2000

Optimal site release strategies and impact of biological control agents on spotted knapweed, Centaurea maculosa.

Sheryl Clark
University of Massachusetts Amherst

OPTIMAL SITE RELEASE STRATEGIES AND IMPACT OF BIOLOGICAL CONTROL AGENTS ON SPOTTED KNAPWEED, *CENTAUREA MACULOSA*

A Thesis Presented

by

SHERYL CLARK

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

MASTERS OF ENTOMOLOGY

September 2000

Department of Entomology

OPTIMAL SITE RELEASE STRATEGIES AND IMPACT OF BIOLOGICAL CONTROL AGENTS ON SPOTTED KNAPWEED, *CENTAUREA MACULOSA*

A Thesis Presented

by

SHERYL CLARK

Approved as to style and content by:

Roy G. Van Driesche, Chair

Anne Averill, Member

Joseph Elkinton, Member

John G. Stoffolano, Acting Department Head
Department of Entomology

ACKNOWLEDGMENTS

I would like to thank my thesis committee chair Dr. Roy Van Driesche for all of his contributions and support. Aside from providing advice, guidance, encouragement and support, he believed in my abilities to accomplish this Masters project. I also want to thank Dr. Joseph Elkinton and Dr. Anne Averill for their valued advice and most of all support and belief. I want to thank Dr. John Buonaccorsi for his patience and guidance throughout the statistical analysis of this project.

Thank you to Dr. Nancy Sturdevant, whose valued friendship, support and belief in my abilities and determination were appreciated. Without your unwavering support, financially and emotionally, this would have been difficult to accomplish. Thank you to Dennis Van Dermeer for all your time and patience while working with me during the field aspects of this project. Thanks also to the Rocky Mountain Research Station of the USDA Forest Service, which provided the financial support for this project.

Dr. Jim Story and Bill Good, University of Montana extension experts on the two root feeding biological control agents with which I was working, have been invaluable sources of advice and information on these two insects.

A special thanks goes out to my parents, Lois and Stan Clark; sister and brother-in-law, Shelagh and Russ Fox; and my brother, Carl Clark, for their unconditional love and support.

Thank you also to a very valued friend, Captain Brian Pedersen. Your unwavering faith and love came at a very needed time in this endeavor.

ABSTRACT

OPTIMAL SITE RELEASE STRATEGIES AND IMPACT OF BIOLOGICAL CONTROL AGENTS ON SPOTTED KNAPWEED, *CENTAUREA MACULOSA*

SEPTEMBER 2000

SHERYL CLARK, BSc., UNIVERSITY OF MONTANA, MISSOULA

MS., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by Professor Roy G. Van Driesche

The release history, the knapweed resource, and physical characteristics at ninety-nine natural enemy release sites were evaluated to determine if the details of these release factors correlated sufficiently well with the probability of establishment of two root-feeding insects (the moth *Agapeta zoegana* L. and the weevil *Cyphocleonus achates* Fahraeus) to be of predictive value. The number of insects released (of given species) at the site, the number of years in which releases were made at the sites, and the number of years since the first release were recorded for the release history. The size, shape and density of the knapweed resource at each site were recorded. The physical characteristics recorded at the site were aspect, elevation, percent slope, soil type, percent bare soil, stand topographic type, percent canopy cover, habitat type, annual rainfall, disturbance factors, land use, presence of fire since release, evidence of flooding since release and species of vegetation (by category and composition) around the site. The factors that were correlated to establishment of the moth, *A. zoegana*, were weed infestation type, site forest structure and soil type. The number of years of releases, the weed infestation type

and elevation were the correlated factors for the weevil, *C. achates.*

The efficacy of larval sampling (in roots) versus adult sampling (on plants) of the detection of the two root feeding insects *A. zoegana* and *C. achates* was determined at the same ninety-nine sites used in the main study. The larval sampling consisted of excavation of fifty-two roots per site and dissection of those roots. The adult sampling for the moth and the weevil consisted of sighting of adults along transects at release sites. A second type of adult sampling, sweep net sampling, was also evaluated in the second field season. Recovery rates for the larval and adult visual sampling methods for the moth, *A. zoegana*, showed that adult visual sampling method was quicker and was an easier method of sampling for the moth. The larval sampling, however, was a more sensitive sampling method for detecting the weevil, *C. achates*. For this species, few recoveries were made based on sightings of adults in either field season.

