Dynamics of leafy spurge (Euphorbia esula L.) infested plant communities influenced by flea beetles in the Aphthona complex (Coleoptera: Chrysomelidae)
by Nikolai Gerard Wiman

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Entomology
Montana State University

Abstract:
Four leafy spurge infested plant communities which were under varying levels of biological control by Aphthona spp. flea beetles were examined in order to gain understanding into how biological control affects plant communities. Very little literature exists as to how introduced natural enemies affect the ecology of the systems to which they are introduced. Four sites were intensively sampled over a two-year period for plant species presence/absence and abundance data, as well as flea beetle densities and species compositions. To better understand the role of the environment in these systems soil was sampled, analyzed and ordinated with species data. Spatial statistics were used extensively to investigate the spatial relationships of all the variables used. To a lesser extent, spatial statistics were used to interpolate sample data to produce contour maps. Other aspects of plant communities were also investigated, including community organization and cover classes.

The results of the study suggest that biological control can at times be considered a disturbance in these plant communities, influencing plant species compositions, species abundances and species richness. However, other factors such as annual precipitation are probably more likely to influence these factors. Biological control worked at different rates at the research sites depending on the environmental conditions as well as the species of flea beetles used. Contour maps indicate that plant species richness tended to be very patchy, and tended to be dynamic between the sampled seasons at the research sites. Plant species richness was often found to be high in areas with moderate cover levels of leafy spurge.

DYNAMICS OF LEAFY SPURGE (*EUPHORBIA ESULA L.*) INFESTED PLANT COMMUNITIES INFLUENCED BY FLEA BEETLES IN THE *APHTHONA* COMPLEX (COLEOPTERA: CHRYSOMELIDAE)

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APPROVAL

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Nikolai G. Wiman

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, english usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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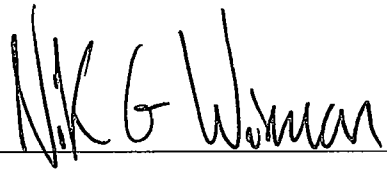
Signature 

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TABLE OF CONTENTS

TABLE OF CONTENTS, CONTINUED

TABLE OF CONTENTS, CONTINUED

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES, CONTINUED

LIST OF FIGURES, CONTINUED

LIST OF CONTOUR MAPS

LIST OF EQUATIONS

ABSTRACT

Four leafy spurge infested plant communities which were under varying levels of biological control by *Aphthona* spp. flea beetles were examined in order to gain understanding into how biological control affects plant communities. Very little literature exists as to how introduced natural enemies affect the ecology of the systems to which they are introduced. Four sites were intensively sampled over a two-year period for plant species presence/absence and abundance data, as well as flea beetle densities and species compositions. To better understand the role of the environment in these systems soil was sampled, analyzed and ordinated with species data. Spatial statistics were used extensively to investigate the spatial relationships of all the variables used. To a lesser extent, spatial statistics were used to interpolate sample data to produce contour maps. Other aspects of plant communities were also investigated, including community organization and cover classes.

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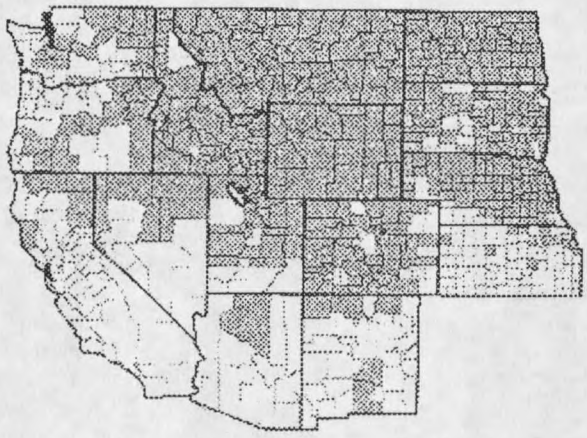
CHAPTER 1

BACKGROUND AND RESEARCH GOALS

Origin and Distribution of Leafy Spurge

Euphorbia esula L. (sensu lato) leafy spurge, is a widespread noxious perennial weed of the family Euphorbiaceae that is invasive on rangelands, grasslands, riparian and wild areas in the United States and Canada (Lajeunesse et al. 1999). Mennonite settlers may have cultivated leafy spurge as an ornamental plant in North America as they were known to do in their native Russia, or may have unintentionally introduced the weed through importation of contaminated seeds or cereal grains (Moore & Lindsay 1952, Best et al. 1980, Dunn 1985). It is also likely that rock and soil discarded onto eastern shores from ship ballasts carried seed or rootstock of *E. esula* to the U.S. (Moore and Lindsay 1952). William Oakes made the first documented collection of leafy spurge in the United States at Newbury, Massachusetts in 1827 (Dunn 1985). Since that time, leafy spurge has spread westward, reaching the western U.S. and Canada by the early 1900's (Lacey et al. 1985). The westward expansion of leafy spurge has been linked to trails used by settlers, railroads as well as waterways, birds, livestock, and distribution routes of contaminated agricultural products such as cereal grains, hay and alfalfa (Best et al. 1980, Dunn 1985). Leafy Spurge now infests approximately 1.1 million hectares of land in the United States (Lajeunesse et al. 1999); approximately 570,609 hectares of land in Montana and North Dakota were infested in 1985 (Lacey et al. 1985). The weed is currently found in every county in Montana, Wyoming and North Dakota (Lajeunesse et al. 1999, see Figure 1).

Figure 1. The distribution of leafy spurge, *Euphorbia esula* in the western United States in 1997. Distribution is based on presence/absence data collected from weed authorities for individual counties. No data were available from several counties (from Lajeunesse et al. 1999).



Ecological Amplitude of Leafy Spurge

Leafy spurge plants can tolerate relatively harsh environmental conditions and occur in both disturbed and undisturbed habitats (Lajeunesse 1999). Leafy spurge has a broad ecological amplitude and the ability to establish in xeric, mesic and hydric environments; sometimes forming pure stands (Nowierski & Zeng 1994, Gassman et al. 1996). The weed is often found in pasture, hayfields, rangelands, forests, badlands, shelterbelts, wildlife management areas and other wildland areas (Bangsund et al. 1993). However, the weed is generally not a problem on agricultural land that is under intense cultivation (Bangsund et al. 1993, Nowierski & Zeng 1994).

Variability of Leafy Spurge

The high degree of phenotypic diversity observed among *E. esula* plants in North America has made the taxonomy of this weed difficult to discern (Shulz-Schaeffer et al. 1987, Nissen et al. 1992). As many as 20 morphologically distinct *Euphorbia* species, subspecies, hybrids, biotypes or varieties have been described from North America by various authorities (Pemberton 1985, Radcliffe-Smith 1985, Nowierski & Pemberton in press). Although as many as 78 biotypes have been described from Eurasia, only 2 species, *E. esula* (sensu stricto), and *E. virgata* (*E. esula* subsp. *tommasiniana* (Bertol.) Nyman), are currently discriminated there (Gassman et al. 1996). North American leafy spurge biotypes are probably a result of one or more hybridization events between Eurasian *Euphorbia* ascensions in view of 1) the high probability of multiple *Euphorbia* spp. introductions (Best et al. 1980, Dunn 1985), 2) the high degree of phenotypic variation exhibited by leafy spurge in North America (Best et al. 1980, Radcliffe-Smith 1985, Crompton 1990), and 3) the broad ecological amplitude of *E. esula* (s.l.), which exceeds that observed in any one Eurasian accession (Nowierski 1999). None of the phenotypic characters used by various authors to identify North American *Euphorbia* accessions have been sufficiently robust to construct a phylogeny of relatedness among the biotypes (Dunn 1985). Other attempts to resolve the taxonomy of the noxious *Euphorbia* spp. have utilized latex triterpenoid and chemical composition (Mahlberg et al. 1987, Harvey et al. 1988 and Torell et al. 1989), epicuticular waxes (Manners and Davis 1984), and cytogenetic analyses (Schultz-Schaeffer & Gerhardt 1987), but were unable to satisfactorily characterize North American leafy spurge biotypes. Restriction

fragment length polymorphism (RFLP) analysis of chloroplast DNA from leafy spurge accessions in North America and Europe indicated that North American leafy spurges are more closely related to each other than to the European accessions that were tested (Nissen et al. 1992). Currently, all exotic and noxious *Euphorbia* accessions and biotypes found in North America are considered to be one highly variable species, *Euphorbia esula* (s.l.) in accordance with Crompton et al. (1990).

Leafy Spurge Life History

From an ecological standpoint, leafy spurge can be considered to be both an early and a late successional plant. Leafy spurge growth begins early in the growing season when shoots emerge from seed and rootstock. Plants continue to develop and proliferate throughout the growing season, flowering and producing seed well into the fall (Best et al. 1980). Root systems of leafy spurge plants are extensive; vertical roots may penetrate the soil to depths up to 9 m, and horizontal roots can extend up to 4.6 m from the parent plant per year (Lajeunesse et al. 1999). As a result, water is less of a limiting factor for leafy spurge than for many other plants. The foliage often remains green and turgid late into the growing season, when most other plants have desiccated or senesced (personal observation 1995-2000). All parts of the plant secrete a milky latex when injured. The latex contains many toxic compounds, some of which had a carcinogenic effect on the epidermal tissues of mice in the laboratory (Upadhyay et al. 1978). The leafy spurge root system is capable of storing energy in the form of nutrients and water for extended periods of time, and removal of the capitulum of the weed through physical or chemical means tends to activate quiescent root buds, thereby stimulating the production

of new shoots (Best et al. 1980). Root buds are present in high numbers on both horizontal and vertical root systems. Buds arising from vertical root structures serve to perennate the parent plants, whereas root buds arising from the horizontal root structures contribute significantly to patch expansion (Best et al. 1980). Long-term control of leafy spurge cannot be achieved without implementing management strategies that negatively impact the root system, due to its extensive and resilient nature.

Leafy spurge seeds are born from a pod containing three seeds, which develop in a cyanthium (Best et al. 1980). On average, leafy spurge plants produce 140 seeds per season (Lajeunesse et al. 1995). Seeds are released explosively from the parent plant and are capable of traveling distances of up to five meters (Best et al. 1980). Fruiting leafy spurge plants that are proximal to waterways can take advantage of currents and periodic flooding to disperse their buoyant seeds (Lajeunesse et al. 1999). Most seed germination occurs within the first two years of dehiscence, although seeds that are buried deep in the soil can remain viable for 8 years or longer (Lajeunesse et al. 1999). Leafy spurge seeds landing in exposed mineral soil are 45 times more likely to germinate than seeds landing in undisturbed vegetation (Best et al. 1980). Seeds are spread by vehicles, animal hair and to some extent by feces from birds, whitetail deer, goats and sheep (Lajeunesse et al. 1995).

Threats to Species Diversity

Ecological repercussions of leafy spurge invasion include, but are not limited to, displacement of native plant species, loss of habitats, and loss of forage for wildlife and livestock (Belcher & Wilson 1989, Nowierski & Zeng 1994). Belcher and Wilson

(1989) found that native plant species richness decreased significantly in leafy spurge infestations on mixed grass prairies in Manitoba, Canada. Habitat loss from leafy spurge infestation is attributed to native plant species displacement, loss of plant structural diversity, and displacement of native microfaunas critical for nutrient cycling (Lajeunesse et al. 1999). The western prairie fringed orchid (*Platanthera praeclara*), a listed endangered plant species of North Dakota grasslands, is now facing extinction due to habitat loss (Sieg & Bjugstad 1992). The displacement of native plant communities by leafy spurge is an indirect threat to several rare and endangered grassland butterfly species including the Dakota skipper (*Hesperia dacotae*), the ottoe skipper (*Hesperia ottoe)*, and the powesheik skipper (*Oarisma garita*) (Lepidoptera: Hesperiidae), which depend on specific native plant species for pollen (Martin 1994, Opler et al. 2001). Many bird species are specialized on the native flora of grasslands as well. One bird species of special concern in Montana and North Dakota is Baird's sparrow (*Ammodramus bairdii*) (US Fish & Wildlife Service 2001). Leafy spurge currently threatens the integrity of many of the habitats of this bird species, and breeding populations are declining drastically with the disappearance of native grasslands (Martin 1994, US Fish & Wildlife Service 2001). Attempts at managing leafy spurge infestations using herbicides may further degrade habitat quality for these sensitive species by selecting against broad-leaved plants, or otherwise altering natural plant species compositions and successions (Maxwell & Fay 1984, Butler 1994, Martin 1994).

Economic Costs Associated with Leafy Spurge Infestations

In recent years efforts have been made to quantify the monetary losses associated with leafy spurge infestations (see Leistritz et al. 1992, Bangsund et al. 1993, Leitch et al. 1994). In the states of North Dakota, South Dakota, Wyoming and Montana, total economic losses from leafy spurge in 1993 were estimated at > $129.5 million from a total estimated 657,435 ha of infested land (Leitch et al. 1994). Estimated impacts were based on lost revenue from the ranching industry, lost revenue from recreational use of wild lands, and loss of soil and water conservation benefits. In Montana, total direct impacts from leafy spurge infestations on wild lands alone were approximately $ 465,000 in 1992 (Bangsund et al. 1993). Leafy spurge is clearly a serious economic threat to the economy of the western United States.

Grazing and Leafy Spurge

Due to the presence of triterpenoids and other compounds contained in the latex, leafy spurge is largely ignored or avoided by wildlife, horses and cattle, likely due to irritating gastrointestinal effects caused by the toxic compounds in the latex (Walker et al. 1994). Sheep and goats do graze on leafy spurge, and have been used in management strategies with some successes. Although sheep generally do not prefer to eat leafy spurge, they can reduce its density in some range situations (Faller 1994). Angora goats can provide excellent control of leafy spurge since they preferentially select the weed from other available forage (Hanson et al. 1993). Leafy spurge infestations can reduce rangeland carrying capacity for cattle to near zero since cattle avoid grazing in areas with

a 10% to 20% cover level of the weed (Best et al. 1980). Where cattle are grazed on low-density leafy spurge infested rangelands, leafy spurge densities may increase due to detrimental impacts on competitive grasses caused by selective grazing (Lajeunesse et al. 1999).

Chemical Control of Leafy Spurge

Traditionally herbicides have been used to control leafy spurge, with some success on small infestations (Alley & Messersmith 1985, Lym 1998). Long-term chemical control on small infestations can be achieved only through diligent, repeated herbicide applications that are timed to prevent seed production and slowly degrade the root system (Lym 1998, Lajeuneese et al. 1999). Chemical control of small infestations can reduce the rate of spread of leafy spurge, since small patches expand in range much more rapidly than large patches, and are most easily treated with an herbicide (Best et al. 1980). However, herbicide management of large-scale infestations is extremely expensive, given the high cost to benefit ratios typical of the marginal lands leafy spurge tends to invade (Bangsund et al. 1996). Intensive herbicide use is not an option for many land managers, who may have justified concerns about the possibility of environmental contamination, development of plant resistance, and ecological degradation (Bangsund et al. 1996, Gassman et al. 1996). One commonly recommended herbicide for leafy spurge control is picloram (4 amino-3, 5, 6-trichcloropicolinic acid, Tordon® or Pinene®), which has been shown to negatively impact forb species and overall plant species diversity on rangeland plant communities in Montana (Maxwell & Fay 1984). For any

large-scale leafy spurge infestation where conservation is a land management goal, exclusive reliance on herbicides is not a valid management strategy.

