sensitive plants.

The activity of a persistent herbicide in the soil will eventually be reduced to the point where sensitive plant species are no longer impacted (Hamaker *et al.* 1967). At that time, however, the gaps created by the original herbicide treatment may be fully occupied by herbicide-tolerant plant species. The result of this selective, delayed, entry is the formation of vegetation that may differ in species composition from that formed after treatment with non-persistent herbicides (Tomkins and Grant 1977) and from untreated vegetation. The longevity of these post-treatment vegetation states is not well known. There is evidence, however, that the first species to colonize a natural disturbance may often exclude or suppress subsequent colonist species for long periods (Connell and Slatyer 1977, Egler 1954). It has been stated that each plant species recovers at rates dependent on the relative availability of their propagules and their life history characteristics (Noble and Slatyer 1980). Plant species also vary greatly in their sensitivity to residual levels of picloram (Jotcham *et al.* 1989). The interaction of these two factors, as well as climate, will determine the eventual temporal pattern of plant species recovery at a particular site.

There is a possibility that certain plant species may never recover to pre-treatment levels due to the existence of stable post-treatment vegetation states. Theories on alternate stable states suggest that once a threshold has been passed in certain vegetation types, transition back to the original vegetation state is unlikely without certain major disturbances or human intervention (Law and Morton 1993, Tausch *et al.* 1993, Laycock 1991). Simply removing the cause of the initial change in vegetation, such as heavy grazing, does not necessarily mean a reversal to the pre-treatment vegetation state. For example, stable vegetation states have been demonstrated for grasslands converted to shrublands by excessive grazing. These vegetation states showed no recovery toward the original vegetation composition after decades of complete removal of grazing (Laycock 1991). The common factors in the formation of stable vegetation states seem to be: 1) a semi-arid or arid environment; 2) an aggressive dominant plant species; and 3) loss of seed source of recovering species. Many of these factors may pertain to vegetation states created after herbicide use in semi-arid rangelands of North America.

The purpose of this study was to examine the long-term effects of picloram on plant species composition at several grassland sites.

2.0 Description of Study Areas

Four separate trials were used to examine changes in the plant community at increasing post-herbicide treatment times. Results reported in chapters 2 and 3 of this thesis were used to provide information at 5- and 1-year post-treatment times, respectively. Herbicide trials originally established in 1992 and 1983 were remeasured to provide information at 3- and 12-year post-treatment times, respectively.

The study was conducted at two discrete grassland sites within the bunchgrass grasslands of the southern interior of British Columbia. The 1- and 3-year sites are located together near Pritchard, B.C., while the 5- and 12-year sites are located 2 km apart in the Lac du Bois grasslands near Kamloops, B.C. Both sites occur in the Bunchgrass (Very-Dry Warm) biogeoclimatic subzone (Lloyd *et al.* 1990). The dominant or codominant species at all sites is *Stipa comata*. The weed *Centaurea diffusa* is common on all untreated experimental areas at the sites. *Elymus spicatus*, *Koeleria macrantha*, and *Poa secunda* are codominant on some areas. Plant taxonomic nomenclature follows Douglas *et al.* (1989). The soils are Orthic Dark Brown Chernozems (Typic Borolls) of aeolian origin with generally fine sandy loam textures. Further site information is given in Table 6.1.

Mean annual precipitation at Kamloops airport (within 200 m elevation and 35 km distance) is 256 mm with peaks in June-August and December-January. At Kamloops, the highest daily maximum temperatures occur in July (28.8° C) and lowest daily mean minimum temperatures occur in January (-9.8° C) (Environment Canada 1984).

The 5-year site is located in an area managed using an 18 month rest-rotation grazing system. This area receives 2 weeks of grazing in early spring followed by 18 months rest, and then 2 weeks grazing in the fall. The 12-year site has been exclosed from grazing since 1959. The 1- and 3-year sites were undisturbed by grazing during the study period.

3.0 Methods

All trials included similar rates of application with picloram, however, the dates of application varied widely (Table 6.1). Herbicide application timing has been shown to be a relatively minor factor on non-target plant species impact (Chapter 3). The date of picloram application has also been shown to be unimportant for control of many grassland weeds. For example, *Centaurea diffusa* (Renney and Hughes 1969, Cranston

Table 6.1 Site descriptions and methodology for remeasured trials.

	1-year site	3-year site	5-year site	12-year site
Original researcher(s)	Newman	Sturko & Woods	Newman	Cranston & Ralph
Treatment year	1994/1995	1992	1991	1983
Year sampled	1995/1996	1995	1996	1995
Picloram rate	0.55 kg ai/ha	0.54 kg ai/ha	0.54 kg ai/ha	0.54 kg ai/ha
Treatment season	Spring	Spring & fall	Summer	November
Number of replications	5	8	3	3
Number of subplots	10	10	10	9
Experimental design	CR	CR	CRB	CRB
Treatment plot size	2 x 11 m	2 x 11.5 m	10 x 18 m	3.5 x 4.5 m
Elevation	450 m	450 m	700 m	700 m
Slope/Aspect	5% S	5% S	0-5% S-SW	2% NW
Lat./Long.	50° 45' N/119° 55' W	50° 45' N/119° 55' W	50° 43' N/120° 23' W	50° 43' N/120° 23' W

CR=completely randomized design; CRB=completely randomized block design

1983a, and b, Hogue 1983), *Centaurea maculosa* (Lacey *et al.* 1989, Duncan and Halstvedt 1994) and *Potentilla recta* (Duncan and Halstvedt 1995) showed no difference in response to different application dates. This lack of effect due to herbicide timing is probably due to the long persistent nature of picloram applied at high rates. Persistence in the soil tends to reduce the influence of initial time of application by spreading the activity of the herbicide over several growing seasons. This exposes all phenological stages of the plant to the herbicide and masks any differential sensitivity at a particular plant growth stage.

Trials were arranged as completely randomized designs or completely randomized block designs with variable numbers of replicates (Table 6.1). A single transect was centered within each plot for sampling purposes. Subplots of 0.1 m^2 (20 x 50 cm) were spaced 0.5 or 1 m along this transect at all sites. Canopy cover was estimated for each vascular plant species using the canopy coverage method (Daubenmire 1959). Sampling for all trials occurred in May 1995 or May 1996.

Soil seed bank samples were taken from picloram-treated and untreated areas at the 5-year site. The soil seed bank was sampled on 17 July 1995 by taking ten 5-cm-diameter soil cores to a 2-cm depth. Surface litter was included in the sample. Seeds of all species were extracted using direct count methods including sieving and flotation (Malone 1969). Extracted seeds were placed on moistened filter paper at 20° C, under a 16 hr light/8 hr dark regime for 3 weeks to determine germinability. Seeds were not pretreated in any manner. Seed viability of ungerminated seeds was not determined.

Soil samples from picloram-treated areas at the 5-year site were bioassayed using picloram-sensitive *Helianthus annuus* to determine if active picloram residues remained (Leasure 1964). Triplicate soil samples to a 25 cm depth were taken for each bioassay and used to fill four 10-cm-diameter pots. Four *Helianthus annuus* seeds were sown per pot, at 2 cm depth, and germinated at room temperature. After four days the pots were transferred to a growth chamber set at a constant temperature of 25 ^{o}C with a 12/12 hr light regime for 3 weeks. After 3 weeks, percentage germination, height growth, and surivival were assessed and compared to results with control soils.

Cover estimates of each plant species and two plant species groupings were analyzed separately. Species density (Hurlbert 1971) was calculated for each plot by totaling the number of unique species identified in the quadrats. Two species diversity indices were calculated for each plot, using canopy cover estimates from the quadrats. The Shannon and Simpson's indices were chosen because they are widely used and have moderate ability to discriminate species diversity differences (Magurran 1988). The Shannon index was calculated using the formula:

$$H' = -\Sigma p_i \ln p_i$$

The value of p_i was estimated as n_i/N (Pielou 1969) where n_i is the cover of a plant species and N is the total cover of all plant species considered in the calculation. Simpson's index was calculated using the formula:

$$D = \Sigma[n_i(n_i - 1)/N(N-1)]$$

using the same definitions of n_i and N. The reciprocal of the Simpson's index ($1/D$) was used for analysis for ease of presentation. Comparison of species density and species diversity measures among sites is not possible due to the variable main plot sizes and the variable number of subsamples used. Both total area sampled and the intensity of sampling have an effect on these calculations (Magurran 1988).

Statistical effects were considered significant at $P \leq 0.05$, however, due to the deficiency of long-term information on this subject, effects at $P \leq 0.10$ are also discussed. In cases of non-significant differences, post-hoc power analyses were conducted to determine if differences could have been detected. Results of post-hoc power analyses were reported as the minimum percentage difference in means which could have been detected at 80% power. Species or groups showing insufficient statistical power to detect a 100% difference in means were not discussed.

Ideally, examination for effects over time should involve simultaneous analysis of all years such as a repeated measures analysis. The variable statistical design of the four trials considered in this study (Table 6.1), however, did not allow for simultaneous analysis because of differences in numbers of subsamples and replicates. Instead, univariate ANOVA was used for separate examination of differences between control and treated plots at each site.

4.0 Results and Discussion

4.1 *Centaurea diffusa*

Centaurea diffusa was the only plant species that showed reduced cover on picloram-treated plots at each site, although this effect was statistically weak ($P \leq 0.10$) at the 5- and 12-year post-treatment sites (Table 6.2, Fig. 6.1). The low replication at the 5- and 12-year sites (Table 6.1) resulted in poor statistical power in general. It is suspected that low power in the experimental design at the 5- and 12-year sites prevented detection of the effect on *Centaurea diffusa* at $P \leq 0.05$.

The apparent longevity of *Centaurea diffusa* suppression is surprising, given that picloram, applied at 0.54 kg ai/ha, is predicted to degrade to non-phytotoxic levels within 2-4 years in the climate of this area (Hamaker *et al.* 1967). In fact, bioassays of the soil at the 5-year post-treatment site revealed no phytotoxic levels of picloram in the top 25 cm of soil after 4 years (Table 2.2, Chapter 2). No evidence of picloram injury such as epinasty, hypertrophy, or fasciation of crown and leaf petioles was observed on any plant.

The results of the bioassay suggest that the reduced abundance of *Centaurea diffusa* at the 5- and 12-year sites is not due to herbicide remaining in the soil. More likely factors are lower availability of *Centaurea diffusa* seed and competitive exclusion

Table 6.2 Canopy cover of vascular plant species (%) at various times after treatment with picloram at 0.54 kg ai/ha at four grassland sites.

Plant species	1-year PT site			3-year PT site			5-year PT site			12-year PT site		
	Control	Picloram		Control	Picloram		Control	Picloram		Control	Picloram	
	(cover %)											
Grasses												
Bromus tectorum	2.0	1.3		2.9	4.6		+	+		-	-	
Elymus spicatus	2.1	1.8		-	-		-	-		10.1	26.3	
Koeleria macrantha	8.3	14.3	**	0.0	+		2.3	1.5		13.0	8.2	
Poa compressa	-	-		+	0.7		1.2	+		-	-	
Poa pratensis	2.8	6.3		2.8	1.2		-	-		-	-	
Poa secunda	7.9	10.3		5.3	4.0		11.3	15.2		6.2	5.6	
Sporobolus cryptandrus	-	-		+	+		1.0	3.4		-	-	
Stipa comata	21.6	26.3		35.2	50.4	*	15.1	42.3	*	3.9	17.4	
Vulpia octoflora	-	-		-	-		0.0	1.1		-	-	
Grass Total	44.5	60.3	**	46.4	61.3	*	31.0	64.3	(*)	33.2	57.5	
Forb												
Achillea millefolium[A]	2.5	0.0	**	5.3	0.0	**	+	+		0.8	1.5	
Androsace occidentalis	0.8	1.1		0.8	1.5	(*)	+	+		-	-	
Antennaria dimorpha[A]	0.7	0.0	**	-	-		10.8	0.5	(*)	0.8	+	
Antennaria microphylla[A]	2.9	0.0	**	+	+		1.2	0.0		4.3	0.6	
Arabis holboellii	0.6	+	**	+	+		1.4	1.0		1.2	1.0	
Arenaria serpyllifolia	1.4	0.6	**	3.7	3.6		+	0.0		-	-	
Artemisia frigida[A]	-	-		-	-		1.2	+		0.9	1.9	
Astragalus miser	4.0	+	**	2.4	0.0	(*)	-	-		-	-	
Balsamorhiza sagittata[A]	+	0.0		-	-		-	-		-	-	
Calochortus macrocarpus	+	+		+	0.0		+	+		1.7	+	*
Castilleja thompsonii	+	+	(*)	-	-		-	-		+	+	

Table 6.2 (continued)