An evaluation of the effects the two root-feeding insects had on spotted knapweed densities was made at thirteen former release sites, in each of the two field seasons of this study. For these thirteen release sites pre-release measurements of the density of the knapweed plant were available. Samples were taken at these sites to determine the average height, number of stems and the number of seed heads per plot. Density of knapweed plants declined at all thirteen sites from the pre-release years (1991-1993) to the post-release evaluation (1997-1998) made in this study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

EFFECTS OF SITE CHARACTERISTICS AND RELEASE HISTORY ON ESTABLISHMENT OF *AGAPETA ZOEGANA* (LEPIDOPTERA:COCHYLIDAE) AND *CYPHOCLEONUS ACHATES* (COLEOPTERA:CURCULIONIDAE), ROOT FEEDING HERBIVORES ATTACKING SPOTTED KNAPWEED, *CENTAUREA MACULOSA*

Introduction

Invasive exotic weeds threaten the natural ecosystems of western United States forests and rangelands. Such weeds invade and damage at least three million hectares per year in western states. Spotted knapweed, *Centaurea maculosa* Lamarck (Asteraceae), occurs across most of the United States, but is most abundant in the northwestern states. It is considered a pest in nine western states and two Canadian provinces and is the most important weed in western Montana, reducing range productivity, wildlife habitat and native plant biodiversity (Story 1984).

Spotted knapweed was most likely introduced into North America in contaminated hay or alfalfa seed in the early 1900s and was first recorded in Montana in the 1920s (French and Lacey 1983). Spotted knapweed is a short-lived perennial forb capable of producing seeds that can germinate throughout the spring and fall. The young plants usually overwinter in the rosette stage and resume growth in the spring (Rees et al. 1996).

Spotted knapweed is highly competitive and easily invades disturbed soil. The plant is adapted to a wide range of environmental conditions and occurs at sites ranging in

elevation from 576 to 3030 meters and in precipitation from 20 to 200 centimeters annually. The largest infestations are on shallow or well-drained soils in locations with 25 to 35 centimeters of annual precipitation. Spotted knapweed is a strong competitor for water and soil nutrients and can suppress seed germination of other species by releasing the allelopathic chemical cnicin into the soil (Lacey et al. 1992). Spread of this species is increased by human activities. Weeds may be caught in undercarriages of many types of machinery such as passenger vehicles, tractors, logging equipment and trains. Movement of contaminated grain and hay has also introduced spotted knapweed into new areas.

The United States Forest Service provides assistance to forest land managers with invasive weed species. Potential methods for control of spotted knapweed include herbicide application, grazing, enhancement of competition from grass species, fertilizer application, fire and release of imported herbivorous insects as biological control agents. The herbicides hexazinone, picloram, clopyralid and 2,4 D provide adequate control of knapweed, but several seasons of treatment are needed to reduce the weed seed bank in the soil (Fay and Davis 1988, McCaffrey and Callihan 1988, Lass and Callihan 1992). Chemical controls, however, can be expensive and are restricted to areas accessible by vehicles. The use of timed, selective grazing can promote the competitiveness of beneficial forage plants that are seeded in spotted knapweed stands (Kennett et al. 1992, Maxwell et al.1992). Nitrogen fertilization by itself as a means of control is impractical and may cause an increase in spotted knapweed density in certain plant communities (Story et al. 1989). Some grass species, however, compete effectively with spotted knapweed when nitrogen fertilizer is applied (Lindquist et al. 1996). Controlled burning has limited potential to reduce spotted knapweed infestations because fire will not carry

through the spotted knapweed stands unless they are very dense or other fine fuels are present. Dense stands can support very intense fires which can scarify the soil. Furthermore, fire does not reduce the seed reserves in the soil (Xanthopoulos 1987).

Biological control agents offer the most promise for permanent control of this noxious weed over large areas of forest and rangeland. Over the preceeding twenty-six years, thirteen agents have been introduced into North America against spotted knapweed (Rees et al. 1996). Two agents are root feeding insects, the cochylid moth *Agapeta zoegana* L. and the curculionid weevil *Cyphocleonus achates* Fahraeus. Other species released against spotted knapweed reduce the seed production of the plant. These are the gelechiidae moth *Metzneria paucipunctella* Zeller, and the tephritid flies *Urophora affinis* Frauenfeld and *Urophora quadrifasciata* Meigen (Julien 1992). Although many species have been released against spotted knapweed, land managers have few tested methods to monitor the establishment, spread, population levels, or impacts on the target plant of most of these organisms.