Biological Control With Flea Beetles in the *Aphthona* Complex

Fleabeetles in the family Chrysomelidae, subfamily Alticinae, are a widely distributed group of leaf beetles best characterized by their stout hind femora and impressive jumping ability. The Alticinae are exclusively phytophagous, and many species are crop pests of worldwide importance (Konstantinov & Vandenberg 1996). The genus *Aphthona* Chevrolat (Coleoptera: Chrysomelidae) contains more than 500 described species (Konstantinov 1998). Associations between *Aphthona* species flea beetles and *Euphorbia* species host-plants are common, more than 40 such associations have been found in Eurasia (Harris et al. 1985). Eurasian *Aphthona* species that showed promise for classical biological control of leafy spurge in North America were subjected to appropriate host specificity testing at the International Institute of Biological Control, European Station in Delémont, Switzerland and the USDA Agricultural Research Service, European Biological Control Laboratory in Montpellier, France. Out of all of the *Aphthona* species involved in host specificity testing, six species received approval for introduction into the United States as natural enemies of *E. esula* (Gassmann et al. 1996, Hansen et al. 1997). During the years of 1988-1996, the United States Department of Agriculture, Plant Protection and Quarantine, Animal and Plant Health Inspection Service (USDA-APHIS-PPQ) coordinated the release and redistribution of approved *Aphthona* spp. in 188 counties in 19 states across the continental United States (Hansen et al. 1997). Five *Aphthona* species are now established to varying degrees in the western

United States, including *A. cyparissiae* Koch, *A. nigriscutis* Foudras, *A. flava* Guillebeau, *A. lacertosa* Rosenhauer, and *A. czwalinae* Weise. One other species, *A. abdominalis* Duftschmidt was also approved and released, but to date has not established in North America (Hansen 1997, Nowierski personal communication 2001). *Aphthona* population sizes and impacts on leafy spurge vary considerably among sites due to preferences of *Aphthona* species for specific environmental conditions, habitat types and *Euphorbia* biotypes (Gassman et al. 1996, Nowierski et al. 1996, Hansen 1997, Nowierski 1999).

Aphthona Species Life History

All five flea beetle species released and established in North America (*A. cyparissiae*, *A. czwalinae*, *A. flava*, *A. lacertosa* and *A. nigriscutis*) are univoltine, vary in length from 3 to 4 mm, and have a host range restricted to the genus *Euphorbia*, subgenus *esula* (Gassman et al. 1996). Adult flea beetles live for 6-12 weeks, during which time they damage leafy spurge plants by feeding on the foliage and the flower bracts. Adults often aggregate on leafy spurge plants, possibly for the purpose of mating. Impacts from herbivory appear to be most concentrated on these aggregation sites (personal observation 1995-2000). Extensive feeding by adult *Aphthona* spp. on leafy spurge plants can result in desiccation in the above ground plant structures, and reduced seed output.

Most of the impact on leafy spurge plants caused by flea beetles is attributed to feeding by the larvae, which damage leafy spurge plants by depleting stored energy in the root structures. The larvae have three instars, which are found on leafy spurge roots where they actively feed for at least 72 days before pupation, although some larvae may

feed for as long as four months (Gassman et al. 1996). First instar larvae hatch late in the growing season from eggs oviposited into the soil around the base of leafy spurge shoots. Most larvae are found within 7.6 cm of the soil surface, where they congregate at feeding sites on root structures of 1-4 mm diameter (Brinkman & Clay 1998).

Risks and Conflicts of Interest

The biological control program against leafy spurge is not without risks and conflicts of interest. The plant family Euphorbiaceae is well represented in North America, and concern regarding possible non-target feeding by leafy spurge biological control agents has been expressed for at least two native annual plant species, *Euphorbia purpurea* Fernald, and *Euphorbia telephiodes* Chapm., both of which are considered rare (Pemberton 1985). However, *Aphthona* species are probably not a serious threat to populations of these plant species for several reasons: 1) only very low levels of feeding on *E. purpurea* and *E. telephiodes* by any of the approved *Aphthona* spp. were observed in laboratory starvation tests (Gassman et al. 1996), 2) low selection for host switching is expected at North American release sites since the preferred host plant, *E. esula* is likely to be abundant at release sites, and will never be eradicated by biological control alone (see Driesche & Bellows 1996), and 3) *Aphthona* spp. require the persistent root structures found only in the perennial *Euphorbia* species for larval development (Maw 1981, Gassman at al. 1996).

Two economically important *Euphorbia* species cultivated in North America, *E. pulcherrima* Willd. (poinsettia), and *E. antisyphilitica* Zucc. (candelilla plant), are not acceptable hosts for *Aphthona* species (Gassman et al. 1996). One naturalized and

potentially economically important *Euphorbia* species, *Euphorbia lathyris* L. (caper spurge), is an accepted host plant for most leafy spurge insects and has been suggested as the primary candidate for a renewable oil resource in the United States (Harris et al. 1985). However, extraction of hydrocarbons from *E. lathyris* is costly, relatively inefficient, and consequently this industry has not been adopted (Harris et al. 1985).

The Need for Ecological Research in Biological Control

Although the biology and life history of host plants and associated natural enemies are well researched for many biological control projects, relatively few research efforts have been undertaken that pertain to the post-release indirect ecological effects of natural enemy introductions, except where projects are associated with controversy and/or severe conflicts of interest. For example, *Rhinocyllus conicus,* the seed head weevil first released in the United States in 1968 for the biological control of musk thistle (*Carduus nutans*), has been shown to feed on a number of native thistles, some of which are considered rare. As a result of the widespread concern regarding the effects of the weevils, direct and indirect ecological effects of *R. conicus* have been well researched (see Reese 1977, Louda et al. 1997, Strong 1997, Louda 1998). However, post-introduction ecological research is the exception rather than the rule. Aproximately 6,000 natural enemies have been released against invertebrate pests, and upwards of 1,000 releases have been made against exotic weeds in the U.S. (Hopper 2001). An inquiry into the literature will yield very little information pertaining to the ecological effects of those introductions. Lack of ecological research on post-introduction biological control of weeds projects is largely attributed to a disparity in collaborative efforts between

entomologists and plant ecologists (Waage 2001). Often, biological control research is funded by land management groups whose main interest is to reduce a given pest population with a natural enemy, with little regard for the ecological effects of the introduction. Consequently, little monetary support for post-introduction ecological monitoring is available. There is a pressing need for studies pertaining to the indirect effects of biological control, especially as biological control programs multiply in response to the advent of new invasive species (Waage 2001). Ecological impact studies have the potential to teach bio-control practicioners valuable lessons that can effectively improve the safety, predictability, precision, and risk to benefit ratios of future biological control programs (Hopper 2001). Thus, the integrity of the practice of biological control of weeds can be maintained, and bio-control can remain available as a tool for future weed management projects.

Research Objectives

The goal of this study was to investigate the indirect effects of biological control of leafy spurge by *Aphthona* spp. on plant ecosystems at the community level. Effects of bio-control were investigated by sampling leafy-spurge infested plant communities with established populations of flea beetles, as well as communities with no or very low leafy spurge cover, in a variety of environments. It was hypothesized that biological control would influence aspects of plant species richness, abundance, productivity, spatial structure and community organization. Efforts were made to gain understanding into how environmental variables may affect plant community responses. The study took place on four sites in Montana and North Dakota during 1999 and 2000. All data were

collected along *Aphthona* spp. impact gradients at USDA-APHIS-PPQ release sites using a system of transects and quadrats. Analyses used in the study to explore ecological relationships of flea beetle biocontrol sites included plant cover analysis, plant species richness analysis, isozyme analysis, canonical correspondence analysis, similarity cluster analysis, species rank-abundance analysis, and geostatistical spatial analysis.

CHAPTER 2

SAMPLING MATERIALS AND METHODS

Research Site Selection and Establishment

Primary research sites were chosen on the basis of several criteria needed to satisfy the needs of the project. Site selection criteria included the availability of release data (including numbers and species of *Aphthona*), as well as permanently marked release points. USDA-APHIS-PPQ bio-control release sites meet these criteria and were selected for this study. The goal was to sample along the plant gradient that is observed when flea beetles impact a patch of leafy spurge by progressing outward from the point of release into the patch. These patterns occasionally occur, since *Aphthona* spp. larval and adult impacts on leafy spurge are the result of aggregated feeding behavior. By sampling transects along these impact gradients, the goal was to collect plant data from a full spectrum of *Aphthona* impact levels and plant successional stages within each site. Regions of reduced leafy spurge cover and/or stunted leafy spurge plants surrounding an *Aphthona* release point within a patch of leafy spurge were assumed to indicate this type of impact, since it was assumed that the range of vigorous leafy spurge plants extended to the stake at the time that flea beetles were released. Therefore, the research sites selected for this study all showed low cover levels of leafy spurge near the release stakes. Four such sites were chosen in Montana and North Dakota. The sites that were chosen all had observable differences in plant species and flea beetle species compositions, and were

selected with the goal of maximizing representation of a variety of environmental types, and plant and flea beetle species compositions.

Transect Establishment

The original intention was to select the direction of one transect randomly from the release point and base all five subsequent transects at a consistent angle from the randomly selected transect. However, this proved impractical for three of the four sites for two reasons; 1) flea beetle impacts tended to occur in a predominant direction, 2) steep banks, rock ledges and fences occurring in the intended sample areas constrained the placement of transects. Therefore, the area of the plant community that could be feasibly sampled using six transects with twelve quadrats each (see sample size and plant sampling sections below) was often restricted. The edges of the site were made as wide as possible using bearings from a compass taken from the release point, given the constraints mentioned. The maximum angle from edge to edge was calculated and divided by six to accommodate the six transects with a 3° buffer zone from lateral transects to the visible edge of the spurge patch on each side, so that the sampled area was conical (Appendix E). The length of each transect was approximately equal to the average distance from the release point to the edge of the spurge patch across the whole sample area. For each transect at a given site one end of the transect measuring tape was always secured to the permanent release stake, and the other end was secured to one of six permanent rebar stakes that were installed at the end of each transect. Quadrat spacing was determined by dividing the total length of transects by 12, so that each

transect had twelve regularly spaced quadrats. One leg of the frame was used to align the frame at each determined quadrat location, in a parallel orientation to the transect tape. In this manner the same quadrat locations were sampled in both field seasons.

Control Transects

Three control transects were established for each primary site per season. Control transects were in all cases located near the primary research sites. These were selected under a different set of criteria than the primary transects. Their primary purpose was to provide a basis for a plant community composition and organization comparison with the primary research sites. In the first field season (1999) areas near the site (within 50 meters) that were devoid of leafy spurge infestation, or represented the lowest cover of leafy spurge within the vicinity were selected. These areas were also selected on the basis of having a similar aspect and slope as the primary site. A starting sample point was then randomly selected by tossing a sledgehammer into the area over my shoulder. The hammer itself was used as the origin, while the handle was used to orient the direction of transects. Although three, rather than six transects were established, they were positioned in the same radiating manner from the random sample point, conserving the same transect length, angles, and quadrat spacing used in the primary site. In the second field season (2000), one transect was randomly located proximately to three sides of the sampled primary sites. This different methodology was implemented to investigate potential edge effects on species compositions and organization.

Plant Sampling

Plant sampling was accomplished using a specially designed pin frame. Originally developed as an alternative to visual estimation of plant cover, pin sampling has also been used by biologists to quantify plant structural diversity, species richness in plants, and species richness in cryptogamic soil crusts (Southwood et al. 1979, Greig-Smith 1983, Magguran 1988, Memmott et al. 1998, Peters & Shaw 1996). The basic principals of pin sampling are as follows: a sample area of finite size within a given plant community of interest may be completely covered, partially covered, or not covered at all by projections of plant biomass within the sample area. If the area of interest is of finite size, it may be thought of as a matrix of thousands or even millions of points, which are either covered or not covered by plant biomass (Magurran 1988). The more a sample area is decreased to approach the size of a point, the likelihood of the sample area being either completely covered or bare increases. If the sample is reduced to an infinitely small point, it will *always* be either covered or not cover by plant biomass. It is impossible to sample an infinitely small point, but pin samples serve as an approximation.

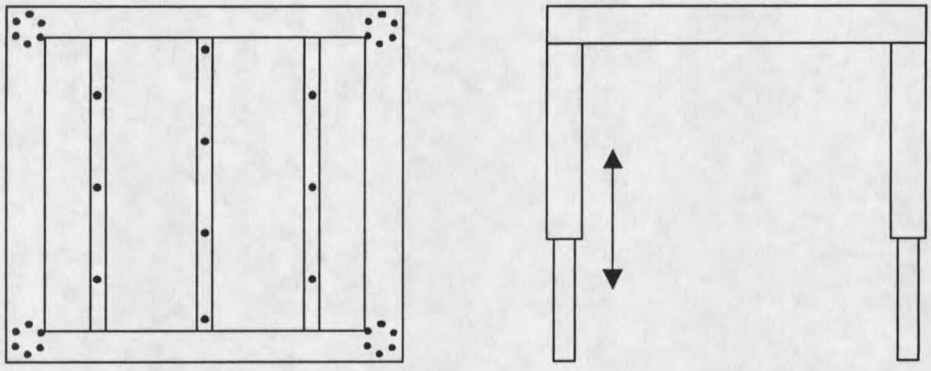
For this study, a 45 cm by 45 cm PVC pipe frame with three 1.27 cm copper cross-tubes equipped with holes to accommodate 10 pins was used as the sampling quadrat (Figure 2). Spacing of holes for the pins were arranged to maximize their independence within the area of the frame, relative to average leafy spurge canopy diameter. Telescoping legs allowed the frame height to be manipulated so that pins were always inserted perpendicular to the plant canopy, and the frame height was always

above the plant canopy. Adjusting the legs independently allowed the frame to stay level when sampling on slopes. A carpenter's level was installed on the frame to allow monitoring of frame orientation. Pins were approximately 2.4 mm in diameter, and made of hard copper. The same pin was inserted sequentially into each hole, and used throughout each site, although pins sometimes had to be replaced if they became bent or damaged.