Plant species	1-year PT site			3-year PT site			5-year PT site			12-year PT site		
	Control	Picloram		Control	Picloram		Control	Picloram		Control	Picloram	
	(cover %)											
Forbs (continued)												
Centaurea diffusa[A]	8.1	+	**	5.6	1.9	*	11.0	1.2	(*)	17.8	1.1	(*)
Cirsium undulatum[A]	-	-		-	-		+	0.0		-	-	
Collinsia parviflora	1.5	2.2		3.9	7.2	(*)	+	tr		tr	tr	
Crepis atrabarba[A]	+	0.0		-	-		+	0.0		0.7	0.0	
Delphinium nuttallianum	+	+	**	1.3	+	**	-	-		0.0	tr	
Draba verna	1.4	1.4		2.5	3.2		+	tr		-	-	
Erigeron compositus[A]	-	-		-	-		+	0.0		-	-	
Erigeron flagellaris[A]	+	0.0		0.0	tr		-	-		-	-	
Erigeron linearis[A]	-	-		-	-		tr	0.0		tr	tr	
Erigeron pumilus[A]	+	0.0	**	1.5	1.0		tr	tr		-	-	
Fritillaria pudica	0.6	+	(*)	1.1	tr		tr	0.0		2.2	tr	
Gaillardia aristata[A]	+	+	**	-	-		-	-		-	-	
Kochia scoparia	-	-		-	-		0.0	tr		-	-	
Lithophragma glabra	+	+		+	+		-	-		-	-	
Lithospermum ruderale	0.5	1.1		1.0	0.6		-	-		-	-	
Lomatium macrocarpum	0.8	+		5.3	2.9		-	-		-	-	
Microsteris gracilis	+	0.0		+	0.0		2.1	tr	**	tr	tr	
Montia linearis	1.0	0.7		1.8	1.1		-	-		-	-	
Myosotis stricta	4.3	+	**	4.0	4.6		0.6	tr		0.6	1.3	
Oxytropis campestris	1.1	+		-	-		-	-		-	-	
Phacelia linearis	+	+		0.0	+	*	-	-		-	-	
Plantago patagonica	+	0.0		+	+		+	0.1		-	-	
Polemonium micranthum	+	+		1.5	1.2		-	-		-	-	
Polygonum douglasii	+	+	*	0.8	+	*	-	-		0.0	tr	

Table 6.2 (continued)

Plant species	1-year PT site			3-year PT site			5-year PT site			12-year PT site		
	Control	Picloram		Control	Picloram		Control	Picloram		Control	Picloram	
	(cover %)											
Forbs (continued)												
Ranunculus glaberrimus	+	0.0	**	+	+		-	-		-	-	
Stellaria nitens	1.1	0.7		1.6	0.9	(*)	-	-		-	-	
Taraxacum officinale[A]	+	0.0		-	-		-	-		-	-	
Tragopogon dubius[A]	0.7	0.0	**	1.0	0.9		+	+		0.0	+	
Verbascum thapsus[A]	-	-		+	0.0		-	-		-	-	
Shrubs												
Artemisia tridentata[A]	2.9	1.6	(*)	-	-		3.0	1.2		9.4	2.5	
Chrysothamnus nauseosus[A]	-	-		-	-		+	+		-	-	
Total Asteraceae	10.6	1.7	**	7.7	1.3	**	16.9	2.7	(*)	16.9	6.7	*
Species density	24.7	16.7	**	26.3	22.0	**	20.0	16.3		16.7	17.0	
Shannon index	2.24	1.86	**	2.37	1.99	**	2.00	1.31	**	2.09	1.92	
Simpson's index	6.30	4.85	**	7.28	5.04	*	6.34	2.51	*	6.44	5.18	

(*), *, ** significant at $P \leq 0.10$, 0.05, and 0.01, respectively.
PT[1]= post-treatment; [A]=Asteraceae; + less than 0.5%

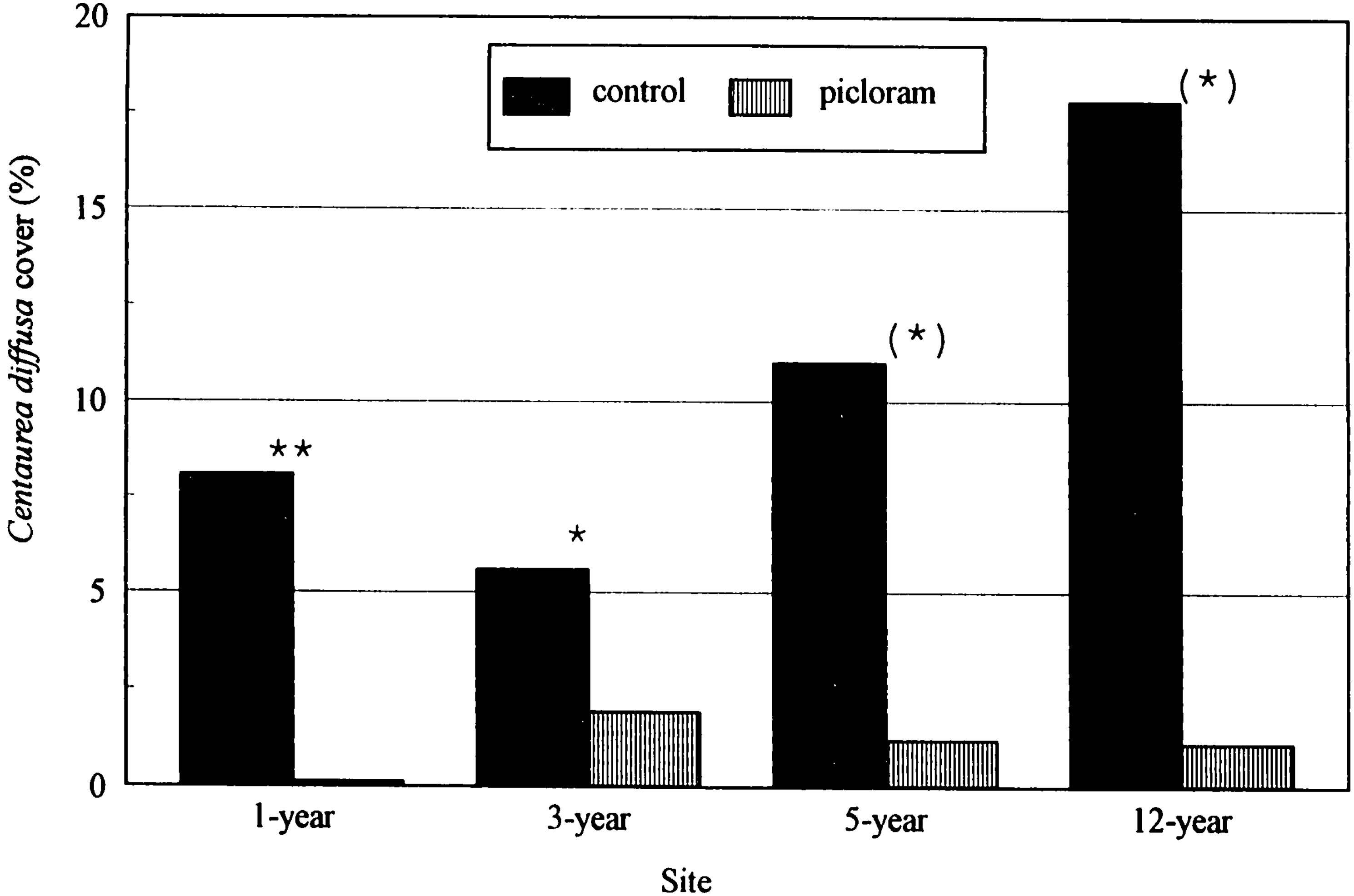

Fig. 6.1 Cover (%) of *Centaurea diffusa* after treatment with picloram at 0.54-0.55 kg ai/ha, measured at sites with different post-treatment times.
(*),*,** significant at $P \leq 0.10$, 0.05, and 0.01, respectively.

by existing vegetation. In fact, examination of the soil seed bank at the 5-year site revealed that the density of *Centaurea diffusa* seeds was seven times lower ($P \leq 0.01$) on picloram-treated areas than control plots (Table 2.4, Chapter 2).

The exclusion of grazing was apparently not an important factor in the long-term reduction of *Centaurea diffusa* because control plots at the exclosed 12-year site had the highest *Centaurea diffusa* cover of the four sites.

There is some evidence, then, that a picloram-treated plant community becomes more resistant to *Centaurea diffusa* invasion, even after soil-active herbicide has been reduced to non-toxic levels. This resistance appears to be retained for at least 12 years. The resistance of unmanaged, grass-dominated communities to invasion by broad-leaved species was also illustrated by Looman and Heinrichs (1973); albeit for a seeded domestic plant species. These authors found that abandoned farmland seeded to crested wheatgrass (*Agropyron cristatum*) was relatively resistant to invasion by other plant species for about 15 years. They also reported that, even after 35 years, native plant species accounted for only 10% of total plant density on these pastures.

On a theoretical level, a somewhat similar example of competitive exclusion was demonstrated by Pound and Egler (1953) on rights-of-way in New York. They reported 15-year prevention of tree invasion using selective herbicide treatments to encourage competitive shrub and herbaceous vegetation. Examples and theories of alternate stable states (Law and Morton 1993, Tausch *et al.* 1993, Laycock 1991) also support the possibility of long-term resistance to weed invasion in altered plant communities. These theories suggest that certain species assemblages, especially those with a strong dominant species in arid to semi-arid climates, may persist for long periods. The mechanism whereby *Centaurea diffusa* is competitively excluded on picloram-treated plots may be related to the theory that the first species to colonize a natural disturbance may often exclude or suppress subsequent colonist species for long periods (Connell and Slatyer 1977, Egler 1954). The evidence presented for long-term resistance to *Centaurea diffusa* invasion requires verification because of the weak nature of the effects at the 5- and 12-year sites. These long-term effects also need to be demonstrated on other sites.

4.2 *Calochortus macrocarpus*

Calochortus macrocarpus was the only other plant species, besides *Centaurea diffusa*, to shów reduced cover on picloram-treated plots at the 12-year post-treatment site (Table 6.2). This reduction was not evident at any other site, suggesting that the picloram treatment did not directly initiate this effect. In fact, *Calochortus macrocarpus* showed no sensitivity to picloram in other components of this study (Chapters 2 and 3). It is possible that the reduction in *Calochortus macrocarpus* cover at the 12-year site is a secondary effect; a consequence of changes in the abundance of other plant species. One explanation is that the 5 years of domination by grassy vegetation on picloram-treated plots may have reduced the colonization success of *Calochortus macrocarpus*.

Post-hoc power analyses of all other plant species at the 12-year site revealed insufficient power to detect reasonable changes. Therefore, no conclusions can be drawn regarding the effect of picloram on other plant species at the 12-year site. Similarly, none of the non-significant differences at the 5-year site provides useful information because of a lack of statistical power (Table 6.2).

4.3 Asteraceae

All Asteraceae, except for *Centaurea diffusa*, were analyzed as a group in order to provide comparable information to previous research on this topic. A total of 17 Asteraceae were recorded across all sites, however, the maximum number at any one site was 12. Only *Achillea millefolium*, *Antennaria microphylla* and *Tragopogon dubius* were present on all sites. The Asteraceae family is known to be highly sensitive to picloram (Chapter 3) and is the most prevalent herbaceous family in these grasslands (Table 6.2).

Asteraceae were less abundant on plots treated with picloram at all sites (Table 6.2, Fig. 6.2). These effects were statistically strong ($P \leq 0.05$) at all sites except the 5-year site. Individual plant species analyses revealed that *Achillea millefolium* and *Antennaria dimorpha* were the most common Asteraceae affected by picloram across the four sites (Table 6.2). *Antennaria microphylla*, *Artemisia tridentata*, *Erigeron pumilus* and *Tragopogon dubius* were affected at single sites only (Table 6.2). The majority of significant individual effects on Asteraceae occurred at the 1-year site. This suggests that individual Asteraceae species recover quickly. The significant effect on Asteraceae as a group at the 12-year site, however, suggests that recovery to the pre-treatment composition may not be complete after 12 years, despite apparent rapid recovery of individual species.

These results generally disagree with those of Lacey *et al.* (1989) who reported no effect of picloram at 0.28 kg ai/ha on a group of Asteraceae at 1 year post-treatment. There is a possibility that Lacey's lower rate of picloram may have led to the differences in results, however, a picloram rate of only 0.15 kg ai/ha was shown to reduce the cover of seven of eight composite plant species at a 1-year post-treatment site (Table 3.2, Chapter 3). It is more likely that low statistical power in the Lacey *et al.* (1989) study resulted in an inability to properly test for the effects on Asteraceae. This points out the importance of conducting statistical power analyses whenever statements are made regarding non-significant differences. Lacey *et al.* (1989) did, however, report similar sensitivity of *Achillea millefolium* to picloram. *Achillea millefolium* appears to be extremely sensitive to picloram and was non-existant on the 1- and 3-year sites (Table 6.2).