Successful establishment of newly released beneficial species is a requirement for biological control. Several attributes influence the ability of a newly released species to establish, including number released, frequency of release, genetic diversity of the released population, climatic adaptability, ability to meet its nutritional and reproductive needs in the surrounding area, and phenological synchronicity of the host and the agent (Van Driesche 1993). Experimental evaluations of the importance of these factors are limited. The gorse thrips, *Sericothrips staphylinus* Haliday, was released against gorse, *Ulex europaeus* Linnaeus, in New Zealand in release numbers of 10, 30, 90, 270 or 810 to determine if the number of individuals released affected establishment of the insect in the

field. It was found that eight releases of 90 individuals over the course of a year was more effective than one large release of 1000 individuals (Memmott et al. 1998). However, the probability of establishment of several biological control agents, i.e., *Galerucella salicaria* L., *Galerucella pusilla* Duftschmidt and *Hylobius transversovittatus* Goeze, released against purple loosestrife, *Lythrum salicaria* L., could not be correlated with local climate, number of insects released, use of laboratory-reared versus field-collected insects, or the use of cages versus non-confined insects (Hight et al. 1995). There is a need for more research on the relationship between site features and the probability of establishment to promote more efficient conduct of biological control projects.

Previous studies of spotted knapweed biological control agents have dealt with the biology of the species that have been released, as well as their establishment and spread in the field (Myers and Harris 1980; Neuenschwander 1984; Story 1985; Story and Nowierski 1984, 1985; Muller et al. 1988; Story et al.1987, 1991, 1994, 1996). Other studies have examined factors affecting the knapweed itself, such as the influences of canopy cover, seeding depth and soil moisture on seedling emergence of spotted knapweed (Spears et al. 1980).

The goal of this study was to develop an understanding of how insect release history, plant stand features and release site features affect the success of establishment of two root-feeding insects attacking spotted knapweed, the moth *A. zoegana* and the weevil *C. achates*. This information will provide land managers with guidance on how to more effectively use these insects as control tools by increasing the rate of establishment through selection of better release sites.

Materials and Methods

Study Design

To determine if any features of the history of the natural enemies' release numbers, weed stand features or release site characteristics correlated significantly with probability of establishment, we measured three release history factors, three weed stand features and twenty site characteristics at a set of release sites, while simultaneously sampling to detect establishment of the released insects. The three release history factors were the total number of insects released (of a given species), the number of years in which releases were made at the site and the number of years between the first release at the site along with the year of sampling in our study. The spotted knapweed resource at the site was characterized by the three parameters of patch size of the local spotted knapweed infestation, knapweed infestation type and knapweed density. Site features measured were (1) habitat type, (2) elevation, (3) percent slope, (4) site aspect, (5) topographic type, (6) forest structure at the forested sites, (7) disturbance factors, (8) land use category, (9) forest canopy cover, (10) percent bare soil, (11) annual precipitation, (12) soil type, (13) forest type, (14) vegetation type, (15) percent vegetation cover, (16) five most abundant herbaceous species, (17) five most abundant shrub species, (18) presence of forest insects and diseases in the forested sites, (19) signs of recent flooding and (20) signs of recent fire. Of these factors, the first twelve variables were subjected to statistical analysis; other data were not analyzed but are available as part of the site descriptions as per the United States Forest Service Timber Management Data Handbook.

These release histories, weed stand features and site features were measured at forty-four sites in 1997 and forty-two different sites in 1998 where the moth *A. zoegana*

had been released. The same variables were measured at twenty-three sites in 1997 and twenty-two different sites in 1998 at locations where the weevil *C. achates* had been released. More *A. zoegana* release sites were available for study because this insect has been available for release for fifteen years, compared to only ten years for *C. achates* (Story et al. 1994, 1996). In addition, laboratory rearing of *A. zoegana* has been more successful than for *C. achates*, which has allowed more releases of the former to be made.

