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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A thesis submitted in partial fulfillment
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Master of Science

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Entomology

MONTANA STATE UNIVERSITY
Bozeman, Montana

August 2001

APPROVAL

of a thesis submitted by

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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ACKNOWLEDGEMENTS

The author would like to thank the people who contributed in various ways to the production of this work. My major professor, Dr. Robert Nowierski, provided me with unfailing financial support, advice and a critical review of this thesis. Dr. David Weaver, who served on my committee, provided unlimited advice on all matters of this work, and provided me with support concerning the spatial analyses. Dr. Matt Lavin, who also served on my committee, acted as a mentor of botany, and checked all of my plant species identifications. Collin Bonnet assisted me in the field in the 1999 season. Bryan Fitzgerald advised me on all matters of design and implementation of this project, and assisted me in the field in the 2000 season.

Appreciation is also extended to Cathy Seibert, of the Montana State Herbarium, who helped me with the more difficult plant identifications. Harold Ziolkowski of the USDA-APHIS laboratory in Bozeman spent a good deal of time helping me find acceptable research sites in Montana. I would like to acknowledge Dale Maus, who allowed me to have two research sites on his land in North Dakota. I would also like to thank the entomology student body for sharing their humor, knowledge, comradery and pain with me. Thanks to all the professors at MSU who teach with excellence, and provide students with inspiration. And finally, a big thanks goes out to my family, especially my mother, for supporting me in this endeavor.

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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.

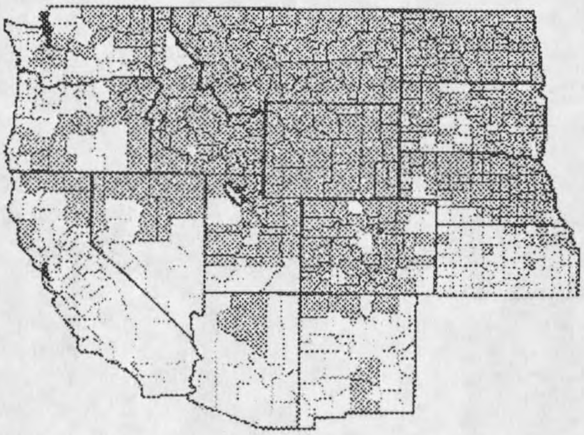
CHAPTER 1

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-trichloropicolinic 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