Plant species richness and plant cover was sampled once at each site in 1999 and 2000 at the approximate time of maximum green standing crop. At each determined sampling location, the pin frame was aligned parallel to the transect tape, using one leg to plant it in the correct position. The frame was maintained as level as possible, so that pin trajectories were perpendicular to the plant canopy. Often, variation in slope and plant canopy height required compensatory adjustments to the length of one or more of the adjustable legs of the frame in order to maintain a consistent level orientation throughout the sampling of sites. As pins were lowered sequentially through the numbered holes, each plant species that intercepted the pin on its normal trajectory toward the soil surface was recorded on data sheets using a two-character code. A reference specimen was then collected from a nearby area, labeled with the appropriate code and carried for reference purposes. After sampling of a given site was completed, each species represented in the portable reference collection was collected, labeled and pressed for later identification. Obstructions occasionally prevented pins from reaching the soil surface. This occurred most often when horizontally oriented broad-leaved dicots such as *Balsamorhiza saggitata* blocked the pin's trajectory, or quadrat locations required frames to be located

over dense woody shrubs such as *Artemesia tridentata.* When this occurred, pins were allowed to remain in place and were not forced to the soil surface.

Figure 2. Top and side view of the pin-frame used for the study showing the locations of the holes in the copper tubing for pin insertions (top view), and the telescoping legs (side view).



Plant Identification

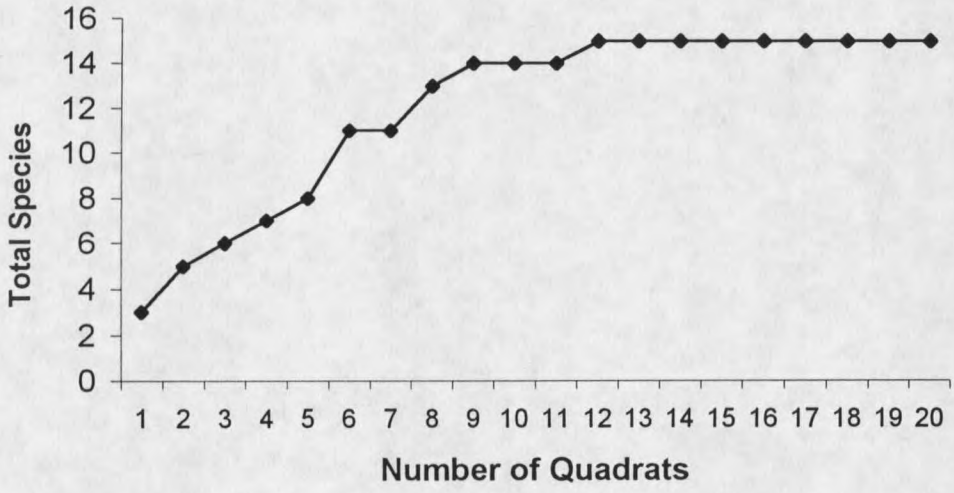
Several floral references were used to key out and cross-reference plant species including Grass Flora of Montana (Rumley & Lavin 1999), Vascular Plants of Montana (Dorn 1984), Illustrated Flora of the Northern United States and Canada (Britton & Brown 1970), and Key to The Grasses of the Pacific Northwest Based Upon Vegetative Characters (Hitchcock 1969). Plant specimens in the study collection that could not be positively identified by the author were identified with the assistance of C. E. Seibert and M. Lavin (Herbarium, Montana State University, Bozeman). Several plants, which were immature or otherwise not represented in the flowering stage, could not be positively identified. Other resources were used to determine whether each species in the study

collection was native or exotic to North America (Looman & Best 1979, Dayton 1988, Whitson et al. 1992, see Appendix A).

Determination of Sample Size

Peilou's pooled quadrat technique (Magguran 1988) was used to determine the approximate sample size necessary to encounter most species present in a xeric leafy spurge infested plant community using the pin frame and a system of transects. A xeric site with a patchy distribution of leafy spurge and an established population of flea beetles was sampled for this analysis. The pin frame was used in multiple random transects to record the species encountered. The analysis indicated that for this particular site (Greycliff, MT), and probably for other xeric leafy spurge-infested sites, most plant species in the leafy spurge infested environment would be encountered by 12 frames (Figure 3). However, few plant species were found at this site (s =15), and more species were thought likely to exist in more mesic leafy spurge infestations. Thus, a 12 quadrat sample size (120 pin) would likely prove inadequate for capturing the majority of plant species in environments with higher plant species richness. Furthermore, a much larger sample size was thought appropriate in order to achieve an adequate estimate of plant species richness for a variety of environmental types, and to provide adequate samples for statistical analyses, especially for spatial interpolation. Therefore, 72 quadrats (720 pins) arranged into six transects were sampled for species richness at each primary research site. Since spatial interpolation was not an objective for the control sites, 36 quadrats were positioned within three transects near each of the four study sites.

Figure 3. Peilou's pooled quadrat method calculated for Greycliff fishing access approximately 32 km WNW of Bozeman, MT. The point at which the curve flattens out shows the approximate sample size necessary to encounter most species in the leafy-spurge infested environment.



Soil Sampling

The upper soil horizon (depth ≅ 10.2 cm) was sampled next to alternating quadrat positions, and at the release stake so that 36 total samples were collected per site. Each soil sample was a composite of six sub samples collected from an area roughly equal to the size of the sampling frame (≅ 2,025 cm^2). Half of the samples (19 per site) were sent to the MSU Soil Analytical Laboratory (Land Resources and Environmental Science Department, Bozeman, MT) to be dried, ground and analyzed. Analyses were selected that were thought likely to contribute to the variability in an ecological data set (Table 1).

Table 1. Soil properties analyzed and their hypothesized relevance to plant community interactions in the study.

Abiotic soil factor	Classification	Role in plant physiology/ ecology
K mg/Kg	macronutrient	protein synthesis, H_20 balance
Mg mg/Kg	macronutrient	enzymatic activity, component of chlorophyll
% $CaCo_3$	mineral	affects soil pH, breaks down into CO_3 and Ca^{++}; important for cell structure, enzymatic activity, organic compounds
Fe mg/Kg	micronutrient	enzymatic activity, component of cytochromes
% C	macronutrient	major component of all organic compounds
% organic C	org. nutrient	correlated with total organic matter in soil, nutrient potential
% N	macronutrient	component of nucleic acids and proteins; plays an important role in community organization and succession; [1] plays a role in leafy spurge adventitious root bud development [2]
% sand, silt, clay	substrate	effects nutrient leaching, habitat suitability for plant species
P mg/Kg	macronutrient	component of ATP, nucleic acids
1/3 & 15 bar % H_20	soil property	ability for soils to hold H_20; effects habitat suitability for plant species

[1] (Tilman 1984), [2] (Best et al. 1980)

Flea Beetle Sampling

Adult flea beetles were sampled along transects once in both field seasons at the primary research sites using a sweep net. Each transect was divided into three equal sized zones, and ten sweeps were made over the vegetation that had been previously sampled for plant species richness. Flea beetle adults from the sweep net samples were identified as *A. lacertosa, A. czwalinae*, *A. flava* and *A. nigriscutis/cyparissiae* using morphological characters (see also isozyme analysis below).

Larval/pupal *Aphthona* spp. densities were sampled early in the 2000 field season using a golf-hole cutter according to Brinkman and Clay (1998) and Mundal (personal communication, 2000). Six soil cores were collected at alternating quadrat positions along transects, so that 36 total cores were collected at each site. Samples were taken at the crown of the nearest neighboring leafy spurge plant adjacent to each sample location. Each core sample captured approximately 400 cm^2 of soil, roots, and organic debris from the upper 12 cm of the soil horizon. Individual cores were sealed in cardboard containers with collection vials attached to the tops. Adult beetles were brought into the laboratory and allowed to emerge into the collection vials over a period of six weeks.

Isozyme Starch-Gel Electrophoresis of Flea Beetle Species

Morphological characters alone can be used to reliably identify *A. flava* due to their large size and copper color, although the distinctive color is lost after freezing (personal observation). The two black species, *A. lacertosa* and *A. czwalinae* are distinguished only on the basis of the color of the hind femur and morphology of the

genetalia. As a result, identification of these species can be difficult and time consuming, and *A. lacertosa* and *A. czwalinae* have often been released as mixed populations (Hansen et al. 1997). Furthermore, hybridization has been reported under laboratory conditions between *A. czwalinae* and *A. lacertosa* (Mundal & Carlson 1994). Although 10 % of the hybridized eggs survived, it is not known whether they developed into fecund adults, or whether hybridization occurs under field conditions. The two brown colored flea beetle species, *A. nigriscutis* and *A. cyparissiae*, are morphologically indistinguishable from each other. Nowierski et al. (1996) and Nowierski & Fitzgerald (unpublished data) found that all five species are genetically distinct, and can be positively identified using isozyme starch gel electrophoresis. Although isozyme electrophoresis is not a direct genetic analysis, genetic variability can be explored indirectly through isozymes, since isozyme-allozyme proteins are synthesized indirectly from DNA through the process of translation and transcription (Pasteur et al 1988). Isozyme analysis was undertaken in this study in order to positively identify the flea beetle species at the research sites, to determine their relative frequencies and densities and to attribute direct and indirect impacts of flea beetles to specific species.

Isozyme Methodology

Adult flea beetles used for isozyme analysis were collected from the sites using a sweep net. Fifty sweeps were made in the general area around the site, but not within the sampling area itself. Beetles were kept alive in containers until they could be frozen and stored at –60.22° C. Beetles were randomly selected from the containers and then individually ground on ceramic plates using three drops of extraction buffer (HCL

solution). The resulting slurry from each beetle was soaked into two filter paper wicks, so that each individual could be electrophoresed simultaneously on two potato-starch gels (11.5 % starch, #S-4501 Sigma), each using either a lithium hydroxide (LiOH, "R") or a tris-citrate (TC pH 8.0) buffer system. Eighteen unknown beetles from each site were run against four to five known species on each gel. Known species were from populations previously identified using isozyme analysis by Bryan Fitzgerald (Department of Entomology, Montana State University). Both gels were run under refrigeration at approximately 9 V for 5-7 hours and then cut into 1.2 mm slices. Each slice was individually stained for one of six enzymes (see Table 2 for enzymes and buffer systems). Gel slices were then incubated at 37° C until stained bands were readily apparent.

Table 2. Isozyme stains, buffer systems and number of loci used in the analysis. Recipes for stains and buffers were modified from Pasteur et al. (1988).

Enzyme system	Abbreviation	No. loci	Buffer system
glucose phosphate isomerase	GPI	1	TC pH 8.0
isocitrate dehydrogenase	IDH	2	TC pH 8.0
malate dehydrogenase	MDH	2	TC pH 8.0
mannose-6-phosphate isomerase	MPI	1	LiOH ("R")
aspartate amino-transferase	AAT	1	LiOH ("R")
α- and β-Esterase	EST	1	TC pH 8.0

CHAPTER 3

QUANTITATIVE METHODOLOGY

Plant Species Richness, Cover and Productivity Methods

Plant species richness was calculated as the total number of species encountered by pins in a given quadrat or site. Cover estimates were based on the number of encounters of plants for a given cover class with the sample pins divided by the total number of plant encounters at the given resolution (i.e., site or quadrat). Total cover estimation calculations regard the percentage of the total number of plant encounters composed of each cover class. Likewise, percent cover of native species reflects the abundance of native species with regards to total plant encounters. The sign test was used to test the null hypothesis that there were no differences between the cover estimates for the samples paired between 1999 and 2000 (i.e., repeated measures on richness and cover classes). The sign test was chosen over parametric methods such as the student's t-test because the sign test is insensitive to autocorrelation, which is a common problem in any data taken from linear transects (Sokal & Rohlf 1981, Stohlgren et al. 1998; see results).

One issue with vertical pin samples is that the quantity of plant biomass has an effect on the frequency of pin encounters. With higher levels of plant biomass, encounters of plant biomass with sample pins are more frequent. Conversely, at lower levels of plant biomass pin encounters with plant biomass are less frequent. Therefore, the total number of plant interceptions with sample pins was used as a measure of plant

productivity in the study. However, for reasons discussed below, this measure of productivity is relatively subjective and is used only for comparison of sites between years.

Aspects of the data taken directly from or derived from vertical pin samples are subject to some degree of error, since plants add biomass in different spatial dimensions. Plants that add biomass predominantly in the horizontal plane would intercept pins, which are oriented vertically, more often than plants with vertically oriented biomass. As a result, the opportunity for intercepting a vertical sampling pin is not equal for all plant species. However, this problem pertains mostly to analyses regarding species abundances (i.e., % cover & productivity), rather than plant species richness, since the likelihood of encountering species with a pin at least one time is more equal among species, assuming that an adequate sample size has been taken.

Plant Species Distribution Model Fitting

Preston (1948) was among the first to observe the tendency of species/abundance distributions from a sampled community of related taxonomical units to consistently fit a log family of distributions. This discovery, called the canonical lognormal hypothesis, is perhaps one of the most empirically supported principals in ecology (Sugihara 1980). Preston generated log series curves by plotting the abundance of Lepidoptera species caught in light traps using $\log_2$ along doublings (octaves or abundance classes). In such analyses the first octave is the log of the abundance of species represented by one individual, the second octave is the log of the abundance of species represented by 2-4

individuals, the third by 4-8 individuals and the fourth by 8-16 individuals etc., until every species abundance is accounted for in a given community (Preston 1948). The logarithmic scale is applied to species abundances because population responses to environmental changes tend to be geometric rather than arithmetic (Hughes 1984). Although Preston (1948) used Log_2, any log base variant is valid (Magurran 1988). Lognormal distribution curves are most commonly observed in ecological studies where sample sizes are large enough to represent most species, and taxonomically related species are sampled (Whittaker 1975, Sugihara 1980, May 1981). Lognormal community distributions contain a large number of rare species, are dominated by a few abundant species, and have a low number of very abundant species. Other observed species distributions include the geometric series, the log series, the broken-stick series and the truncated log series (Magurran 1988).

Considerable debate exists as to whether there is a basis for direct biological interpretation for the observation of lognormal and other distributions among species of various communities, or whether such distributions are statistical artifacts that offer indirect biological interpretation only (MacArthur 1957, Whittaker 1972, May 1975, May 1980, Sugihara 1980, Ugland & Gray 1982, Magurran 1988, Hughes 1986, Lande 1996). MacArthur's (1957) broken stick hypothesis, Peilou's (1975) resource opportunity model and Sugihara's (1980) sequential breakage hypothesis are all attempts to attach biological meaning to some commonly observed distributions. However, since such hypotheses are often regarded as speculative, and since they are difficult to prove, none have gained widespread acceptance (Routledge 1980, Hughes 1986). Thus the debate remains largely unresolved. May (1981) and Ugland & Gray (1982) have argued convincingly

that a lognormal distribution is expected to occur in any sampled community that is in a dynamic equilibrium state, which is typical of climax communities. In a large group of species, many independent factors such as emigration, immigration, competition, trophic level interactions and abiotic environmental variables all contribute to the variation in the relative abundances of species. When a community is in a dynamic equilibrium state, interaction between these factors is expected to be complex to the point where interaction results can be considered random multiplicative products. When the Central Limit Theorem is applied to any heterogeneous data assemblage that is a product of complex, random interactions, a lognormal distribution is expected (Whittaker 1972, May 1975, May 1980). This argument is a statistical one, and it holds not only for ecological data, but also for other large data sets; including the distribution of wealth in the United States, and GNP among nations of the world (May 1981).