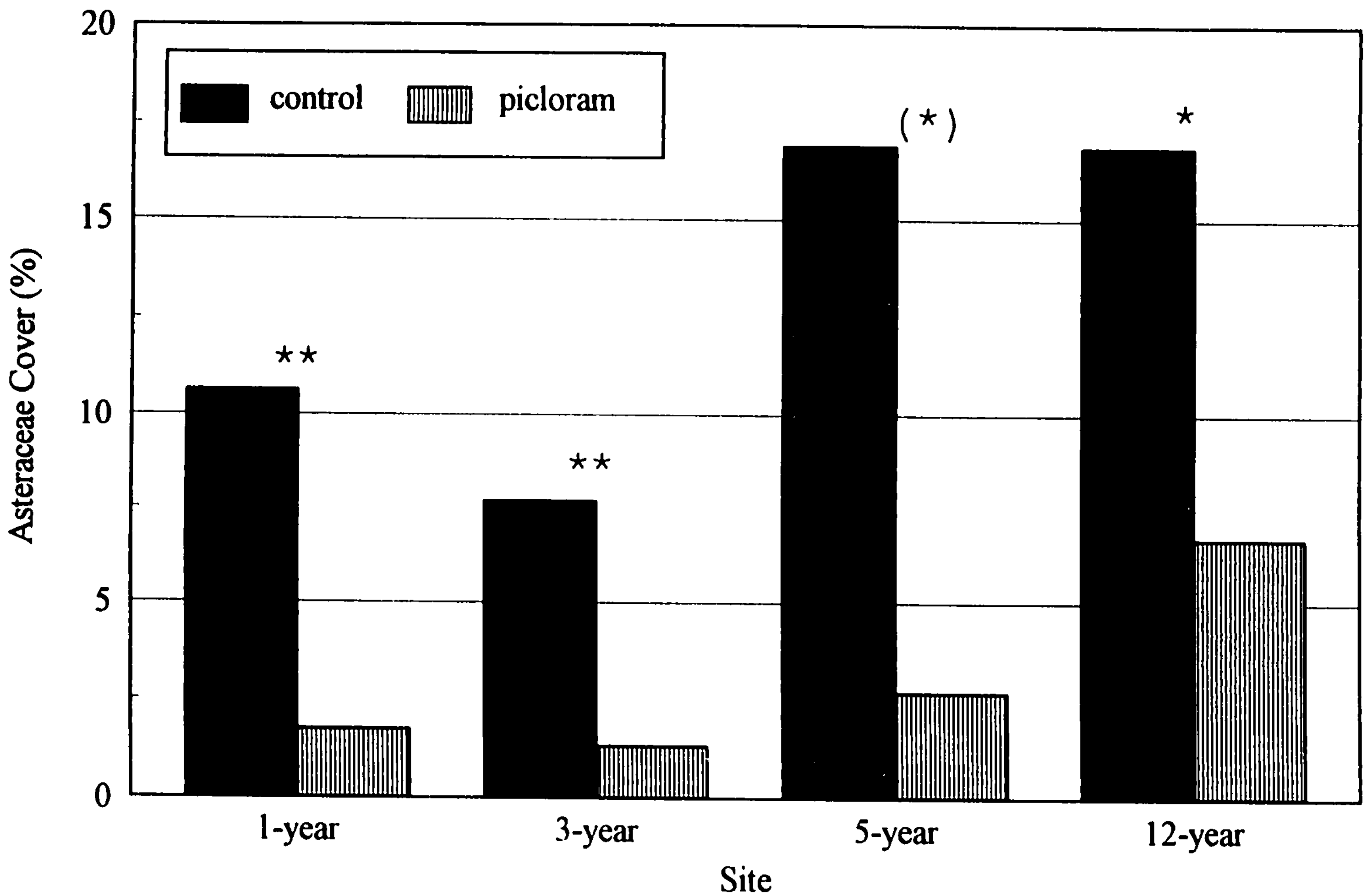

Fig. 6.2 Cover (%) of Asteraceae after treatment with picloram at 0.54-0.55 kg ai/ha measured at sites with different post-treatment times.
(*),*,** significant at $P \leq 0.10$, 0.05, and 0.01, respectively.

The fact that *Centaurea diffusa*, as well as all other Asteraceae as a group, both showed long-term reductions after picloram treatment, provides a strong indication of the harmful impact of this herbicide on members of the Asteraceae family. The long-term reduction of Asteraceae after picloram treatment may pose problems in areas where conservation values are paramount. This will be a particularly important consideration if rare Asteraceae occur in the area.

4.4 *Stipa comata* and Other Grasses

Stipa comata did not respond to the picloram treatment at the 1-year post-treatment site despite sufficient power to detect a 40% difference between the means (Table 6.2, Fig. 6.3). Grasses considered as a group, however, did respond to picloram at the 1-year post-treatment site (Table 6.2, Fig. 6.4). This suggests that grasses such as *Koeleria macrantha* and *Poa secunda* may be quicker than *Stipa comata* to respond to the resources released by the mortality of picloram-sensitive plants. Individual analyses revealed that *Koeleria macrantha* was the only grass species significantly more abundant on picloram-treated plots at the 1-year site.

Both *Stipa comata* considered separately, and the grasses as a group, had greater cover when treated with picloram, compared to control plots, at the 3- and 5-year post-treatment sites (Figs. 6.3 and 6.4), although this effect was statistically weak ($P \leq 0.10$) for grasses at the 5-year post-treatment period. It appears that *Stipa comata* initially is slow to respond but eventually responds strongly for at least 5 years. No significant difference was detected at the 12-year post-treatment site despite an apparently large difference in the means of the grass group. Post-hoc power analysis revealed that insufficient statistical power existed to properly test for this effect.

There is evidence that *Stipa comata* maintains an increased cover on picloram-treated plots for at least 5 years on the grassland sites examined in this study. The increase of *Stipa comata* is the most likely cause of the reduced invasion of *Centaurea*

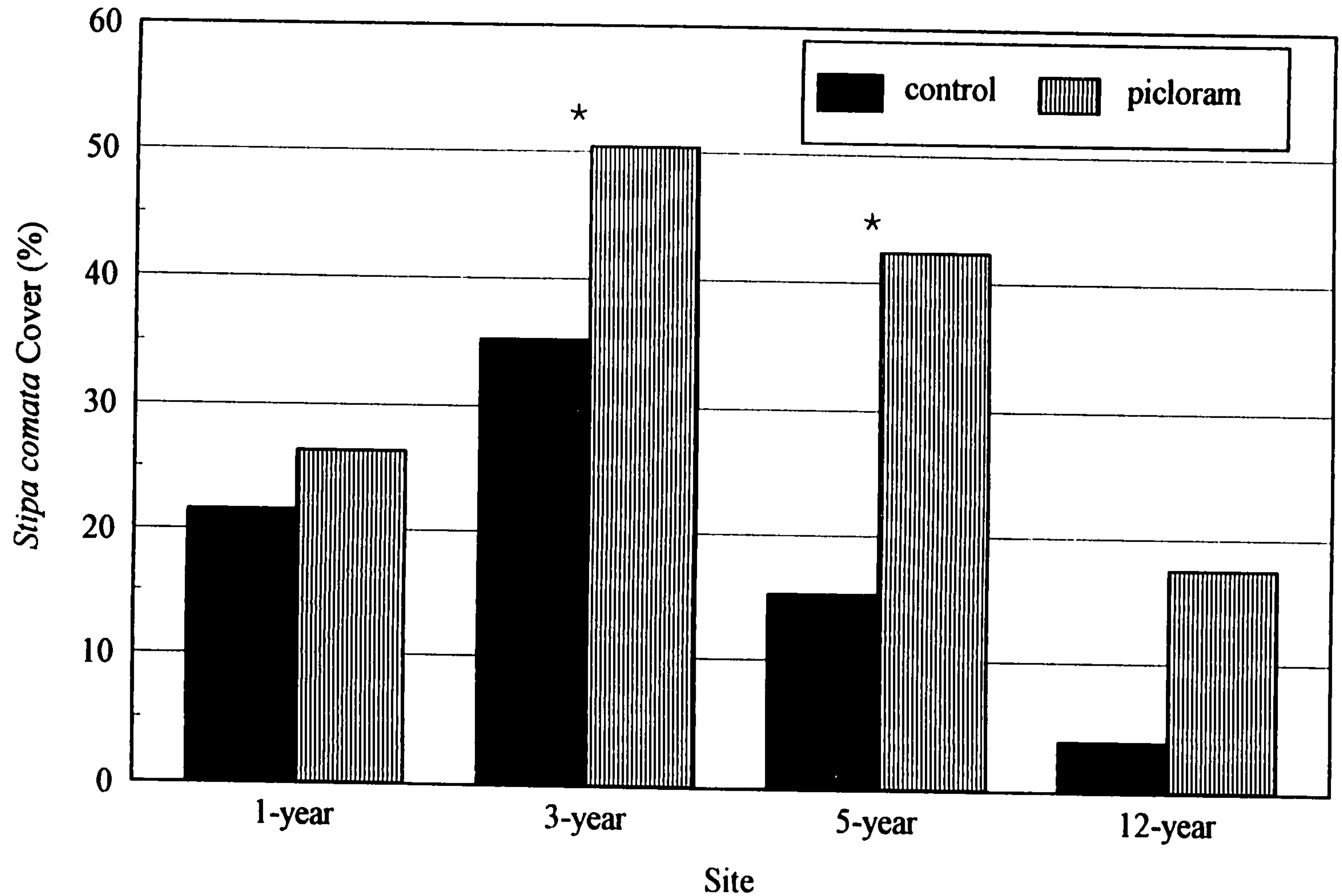

Fig. 6.3 Cover (%) of *Stipa comata* after treatment with picloram at 0.54-0.55 kg ai/ha, measured at sites with different post-treatment times.
* significant at $P \leq 0.05$

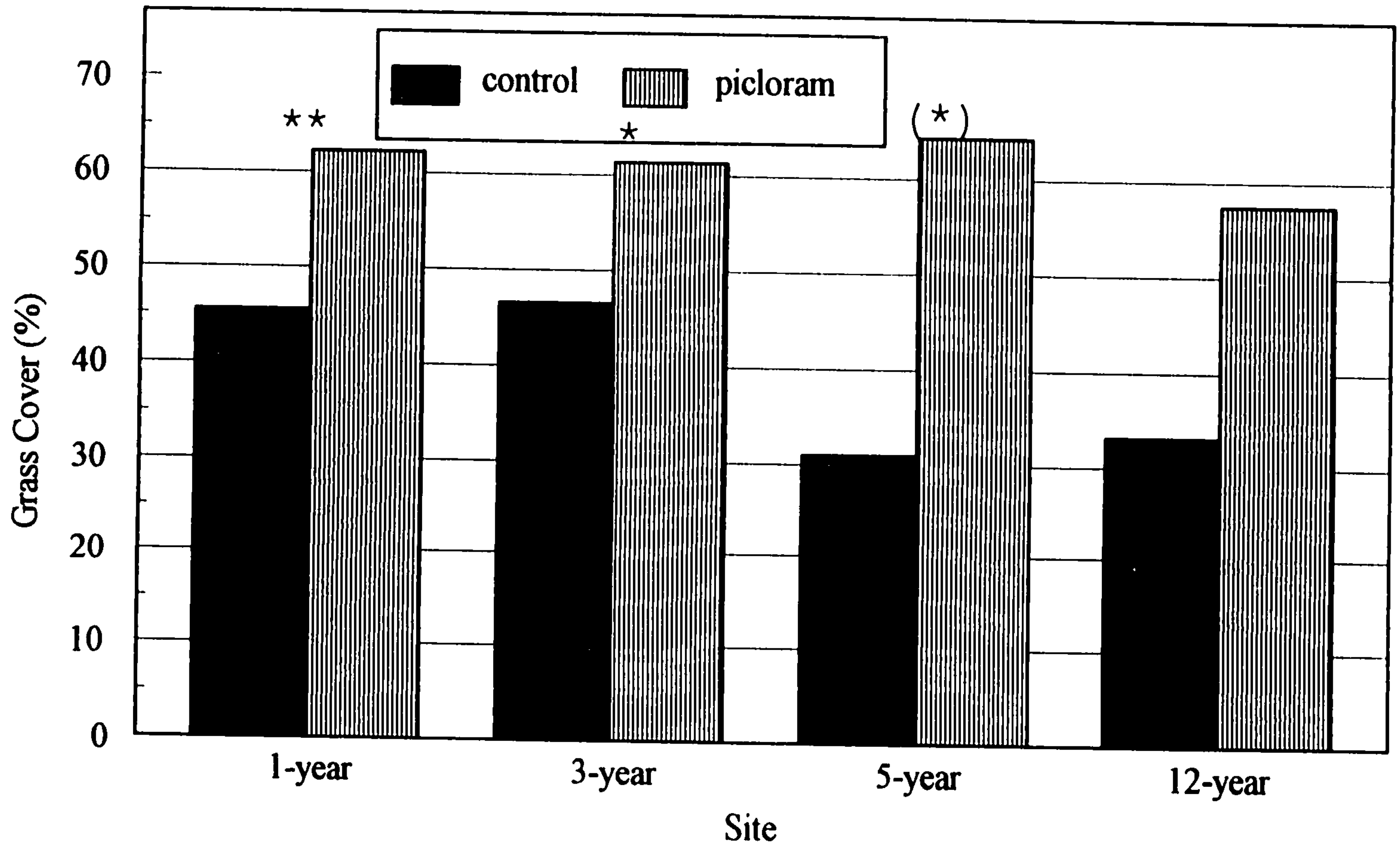

Fig. 6.4 Cover (%) of grasses after treatment with picloram at 0.54-0.55 kg ai/ha, measured at sites with different post-treatment times.

(*),*,** significant at $P \leq 0.10$, 0.05, and 0.01, respectively.

diffusa after dissipation of soil residual picloram, although the exact mechanism is unclear.