Study Sites

The study was conducted in 1997 and 1998, with a portion of each natural enemy's release sites being visited in each year. For *A. zoegana*, eighty-six sites were examined, with seventy-six in Montana, seven in Idaho and three in Washington (Appendix A). For *C. achates*, there were forty-five sites, thirty-five in Montana, nine in Idaho and one in Washington (Appendix B). These releases were selected for the study from pooled records of all previous releases of *A. zoegana* and *C. achates* made by the U.S. Forest Service or state or county agencies. Sites were selected without any knowledge of establishment of the biological control agents to obtain the greatest variation in release history and to maximize the number of sites for *C. achates*, the less commonly released of the two study insects.

Release History, Plant Stand Features and Release Site Features

Release History

Release records of land managers who made releases were examined, and for each site the total number of each insect species released was recorded, as was the number of years since the first release at the site and the total number of years in which releases were made at the site.

Plant Stand Features at the Site

The size of the spotted knapweed infestation at each site was recorded as less than one half of a hectare, one half of a hectare to two hectares, two and one half hectares to four hectares and more than four hectares. The area of weed infestation was taken from notes recorded by the land managers in the year of the first release, or if this information was not available, from our own assessment at the time of this study. Accuracy of patch size assessments was checked by plotting the infestation boundaries on a topographic map. The shape of each spotted knapweed infestation was categorized by visual assessment as patchy, linear or continuous. The patchy sites were areas where the spotted knapweed plants occurred in clumps throughout the infested area, intermixed with other vegetation. The linear patches were typically along roadsides or in land areas where the infestation could only be linear in nature because of topographic constraints. Continuous infestations were areas where spotted knapweed was the dominant vegetation, but not along roadsides or in areas were the topographic constraints made the infestations linear. Therefore, the continuous and linear knapweed classifications are mutually exclusive. We categorized the infestations as stated above to prevent problems where the infestations could fit into more than one category. The density of spotted knapweed at each site was categorized as 1 to 5, 6 to 10, or greater than 10 post-rosette plants per 0.09 m^2. The density was measured by counting the number of spotted knapweed plants that had an erect stem within an area defined by a 0.09 m^2 wire hoop. Sixteen samples were taken per site, located at 3.0, 6.0, 9.0 and 12.1 meters from the release point at center of the site (marked by a reference stake) along lines arranged in the cardinal directions.

Physical Characteristics at the Release Site

Habitat Type. Habitat types of the sites (Appendix C) were determined based on the potential climax vegetation present at each site, using standard forest habitat type categories for Montana (as per Pfister et al. 1977).

Site Elevation. Elevation is the site's altitude above sea level and this value was taken from the land managers' records and cross checked by examining local topographic maps and using an altimeter at each site.

Site Slope. Site slope is the degree of incline (as a percentage) that the land deviates from flat terrain. The site slope was measured with a relaskope at the point of insect release, one measurement pointing up the slope from the release and the other pointing down the slope. The two measurements were then averaged to obtain the slope value for the site.

Site Aspect. The site aspect is the direction or the lay of the land in which the majority of the site faces. Site aspect was determined from contour maps and by taking compass readings directly down slope from points at the site, especially the original point at which insects were released. Aspect of each site was classified for the analysis as north, south, east, west, northeast, northwest, southeast or southwest.

Site Topographic Type. The site topographic type is the general physiographic description for the major land features that surround the site. The scale of recognition is of major ridges and main valleys. The classifications used were valley bottom, concave slope, even bench or even slope, convex slope, ridge-top and sheer cliff.

Site Forest Structure. The site forest structure is a description of the distribution of the tree size classes at or immediately surrounding the site. The site vegetation structure classification categories used were even-age, uneven-age, two-storied, mosaic and no structure (i.e., no forest) if the site was in a grassland area. Descriptions of categories are provided by the Timber Management Data Handbook (Anon., 1991).

Disturbance Factors. The disturbance factors measured were occurrences such as obvious grazing, logging, roads, fire, cultivation, flooding, construction, or the absence of such disturbances at a site. The disturbance factors for the analysis were scored as either positive or negative for each site.

Land Use Factors. The land use variables were intended to characterize how the site was being used and included grazing land, timbered land, wildlife-use areas, road right-of-way, wetland, recreation, or mining.

Canopy Cover. Canopy cover was forest overstory density, as measured by a spherical densitometer (Lemmon 1956). Measurements were taken at the point of insect release, facing north, south, east and west, and then averaging the four values.

Forest Type. The forest type at a site was determined by the dominant tree species at the site. A list of all tree species in the forest stand was recorded.