Conversely, if a dynamic-state equilibrium plant community is disrupted by a significant disturbance, interactions between variables influencing species abundances may simplify (Whittaker 1972). Individual plant species are disproportionately affected by disturbances since they are unequally adapted to survive under conditions imposed by the disturbances (Whittaker 1965, Tilman 1985). Some plant species would therefore be expected to decrease the community, while other species would be expected to increase. The resulting plant community would likely not have as many coexisting and interacting species as the climax community. Thus, the effects of large disturbances may at times

simplify species interactions in plant communities, and application of the Central Limit Theorem to such communities leads to the expectation that they will not conform to a lognormal distribution (May 1981, Ugland & Gray 1982). Indeed, there are many examples of species distributions from disturbed or annual communities which tend to have multiple modes, and do not conform to a single lognormal curve (May 1981, Ugland & Gray 1982, Hughes 1984). A combination of two or more non-log normally distributed data sets from the same community from different points in time will usually fit a lognormal distribution (Magurran 1988).

Species distributions at times fit the log series model, a relative of the lognormal model, equally well or better, than they fit the lognormal model (Magurran 1988). These distributions are characterized by a small number of abundant species and a high number of rare species, so that the class containing one taxonomic unit has the highest abundance of any classes, and is followed by a normal distribution of more common species (Hughes 1986). Data that fit the log-series distribution are thought to come from small, disturbed, or early successional communities, although Hughes (1986) has argued that log-series distributions may result from misidentification of species, or inadequate sample sizes.

Species-abundance analyses were used in this project to test the hypothesis that species distributions in primary sites reflect the level of disturbance caused by biological control of leafy spurge with *Aphthona* spp. Sites with low intensities of biocontrol and dynamic, stable plant communities were expected to show lognormal plant species

distributions. Sites with high intensities of biological control, and therefore unstable populations of leafy spurge, were not expected to show lognormal distributions. Several assumptions are made in order to test these hypotheses. One assumption is that the level of biological control can be ascertained by monitoring flea beetle populations and leafy spurge density over the two field seasons in combination with available historical flea beetle release information. One other assumption is that biological control of leafy spurge by *Aphthona* spp. can at times be considered a disturbance in a plant community. This second assumption seems reasonable considering the rapid, drastic reductions in spurge cover due to high populations of *Aphthona* spp. seen at some biocontrol sites, including at least one primary research site in this analysis. Relatively rapid reductions in leafy spurge plants caused by the impacts of fleabeetles would be expected to affect the entire plant community, since previously occupied niche space would be made suddenly available for colonization.

Species-abundance analyses were conducted for control sites to test the hypothesis that community organization is different between primary sites and surrounding plant communities where leafy spurge invasion and biological control by *Aphthona* spp. are less influential. This analysis was undertaken with the intent to provide insight into how much disturbance can be attributed to leafy spurge invasion, and how much can be attributed to biological control effects in the primary sites.

Plant Species Distribution Model Fitting Methods

Ranked plant species lists were created for each site in spreadsheets. The natural log of abundances was plotted in series from most abundant to least abundant species

(rank/abundance plot) in order to predict the most appropriate model (Magurran 1988). Expected values were calculated for potential best-fit models using a series of calculations within the spreadsheets. Expected values for the lognormal model were calculated using Equation 1. Expected values for the log series model were calculated using Equation 2.

Equation 1. The lognormal model used to calculate expected plant species distributions (from Magurran 1988, Ludwig & Reynolds 1988).

$$S(R) = S_o e^{(-a2\,R2)}$$

where $S(R)$ = the expected number of species in the R^{th} octave,

S_o = the number of species in the modal octave, and

$a = (2\sigma^2)^{½}$ (the inverse width of the distribution).

Equation 2. The log series model used to calculate expected plant species distributions (from Magurran 1988).

$$\frac{\alpha\chi}{2}, \frac{\alpha\chi^2}{3}, \frac{\alpha\chi^3}{4}, \frac{\alpha\chi^4}{n} \ldots \frac{\alpha\chi^n}{n}$$

where $\alpha = \frac{N(1-\chi)}{\chi}$, and

χ is estimated by iteration of $\frac{S}{N} = \frac{[(1-\chi)]}{\chi}\ [-\ln(1-\chi)]$,

where S = total number of species,

N = total number of individuals, and

n = number of individuals in the *i*th octave.

Observed and expected values were plotted along octaves according to Preston (1948), Ludwig & Reynolds (1988) and Magurran (1988) to graphically display the relationships between the observed and expected models. Lognormal and log series expected values were calculated for each site, although additional models were calculated for some sites when deemed appropriate, including the geometric series, the truncated lognormal and the broken stick series. However, none of these ever came close to describing the distribution of species and are not discussed further (see Magurran 1988).

Chi-square (χ^2) tests were used to determine the goodness of fit between observed and expected distributions in the traditional manner (Ludwig & Reynolds 1988, Magurran 1988). However, since the statistics yielded by chi-square tests on small distributions are often unreliable (Sokal & Rohlf 1981, Fingleton 1984, D'Agostino 1986, Hagenaars 1990), an additional test of fit was used, the Kolmogorov-Smirnov two-sample test (Sokal & Rohlf 1981, Daniel 1990). Compared with the chi-square test, the Kolmogorov-Smirnov test is more sensitive to trends in the shape of a distribution and is less sensitive to smaller sample sizes (Daniel 1990). The Kolmogorov-Smirnov test was used in conjunction with chi-square tests to determine the best fitting model, especially when chi-square test statistics were suspicious. Chi-square and Kolmogorov-Smirnov tests were performed in STATISTICA (non-parametrics/distribution fitting module, StatSoft 1988).

Occasionally, octaves did not contain any species, which is a common problem in such analyses (Feinberg 1970). This presents a problem for chi-square testing, since zeros create high chi-square values regardless of the magnitude between observed and

expected values (Sokal & Rohlf 1981, Daniels 1990). Most researchers have dealt with this problem by collapsing categories, which is not necessarily a justified practice (Feinberg 1970, Hagenaars 1990). Other strategies include the addition of a small constant to the empty octave or to all octaves, or the use of alternative chi-square test statistics (Fingleton 1984, D'Agostino 1986, Hagenaars 1990). Following the recommendations of Hagenaars (1990), zeros were dealt with in a variety of ways and the respective outcomes were compared. Collapsing categories tended to drastically improve the fit of the model in some cases, but not at all in others. Adding a constant to only the empty category always significantly improved the fit. To be consistent and conservative, the value 0.5 was added only to octaves containing zero observations in the distributions. The Kolmogorov-Smirnov test is not as sensitive to zero values, so this adjustment was done for the benefit of chi-square goodness of fit tests.

Multivariate Analyses

Community level ecological studies often attempt to explain the variation in the occurrence and abundance of taxa in both space and time with respect to environmental variables. Consequently, ecological data sets commonly consist of matrices of abundances of taxa × sampling units (sites, quadrats etc.), and environmental factors × sampling units (Palmer 1993, ter Braak 1995). Such data are multivariate; each statistical sampling unit is characterized by many attributes (Jongman 1995). Multivariate data sets are bulky; and they are often accompanied by outliers, noise, redundancy, and information that is not directly interpretable (Jongman 1995). Human beings are

capable of understanding data relationships in no more than a few dimensions, so statistical tools must be employed to uncover underlying ecological themes in multidimensional data sets. Ecologists use two principal approaches for multivariate data analyses. These include classification analyses (e.g., cluster analysis, see below) which assume species assemblages fall into distinct groups that can be classified, and ordination analyses, which assume that species assemblages vary gradually and can be arranged along environmental gradients (Palmer 1993, ter Braak 1994, Jongman 1995). Ordination methods are further subdivided into direct gradient methods, which evaluate species and environment data in a single analysis, and indirect gradient methods, which infer environmental gradients by analyzing species data only (ter Braak 1995). Direct and indirect ordination methods further vary as to whether the underlying model used for species responses is linear, nonlinear or a unimodal function (ter Braak 1994). Two multivariate analyses, canonical correspondence analysis and cluster analysis, were used in this project to explore ecological interactions between environmental factors and plant species compositions in *Aphthona* spp. biocontrol sites.

Canonical Correspondence Analysis

Canonical correspondence analysis (CCA) is a gradient ordination technique and a special case of multivariate least-squares regression (Palmer 1993, ter Braak 1994). Because CCA performs well despite skewed species distributions, samples taken from unique designs, quantitative noise and intercorrelation among variables, it has been increasingly embraced by ecological researchers (Palmer 1993, ter Braak 1994, ter Braak 1995, Jongman 1995). CCA is a direct gradient analysis technique; species and sites are

arranged along environmental gradients using a set of sampled environmental factors that are hypothesized to contribute significantly to the abundances of species (ter Braak 1995). The underlying model in CCA assumes that species exhibit a unimodal response along environmental gradients (ter Braak 1994), though this assumption is tolerant of minor violation (Palmer 1993).

The CCA algorithm is closely related to that for correspondence analysis (CA), an indirect gradient ordination method, but has additional steps for integrating environmental variables (Palmer 1993). Species response curves are standardized at various times during the algorithm so they remain comparable, and do not approach zero (ter Braak 1994). One simple way to standardize variables is by subtracting the mean and dividing by the standard deviation (centering and normalizing), although the method proposed by Hill (1979) may make the ordinations more interpretable ecologically (Palmer 1993, McCune & Mefford 1999). In the first iteration of the CCA algorithm, sites are assigned an arbitrary score, the value of which will not affect the outcome of the analysis, called the LC (linear combination of variables) score, and species are assigned scores based on the weighted average (WA scores) of the LC scores. WA scores are then assigned as weighted averages of species scores, and re-standardized so that they do not approach zero. A multiple linear least-squares regression is carried out using WA scores as the dependent variables and environmental variables as the independent variables. The value predicted by the regression equation becomes the new LC score, and the algorithm repeats until LC and WA scores no longer change between iterations (see Palmer 1993, ter Braak 1994, ter Braak 1995 for a more thorough discussion of the CCA algorithm).

The point at which LC and WA scores no longer change between iterations represents the maximum ability for environmental data to predict species responses.

Once LC and WA scores have stabilized (usually after many iterations), either score can be plotted along the coordinate axes according to their affinity for the measured environmental variables. The first CCA axis, or first eigenvector, represents the linear combination of variables that explains the maximum variance in species WA scores. Successive axes are orthogonal and independent, each represents the next best unique linear combination of variables that explain species WA scores. The eigenvalue associated with each eigenvector is a measure of how well the axis can separate the species along environmental gradients, and will decrease for each successive axis (McCune & Mefford 1999). On the joint-plot ordination diagram, site and species scores from one axis are plotted against those of another axis. Eigienvalues are often extremely low past the third axis and are consequently of little value, since they explain very little species variability and have questionable interpretation (ter Braak 1994).

CCA Methods. CCA was used in this study to explore the relationships of plant species and sampled quadrats with the sampled environmental factors at the four primary research sites. The program PC-ORD (McCune & Mefford 1999) was used to run CCA analysis for all primary site species richness data that was paired with analyzed soil data in the 1999 field season (n=18/site). In order to utilize CCA at the resolution of interest (i.e., species-environment interactions under various levels of biocontrol), quadrats (frames) were treated as sites (Palmer 1993) and species richness and abundance data collected from each of the 10 pins were treated as samples. The scaling method from Hill

(1979) was used to standardize and re-standardize LC and WA scores. This scaling results in species response curves that rise and fall over a distance of approximately ± 2 standard deviations from the mean (McCune & Mefford 1999). The CCA algorithm requires two matrices, a main matrix with sites × species and a second matrix with sites × environmental factors. In this case, the main matrix initially contained 72 frames × 56 plant species and the second matrix contained 72 frames × 16 environmental variables. Available environmental variables included the 13 analyzed soil factors, elevation data (obtained from an Ag-Navigator GPS unit), and beetle densities (both soil and sweep samples).

When environmental data were initially examined in the correlation matrices, a great deal of intercorrelation was found among the variables (Table 3). This was to be expected, considering geological and ecological processes affecting these factors are often related (e.g., both sand and silt can be expected in a flood deposit), and soil resources are naturally inversely related (Tilman 1984). Redundancy of environmental variables creates a multicolinearity problem in CCA since these variables are used in a multiple regression analysis to attempt to explain the distribution of species and sites (ter Braak 1995, McCune & Mefford 1999). Multicolinearity impedes interpretation of the CCA ordinations, and causes errors in the calculation of the canonical coefficients (which are essentially regression coefficients), resulting in general instability of the model (Palmer 1993, ter Braak. 1995). Thus, it was necessary to remove some highly redundant variables in order to alleviate this problem. Of the 16 available environmental variables, 8 were eliminated on the basis of redundancy and multicolinearity (i.e., erroneous

canonical coefficients), including % silt, % sand, K, total C, organic C, ⅓ bar H_20, 15 bar H_20 and elevation. One environmental variable, flea beetle density (from soil core samples), was removed from the set because it was determined to be irrelevant, as evidenced by very low canonical coefficients on the first 3 axes (axis 1= 0.001, axis 2= 0.015 and axis 3= 0.149). Model behavior was examined after each variable was removed sequentially, and in various combinations, to arrive at a set of environmental variables that had minimal redundancy and maximal explanatory power. The resulting second (environmental) matrix now had 72 frames × 7 environmental variables, while the main (species) matrix was unchanged. The seven conserved environmental variables were Mg, $CaCO_3$, Fe, N, Clay, P and density of *Aphthona* spp. adults collected from vegetation using a sweep net.

Although a few correlations do exist among this set of environmental variables, the original problem with calculation of canonical coefficients was resolved. Further removal of environmental variables resulted in drastically lower eigenvalues, suggesting that this set of 7 variables, while somewhat intercorrelated, are not redundant in the context of the analysis. Furthermore, it has been demonstrated by Palmer (1993) that CCA handles some intercorrelation very well.

PC-ORD gives 3 options for axis scaling including: optimization of rows (frames) so that site scores are linear combinations of mean WA scores; optimization of columns (species) so that species scores are weighted mean site scores; or a compromise between optimization of rows and columns. Rows (frames) were optimized for ordinations as recommended by ter Braak (1995) when Hill's (1979) scaling method is used. PC-ORD also gives the option of plotting LC or WA scores on the ordination. Although WA

scores are traditionally plotted, LC scores were used in this analysis following the recent recommendations of Palmer (1993) and ter Braak (1994). Another option available in PC-ORD program allows for testing of the following null hypotheses; 1) that there is no linear relationship between the main and second matrices, or 2) that there is no structure in the main matrix (and therefore no linear relationship between matrices), using forms of the Monte Carlo permutation test. However, Monte Carlo tests should only be used when sites are located randomly with respect to environments, and are spatially discreet (Palmer 1993). Clearly these assumptions are not met in this design, where sites were 72 quadrats from 4 areas. Therefore, Monte Carlo randomization tests were not used in this analysis.