4.5 Species Density and Diversity

Species density was significantly lower on picloram-treated plots at the 1- and 3-year sites but had recovered on the 5- and 12-year sites (Table 6.2). Simpson's index and the Shannon index were lower on picloram-treated plots at the 1-, 3- and 5-year sites but had recovered on the 12-year site. The non-significant differences at the 12-year site provide reliable information because sufficient power existed to detect changes of 36%, 22%, and 64% of the control mean for species density, Shannon index, and Simpson's index, respectively. It can therefore be conclusively stated that plant species diversity returns to pre-treatment levels between 5 and 12 years post-treatment at these sites.

These results concur with those of other components of this study showing that species density recovers rapidly, while the Simpson's and Shannon indexes of species diversity are slower to recover (Chapter 4). Rice *et al.* (1992) provided graphical data showing similar declines and recoveries in species numbers in Montana after treatment with 0.28 kg ai/ha picloram. Their data on the Shannon index, however, did not show the slower recovery demonstrated in this study, although this was difficult to assess without the provision of statistical tests.

The insensitivity of species density count and the two species diversity indices in detecting long-term changes of *Centaurea diffusa*, *Calochortus macrocarpus* and Asteraceae suggests that these calculations do not provide complete understanding when used alone. Enumeration of species information into a single value has proven to be misleading in this study. In addition, the relationship of species density and species diversity index values to actual plant community function is poorly known (Hurlbert 1971). This limits any meaningful discussion about changes in species diversity values.

For example, Tilman and Downing (1994) provided evidence that more diverse plant communities possess greater ability to recover after drought. This information is less informative, however, than their description of the actual plant species that contributed to the increased ability to recover.

5.0 Conclusions

The evidence presented for the long-term effects on some plant species and groups is weak although other effects are quite conclusive. Inadequate statistical power at the 5- and 12-year sites resulted in no information regarding plant species that showed non-significant long-term response to picloram. These problems occurred because the longer-term research trials used originally were not designed to examine non-target plant species responses. In addition, the effect of post-treatment time could not be tested directly because the variable experimental designs of the four trials required separate analyses. The results of this study must, therefore, be used cautiously. Nonetheless, long-term data in field ecology trials are rare and require tremendous commitment of resources to obtain. Valuable insight into the long-term effects of herbicides has been gained through this study despite the many problems.

Authors of other field-based studies have indicated that recovery of the original plant species composition is rapid, and occurs within 2-5 years after treatment with herbicide (Malone 1972, Murray *et al.* 1991, Rice *et al.* 1992). The present study has uncovered the possibility that long-term vegetation changes may occur in response to picloram treatment. Similar long-term changes were not demonstrated using species diversity parameters, however, these parameters appear to be less useful for detecting important changes.

The long-term reduction of the invasibility of two grassland communities by *Centaurea diffusa* following treatment with picloram has been demonstrated. This suggests a benefit of herbicide treatment that is not often considered. The suppression of a weed at levels not considered to be sufficient for traditional weed control purposes may, nonetheless, have ecological benefits. The long-term reduction of the abundance of native Asteraceae and *Calochortus macrocarpus* has also been demonstrated. This may have negative ecological consequences that may override the positive effects of long-term weed control.

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CHAPTER 7. DEATH OF DOMINANT PLANTS AS A MECHANISM FOR SPECIES RECOVERY AFTER HERBICIDE-INDUCED LOSSES IN A GRASSLAND COMMUNITY

1.0 Introduction

Plant communities often become dominated by grassy vegetation after treatment with picloram (4-amino-3,5,6 trichloro-2-pyridinecarboxylic acid) because mature grasses are generally tolerant of this herbicide (Renney and Hughes 1969, Tomkins and Grant 1974). The recovery of plant communities treated with a persistent herbicide, such as picloram, can occur initially only by species that are tolerant of the herbicide. Many authors have found that the gaps left by herbicide-sensitive species are quickly filled by vegetative expansion of the tolerant species and sometimes by establishment from their seed (Hyder and Sneva 1956, Sturges 1986). This process results in a vegetation state that is different from naturally occurring vegetation and that may be persistent (Egler 1954, Tomkins and Grant 1977). The herbicide-tolerant species may continue to have an advantage over herbicide-sensitive species because of well dispersed and proximate seed sources and the availability of vegetative sources for expansion.

It is not known to what extent, and at what rate, the dominance attained by herbicide-tolerant species is lost over time. In fact, Connell and Slatyer (1977) provided compelling evidence that the first species to colonize a natural disturbance may often exclude or suppress subsequent colonist species for long periods (see also Egler 1954). Alternatively, many authors of field-based studies have reported that recovery of the original plant species composition is rapid, occurring within 2-5 yrs after treatment with herbicide (Malone 1972, Murray *et al.* 1991, Rice *et al.* 1992).

It has been hypothesized that plant diversity can be maintained in a plant community through the continuing death of dominant individuals (Grubb 1977). Gaps created by the loss of these plants provide areas of increased resource availability that significantly enhance the chances of seedling recruitment. The seedlings that colonize the gaps may be of a different species than the dead plant, leading to increased diversity. Connell and Slatyer (1977) suggested that, even though earlier species may continue to exclude or suppress later ones for long periods, the former eventually are damaged or killed and are then replaced. Individual perennial grass plants are known to vary in longevity from about 3 to 20 years (Canfield 1957, Looman and Heinrichs 1973). Opportunities for the recruitment of seedlings or for vegetative expansion may occur when these plants die. If resources such as light, nutrients, moisture or space created in the gaps are not captured by plants of the same species that died, then the community is unstable and new species may be introduced (Canfield 1957, Looman and Heinrichs 1973, Holechek *et al.* 1995).

The objective of this study was: to determine if death of a dominant picloram-tolerant plant allows for the establishment of picloram-sensitive plants in the resulting gap, on sites previously treated with picloram.

2.0 Study Area

The study was conducted on three low-elevation grassland sites in the southern interior of British Columbia, Canada. All sites occur in the Bunchgrass (Very Dry-Warm) biogeoclimatic subzones (Lloyd *et al.* 1990) and are dominated by the grass *Stipa comata*. *Elymus spicatus* is expected to dominate these sites at their potential natural community (Tisdale 1947) but is not abundant at any of the sites. Plant taxonomic nomenclature follows Douglas *et al.* (1989). The soils are generally Dark Brown Chernozems (Typic Borolls) of aeolian origin with fine sandy loam textures.

The elevations are 700 m at the Redhill (50° 43'/120° 23') and Scheidam (50° 45'/120° 13') sites and 550 m at the Daryll site (50° 45'/119° 55'). Aspects are flat, 5% southeast, and 3% south at the Redhill, Scheidam, and Daryll sites, respectively. Mean annual precipitation at Kamloops airport, which is within 250 m elevation and 35 km distance of all sites, is 256 mm with peaks in June-August and December-January. At Kamloops, the highest daily maximum temperatures occur in July (28.8° C) and lowest daily mean minimum temperatures occur in January (-9.8° C) (Environment Canada 1984).

The Redhill and Scheidam sites were fenced to exclude livestock while the Daryll site remained unfenced. Before fencing, the Redhill site was used for moderate spring/fall grazing on an 18-month rest-rotation system and the Scheidam site was used primarily for summer grazing by horses and steers. The Daryll site continued to be lightly used by bulls in the spring throughout the period of the study. Although some light use of grasses was observed on the experimental area, no plots were disturbed by trampling or grazing at the Daryll site.

3.0 Methods

Sites were selected that were known to have had previous treatment with picloram and showed obvious dominance by *Stipa comata*. Such areas are relatively common wherever *Centaurea diffusa* has been controlled within these grasslands. Verification of the herbicide treatment was provided by checking herbicide application records or by personal communication with the agency responsible for management of the areas. Distinct boundaries between *Stipa*-dominated grassland and *Centaurea*-dominated grassland occurred at each site due to the pattern of initial herbicide application.

Soil samples from untreated and treated areas on each site were bioassayed using picloram-sensitive *Helianthus annuus* to determine if phytotoxic picloram residues remained (Leasure 1964). Triplicate soil samples to a 25-cm depth were taken for each bioassay and used to fill four 10-cm-diameter pots. Four *Helianthus annuus* seeds were sown per pot, at 2 cm depth, and germinated at room temperature. After 4 days the pots were transferred to a growth chamber set at a constant temperature of 25 ^{o}C with a 12/12 hr light regime for 3 weeks. After 3 weeks, percentage germination, height growth, and surivival were assessed and compared to untreated soils.

At the Redhill and Scheidam sites, 40 *Stipa comata* plants were systematically selected, about 1.5 m apart, along each of four 18-m transects, also spaced 1.5 m apart. Only 20 *Stipa* plants were selected at the Daryll site. One-half of the plants at each site were randomly chosen for individual treatment with glyphosate (N-[phosphonomethyl]glycine). The herbicide treatment was applied in late April, 1995 and resulted in complete kill of all treated plants within a few weeks. During treatment, neighbouring plants were shielded by a conical paper barrier to prevent damage from overspray of the herbicide.

Information was collected on neighbouring plants within a 56-cm diameter circular plot (0.25 m^2), centered on each selected *Stipa comata* plant (dead or alive). The outline of the dead *Stipa comata* plants remained distinct throughout the study period because of slow litter decomposition. The taxonomic identification, distance and direction from the central plant, and dimensions of all seedlings and mature plants within the plots were recorded during May 1995 and June 1996. The basal areas of juvenile and mature *Stipa comata* plants were estimated by taking two basal diameters at right angles and using the average diameter for the calculation. Flowering culms of mature *Stipa comata* plants were counted to provide an index to plant vigour.

Soil moisture tension was monitored on a subset of plots for two growing seasons at three depths (5, 10, and 15 cm) using gypsum blocks buried within 5 cm of the central reference *Stipa comata* plant. Eight plots (four control and four glyphosate-treated) were monitored at each of the Redhill and Scheidam sites.

Pre-treatment canopy cover (Daubenmire 1959) of all vascular plant species was estimated at each site during the first week of May 1995 to provide a description of the plant community in the picloram-treated areas. Adjacent, untreated, *Centaurea*-infested areas were also sampled for comparison. Twelve 20 x 50-cm quadrats (0.1 m^2 were systematically located along each of four transects for a total of 48 quadrats in both the treated and untreated portions of each of the three experimental areas. The numbers of unique plant species identified in the quadrats were totaled by transect to calculate species density (Hurlbert 1971). Canopy cover estimates were used to calculate the Shannon index of species diversity (Magurran 1988), using the formula:

$$H' = -\Sigma p_i \ln p_i$$

The value of p_i was estimated as n_i/N (Pielou 1969) where n_i is the cover of a plant species and N is the total cover of all plant species considered in the calculation. T-tests were used to determine if species density and the Shannon index differed on picloram-treated and adjacent untreated areas at the Redhill, Scheidam and Daryll sites.

3.1 Analyses

The experiment has a completely randomized one-factor design with 20 replicates at the Redhill and Scheidam sites and 10 replicates at the Daryll site. Each selected *Stipa comata* plant, including the 0.25-m^2 circular area surrounding the plant is considered a replicate. Sites were analyzed separately using ANOVA to test for significant effects on neighbouring plants due to the glyphosate treatment of central *Stipa comata* plants.

The number of seedlings occurring within 5 cm of the central *Stipa comata* plant was analyzed separately by species for the May 1995 and June 1996 sampling dates. It was expected that seedlings within the 5-cm zone would be strongly influenced by the central *Stipa comata* plant. Basal area growth of *Stipa comata* was determined by calculating the change in basal area from May 1995 to June 1996. This calculation was performed on mature plants occurring within 5 cm of the central *Stipa comata* plant only, which resulted in a reduced number of replicates, because not all plots contained plants with those characteristics. The final number of replicates used in the basal area analysis was 12, 16, and 7 at the Redhill, Scheidam and Daryll sites, respectively. The same plots were also used for a separate analysis of number of flowering *Stipa comata* culms. All analyses were performed using the Statistical Analysis System (SAS Institute 1988). Arcsine transformations or square root transformations were used to adjust for non-normal data where necessary.

4.0 Results and Discussion

4.1 Vegetation Characteristics

Stipa comata was the exclusive dominant plant species, and had a canopy cover of 47% or more at all three sites (Table 7.1). The only other species with substantial abundance was *Poa secunda* which had less than 12% cover at all sites. The majority of the remaining cover was shared by 12 annual and perennial forbs.

The number of species, measured by species density, was similar in picloram-treated and untreated areas. However, the Shannon index was always substantially lower on the picloram-treated areas (Table 7.2). The low Shannon index values are a direct result of dominance by a single species, which reduces the equitability

Table 7.1 Pre-treatment canopy cover (%) of vascular plant species in May 1995 at the Redhill, Scheidam and Daryll sites.