Annual Precipitation. The value used as the average annual precipitation for each site (rainfall or snow) was the 25 year average from the closest weather station.

Soil Type. Soils present at research sites were noted and fell into seven groups: loam, sand, silty clay loam, sandy clay loam, clay loam, sandy loam and silty loam. Soils at individual sites were classified into a soil type using the key to soils

provided by Nimlos (1992). Additional soil properties that were recorded included cover and depth of the organic layer, percent of coarse fragments, soil color and soil pH, but these values were not analyzed.

Vegetation Type. Vegetation type was determined by the dominant plant groups at the site. The classification categories were grassland, shrubland, cropland, riparian, coniferous forest, deciduous forest, mixed forest, or no single major plant type.

Bare Soil. The degree (%) of bare soil was determined by visual estimation for a 3.6 meter radius plot around the release point.

Sampling Methods to Determine Natural Enemy Presence at Sites

Two methods were used to detect each insect species at all study sites: root excavation to detect larvae and visual observation to detect adults. Root samples were collected in spring and early summer before emergence of adults, as both species overwinter as immatures in roots. Visual samples to detect adults were made on a subsequent visit later in the summer, at times chosen to be appropriate for each site given its altitude and location. Quantitative aspects of observed densities are presented elsewhere (Clark et al., in press). For the analyses in this report, each natural enemy was categorized either as established (if detected) or not established, at each study site.

Root samples were taken on the initial visit to each site, along with the measurement of each site's physical features. The site was then revisited in July, August or September, depending on site altitude and location, at a biologically appropriate time (Rees et al. 1996), and plants were examined visually by an observer to detect adults of the study insects while walking transects through the knapweed infestation.

Root Sampling for Larvae of Both Species

Four transects were established (north, south, east, west) radiating from the central release point and one plant was excavated every 0.90 m from the central release point out to a distance of 11.8 meters, for a total of fifty-two roots sampled per site. Roots were collected in June or early July before emergence of overwintering larvae of either species. Roots were held at 14° C in the laboratory until they could be dissected and the species and numbers of all larvae found recorded.

Visual Sampling for *A. zoegana* Adults

Six 50-meter transects were established at various directions (north, south, west, west, northwest, northeast, southwest or southeast) from the insect release point at each *A. zoegana* release site. An observer walked each transect slowly, scanning the vegetation on either side of the line for adults of *A. zoegana,* which rest on the middle to lower part of the spotted knapweed plant. For each moth sighted, the distance from the origin along the transect and the distance of the moth's position out at 90 degrees from the transect were recorded.

Visual Sampling for *C.achates* Adults

Four transects were established at the major compass directions (north, south, east, west, northwest, northeast, southwest or southeast) from the insect release point, at each *C. achates* site and observations were made at 1.5, 4.5, 7.5 and 10.6 meters along the transect. The directions chosen from the eight possible varied depending on the make-up of the site and the spotted knapweed infestation. At each observation point, the numbers of weevils and plants in a $1m^2$ quadrat, defined by a hoop, were recorded. An observer walked slowly along the transect until reaching a sample point and then slowly

placed the hoop on the ground to prevent disturbing the weevil. *Cyphocleonus achates* is easily disturbed and will fall to the ground and bury itself in the soil or vegetation. As the hoop was placed over the sample area, the vegetation in the quadrant was observed closely to note any *C. achates* present. The observer would then carefully search the sample area for weevils, examining each plant.

Statistical Analysis

To examine the relationship of each variable to the presence or absence of the biological control agents and to test for independence, analyses were carried out using either contingency tables with accompanying Chi-Square or Fisher's exact tests for the categorical variables or using logistic regression for the quantitative variables. The former was done using PROC FREQ in SAS and the latter using Proc LOGISTIC (SAS Institute 1996). In handling the quantitative variables, we tested the adequacy of the logistic model with the Hosmer and Lemeshow goodness of fit test (Hosmer and Lemeshow 1989). For variables where the model failed, the use of a quadratic term or other transformations were considered along with the deletion of some outlying values that might be responsible for the failure of the model.