Similarity Cluster Analysis

Cluster analysis is another way to approach large multivariate ecological data sets. In a biological context, cluster analysis assumes that discrete composition groups can be discerned among sampling units using a classification system such as allelic frequency, species abundance, diversity, etc. (Jongman 1995). Environmental characteristics are inferred by the behavior of clusters. One desired property of cluster analyses is that the relationships between clusters (groups) are plotted simultaneously on a dendogram, where distances separating groups are representative of their dissimilarity (van Tongeren 1995). The way in which relationships between clusters are determined by cluster analysis depends to a large degree on the selection of the linkage method (van Tongeren 1995). A variety of linkage methods are available, including single-linkage (nearest neighbor), complete-linkage (furthest neighbor), average-linkage, Ward's method and

centroid clustering (van Tongeren 1995). Linkage methods differ as to which variables are compared to determine the similarity relationships between clusters.

Two major types of cluster analysis are commonly distinguished. Divisive methods start with all objects as a group, and sequentially subdivide groups out until the algorithm encounters a stopping rule. With divisive methods, major differences are thought to prevail over small ones in the formation of clusters. Conversely, agglomerative methods start with the individual objects, which are combined to form groups. With agglomerative methods, local differences are thought to prevail over general differences in formation of clusters (van Tongeren 1995). Agglomerative methods typically operate using a similarity data matrix.

Morista-Horn Similarity Index

Similarity indices yield a statistic that is reflective of the similarity between two species compositions, based either on the number of species, the abundance of individual species, or both numbers and abundances of species (Magurran 1988, van Tongeren 1995). There are a variety of such indices available, though many are disproportionately affected by either sample size or species richness (Wolda 1983). Wolda (1983) and Smith (1986) investigated the efficacy of a wide selection of similarity indices. Wolda (1983) found that the Morista-Horn index was the only similarity index tested that was not strongly affected by species richness and sample size. Smith (1986) found the Morista-Horn index to be among the best of the available similarity indices. Insensitivity to richness and sample size are desired properties in the context of this project, since one goal of this analysis was to compare similarity of primary site plant communities (n = 72)

Table 3. Raw data correlations among sampled abiotic soil factors examined for CCA. Underlined environmental variables were not used in the ordination to minimize intercorrelation and multicolinearity.

	K	Mg	CaCO3	Fe	Tot. C	Org. C	N	Sand	Silt	Clay	P	⅓Bar H_20	15 Bar H_20
K	1.0	-.427	-.391	.689	.203	.314	.292	.184	-.012	-.331	**.857***	.114	.210
Mg	-.427	1.0	.533	-.522	-.187	-.341	-.382	-.381	.185	.513	-.358	.114	-.153
CaCO3	-.391	.533	1.0	-.586	.035	-.267	-.248	-.802	.680	.773	-.426	.234	-.149
Fe	.689	-.522	-.586	1.0	.312	.477	.430	.394	-.256	-.464	.627	.139	.321
Tot C	.203	-.187	.035	.312	1.0	**.954***	**.952***	-.348	.527	.089	-.039	**.927***	**.948***
Org C	.314	-.341	-.267	.477	**.954***	1.0	**.993***	-.094	.303	-.147	.091	**.823***	**.959***
N	.292	-.382	-.248	.430	**.952***	**.993***	1.0	-.110	.332	-.147	.067	**.811***	**.954***
Sand	.184	-.381	**-.802***	.394	-.348	-.094	-.110	1.0	**-.911***	**-.898***	.404	-.579	-.186
Silt	.012	.185	.680	-.256	.527	.303	.332	**-.911***	1.0	.636	-.214	.674	.374
Clay	-.331	.513	.773	-.464	.089	-.147	-.147	**-.898***	.636	1.0	-.526	.364	-.051
P	**.857***	-.358	-.426	.627	-.039	.091	.067	.404	-.214	-.526	1.0	-.148	-.005
⅓Bar H_20	.114	.114	.234	.139	**.927***	**.823***	**.811***	-.579	.674	.364	-.148	1.0	**.885***
15 Bar H_20	.210	-.153	-.149	.321	**.948***	**.959***	**.954***	-.186	.374	-.051	-.005	**.885***	1.0

to control communities (n = 36). One further advantage of using similarity indices is that the information can be arranged into a similarity matrix and used as the basis for agglomerative cluster analysis (Magurran 1988). This method allows similarity relationships among sites to be expressed concurrently on dendograms.

Cluster Analysis Methods. Cluster analysis was used in this study to explore the similarity of species compositions between all primary and control sites within and between field seasons. The Morista-Horn similarity index was used as the classification system for cluster designation, and was calculated between all discretely sampled communities in the study to produce three different cluster analyses. These included, all sites compared across both field seasons, and a comparison among primary sites and control sites from the two field seasons. Sites were compared among seasons to investigate change in plant communities between the two years. This cluster analysis also allowed for similarity comparisons to be made between the respective sites. Controls were compared with primary sites to investigate the amount of similarity between plant communities influenced by biological control (primary sites) and sites that were not influenced by the flea beetles (controls). Morista-Horn similarity values were calculated in spreadsheets from site-specific species lists that were imported as similarity matrices into the program STATISTICA (StatSoft, Inc. 1998). The form of the Morista-Horn similarity index used in the analysis is given in Equation 3.

Equation 3. The Morista-Horn similarity index (see Wolda 1983).

$$C_{MH} = \frac{2\sum(an_i \times bn_i)}{(da + db)aN \times bN}$$

where an_i = number of individuals in the *i*th species of site A,

bn_i = number of individuals in the *i*th species of site B,

aN = total number of individuals at site A,

bN = total number of individuals at site B,

$da = \sum an_i^2/aN^2$, and

$db = \sum bn_i^2/bN^2$.

STATISTICA was used to perform an average-linkage cluster analyses on each of the three similarity matrices. In average linkage clustering, sample pairs are joined only when they have the highest similarity, and between-group similarity is equal to the average similarity between all possible pairs (van Tongeren 1995). Average-linkage is the most commonly used cluster linkage method in ecological and systematics research (van Tongeren 1995).

Spatial Analysis

Although classical statistical methods are often sufficient to describe ecological variation, such methods do not sufficiently describe the spatial aspects of data (Legendre & Fortin 1989). Most biological theories pertaining to competition, succession, evolution, maintenance of diversity, parasitism, predator-prey interactions etc. make spatial assumptions about data (i.e., elements close to each other in space are influenced

by the same ecological processes). However, spatial assumptions are not always addressed by researchers, and are likely not always met (Legendre & Fortin 1989). Furthermore, assumptions of statistical independence are often erroneously assumed for classical statistics in biological research, especially with regards to gradient and successional analyses (Juhász-Nagy & Podani 1983, Phillips 1985, Legendre & Fortin 1989). Geostatistical methods allow researchers to test and make use of the spatial relationships of ecological data (Isaaks & Srivastava 1989). Originally developed for use in the mining industry, geostatistics has been widely adapted to suit a variety of environmental applications (Jongman 1995). The data used in many geostatistical analyses generally have an X, Y, and a Z variate (although Z may also have a covariate), where X and Y are coordinates for the location of the sampled variable Z, and may be GPS data, geo-referenced location data or simply grid coordinates. In addition to investigation of the spatial aspects of data, the end goal of geostatistical analyses is often directed at mapping the distribution of the Z variate over a given landscape using dispersed rather than exhaustive sampling (Burrough 1995). This can be accomplished by modeling the spatial behavior of data, then using model parameters and other inputs to interpolate (predict) values of the sampled variate at unsampled locations (Isaaks & Srivastava 1989).

Semivariogram Analysis

Spatial structure is commonly investigated using the semivariogram, which has the added benefit of providing a means to model data spatial behavior (Isaaks & Srivastava 1989). The semivariance takes the form $\gamma(h) = \frac{1}{2} \text{var}\,[Z(\chi_1) - Z(\chi_2)]$, and can

be defined as one-half of the variance between the means of samples (χ_1) and (χ_2) at the increment $Z(\chi_1) - Z(\chi_2)$ (Burrough 1995). Semivariances are calculated between samples that are within a range of distances from each other, or lag distances, to plot the semivariogram. All data pairs falling within a lag distance are said to make up the lag class. Mean semivariance at each lag class is plotted on the semivariogram. Spatial structure is confirmed if average semivariances increase as a function of distance, meaning points that are close together are more similar than points that are far apart (autocorrelation). Conversely, when there is no evidence for spatial structure on the semivariogram, points that are far apart are as similar as points that are close together (pure nugget effect). Lag distances should be manipulated to reflect the structure of the sample design. Although regularly gridded sampling patterns require only one lag distance because all points are equidistant, irregular sampling patterns require a series of lag distance inputs to reflect varying distances between sample points (Burrough 1995).

For a single Z variate, model selection is traditionally accomplished using semivariogram analysis (Isaaks & Srivastava 1989). If spatial structure is confirmed and semivariograms are manipulated to reflect the sampling pattern, a model is fit to the data that best describes the spatial variation of Z. There are many models available, and they are broadly categorized into transitional and non-transitional forms (Isaaks & Srivastava 1989). Transitional models initially have a positive slope, but eventually level out at the sill, where semivariance is theoretically equal to the variance of the series. The slope of a model is referred to as the nugget; the distance from the origin to the sill is the range. Examples of transitional models include the spherical model, the exponential model, and the Gaussian model. Non-transitional models such as the linear and logarithmic models

do not have a range or a sill, and generally apply to data with significant trend (Isaaks & Srivastava 1989). Interpolation methods use the nugget, range and sill information (if available), as well as parameters from the semivariogram that are contained in the model to estimate values of Z at unsampled locations (Isaaks & Srivastava 1989).

Semivariograms are also used to explore and model anisotropic effects, which are directional trends in the data. Evidence for anisotropy is detected when semivariograms differ significantly in range and nugget depending on the direction of the azimuths. Anisotropic models are more complicated than isotropic (omnidirectional) models since they include a directional component. Anisotropic models were not used in this study, and are not discussed further. Readers are referred to Isaaks & Srivastava (1989).

Kriging Interpolation

Ordinary kriging is a widely used interpolation algorithm that uses linear combinations of the Z-variate and parameters from the semivariogram model to estimate values of Z at unknown neighboring points (Isaaks & Srivastava 1989, Burrough 1995). Kriging is superior to most interpolation methods because it provides an optimal unbiased estimate for each point, as well as an estimated variance (Isaaks & Srivastava 1989, Burrough 1995). Block kriging is a variant of ordinary kriging that uses linear combinations of available data to estimate mean values within a local area, or block. Each block value is linear combination (average) of the set of points contained in the block (Isaaks & Srivastava 1989).

It is necessary to provide the kriging algorithm with information about the search neighborhood, regarding how many neighboring points to use for interpolating local Z values, as well a direction and distance in which to search for them. In isotropic models the search neighborhood is conceptually a circle surrounding the point to be interpolated. Points that are contained within the circle are used in the estimation of the unknown point. Where samples are from an irregular grid, search neighborhoods should be slightly larger than the average spacing between samples (Isaaks & Srivastava 1989).

Spatial Analysis Methods

Geostatistical analyses were used in this project to investigate the spatial dynamics of plant communities affected by biological control of leafy spurge by *Aphthona* spp. It was hypothesized that geostatistical analysis of primary research sites would demonstrate effects that biological control of leafy spurge by fleabeetles have on the spatial structure of leafy spurge cover and plant species richness. Geostatistical techniques used to accomplish these goals include isotropic semivariogram analysis, isotropic semivariogram modeling, block kriging, cross validation analyses, and contour mapping.

Coordinates. Although an AG-Navigator GPS unit was used at all sites to record X and Y coordinates for every quadrat, these data were not used in the analysis. When plotted, GPS frame coordinates showed remarkable error and inaccurate representation of sample locations. Therefore, sample points at primary research sites were assigned X and Y coordinates based on their relative position on a grid system with transects centered around Y=0 (Appendix E). Use of a coordinate system allowed accurate representation

of the actual sampling locations, although no indication of geographical location or direction is given.

Data Distributions. Z-variate data were examined using summary statistics (mean, variance, skew, kurtosis) and a variety of descriptive plots in the program GS+ (Gamma Design Software 2001) including frequency distributions to check data distributions, and cumulative frequency distributions and normal probability plots to check for normality. Since linear assumptions must be met by data used in kriging, all data sets that showed evidence of non-normality, high variance, and skewed or multi-modular distributions were adjusted using either a square root or log transformation. Due to the presence of zero values in some of the data sets, transformations sometimes required an offset to be calculated, and the value 1 was always used for this purpose. Where transformations were utilized, back transformations were also used in order to conserve original data interpretation.

Outlier Points. Some of the semivariograms distinctively resembled experimental semivariogram models, excepting one or more points that prevented the model from fitting the data. Several aspects of these data points were considered. First, it was necessary to determine which pair of points had the excessively low or high semivariance (since semivariances are means at each lag class), and the relative location of each point. When a pair of points in a short lag class had a high semivariance, all other lag classes within the range were investigated to see whether the same points caused any other discrepancies. If they did, the probability of both data entry error and sampling error

were evaluated. There are several potential sources of sample error in the plant data: Immature grasses and sedges were often difficult to distinguish in the field, and consequently could have been over or underestimated; structural variability among species could have erroneously influenced species abundances; and windy sampling conditions may have influenced pin to plant encounters. When the probability for error as described above was thought high for a given outlier point, the point was dropped for the purpose of modeling.

Semivariograms & Model Fitting. All modeled semivariograms were isotropic, since no significant directional effects were detected. Variability and irregularity in sampling patterns at primary research sites (Appendix E) meant that lag classes had to be calculated independently for each site. One point was selected and distances to all other points calculated using the Pythagorean theorem. Several different points in the site were chosen for these calculations, and each provided a different set of lag values depending on location of the chosen point within the site. Points near the periphery of sites have nearest neighbors at greater distances than points near the origin. Consequently lag classes calculated for these points did not reflect minimal distances between points near the origin. This resulted in too many pairs of points compared at the smallest distances on the semivariogram. When nearest neighbor distances were calculated for points nearest the origin, it was found that the full spectrum of distances was best represented. Thus, lag class distances for all primary research sites were nearest neighbor distances calculated from the first sample point.

When fitting the model to the semivariogram, the first objective was to maximize the range (a_o), or the distance within which sample points are spatially dependent (assuming a positive nugget) (Burrough 1995, Wingle & Poeter 2001). On the experimental semivariogram, range is the distance from the origin to the point where average semivariances for lag classes intercept the sample variance, or the sill ($C_o + C_1$). Range can be adjusted by manipulating the active lag distance around the sill. In each semivariogram, arrays of lag distances taken from the vicinity of the sill were examined for their effect on the range and fit of models. Lag distances were selected that maximized the range in the model so long as model fit was not compromised.