	Site		
	Redhill	Scheidam	Daryll
	----------Cover (%) ----------		
Grasses			
Bromus tectorum	+	+	1.2
Elymus spicatus	-	-	1.6
Koeleria macrantha	-	+	-
Poa secunda	11.4	6.6	5.4
Sporobolus cryptandrus	1.6	-	-
Stipa comata	47.2	69.6	67.4
Forbs			
Achillea millefolium	-	2.3	-
Androsace occidentalis	-	+	+
Antennaria dimorpha	0.6	-	-
Antennaria microphylla	-	+	-
Arabis holboellii	0.5	+	+
Calochortus macrocarpus	+	+	-
Centaurea diffusa	0.6	-	2.0
Collinsia parviflora	+	2.0	5.2
Draba verna	+	4.4	1.8
Erigeron pumilus	+	-	-
Fritillaria pudica	+	+	+
Lithophragma glabra	-	+	-
Lithospermum ruderale	-	+	-
Microsteris gracilis	+	-	+
Montia linearis	-	+	1.1
Myosotis stricta	+	1.4	7.4
Plantago patagonica	+	+	-
Polemonium micranthum	-	-	+
Polygonum douglasii	-	+	+
Ranunculus glaberrimus	-	+	-
Stellaria nitens	-	-	+
Taraxacum officinale	-	-	+
Tragopogon dubius	-	+	-
Verbascum thapsus	-	-	1.3
Shrubs			
Artemisia frigida	1.1	-	-
Artemisia tridentata	+	-	-

+ Less than 0.5%

Table 7.2 Pre-treatment species density (no./transect) and plant diversity (H') in May 1995 at the Redhill, Scheidam, and Daryll experimental sites with picloram treatment history and on adjacent areas without picloram treatment history.

	Species density			Shannon index			
	On site	Adjacent	Prob.>t	On site	Adjacent	Prob.>t	
	--- (no./transect) ---			-------- (H') ---------			
Redhill	11.0	10.0	0.4454	0.64	1.14	0.0012	**
Scheidam	12.5	14.5	0.0924	0.63	1.24	0.0006	***
Daryll	13.5	11.8	0.2002	0.74	1.34	0.0012	**

, * significant at $t \leq 0.01$ and $t \leq 0.001$, respectively component of the index. Plant community composition on the grass-dominated sites is substantially different, however, as indicated by the lower Shannon index.

4.2 Picloram Activity in the Soil

Bioassays using *Helianthus annuus* established that not enough picloram remained in the soil to produce phytotoxic symptoms at the three sites. There were no differences (t>0.05) in *Helianthus annuus* germination, 3-week height growth, or 3-week survival between soil collected from the sites and an adjacent control soil (Table 7.3). No evidence of picloram injury such as epinasty, hypertrophy, or fasciation of crown and leaf petioles was observed on any plant. The negative results of these tests indicate that plant species with similar, or greater sensitivity to picloram than *Helianthus annuus* are able to establish on these soils without suffering detrimental effects. Therefore, herbicide effects can be discounted as a potential cause for the absence of certain species of seedlings on the plots.

4.3 Treatment Effects Due to *Stipa comata* Death

4.3.1 Seedling Emergence

The death of *Stipa comata* plants resulted in significant increases in seedling counts on the area immediately surrounding the dead plants. This effect was generally (two of the three sites) only weakly expressed ($P \leq 0.10$) at the May 1996 sampling date (Table 7.4), but increased in strength ($P \leq 0.05$) by the June 1996 sampling date (Table 7.5). The one-month period between these sampling dates appears to have been a critical time for seedling emergence near dead *Stipa comata* plants because of the large increases recorded during this period.

Both total seedling counts and seedling counts of individual species were significantly higher on plots with a dead *Stipa comata* plant center. The most

Table 7.3 Pre-treatment germination (%), 3-week height growth (cm), and 3-week survival of *Helianthus annuus* grown in soil collected at Redhill, Scheidam and Daryll experimental sites (On site) with picloram treatment history and on adjacent areas without picloram treatment history.

	Germination			Growth			Survival	
	On site	Adjacent	Prob >t	On site	Adjacent	Prob >t	On site	Adjacent
	--------(%) --------			------- (cm) -------			--------(%) --------	
Redhill	58	83	0.2508	10.6	9.6	0.5518	100	100
Scheidam	83	50	0.2051	9.3	8.2	0.2051	100	100
Daryll	90	75	0.3739	8.1	10.1	0.3153	100	100

Table 7.4 Numbers of seedlings of vascular plant species occurring within 5 cm of a reference *Stipa comata* plant in May 1996 following glyphosate-treatment of the reference *Stipa comata* (Stco) plant in April 1995.

	Redhill		Scheidam		Daryll	
	dead Stco	living Stco	dead Stco	living Stco	dead Stco	living Stco
	count					
Grasses						
Bromus japonicus	-	-	0.25	0.00**	-	-
Bromus tectorum	0.00	0.05	-	-	0.90	0.50
Poa secunda	0.20	0.45	0.30	0.10	0.10	0.00
Stipa comata	0.40	0.45	1.15	0.75	0.80	0.90
Vulpia octoflora	0.10	0.00	0.30	0.15	-	-
Forbs						
Achillea millefolium	-	-	0.05	0.10	-	-
Androsace occidentalis	-	-	0.15	0.05	-	-
Antennaria dimorpha	0.05	0.05	-	-	-	-
Arabis holboellii	0.15	0.40	-	-	-	-
Centaurea diffusa	0.10	0.05	-	-	0.40	0.80
Collinsia parviflora	-	-	1.60	1.25	0.40	0.00
Draba verna	-	-	0.30	0.25	0.10	0.00
Erigeron pumilus	0.00	0.05	-	-	-	-
Kochia scoparia	0.30	0.35	-	-	-	-
Microsteris gracilis	0.15	0.10	0.00	0.05	-	-
Montia linearis	-	-	0.80	0.10(*)	0.10	0.10
Myosotis stricta	0.05	0.05	0.15	0.05	0.30	0.00
Plantago patagonica	0.05	0.15	0.75	0.75	-	-
Polygonum douglasii	-	-	-	-	0.00	0.10
Tragopogon dubius	-	-	0.15	0.00(*)	0.20	0.10
Monocarpic forbs	0.65	0.70	3.90	2.50	1.50	1.40
Shrubs						
Artemisia frigida	0.55	0.05(*)	0.05	0.00	-	-
Chrysothamnus nauseosus	0.10	0.10	-	-	-	-
Total	2.20	2.30	6.00	3.60(*)	3.30	2.80

n=20 at Redhill and Scheidam sites; n=10 at Daryll.

(*) ,*, ** significant at $P \leq 0.10$, 0.05 and 0.01, respectively

Table 7.5 Numbers of seedlings of vascular plant species occurring within 5 cm of a reference *Stipa comata* plant in June 1996 following glyphosate-treatment of the reference *Stipa comata* plant (Stco) in April 1995.

	Redhill		Scheidam		Daryll	
	dead Stco	living Stco	dead Stco	living Stco	dead Stco	living Stco
	count					
Grasses						
Bromus japonicus	-	-	0.05	0.00	-	-
Koeleria macrantha	-	-	0.10	0.00	-	-
Poa secunda	0.65	1.35*	0.25	0.60	0.40	0.30
Sporobolus cryptandrus	0.45	0.35*	-	-	-	-
Stipa comata	2.30	1.40	4.00	2.65*	3.40	2.20(*)
Vulpia octoflora	0.05	0.15	0.25	0.10	0.10	0.00
Forbs						
Achillea millefolium	-	-	0.10	0.15	-	-
Antennaria dimorpha	0.00	0.05	-	-	-	-
Arabis holboellii	0.25	0.40	-	-	-	-
Calochortus macrocarpus	0.05	0.00	0.05	0.00	-	-
Castilleja thompsonii	0.05	0.35	-	-	-	-
Centaurea diffusa	0.15	0.05	-	-	0.20	0.00
Echium vulgare	0.05	0.00	-	-	-	-
Erigeron pumilus	0.00	0.05	-	-	-	-
Kochia scoparia	0.40	0.25	-	-	-	-
Plantago patagonica	0.05	0.05	1.15	0.70	-	-
Tragopogon dubius	-	-	0.15	0.00	0.20	0.00
Verbascum thapsus	-	-	0.30	0.05	1.20	0.30*
Monocarpic forbs	0.65	0.35	1.60	0.75(*)	1.60	0.30**
Shrubs						
Artemisia frigida	0.05	0.00	0.15	0.00(*)	-	-
Artemisia tridentata	0.50	0.20	-	-	-	-
Chrysothamnus nauseosus	0.10	0.05	-	-	-	-
Total	5.10	4.70	6.55	4.25**	5.50	2.80**

n=20 at Redhill and Scheidam sites; n=10 at Daryll

(*) ,*, ** significant at $P \leq 0.10$, 0.05 and 0.01, respectively consistent response at all sites was by *Stipa comata* seedlings. Abundance of these seedlings in June was 64, 51, and 55% greater near dead *Stipa comata* plants at the Redhill, Scheidam and Daryll sites, respectively (Table 4). *Stipa comata* appears to be well adapted to take advantage of gaps caused by death of mature plants. Looman and Heinrichs (1973) reported that *Stipa comata* occurred in many of the aging *Agropyron cristatum* pastures that were sampled. The death of aging *Agropyron cristatum* plants is a likely mechanism for allowing *Stipa comata* to invade these pastures. Seeds of *Stipa comata* have been reported to be persistent for 1 or 2 years in the soil seed bank (Hassan and West 1986, Hume and Archibold 1986) and have the ability to drill into the soil through hygroscopic processes acting on a long spiral-shaped awn (Peart 1979). More important, however, is the abundance of seeds dispersed by the mature *Stipa comata* plants that compose the majority of the cover at the three experimental sites. Mature, seed-bearing *Stipa comata* plants were recorded in all plots in the study (Table 7.1). The ready availability of seed confers a tremendous advantage on this species in the colonization of gaps produced by dead plants. Furthermore, this species is known to germinate better at higher temperatures (27° C) than other native grasses (Smoliak and Johnston 1967). This increases the likelihood of germination in gaps as compared to areas near living *Stipa comata* plants since insolation is greater at the soil surface of vegetation gaps.

The introduced species *Tragopogon dubius* occurred exclusively near dead *Stipa comata* plants in both May and June of 1996 at the Scheidam site (Tables 7.4 and 7.5). This species was not abundant on any experimental site (Table 7.1). However, several weedy characteristics may have contributed to its response to the experimental treatment. *Tragopogon dubius* is capable of long-distance dispersal of seeds (>250 m), although seeds do not remain viable in the soil for more than 1 or 2 yrs (Gross and

Werner 1982). In this study, *Tragopogon dubius* was observed to produce seeds over an extended period of time (June through September or October). These traits allow season-long "testing" for vegetation gaps over wide areas.

Verbascum thapsus, also an introduced species, was four times more abundant near dead *Stipa* plants than near live plants at the Daryll site in June (Table 5). In contrast to *Tragopogon dubius*, *Verbascum thapsus* seeds have no specialized structures for long-distance dispersal. Eighty percent of *Verbascum thapsus* seeds fall within 7 m of the parent plant (Gross and Werner 1978). This species is known to have seeds that can remain viable in the soil for up to 80 years (Kivilaan and Bandurski 1973), which gives it a distinct advantage in the "race" to colonize vegetation gaps. Seeds of *Verbascum thapsus* were probably present in the soil seed bank at the time of treatment application. This provided an immediate seed source for colonization of the gaps created by the death of *Stipa comata*.

Montia linearis, an ephemeral annual species, was the only native forb to show increased seedling emergence in response to the *Stipa comata* death treatment. This species was eight times more abundant near dead *Stipa comata* plants than near live plants in May at Scheidam (Table 7.4). This trend was not observed at Daryll on the same date and, therefore, the response is not completely reliable because of the inconsistency. Several other ephemeral annual forbs (*Androsace occidentalis*, *Collinsia parviflora*, *Draba verna*, *Microsteris gracilis*, *Myosotis stricta*) did not respond to the treatment. These species were recorded in May, but not in June, because they were mature or senescent by June.

It is important to note that emergence of most perennial forb seedlings did not increase in the gaps caused by the death of *Stipa comata*. The perennial Asteraceae *Achillea millefolium*, *Antennaria dimorpha*, *Antennaria microphylla*, and *Erigeron* *pumilus* are especially sensitive to picloram (Chapters 2 and 3) and can be greatly reduced in areas treated with this herbicide. These species were apparently unable to take advantage of the resource opportunity created by the death of *Stipa comata* under the environmental conditions prevailing from May 1995 through June 1996.

The half-shrub *Artemisia frigida* was the only non-grassy perennial species to respond to *Stipa comata* death. This species was 10 times more prevalent on Redhill plots with dead *Stipa comata* centers in May (Table 7.4). By June, however, overall counts of *Artemisia frigida* were reduced and no significant treatment effect existed. It appears that there is an interaction between climatic conditions and emergence in response to *Stipa comata* removal at this site. Most likely, conditions were favourable for emergence of this species in the gaps in May, but establishment was thwarted by subsequent unfavourable conditions. The trend of increased emergence (Table 7.5) near dead *Stipa comata* plants was also evident at Scheidam in June, providing support to the results at Redhill in May. *Artemisia frigida* is well known as one of the first perennials to establish on disturbed sites (Shantz 1917, Bai and Romo 1996). Looman and Heinrichs (1973) reported it to be present in all 85 *Agropyron cristatum* pastures they sampled in southern Alberta and Saskatchewan; from 5-year-old pastures to 38-year-old pastures. A mature *Artemisia frigida* plant can produce up to 190,000 seeds annually (Wilson 1982) and the seeds can remain viable in the soil for 3 - 5 yrs (Bai and Romo 1994). Bai and Romo (1994) also suggested that seedling emergence may occur at any time during the growing season, which provides the opportunity for a quick response to the creation of vegetation gaps.