Our main objective was to find which combinations of the predictor variables such as site characteristics, release history and plant stand features best predicted the presence or absence of the biological control agents. This was done by trying to fit a logistic model for the probability that the agent was present as a function of multiple predictor variables. It was important that categorical variables be properly treated through the use of dummy variables, so we used PROC CATMOD in SAS, which allows for fitting of logistic models involving both categorical and quantitative variables

automatically without the user having to define dummy variables explicitly. However, PROC CATMOD does not test for lack of fit. PROC LOGISTIC, on the other hand, does an automatic assessment of lack of fit of the model, but the user must explicitly define dummy variables to deal with categorical predictors. The original intent was to use PROC CATMOD to suggest some good combinations of predictors and then assess the fit of the model by the use of PROC LOGISTIC in SAS.

Only those variables with p values of less than 0.5 from the individual tests of independence were considered in the multiple predictor models. Unfortunately, even with this criterion, it was impossible given the number of sites and the pattern of the presence or absence response to fit the models that included all of the potential predictors. Models were then considered that were based only on some subsets of predictors. PROC CATMOD was used to model these smaller data sets. If the data did not fit within the model, we employed an ad-hoc approach using selected subsets of the variables, or collapsing of the categories used in defining some of the categorical variables, or examining the association among the different predictor variables to identify variables to delete from the models. Associations among variables were examined by the use of contingency tables for pairs of categorical variables and through correlation for quantitative variables. If two predictors were found to be strongly associated, then both were not considered in the model (as doing so leads to fitting problems).

The PROC MEANS procedure was run on all variables to determine a high percentage of presence or high percentage of absence of the insect and to determine which grouping within each variable was the possible predicting group (Hosmer and Lemeshow 1989). The PROC MEANS procedure can calculate descriptive statistics

separately for groups of statistics and can also produce simple descriptive statistics for numeric variables.

Results

Agapeta zoegana

Release History

For *A. zoegana*, the number of moths released per site varied from 49 to 1945. Establishment rates were highest at sites receiving the greatest number of moths (> 501), with 69% (11/16) of such sites having moths established (Figure 1.1A). Establishment rates, however, were not significantly different across the range of numbers of insects released per site present in this study ($\chi^2 = 0.16$, df = 5, $p = 0.22$).

Sites receiving releases in four or more years had the highest rates (100%) of establishment (Figure 1.1B). No statistically significant relationships, however, could be detected for this variable from the study sites ($\chi^2 = 2.25$, df = 4, $p = 0.69$).

The parameter "number of years since the first release" had its highest rate of establishment at seven years (80%) (Figure 1.1C). No statistically significant relationships, however, could be detected for this variable from the study sites ($\chi^2 = 6.33$, df = 7, $p = 0.50$).

Plant Stand Features

For *A. zoegana*, the highest rate of establishment was at sites with infestation sizes greater than four hectares, with 79% (11/14) of such sites having moth establishment (Table 1.1). Establishment rates, however, were not statistically significant across the infestation sizes present in this study ($\chi^2 = 1.68$, df = 1, $p = 0.19$).

The continuous infestation type had the highest rate of establishment (68%, 25/37) (Table 1.1) and this difference was statistically significant ($\chi^2 = 8.77$, df = 2, $p = 0.01$).

Among plant density categories, the highest rate of establishment occurred in the density range of 11 to 20 stems per 0.09 m^2 (66%, 21/32) (Table 1.1). No statistically significant relationships, however, could be detected for this variable ($\chi^2 = 0.25$, df = 3, $p = 0.97$).

Physical Characteristics

For *A. zoegana*, the physical characteristics that were significant ($p \leq 0.05$) were soil type and forest structure. The highest probability of establishment occurred at sites with clay loam (100%) and sandy clay loam (100%) soils ($\chi^2 = 12.71$, df = 6, $p = 0.05$). Forest structure was also significantly related to establishment probability, with the highest rate of establishment being in even age forests (85%) ($\chi^2 = 8.33$, df = 4, $p = 0.04$). Other physical factors that, while not significant, appeared to be partially correlated to establishment were site slope, precipitation and the presence of disturbance factors. Other factors measured had no relationship to establishment (Table 1.2).

Analysis of Multiple Factors

From among the potential predictor variables, an attempt was made to identify a set that collectively had higher predictive value than any single variable. A model was constructed that incorporated the two most significant quantitative variables (the number of releases and precipitation) and the three most significant categorical variables (soil type, infestation type and forest structure). The model ran with five variables, but when using a backward selection process, only infestation type and forest structure were found to be significant. The joint effects of these two factors are seen by comparing the

percentage establishment for each combination of forest structures and infestation type (Table 1.3). The highest probability of establishment was at sites with continuous knapweed regardless of the site forest structure classification of the forest surrounding the release site (even aged, two storied, mosaic, no forest). Releases at linear roadside strips of knapweed were least likely to result in establishment.