In addition to maximization of range, models were selected by their ability to accurately represent the semivariogram distributions. Model fit can be assessed using the regression coefficient (r^2 value), although this is not considered a robust test of fit. A more precise measure of fit is provided by the RSS (residual sums of squares) value (Isaaks & Srivastava 1989). The better a model fits a given semivariogram, the lower the RSS value will be. Although r^2 values were considered during model selection, most emphasis was placed on the maximization of the range and minimization of RSS values.

Block Kriging. Block kriging (2 × 2 blocks) was used to interpolate average Z values over the areas of interest, since quadrats (which cannot be regarded as points) were used as sample units. The form of the interpolated surface is affected by the size and shape of the interpolation grid (Burrough 1995), and GS+ interpolates over a regular (square) grid as the default. However, interpolation over a square or rectangular grid does not complement the layout of transects in this project, since the corners lack any

data points. Therefore, a polygon was used to exclude areas on the grid that were totally lacking data points. Polygons were created so that interpolation surfaces extended five meters beyond the last data point in each transect, and extended to a distance equal to half of the angle between transects on each side. Average sample spacing over the interpolation surface was estimated using a formula adapted to the sample design from the search radius equation given in Isaaks & Srivastava (1989) (Equation 4). The mean sample separation distance was increased by + 1-2 m and used as the search radius. To ensure that resolution was not lost in areas where samples were very close together due to too many neighboring points being analyzed, eight neighbors were used.

Equation 4. Formula used to calculate mean sample separation distance at the research sites. The results were used as a baseline for the search radius input in block kriging.

$$\text{Mean sample separation distance} \approx \frac{[\pi R^2 \times (D/360)]^{-2}}{N}$$

where R = length of the interpolation polygon,

D = total degrees between sides, and

n = total number of samples (72).

Cross Validation Analyses. Cross validation is an extremely valuable tool for evaluating the performance of models using available data (Isaaks & Srivastava 1989). By removing a sampled data point from the spatial domain, and then estimating the value for that point by interpolating from other known sample points, the performance of the model can be assessed. In GS+ a regression line is applied to this data as a measure of fit between observed and predicted values. Cross validation analysis was used extensively

to evaluate the effect of choosing different models and parameters on the interpolated values. The semivariogram model was never refit as a result of cross-validation analysis, but slight adjustments were made to the search neighborhood if they improved the predictions for known points. Although common in practice, it is not reasonable to refit models based on cross validation analyses, since it is never known exactly how a model behaves at points where no data exist (Isaaks & Srivastava 1989).

Behavior of the interpolation surface was evaluated in GS+ using contour maps of estimated Z standard deviation values (Isaaks & Srivastava 1989, Burrough 1995). This is possible since kriging estimates a variance function for each point (or block). These maps show where interpolated values are likely to be inaccurate (high standard deviations), owing to semivariogram model and/or kriging parameters. As a result, modeling and kriging parameters were sometimes adjusted to compensate for estimation deficiencies.

Mapping. Kriging interpolation output from GS+ was saved as a SURFER (Golden Software 2001) grid file. The grid information was imported into SURFER to create high quality contour maps. A blanking polygon file was created for each site and overlaid onto the maps so that estimated values extended only 2 m past the range. A high degree of smoothing was applied to all contour maps, in an effort to minimize meaningless contour projections. Maps created on the same Z variable between years at each site were put onto the same scale (legend), so that they were directly comparable.

CHAPTER 4

SITE SPECIFIC RESULTS

Site 1, Medora ND

The Medora 1site was located on private land approximately 6.5 km north of interstate 90 on Camel's Hump Lake Road near Medora, North Dakota. The site was close to the eastern border of Theodore Roosevelt National Park, North Unit, in Golden Valley county (46.98809° N, 103.8392° W). Approximate elevation of the site was 852.42 m. The environment at the site was xeric, with sandy loam soils and mixed-grass prairie species compositions (Appendix C, Table 4). Mean annual precipitation in nearby Wibaux, MT was 38.65 cm in 1999, and 24.82 cm in 2000 (High Plains Regional Climatic Data Center, 2001). The sampled area was on a gentle hill nestled between gumbo buttes. Although livestock grazing was not observed on the site during the years of this study, it was assumed that there had been some amount of grazing in the past. Wildlife grazers in the area include white-tailed and mule deer. The six established transects were 18.66° apart, 30 m long, with twelve quadrat locations on each transect at 2.25 m increments. Total area encompassed within the site was approximately 879.64 m^2. Sampling took place on June 29, 1999 and June 27, 2000.

Species Productivity, Frequency and Richness

Plant productivity, or the number of plant encounters with sample pins, did not change significantly between field seasons (sign test, $p > 0.05$; see Table 4). Of the

commonly encountered species, two species, *Aristida longiseta* and *Stipa comata*, showed increases in pin-encounter frequency in 2000, but these increases were non-significant (sign test $p > 0.05$). *Poa pratense* was the only common species that declined in pin-encounter frequency in 2000, but the decrease was not significant (sign test, $p > 0.05$). Notably, *Besseya wyomingensis*, which was encountered 42 times in 1999, was not encountered in the 2000 samples. Plant species richness did not change significantly between the field seasons (sign test, $p > 0.05$). Most species encountered at this site in 1999 were also encountered in 2000. Only 14 plant species were encountered in the control site in 1999, whereas 25 species were encountered in the control site in 2000.

Table 4. Plant species list ranked by total number of encounters of each species with sample pins during 1999 and 2000 at the Medora 1 site.

Genus	*species*	1999	2000	Total
Poa	*pratensis*	908	867	1775
Stipa	*comata*	118	212	330
Bromus	*inermis*	117	132	249
Aristida	*longiseta*	40	174	214
Stipa	*viridula*	94	109	203
Symphoricarpos	*occidentalis*	91	95	186
Koeleria	*nitidia*	71	63	134
Euphorbia	*esula*	93	27	120
Aster	*pansus*	34	44	78
Panicum	*capillare*	48	26	74
Astragalus	*adsurgens*	39	30	69
Rosa	*woodsii*	31	18	49
Besseya	*wyomingensis*	42	0	42
Artemesia	*ludoviciana*	19	20	39
Artemesia	*frigida*	11	16	27
Psoralea	*agrophyla*	4	10	14
Poa	*compressa*	12	0	12
Grindelia	*squarrosa*	0	9	9
Vicia	*americana*	8	1	9
Melilotus	*officinalis*	3	6	9
Echinacea	*angustifolia*	4	2	6
Tragopogon	*dubius*	4	2	6

-continued-

-Table 4, continued-

Bromus	*japonicus*	2	2	4
Erysimum	*asperum*	1	1	2
Tradescantia	*occidentalis*	2	0	2
Anemone	*cylindrica*	1	0	1
Totals	(productivity)	1797	1866	3663

Cover Analysis

The Medora 1 site showed relatively low cover levels of leafy spurge, forbs, and bare ground, and high cover levels of grasses during both field seasons (Appendix B). The lowest leafy spurge densities were nearest the flea beetle release stake in both field seasons (see Figures 4 & 5). Assuming leafy spurge cover was relatively high near the release stake at the time of flea beetle release, flea beetles have had a striking impact at this site. Mean encounters of leafy spurge, grasses and forbs per frame were not significantly different between the two field seasons (sign test, $p > 0.05$). Mean encounters of native grass species increased significantly from the 1999 to the 2000 field season (sign test, $p < 0.01$). Additionally, more pins failed to intercept any plants in 2000 (Appendix B).

Cover levels measured in 2000 were similar to those measured in 1999 (Appendix B), except that the increase in cover of native grass species as a function of distance from the release stake was more pronounced in 2000 (Figure 5). This was likely due to reduced leafy spurge cover levels from those observed in 1999. Mean cover levels of grasses and forbs at the 12 distances were similar between field seasons (sign test; $p > 0.05$). Grasses continued to represent most of the plant cover in 2000, while low cover levels of leafy spurge and forbs were maintained. In neither of the sampled years did any

cover class show substantial fluctuation in amplitude as distance from the flea beetle release point increased, with the exception of percent cover of native species (Figures 4 & 5).

Figure 4. Mean plant cover levels plotted as a function of distance from the flea beetle release point for the Medora 1site in 1999.

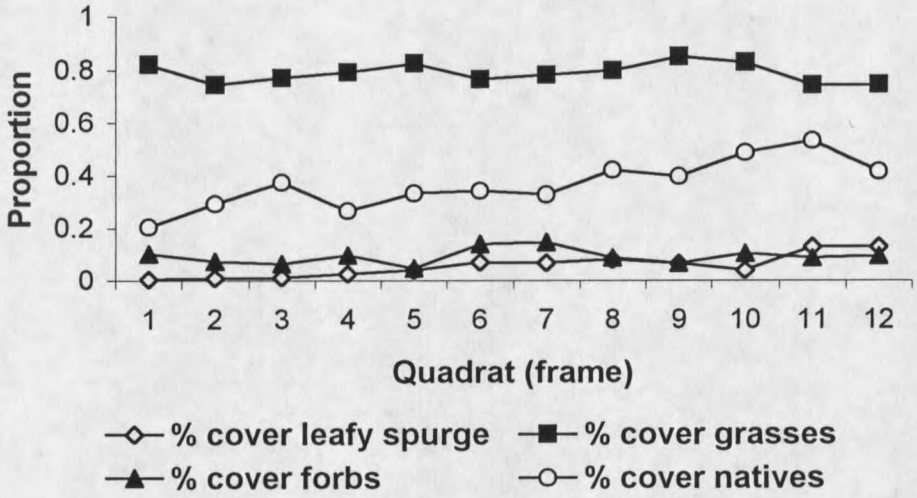
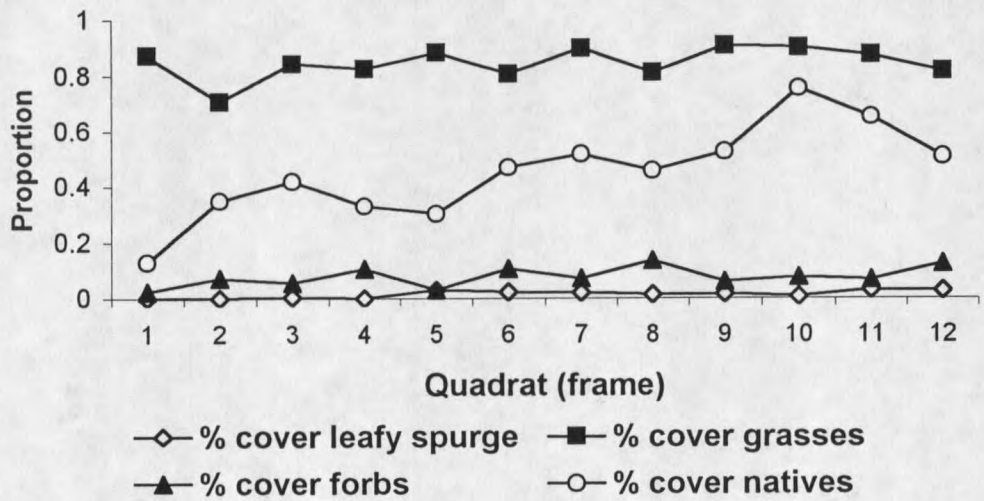


Figure 5. Mean plant cover levels plotted as a function of distance from the flea beetle release point for the Medora 1site in 2000.



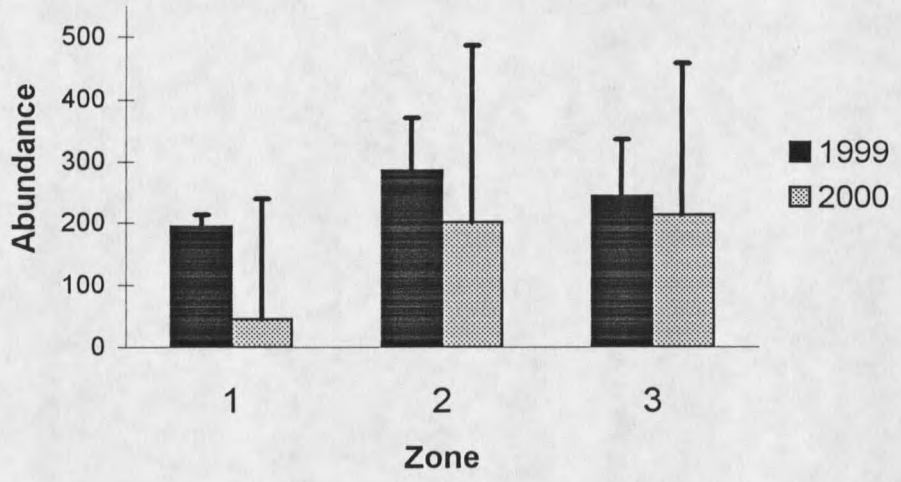
Plant cover attributes at the control sites varied wildly between the two field seasons, likely due to the varying locations of the control sites between the 2 field seasons (Appendix B). Control site cover levels from 1999 were remarkably different from the cover levels measured at the primary site; there were nearly half as many species, and half as much total cover of forbs (Appendix B). In the 2000 field season species richness and cover levels at control site were comparable to the primary site, except that the control had a higher cover level of native species.

Flea Beetle Populations

A total of 2000 flea beetles (species unknown) were originally released at the Medora 1 site in 1995 (O'Brien, personal communication 1999). *Aphthona* spp. adults collected at the site in 1999 in the sweep net samples totaled 2,536 beetles. A total of 1,610 adult flea beetles were collected from the vegetation in the sweep net samples in 2000. Each flea beetle sample zone was 10 m long, and was sampled with 10 sweeps. Flea beetle densities were not significantly different between zones in 1999 (ANOVA; $p > 0.05$), although they were significantly different between zones in 2000 (ANOVA, $p < 0.05$, see Figure 6). Flea beetle densities were significantly lower in 2000 compared with 1999 (ANOVA, $p < 0.05$). Flea beetle densities were significantly different between zones and years (ANOVA, $p < 0.05$). In both 1999 and 2000, 98 % of adult fleabeetles collected were morphologically identified as *A. lacertosa*, and the remaining 2 % were brown flea beetles (either *A. nigriscutis* or *A. cyparissiae*). The results of isozyme analysis of 86 individuals showed that 16.27 % of the sampled flea beetle population was *A. nigriscutis,* and the remaining 83.72 % were *A.*

lacertosa. No other species were found, so it is probable that brown flea beetles collected within the zones and morphologically identified were *A. nigriscutis.* In the emergence experiment, 70 total flea beetles emerged from the soil cores collected from the site on May 9, 2000 into the collection vials from each sample container (N= 37).

Figure 6. Abundance of adult *Aphthona* spp. in each sample zone at the Medora 1 site in 1999 and 2000. Error bars indicate standard errors of means.



Plant Species Distributions

Plant species abundance data from the 1999 field season showed a bimodal distribution, and did not fit any one of the available models (Figure 7). Two empty octaves (octaves 2 & 8) were adjusted with the value 0.5, although this did little improve chi-square goodness of fit statistic. There was a high abundance of rare species (octave 1), but also a high abundance of common species (octave 7) represented in this distribution. The distribution does not approximate a form expected from a dynamic equilibrium plant community; rather, the observed distribution may indicate instability.