Poa secunda was the only plant species measured that showed greater seedling emergence near living *Stipa comata* plants. In June, seedlings of this species were twice as abundant near living *Stipa comata* as near dead *Stipa comata* plants at Redhill

(Table 7.4). Although not statistically significant ($P>0.10$), this trend in the means was also evident at Scheidam in June, suggesting that the response at Redhill was not completely anomalous. *Poa secunda* is a short-lived grass of low stature that takes advantage of the period of favourable soil moisture after spring thaw. This species is normally found in the interspaces of larger perennial grasses such as *Stipa comata* and *Elymus spicatus*. It is possible that *Poa secunda* may require the shade of other plants for germination and emergence. The gaps caused by dead *Stipa comata* plants, with higher insolation than near living *Stipa comata*, may not be the optimum micro-environment for the germination and emergence of *Poa secunda*.

4.3.2 Basal Area Growth and Flowering Culm Production

The basal area of *Stipa comata* plants neighbouring the reference *Stipa comata* plant was measured before and after treatment to determine if vegetative growth was occurring in response to the treatment. Vegetative growth for the 1-year period (June 1995 - June 1996) appeared higher near dead *Stipa comata* plants at all sites, but this was not significantly different ($P>0.05$) from growth recorded near living *Stipa comata* plants (Table 7.6). High natural variability in plant basal area may have resulted in a low power to detect true differences in basal area means.

The flowering culms of living *Stipa comata* plants were counted to determine if the vigour of neighbouring plants increased near dead *Stipa comata* plants. Flowering culms were more numerous on neighbouring *Stipa comata* near dead *Stipa comata* plants at the Redhill site (Table 7.6). *Stipa comata* plants on these plots produced five times more flowering culms than plots with living *Stipa comata* centers. A somewhat similar (but non-significant) response occurred at the Scheidam and Daryll sites.

Table 7.6 Basal area growth (cm²) and flowering culm production (no./plant) of *Stipa comata* plants in proximity to a reference *Stipa comata* plant in June 1996 following glyphosate-treatment of the reference *Stipa comata* (Stco) plant in April 1995.

	Redhill		Scheidam		Daryll	
	dead Stco	living Stco	dead Stco	living Stco	dead Stco	living Stco
	------------------- (cm²) -------------------					
Basal area	0.60	-0.91	1.44	0.21	3.26	-0.28
	------------------- no./ plant -------------------					
Flower culms	4.50	0.83*	4.00	3.00	7.29	3.86

*significant at $P \leq 0.05$

4.4 Species Composition of Seedlings

A total of 29 species of seedlings were identified and counted on the plots at the three sites over the two sampling dates (Tables 7.4 and 7.5). Species composition based on seedling counts was compared to species composition based on the cover of mature plants taken in May 1995. This was done to determine whether potential regeneration of plant species (measured by seedling counts) matched existing parent plant species (measured by cover of mature plants). There was a general correspondence between species composition of seedlings and species composition of mature plants at all sites for the most abundant species. This provides some evidence of vegetation stability (Figs. 7.1a, 7.1b and 7.1c). No doubt, seed available from mature plants was a major factor leading to increased incidence of seedlings of the same species.

4.5 Soil Moisture Tension

Soil moisture tension was monitored within the top 15 cm of soil in order to determine if this parameter could be linked to seedling germination and emergence. Soil moisture tension was not affected by the *Stipa comata* death treatment until well into August, 1995. Soil moisture tension at the Redhill site was affected more often than at Scheidam (Table 7.7). Soils at the 10- and 15-cm depths were wetting up more slowly near dead *Stipa* plants on 23 August, 30 August and 1 September at Redhill. At the same site, soils in the surface 5 cm were drying more quickly on 7 September and 11 October. A similar effect occurred at Scheidam on 6 September at the 10-cm depth.

It is apparent that micro-environmental changes due to the loss of plant foliage are important factors in increased soil moisture tension in the top 15 cm of the profile. Factors such as increased insolation of the surface and increased air movement through the gaps may explain why surface soils dried more quickly in September and October

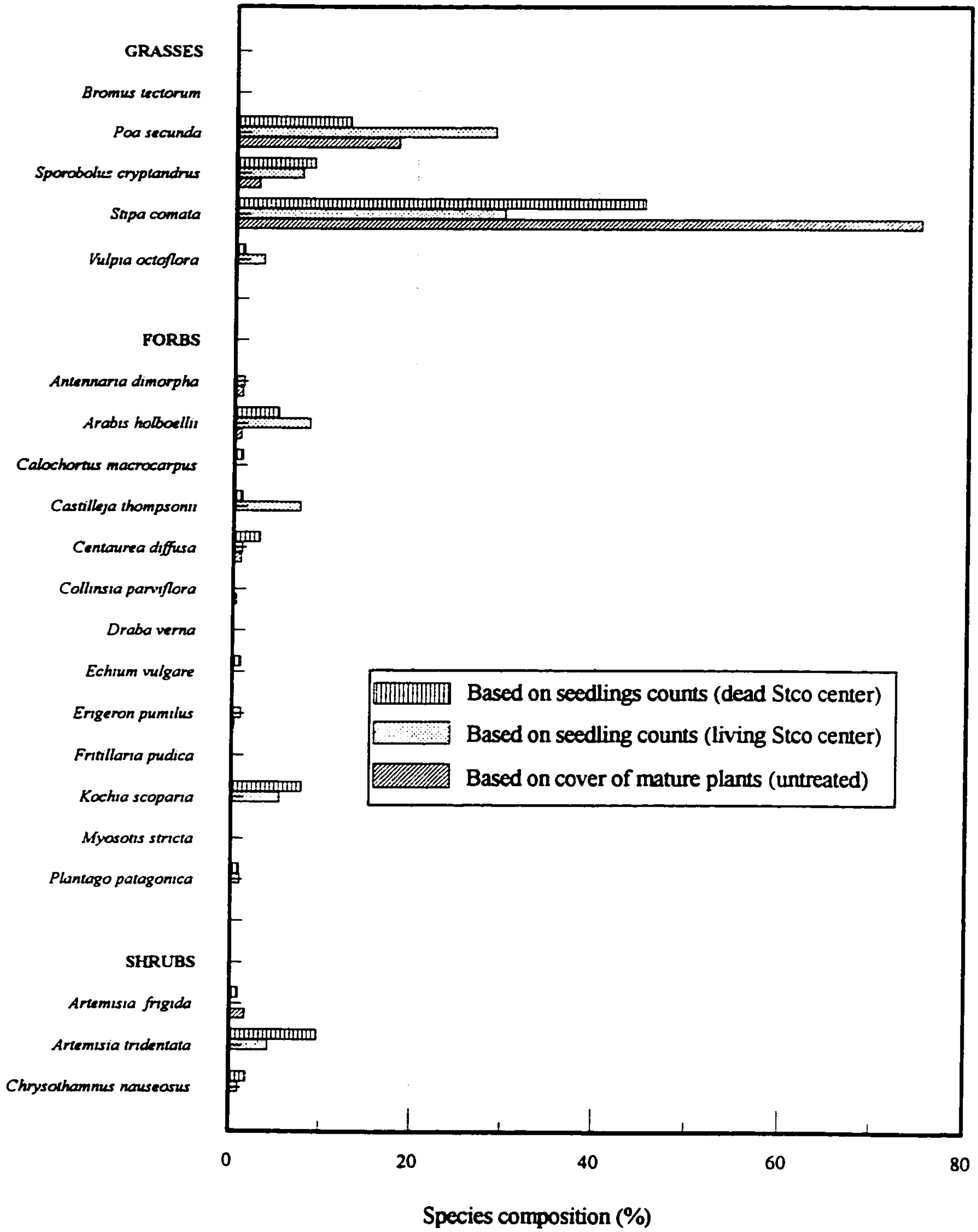

Fig. 7.1a Species composition (%) based on seedling counts in May 1996 following glyphosate-treatment of the reference *Stipa comata* (Stco) plant in April 1995 at the Redhill site. Species composition based on estimated cover of mature, untreated plants in May 1995 provided for comparison.

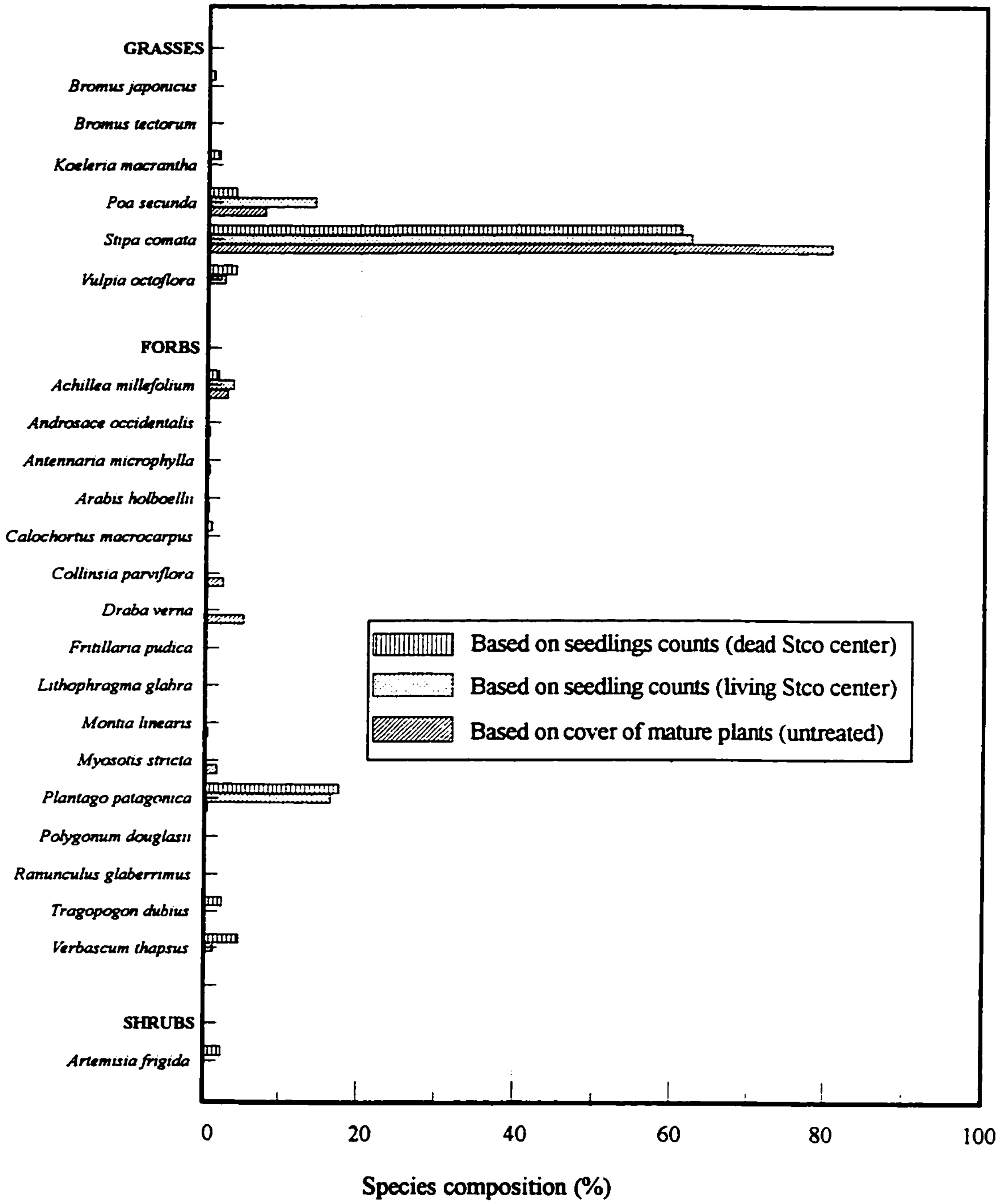

Fig. 7.1b Species composition (%) based on seedling counts in May 1996 following glyphosate-treatment of the reference *Stipa comata* (Stco) plant in April 1995 at the Scheidam site. Species composition based on estimated cover of mature, untreated plants in May 1995 provided for comparison.

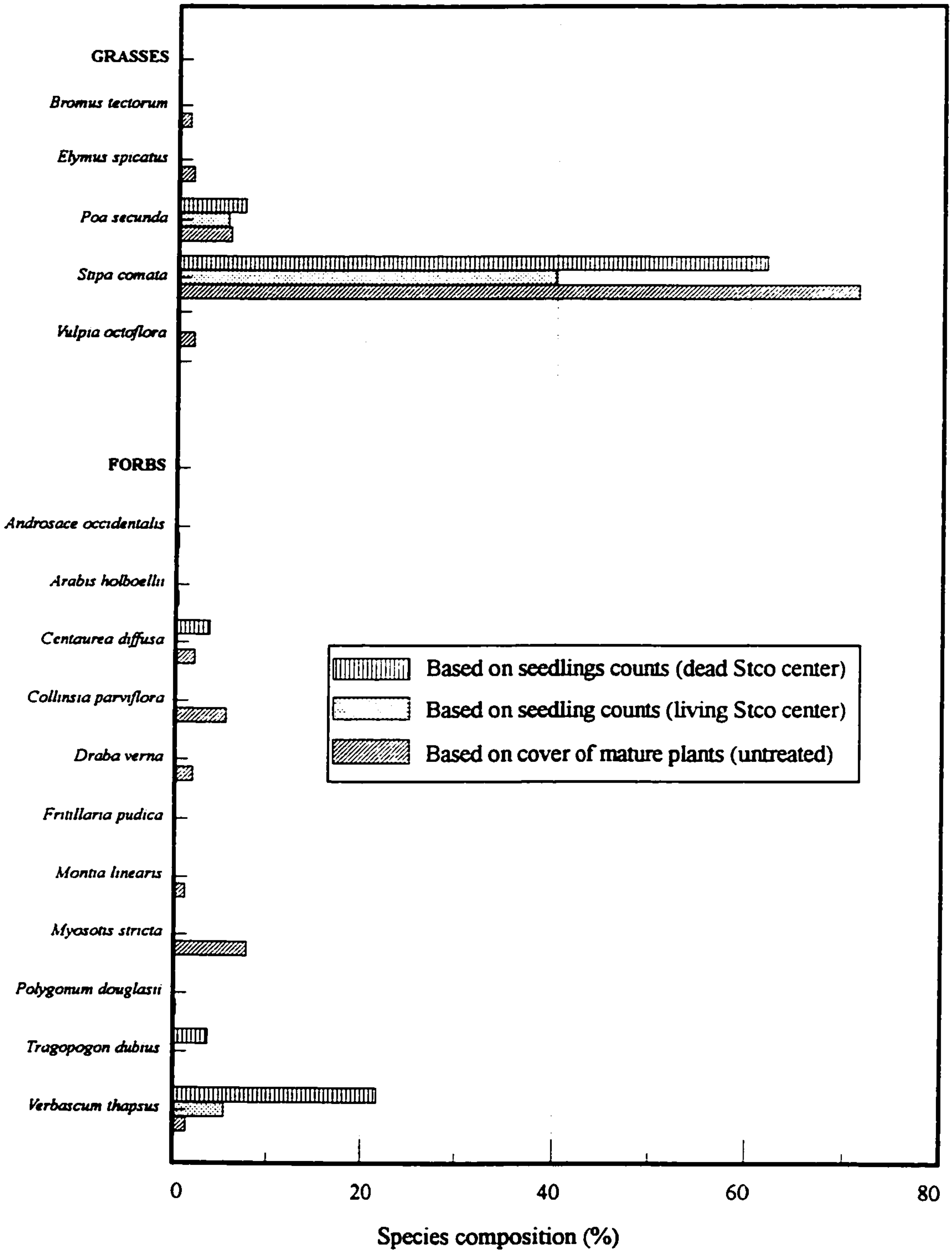

Fig. 7.1c Species composition (%) based on seedling counts in May 1996 following glyphosate-treatment of the reference *Stipa comata* (Stco) plant in April 1995 at the Daryll site. Species composition based on estimated cover of mature, untreated plants in May 1995 provided for comparison.

Table 7.7 Soil moisture tension (MPa) at 5-, 10-, and 15-cm depths following glyphosate treatment of reference *Stipa comata* (Stco) plants in April 1995.

Date	Probe depth	Treatment dead Stco	Treatment living Stco	Significance	
	---- (cm) ----	--------- (MPa)[1]	------	Prob. > F	
Redhill					
17 Aug 1995	5	0.045	0.040	0.134	
	10	0.060	0.040	0.071	(*)
	15	0.060	0.048	0.094	(*)
23 Aug 1995	5	0.128	0.103	0.405	
	10	0.125	0.078	0.074	(*)
	15	0.105	0.068	0.149	
30 Aug 1995	5	1.155	0.888	0.424	
	10	1.160	0.615	0.067	(*)
	15	0.793	0.320	0.118	
1 Sept 1995	5	1.680	1.495	0.356	
	10	1.585	1.320	0.367	
	15	1.403	0.753	0.032	*
7 Sept 1995	5	0.213	0.163	0.019	*
	10	1.655	1.460	0.262	
	15	1.680	1.600	0.356	
11 Oct 1995	5	0.775	0.588	0.088	(*)
	10	1.308	1.168	0.647	
	15	1.548	1.680	0.356	
Scheidam					
6 Sept 1995	5	0.093	0.083	0.190	
	10	0.140	0.095	0.018	*
	15	0.300	0.407	0.723	

(*) ,**significant at $P \leq 0.10$ and 0.05, respectively. [1]MPa=Mega pascal
Data presented for dates showing significant effects only.

on plots where *Stipa comata* plants were killed. It is more difficult to explain why soils wetted up more slowly in August and September at the Redhill site. One possible explanation is that living *Stipa comata* plants act as funnels to intercept and concentrate rainfall near the base of the plant. This phenomenon was demonstrated for soil moisture under *Elymus spicatus* in the bunchgrass grasslands near Kamloops, B.C. (Ndawula-Senyimba *et al.* 1971). The death of a plant may result in reduced wetting up of soils near the plant because of the loss of the funneling action of the plant canopy.

The lack of significant response earlier in the year (May - July) may indicate that soil water use by living *Stipa comata* plants negates any gains in precipitation intercepted by intact plant canopies. *Stipa comata* generally enters summer dormancy by July in these grasslands (Pitt and Wikeem 1990), after which time soil water use by *Stipa comata* is minimal. This may allow soil moisture differences due to canopy architecture to become evident. Alternatively, soil moisture response may simply be delayed. Tabler (1968) found no response in gravimetric soil moisture samples for the first 3 years after sagebrush control with herbicides.

5.0 Conclusions

Only one native perennial species, *Artemisia frigida*, showed increased seedling emergence near dead *Stipa comata* plants. This species responded rapidly in the gaps resulting from plant death. Although there was a general increase in seedling emergence of other species on treated plots, there was little evidence to suggest that this response will result in increased colonization of picloram-sensitive species. Perennial forb seedlings were particularly unresponsive to the creation of gaps caused by the death of *Stipa comata* plants. Picloram-sensitive native plant species such as *Antennaria dimorpha* did not show increased seedling emergence near dead *Stipa comata* plants. *Stipa comata* is the most likely plant to colonize gaps created by death of other *Stipa comata* plants. Such self-replacement suggests that the stability of the grass-dominated community created by picloram treatment is relatively high.

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CHAPTER 8. SUMMARY AND CONCLUSIONS

1.0 Introduction

This research project has demonstrated some effects of the herbicide picloram on non-target plant species in the bunchgrass grasslands of the southern interior of B.C. The research has also provided an evaluation of the impact of some alternative herbicide practices on non-target plant species. The results provide rangeland weed managers with solutions that integrate weed management objectives with biodiversity objectives.

1.1 Objectives Restated

The objectives of this research were:

1) to quantify the response of non-target plant species to the herbicide picloram in the bunchgrass grasslands of southern B.C.;

2) to evaluate alternative herbicide practices aimed at mitigating damage to non-target plant species;

3) to determine the efficacy of these alternative methods on control of *Centaurea diffusa*; and,

4) to examine a mechanism for the recovery of non-target plant species following picloram damage.

These objectives were addressed using a combination of descriptive, manipulative and retrospective experiments.

2.0 Objective 1: Quantification of Response of Non-Target Plant Species to Picloram

2.1 Short-term Effects

This study has provided evidence of short-term effects on non-target plant species following treatment with picloram at the currently recommended application rate. It has also provided a quantification of the extent of damage that can be expected on 24 plant species common to the bunchgrass grasslands of southern interior B.C.

Picloram applied at the currently recommended application rate resulted in reduced cover of 14 of 24 relatively common non-target plant species 1 year after treatment (Chapter 3). These effects were observed on two sites (three blocks), and in two different treatment years. A number of other plant species may also have been affected but there was insufficient statistical power to test species with sparse distribution.

Six of the affected non-target plant species, primarily in the Asteraceae family, were eliminated after 1 year. Plant species density (a plot-based measure of species richness) declined by one-third following treatment (Chapter 4). Species diversity was reduced by approximately 20%. There was no evidence of plant diversity recovery after 2 years on the same sites.

2.2 Longer-term Effects

The quantification of the longevity of non-target species impacts is less certain. Effects on plant species abundance were longer lasting than the effects on plant diversity. Some evidence demonstrated suppression of *Centaurea diffusa*, *Antennaria dimorpha*, and *Microsteris gracilis* for 5 years on three areas (Chapter 2). *Stipa comata* maintained increased cover for at least 5 years. There was an indication that even longer-term effects are possible on some species. *Centaurea diffusa*, *Calochortus macrocarpus*, and the Asteraceae as a group, remained suppressed 12 years after treatment with picloram at the currently recommended application rate at one site (Chapter 6).

The evidence presented for the long-term effects on some plant species and groups is not conclusive. Inadequate statistical power at the 5- and 12-year sites resulted in an inability to test plant species with sparse distribution. As a result, it was necessary to report statistical significance at $P \leq 0.10$ rather than the standard $P \leq 0.05$ to reduce the risk of accepting the null hypothesis when it is false. These problems occurred because the longer-term research trials used were retrospective in nature and not originally designed to examine responses of non-target plant species. The results of this component of the study, therefore, must be used cautiously. Nonetheless, long-term data in field ecology trials are uncommon and require a tremendous commitment of resources to obtain. Insight into the long-term effects of herbicides has been gained through this component of the study despite the problems.

There was only slight evidence of long-term plant species extirpation following treatment with picloram. However, this result is only applicable to the treatment of small areas because of the small plot size used in the experiment (2 x 11 m). The speed of recovery of an extirpated plant species on a particular site will likely depend on the proximity of the nearest seed source. Therefore, the recovery of an extirpated plant species within larger treated areas generally will be slower than within smaller treated areas. Other factors, such as the availability of viable seed in the soil seed bank, will modify this relationship. The potential for long-term plant species extirpations following the treatment of large areas has not been addressed.

Evidence was provided that plant diversity reductions persist for at least 5 years (Chapters 2 and 6). Recovery of plant diversity appears to occur between 5 and 12 years following treatment with picloram at the currently recommended application rate. In this study, plant diversity declines were longer lasting than reductions reported in other similar studies. There is no doubt that some of the disagreement between the conclusions of the present study and those of previous studies is a reflection of the differences in grazing use, vegetation types and climates examined. The present study has demonstrated that variations in plant diversity responses occur following picloram treatment, even within a relatively homogenous group of research sites with similar ecological classification (Chapter 4). These site-specific responses are likely due to differences in the pre-treatment proportion of picloram-sensitive species. Picloram can be expected to have a greater impact on plant diversity on sites with a greater pre-treatment proportion of picloram-sensitive plant species.

The effect of climate on the longevity of picloram activity in the soil is well known. Precipitation and over-winter temperature have been reported to be major factors determining the degradation rate of picloram in soils. In theory, species diversity should recover faster in climates with relatively higher precipitation and milder winters. Therefore, it is not surprising that differences in the longevity of plant diversity effects have been reported from studies with different plant species composition and climates.

3.0 Objective 2: Evaluation of Methods to Mitigate Damage to Non-target Plant Species

In this component, standard operational practices were compared to alternative practices with the objective of reducing the impact of picloram on non-target plant species.

3.1 The Use of Reduced Application Rates of Picloram

Picloram applied at 0.05 or 0.15 kg ai/ha resulted in less reduction in cover of most plant species than at the 0.55 kg ai/ha standard operational rate (Chapter 3). The low rate (0.05 kg ai/ha) had no effect on species density and the Shannon index of species diversity (Chapter 4). The intermediate rate (0.15 kg ai/ha) was substantially less effective in reducing species density than the standard operational rate, but only marginally less effective in reducing species diversity.

Weed control of *Centaurea diffusa* was not as complete at the reduced application rates. The intermediate rate provided 84% control of *Centaurea diffusa*, 14% less than the standard rate (Chapter 3). The low rate did not provide sufficient control of *Centaurea diffusa* for weed management purposes. Therefore, the 0.15 kg ai/ha rate appears to be a reasonable compromise between *Centaurea diffusa* control and reduced injury to non-target plant species

The study has demonstrated that no species was eliminated following treatment with the two lower herbicide rates, while treatment with 0.55 kg ai/ha picloram resulted in the short-term loss of six species. This may be the most important benefit to using lower rates of picloram. Recovery of a herbicide-sensitive species within a treated plant community is likely to occur more rapidly if scattered residual plants remain. These plants can provide dispersed sources of vegetative and reproductive material. In the absence of these plants, recovery will be slower because plant propagules must then be dispersed from the untreated periphery.

3.2 The Use of an Alternative Herbicide, Clopyralid

Clopyralid was less damaging than picloram to ten of the eleven affected species that showed a response to herbicide type (Chapter 3). Treatment with clopyralid resulted in no effect on species density and less reduction in species diversity (Shannon index) than treatment with picloram (Chapter 4). The vegetation in the bunchgrass grasslands of southern interior B.C. appears to be less susceptible to damage from clopyralid than picloram. It should be noted, however, that the pre-treatment proportion of picloram-sensitive vs. clopyralid-sensitive species will vary at different grassland sites.

3.3 The Use of Alternative Application Dates

Altering the date of herbicide application was an ineffective method of ameliorating herbicide impacts on non-target plant species. The application date of the picloram or clopyralid treatment had no effect on species density or species diversity. Six plant species were sensitive to the herbicide application date, but the response to the treatment date was mixed. This result was expected since most plant species are known to possess unique characteristics, including phenological development. The phenological stages of the 40-50 plant species at the experimental sites are unlikely to be completely synchronous. Therefore, some species will benefit while others are negatively affected by treatment at any one application date. The benefits of alternative dates appear to be minimal, especially considering that late growing season (summer and fall) treatments may allow *Centaurea diffusa* to complete seed maturation after herbicide treatment.