Plant species abundance data from the 2000 field season was well fit with the log series model (χ^2, $p < 0.90$, Figure 8). Thus, plant community organization changed between the field seasons, from a distribution indicative of disturbance in 1999, to a distribution suggestive of a more stable plant community in 2000.

Figure 7. Best-fit model for species abundance data from the Medora 1 site in 1999 ($\chi^2 = 11.43$, $p < 0.17$; $D_{max} = 0.44$, $p > 0.10$).

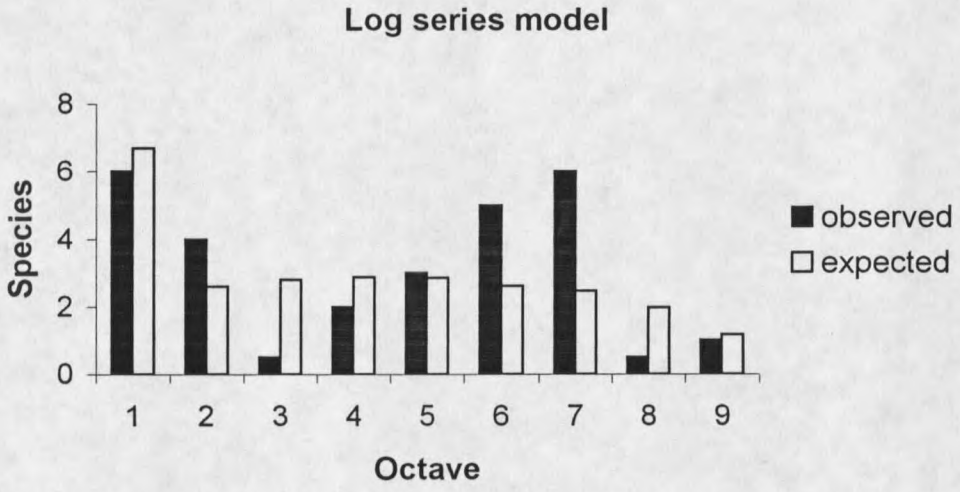
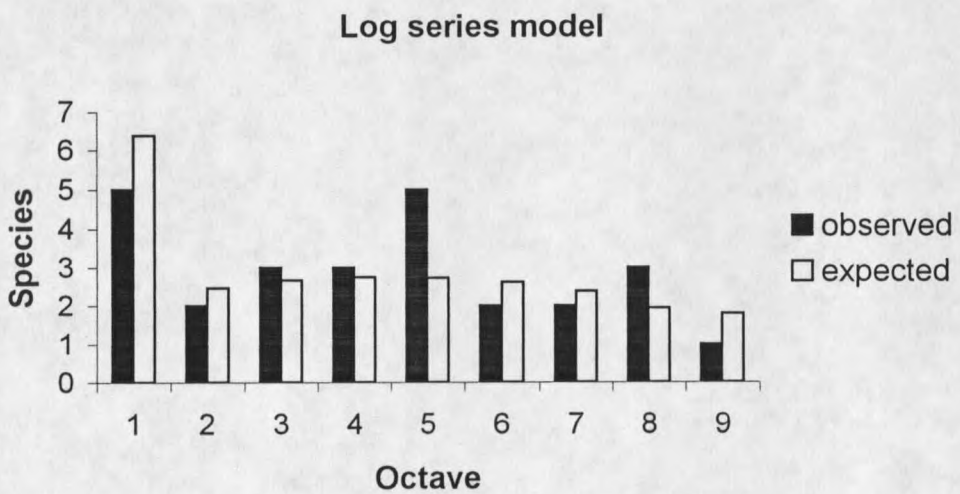


Figure 8. Best-fit model for species abundance data from the Medora 1 site in 2000 ($\chi^2 = 3.45$, $p < 0.90$; $D_{max} = 0.44$, $p > 0.10$).



Species abundance data from the control site in the 1999 field season, like the primary site distribution, followed a bimodal distribution, and consequently did not fit any available models (Figure 9). Octaves 5 and 7 did not contain any species, and were adjusted by the value 0.5. This data came from an area free of leafy spurge, so instability is not attributed to biological control or leafy spurge infestation. However, a disturbance effect may be expected since horses grazed extensively in the control sample area. The control data from the 2000 field season fit the log normal model (χ^2, $p < 0.72$) suggesting that the plant community immediately surrounding the primary site was in a dynamic equilibrium state, and are not likely under the influence of any significant disturbance (Figure 10).

Figure 9. Best-fit model for species abundance data from the Medora 1 control site in 1999 ($\chi^2 = 16.45$, $p < 0.02$; $D_{max} = 0.50$, $p > 0.10$).

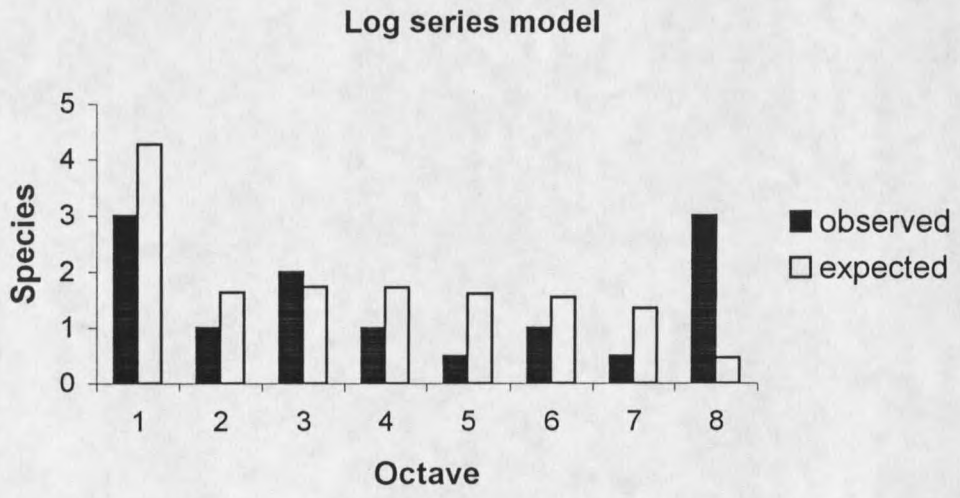
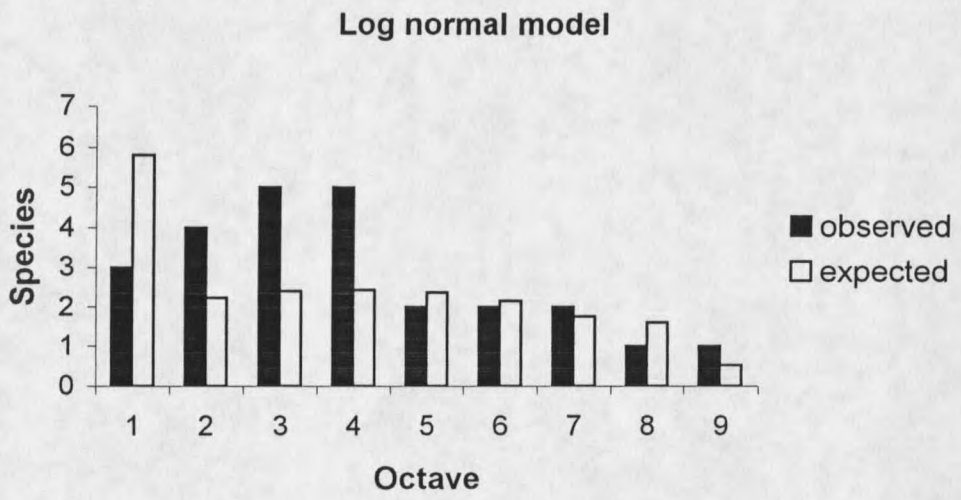


Figure 10. Best-fit model for species abundance data from the Medora 1control site in 2000 ($\chi^2 = 5.33$, $p < 0.72$; $D_{max} = 0.44$, $p > 0.10$).



Autocorrelation

Much of the plant data collected along the six transects showed some degree of autocorrelation in the experimental semivariogram analysis (Table 5). A pure nugget effect, or complete lack of autocorrelation, was found in several variables, such as flea beetle emergence data, distribution of forbs in 1999, total native species in 1999, and native forbs in 1999. Transformations (either log or √) were applied to improve normality in the data, for the benefit of semivariogram analysis. Models were fit based on maximization of the range and minimization of residual sums of squares. Most best-fit models were spherical transitional models, although native grasses from 2000 showed a linear trend. The distribution of leafy spurge and plant species richness were interpolated to produce contour surface maps, which are discussed separately below.

Table 5. Autocorrelation among the variables analyzed in semivariograms at the Medora 1 site from 1999 and 2000.

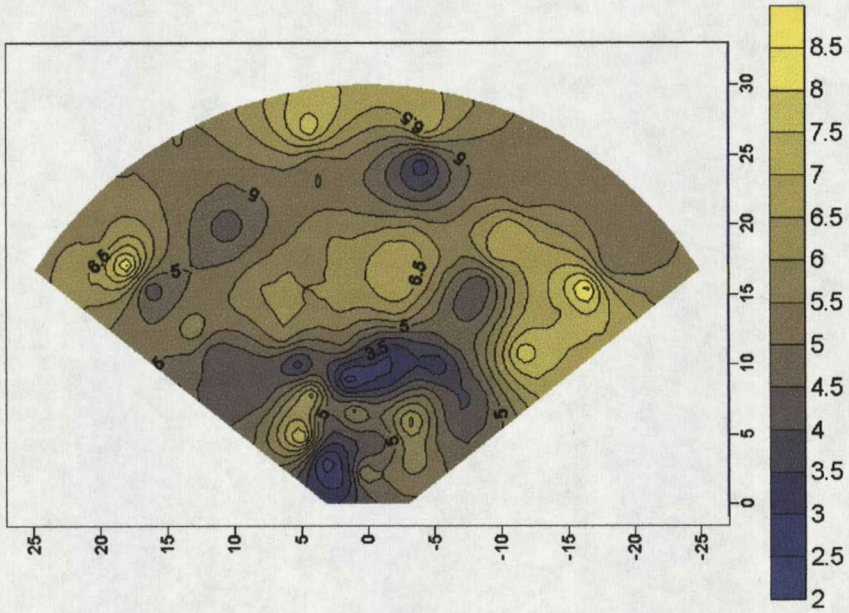
Variable	Model	Trans.	Range (A_o)	r^2	RSS
Aphthona emerg.	nugget	---	---	---	---
Forbs 99	nugget	---	---	---	---
Forbs 00	spherical	√	17.20	.471	.089
Grasses 99	spherical	Log	26.52	.637	.012
Grasses 00	spherical	√	31.12	.610	.890
Tot. natives 99	nugget	---	---	---	---
Tot. natives 00	spherical	√	12.00	.687	.660
Nat. grasses 99	spherical	Log	41.00	.303	.693
Nat. grasses 00	linear	√	6.31	.749	2.87
Native forbs 99	nugget	---	---	---	---
Native forbs 00	spherical	√	4.13	.400	.400

Interpolated Z Variates

Species richness. A square root transformation was applied to normalize the data. Although spatial structure was evident in the semivariogram, quality of the model was thought compromised by two influential outlier points within short lag distances. These two outliers came from frames containing *Agropyron* grass species, which were difficult to distinguish under field conditions, and might have been easily confused when not in a flowering state. Therefore, these points were thought to be extreme values and probably a result of sampling error. When the two points were removed from the data set, the exponential model provided a good fit to the mean semivariance values (A_o = 6.97, r^2 = 0.831, RSS = 7.326×10^{-04}). Standard error of predicted versus actual values

in cross validation analysis was ± 1.479. In the areas of highest interest (i.e. between transects) the mean standard deviation of kriged values was < 0.5. Results of the block kriged interpolation surface are presented in Map 1. The map shows a patchy pattern of plant species richness, with arcing contours which may reflect patterns of flea beetle dispersal from the release point.

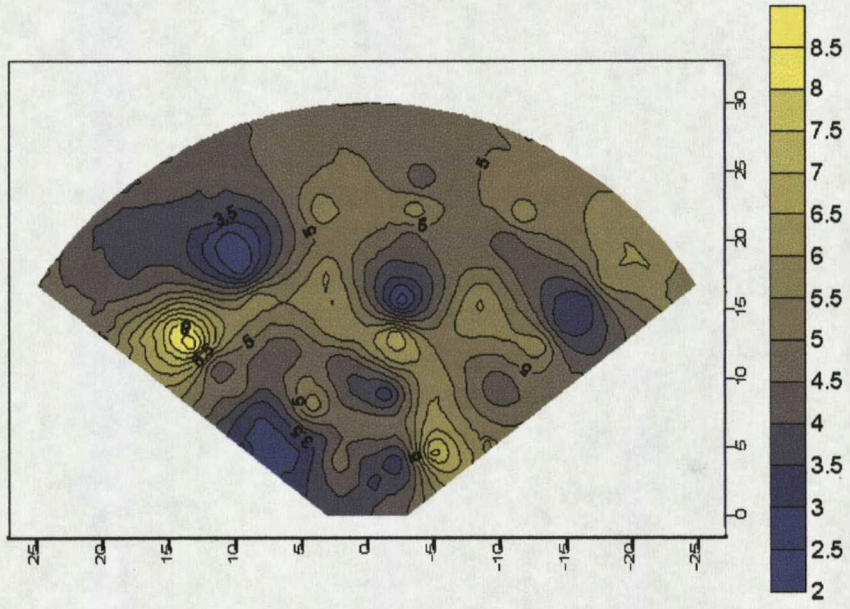
Map 1. Plant species richness contour map produced for the Medora 1 site in 1999 by block kriging. Contours reflect the mean number of species per quadrat.



A square root transformation was applied to the species richness data set from 2000 samples to improve normality. Spatial continuity was evident in this data set; the spherical model readily fit the semivariance values at the maximum range at lag distance 6.0 m (Ao = 12.97, r2 = 0.694, RSS = 4.532×10^{-03}). The standard error of predicted values in cross-validation analysis was ±1.240. Results of the block kriged interpolation

surface are presented in Map 2. Again, the general pattern of plant species richness is patchy. However, the contour shapes either transformed or moved from their position in 1999. Some of the areas containing richness hotspots in 1999 (Map 1) were not rich with species in 2000.