4.0 Objective 3: Efficacy of Alternate Methods on Control of *Centaurea diffusa*

There is evidence that picloram application rates of 0.05 and 0.15 kg ai/ha are effective at suppressing *Centaurea diffusa* densities for 2 years. There is also evidence that clopyralid at 0.15 kg ai/ha provides the same level of *Centaurea diffusa* suppression as picloram applied at 0.55 kg ai/ha, for at least the first 2 years after treatment.

5.0 Objective 4: Examination of a Mechanism for the Recovery of Non-target Plant Species Following Damage from Picloram

The intent of this component of the study was to increase our understanding of plant species recovery in an ecosystem treated with picloram.

Stipa comata is the most likely plant to colonize gaps created by death of other *Stipa comata* plants (Chapter 7). Such self-replacement suggests that *Stipa comata*-

dominated plant communities created by picloram treatment are relatively stable. Additional evidence of the stability of these communities was provided by data showing 5- and 12-year persistence of increases in *Stipa comata* cover (Chapter 6).

Only one native perennial species, *Artemisia frigida*, showed increased seedling emergence near dead *Stipa comata* plants. Although there was a general increase in seedling emergence of other species on treated plots, there was little evidence to suggest that this response will result in increased colonization of picloram-sensitive species. Evidence was provided that, in general, perennial forb seedlings did not initially colonize gaps caused by the death of *Stipa comata*.

6.0 Management Implications

Six management options are considered for the control of *Centaurea diffusa* infestations under each of three different scenarios. Two of the options (5 and 6) are derived from the results of this study.

6.1 Scenario I: Grassland Park Heavily Infested with *Centaurea diffusa*

Should *Centaurea diffusa* be controlled in an area where the maintenance of species diversity is a major objective? If so, what techniques will minimize damage to non-target plant species?

Option 1: No Action

Centaurea diffusa has a detrimental effect on native plant species due to its competitive nature. Once established, this weed can dominate a site. The abundance of *Centaurea diffusa* on untreated control plots demonstrates the tendency for this weed to become dominant (Chapters. 2 - 7). There is little evidence that this weed causes reductions in species richness (measured as species density); however, species diversity can be reduced (Chapter 2).

A decline in the abundance of *Centaurea diffusa* is not likely to occur quickly without management intervention. This was illustrated in an area near the study sites that remains heavily infested with *Centaurea diffusa* despite 25 years of grazing exclusion (Chapter 2).

This option fails to meet both weed management and plant diversity objectives.

Option 2: Biocontrol

Several biocontrol agents are available against *Centaurea diffusa* but successful reductions in weed abundance have yet to be demonstrated. Biocontrol of *Centaurea diffusa* may require 20 years or more, without a guarantee of success (Harris 1984).

This option is likely to have the least direct negative impact on plant diversity. However, this option currently has an unknown certainty of success against *Centaurea* *diffusa* and cannot be expected to produce quick results. As a result, plant diversity declines due to dominance of *Centaurea diffusa* may remain.

Option 3: Manual Control Methods

Manual methods, such as hand-pulling and mowing, are appropriate for weed control in smaller areas; however, these methods are many times more expensive than other methods. In a study examining the relative costs of various methods of *Centaurea diffusa* control, the cost of hand-pulling was $8600 per ha over 5 years compared to $110 per ha using picloram (Woods *et al.* 1996). The expense of this option is a serious limitation to its regular use.

Option 4: Treatment using the Currently Recommended Rate of Picloram

Treatment using the currently recommended rate of picloram in this study caused plant species extirpations for short periods, and resulted in 2- to 12-year reductions in species diversity (Chapters 2-7). The potential also exists for longer-term extirpation of rare, picloram-sensitive plant species if the treatment area is large.

Centaurea diffusa suppression after picloram treatment persisted for 5 years, and perhaps as long as 12 years. This option best satisfies weed control objectives but is the most damaging to plant diversity.

Option 5: Treatment using Lower Rates of Picloram

Lower rates of picloram, applied in the spring, provided at least 2 years of *Centaurea diffusa* control while preventing plant species extirpation and minimizing plant diversity reductions.

Option 6: Treatment using Clopyralid

Spring application of clopyralid at 0.15 kg ai/ha provided at least 2 years of *Centaurea diffusa* control while preventing plant species extirpation and minimizing plant diversity reductions.

Conclusion

It is evident that choices 5 or 6 provide the best balance between protection from the negative impacts of *Centaurea diffusa* and protection from the negative impacts of herbicide on non-target plant species. These two options are more likely to provide quick and successful weed control than option 2. They are also far less costly and they are applicable to larger areas than option 3.

6.2 Scenario II: Privately-owned Rangeland Heavily Infested with *Centaurea diffusa*

What is the best method to control *Centaurea diffusa* in order to provide increased forage production on areas where the maintenance of plant diversity is not a primary objective?

The practice of large-scale herbicide treatment of *Centaurea diffusa* infestations is generally regarded to be uneconomical when based on increased forage values alone (Cranston *et al.* 1983, Griffith and Lacey 1989). These authors concluded that 20 - 30 years would be needed to repay the cost of picloram treatment, while only 7 years of control of *Centaurea diffusa* could be expected.

Options 1 through 3 are not viable methods for providing rapid and relatively inexpensive forage on large areas. Option 2 (biocontrol) may be the only choice in situations where it is uneconomical to treat *Centaurea diffusa* with herbicide.

In circumstances where it is economical to treat *Centaurea diffusa* with herbicide, Option 4 (currently recommended picloram application rate) may be the only viable option. This study, and many other previous studies, have demonstrated that forage grasses increase manyfold following treatment with picloram at the currently recommended application rate. This study has also provided some evidence of long-term suppression (5-12 years) of *Centaurea diffusa*, and long-term increases of *Stipa comata* following treatment. However, Option 4 caused relatively long-term declines in plant species diversity and there remains a possibility of long-term plant species extirpation when large areas are treated. Nonetheless, there is currently little evidence showing significant damage to community function as a result of plant diversity changes at the degree demonstrated in this study.

Although options 5 (lower rates of picloram) and 6 (clopyralid) provided at least 2 years of *Centaurea diffusa* control, it is unlikely that they will provide the same long-term benefits as the full application rate of picloram, due to the expected decrease in soil persistence.

Options 1 through 3 are not viable methods for providing rapid and relatively inexpensive forage on large areas. Option 2 (biocontrol) may be the only choice in situations where it is uneconomical to treat *Centaurea diffusa* with herbicide

6.3 Scenario III: Publicly-owned Rangeland Heavily Infested with *Centaurea diffusa*

What is the best method to limit the spread of *Centaurea diffusa* to uninfested areas (containment) while maintaining the biodiversity objectives required on public land?

British Columbia's *Centaurea diffusa* management program consists of treating satellite infestations and rights-of-way to limit the spread to uninfested areas (containment program). The widespread chemical control of large infestations of *Centaurea diffusa* is not practiced on public land in B.C. Biocontrol (Option 2) is currently being attempted on large infestations. Option 3 (hand pulling) is used on areas where herbicide use is restricted or strongly opposed.

Chemical control is currently the best option for fulfilling the weed containment function. Option 4 may be the best choice for spot treatment of small areas when repeated treatment is infrequent. If relatively large areas are treated, or treatment of the same area is more frequent, then Options 5 and 6 should be considered.

Options 5 and 6 are unlikely to provide the same long-term suppression of *Centaurea diffusa* as Option 4, due to the expected decrease in soil persistence. However, options 5 and 6 do not result in the same declines in species diversity as Option 4.

6.4 Consideration of Picloram-Sensitive Rare Plant Species

In the ideal situation, a complete plant species list should be developed prior to treatment of any natural area with herbicide. This list can be used to predict the impact of the herbicide treatment on the plant community when used in conjunction with information on the sensitivity of each plant species. Additional information on the reproductive capacity and dispersal characteristics of individual species will aid in predicting the longevity of any impacts. In most circumstances, however, this procedure would create an unrealistic workload for rangeland weed managers.

There are some circumstances when the development of a pre-treatment species list may be justified; such as when a significant threat exists for plant species extirpation. The development of a pre-treatment species list would enable weed managers to determine if highly sensitive, rare plant species existed on the area to be treated. If the species of concern occurred on the site to be treated, then Option 5 or 6, or Option 3 may be the best choice.

This strategy requires the development of maps showing the probable distribution of highly sensitive, rare plant species in the management area. These maps could be provided to weed managers who would ensure that pre-treatment lists are developed when treatment areas occur within the known distribution of such species.

7.0 Conclusions

This study has provided the rangeland weed manager with viable choices that will maintain control of *Centaurea diffusa* while reducing damage to non-target plant species. Ideally, an understanding of the total plant community response to herbicides is necessary to avoid unexpected outcomes. This can occur only if pre-treatment plant species lists are developed, and are used in conjunction with information on the herbicide sensitivity of each plant species.

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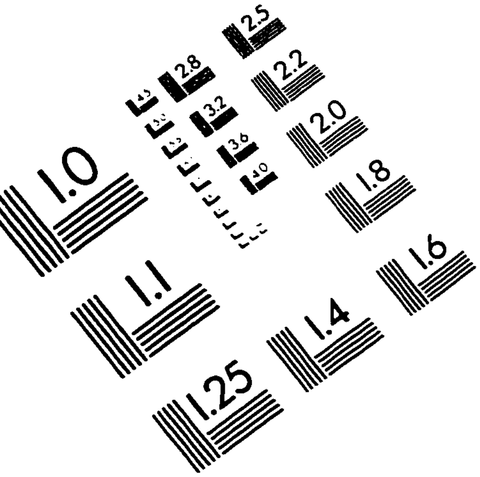
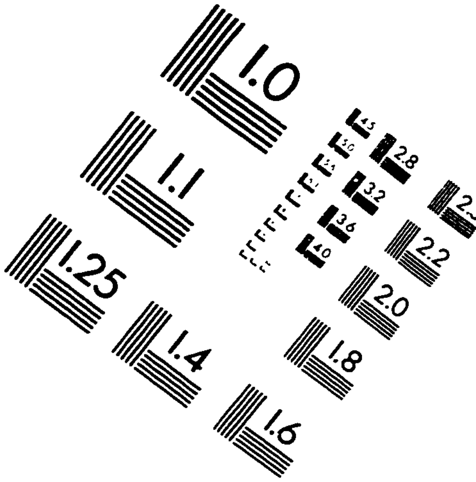
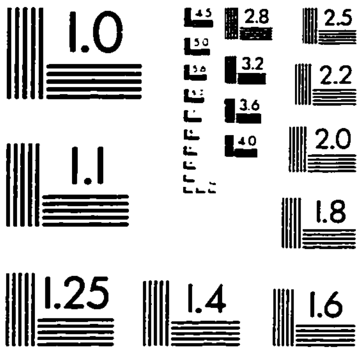
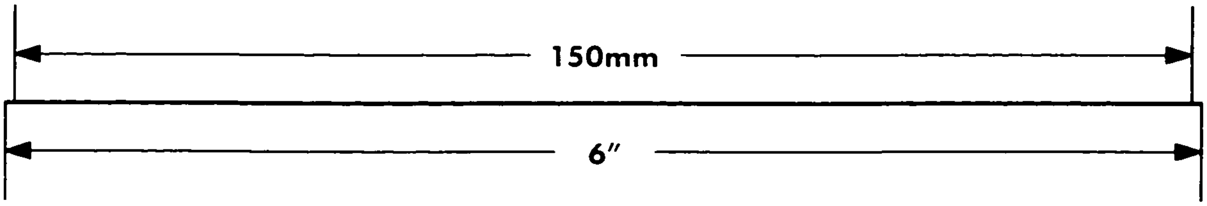
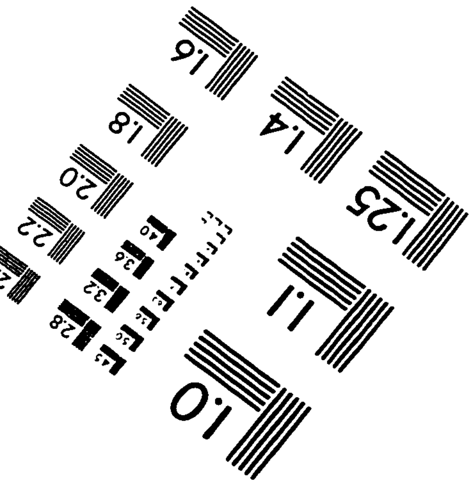
IMAGE EVALUATION TEST TARGET (QA-3)

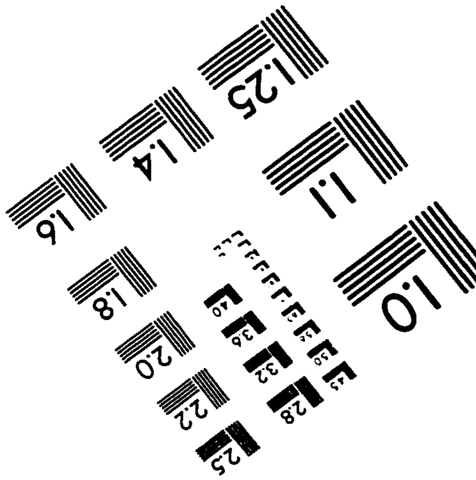












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