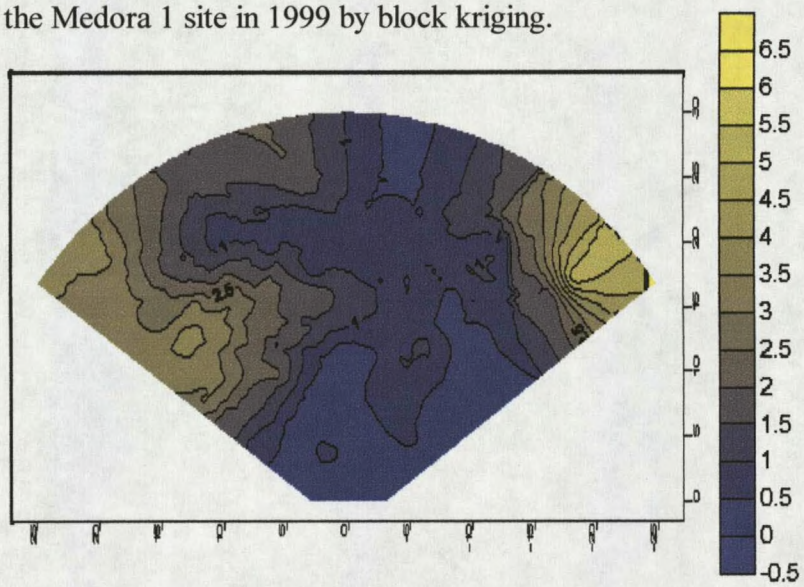
Map 2. Plant species richness contour map produced for the Medora 1 site in 2000 by block kriging. Contours reflect the mean number of species per quadrat.



Leafy Spurge Encounters. All values in the 1999 data set were offset by the value 1 and a square root transformation was applied. The offset was required in this data set due to the presence of zero values. Spatial continuity was evident in the data as the range was maximized at the lag distance of 15.89 (m), where semivariance values were best fit by the spherical model (Ao = 39.09, $r^2 = 0.671$, RSS = 0.0589). Kriging estimates for known values were mostly underestimated slightly where actual values were high, as evidenced in cross-validation analysis (regression coefficient = 0.771, SE prediction = ±

0.062). Results of the block kriged interpolation surface are presented in Map 3. Unlike plant species richness, the distribution of leafy spurge was not patchy. Leafy spurge was predominantly distributed on the periphery of the site. Beetle impacts are apparent in areas where leafy spurge densities are low, such as in the middle lane of the sample area, and near the flea beetle release point.

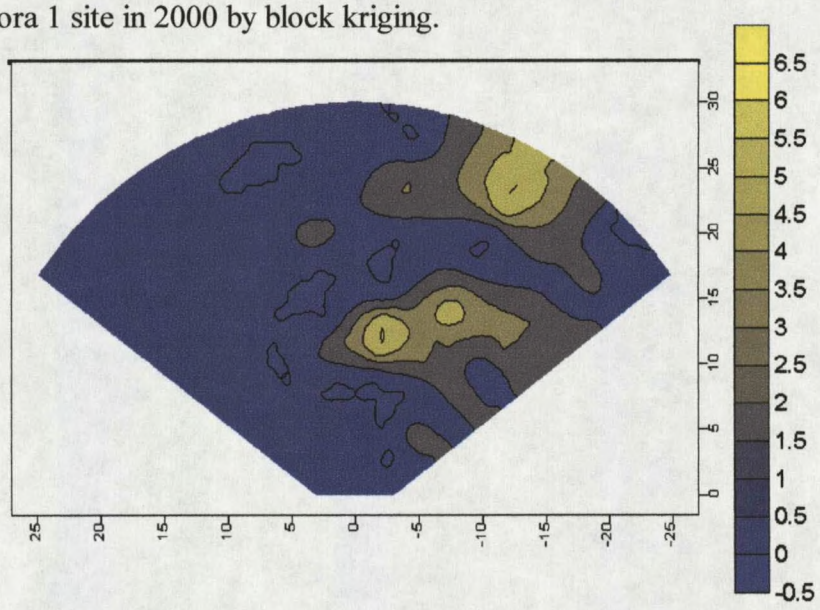
Map 3. Contour map of mean leafy spurge encounters per frame produced for the Medora 1 site in 1999 by block kriging.



The leafy spurge encounter data set from 2000 was offset by the value 1, and a square root transformation was applied in an attempt to improve normality of the data. One outlier point (pt. 11) was removed from the analysis. Maximum range was best represented at an active lag distance of 11.39 m, where the spherical model provided the best possible fit ($A_o = 11.63$, $r^2 = 0.715$, RSS = 4.184×10^{-3}). Standard error of predicted vs. known points was ± 0.503, regression coefficient = 0.771 (cross validation). Results

of the block kriged interpolation surface are shown in Map 4. All of the leafy spurge on the left side of the sample area had been completely removed by flea beetles. Beetle impacts were also apparent on the right side of the site. Some new leafy spurge infestations were also apparent in 2000.

Map 4. Contour map of mean leafy spurge encounters per frame produced for the Medora 1 site in 2000 by block kriging.



Site 2, Medora ND

The Medora 2 site was located on private land approximately 5 km north of Interstate 80 on Camel's Hump Lake Road, near Medora, North Dakota (46.99709° N, 103.83544° W). The site is approximately 2 km north of the Medora 1 site. Approximate elevation of the site was 813 m above sea level. This xeric site was located on an old road-cut within a minor drainage. The soil was characterized as a silty/clay type with a high level of Mg (644.33 mean mg/kg ± 91.87 SEM) (Appendix C). The topography was varied at this site, so sampling was constrained. A sharp drop-off is located just behind the release stake (old bridge connection), a steep hill sloped upward to the west, and another hill sloped downward to the east. Climatic data was the same as that for Medora site 1. Although the sampled area on the slope was xeric, 10 m below the release stake in the bottom of the drainage the environment changed to hydric. Cattle were observed grazing on this site early in the 2000 growing season. The six transects were 20^0 apart, 24m in length and had 12 samples at 2m increments (Appendix E). The total area comprising the site was approximately 603.20 m^2. Sampling took place at the Medora 2 site on June 30, 1999 and June 28, 2000.

Plant Species Productivity, Frequency, and Richness

Total plant productivity decreased by more than one-half in 2000 compared to 1999 (sign test, $p < 0.05$, see Table 6). Major community reorganization took place between 1999 and 2000 in terms of species proportions among the species that were most commonly encountered. *Bromus inermis*, which was encountered 517 times in 1999, was not found at the site in 2000. The most common species, *Agropyron smithii*, decreased in

frequency by 117 encounters in 2000, but this decrease was not significant at the 0.05 level (sign test, $p = 0.07$). Other species such as *Bromus tectorum*, *Artemesia ludoviciana*, *Stipa comata* and *Poa compressa*, that were absent or rare in 1999, were frequently encountered in 2000. The frequency of encounters of many of the rare species also varied considerably between the field seasons. Despite these major plant species organizational changes, total plant species richness decreased overall by only two species from 1999 to 2000 (27 species were found in 1999, 25 were found in 2000). However, species richness per frame declined significantly between the field seasons (sign test; $p < 0.05$). Control sites had 5 and 8 less species than primary sites in 1999 and 2000, respectively.

Table 6: Plant species list ranked by total number of encounters of each species with sample pins during 1999 and 2000 at the Medora 2 site.

Genus	species	1999	2000	Total
Agropyron	*smithii*	466	349	815
Euphorbia	*esula*	676	0	676
Bromus	*inermis*	517	0	517
Bromus	*tectorum*	5	270	275
Artemesia	*ludoviciana*	66	115	181
Stipa	*comata*	0	113	113
Poa	*compressa*	0	102	102
Melilotus	*officinalis*	56	30	86
Artemesia	*frigida*	58	11	69
Poa	*pratensis*	54	12	66
Agropyron	*cristatum*	30	6	36
Erysimum	*asperum*	0	31	31
Schedonnardus	*paniculatus*	24	0	24
Symphoricarpos	*occidentalis*	10	14	24
Agropyron	*dasystachyum*	20	0	20
Convolvus	*arvensis*	3	12	15
Stipa	*comata*	1	12	13
Lactuca	*serriola*	9	3	12

-continued-

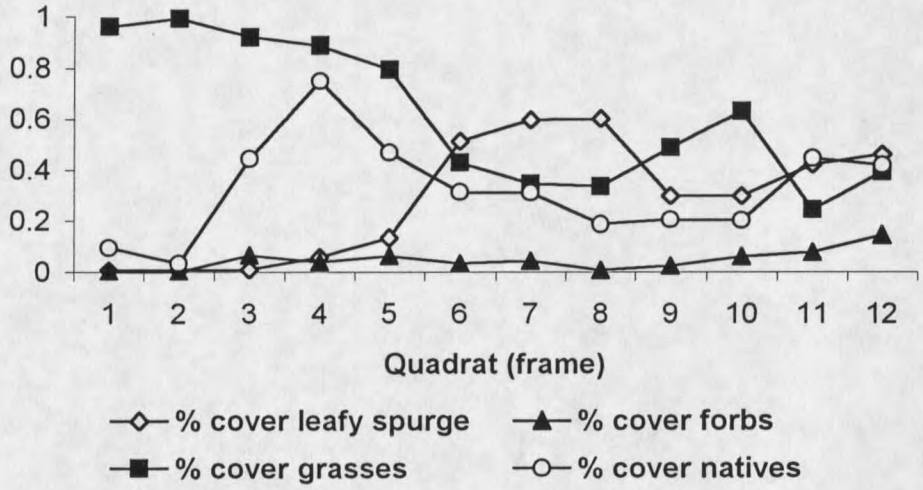
-Table 6. continued-

Stipa	*viridula*	5	6	11
Tragopogon	*dubius*	7	3	10
Taraxacum	*officinale*	5	4	9
Bromus	*japonicus*	6	0	6
Sphaeralcea	*coccinea*	6	0	6
Rosa	*woodsii*	4	0	4
Descurainia	*sophia*	1	3	4
Liatris	*punctata*	1	2	3
Solidago	*rigida*	0	3	3
Lepidium	*virginicum*	2	0	2
Echinacea	*angustifolia*	1	0	1
Potentilla	*gracilis*	1	0	1
Linum	*rigidum*	1	0	1
Ratibida	*columnifera*	0	1	1
Totals	(productivity)	2035	1102	3137

Cover Analysis

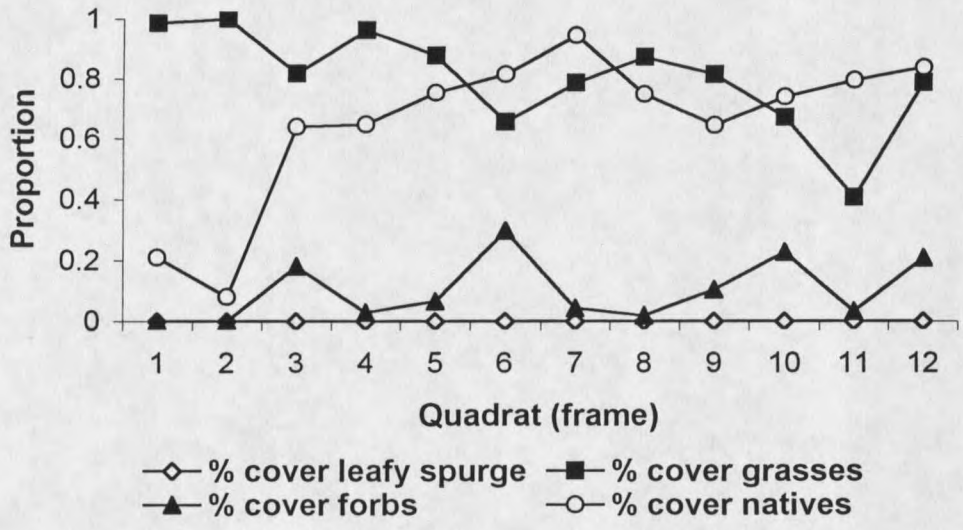
This site showed a significant reduction in leafy spurge cover over the two field seasons (sign test, $p < 0.01$). In 1999 leafy spurge covered 31.87 % of the sampled area, but in 2000 not one green leafy spurge plant could be found within the sampling area of the site. Initial level (1999) of bare ground cover was high, but the sampled area contained more than twice as much bare ground in 2000 (Appendix B). Mean number of grasses and forbs encountered per frame did not change significantly between 1999 and 2000 (sign test; $p > 0.05$). Likewise, mean number of native grasses and forbs encountered per frame did not change significantly between field seasons (sign test, $p > 0.05$).

Figure 11. Mean plant cover levels plotted as a function of distance from the flea beetle release point for the Medora 2 site in 1999.



Flea beetle impacts on leaf spurge resulted in significant mean cover level fluctuations as a function of distance from the original release point in 1999 (Figure 11). In areas of the site where flea beetle impacts were the oldest (i.e. the first few meters from the release stake), there were high cover levels of native species and grasses. Grass and native species cover levels declined at greater distances from the release stake, where leafy spurge cover levels increased. Forb cover levels stayed at relatively low levels independent of distance from the release point. In contrast, in 2000 mean cover levels of forbs changed in response to reduced leafy spurge cover (Figure 12). The cover of all grass species combined and the native grass species tended to increase in the areas where leafy spurge had been dominant in the previous year (1999). At distances where mean forb cover levels increased (i.e. 3, 6 and 10 m), grass cover levels correspondingly decreased.

Figure 12. Mean plant cover levels plotted as a function of distance from the flea beetle release point for the Medora 2 site in 2000.



Flea Beetle Populations

A total of 1,000 flea beetles (species unknown) were introduced to this site in 1995 (O'Brien, personal communication 1999). In 1999, 2,501 total adult *Aphthona* spp. were collected from the vegetation adjacent to transects using a sweep net. Each flea beetle sample zone was 8 m long and was sampled with 10 non-overlapping sweeps. Flea beetle densities were significantly different between the three zones in 1999 (ANOVA, $p < 0.01$), but were not significantly different in 2000 (ANOVA, $p > 0.05$). Flea beetle densities were significantly lower in 2000 compared with 1999 (ANOVA, $p < 0.01$). Flea beetle densities were also significantly different between years and zones (ANOVA, $p < 0.01$; see Figure 13). Using morphological characters it was determined that 98 % of *Aphthona* adults collected in 1999 were *A. lacertosa*, and the remaining 2 % were either *A. nigriscutis* or *A. cyparissiae*. The lowest densities of *Aphthona* spp. adults

were nearest the release point in both years (zone 1), where leafy spurge was at the lowest density.

A total of 823 adult flea beetles were collected in the 2000 sweep net samples; 92 % of these adult flea beetles collected were *A. lacertosa,* 4 % were *A. czwalinae* and 1 % were either *A. nigriscutis* or *cyparissiae.* Since all green leafy spurge foliage within the boundary of the site had been eliminated by flea beetles in 2000, beetles were observed clinging to a variety of plant species, although no non-target feeding was observed. Highest concentrations of flea beetles were found in zones 2 and 3 in 2000. Results of isozyme analysis of 36 individuals showed that the flea beetle population was composed of 94.4 % *A. lacertosa* and 6.6 % *A. nigriscutis.* A total of 85 fleabeetles emerged from the soil cores collected on May 10, 2000.

Figure 13. Abundance of adult *Aphthona* spp. in each sample zone at the Medora 2 site in 1999 and 2000. Error bars indicate standard errors of means.