Cyphocleonus achates

Release History

For *C. achates*, the number of moths released per site varied from 25 to 750. Establishment rates were highest at sites receiving the greatest number of weevils (> 200), with 54 % of such sites having the weevil established (Figure 1.2A). Establishment rates were not, however, statistically significant across the range of numbers of insects released per site in this study ($\chi^2 = 0.21$, df = 2, $p = 0.72$).

Sites receiving releases in three years had the highest rates (67%) of establishment (Figure 1.2B) and this difference was statistically significant ($\chi^2 = 5.89$, df = 2, $p = 0.05$).

The number of years since the first release was made had the highest rate of establishment at six years (100%) (Figure 1.2C). No statistically significant relationships, however, could be detected for this variable from the study sites ($\chi^2 = 6.26$, df = 5, $p = 0.28$).

Plant Stand Features

For *C. achates*, the highest rate of establishment was in the infestation size of two and one half hectares to four hectares, with 100% of such sites having weevil establishment (Table 1.1). This difference was not statistically significant ($\chi^2 = 3.57$, df = 1, $p = 0.06$).

The continuous infestation type had the highest rate of establishment (42%) and this difference was statistically significant ($\chi^2 = 7.33$, df = 2, $p = 0.04$).

The highest rate of establishment for plant infestation density categories was in the density range of 6 to 10 post-rosette plants 0.09 m^2 (50 %), but this difference was not statistically significant ($\chi^2 = 5.54$, df = 3, $p = 0.14$).

Physical Characteristics

For *C. achates*, the only physical characteristic that was significant ($p \leq 0.05$) was elevation. The highest probability of establishment occurred at sites that were 910 to 1515 m in elevation (54%) ($\chi^2 = 6.97$, df = 2, $p = 0.03$). Other physical factors that, while not significant, appeared to be partially correlated with establishment were aspect, site forest structure and land use using the criterion of a probability less than 0.25 (Table 1.4). These factors were selected for further analysis using multiple factor analysis. Establishment rate was highest at north-facing sites (50%) ($\chi^2 = 4.31$, df = 3, $p = 0.23$) and at sites with uneven age forests (100%) ($\chi^2 = 5.16$, df = 4, $p = 0.16$). Establishment was highest at sites with developed recreational land use (55%) ($\chi^2 = 8.48$, df = 5, $p = 0.13$). Other factors measured had no relationship to establishment (Table 1.4).

Analysis of Multiple Factors

The attempt to find a set of two or more of the study variables that might be more predictive of establishment probability than any single factor was based on examining a series of two factor models.

Logistic fits for both elevation and precipitation failed the Hosmer-Lemshow goodness of fit test, indicating a logistic regression model involving these variables in a linear fashion was not appropriate. For elevation, this problem was alleviated with the use

of a quadratic model, which resulted in a fitted model of $y = -2E\text{-}06x^2 + 0.0036x - 1.4456$ for the probability of establishment as a function of elevation (Figure 1.3). For precipitation, the data were grouped by precipitation level and the probability of establishment plotted versus the mean precipitation for the group. However, this relationship showed little correlation and precipitation was dropped from further consideration.

Subsequently, we considered four two-factor models, each one combining elevation with one additional variable from among the other four, most predictive single categorical factors (i.e. knapweed infestation size, knapweed infestation type, knapweed plant density category and forest structure). Because of the limited number of sites involved in this study for this insect only the model with elevation and knapweed density could be fitted. Knapweed density was not significant with elevation in this model in the quadratic format.

Discussion

While it is widely accepted that habitat should have important effects on natural enemy success, few studies have successfully measured site characteristics and found them to predict establishment probability. The only major habitat characteristic that has been well studied is climate, in terms of temperature, precipitation and length of the growing season (Cruttwell and Fletcher 1991, McClay 1996).

With regard to spotted knapweed, most land managers currently believe that the best locations to release biological control agents are sites with south-facing aspect, low elevation, moderate slope, high plant density, and large areas of continuous unshaded weed infestations and disturbed soil. These features describe sites favorable to the plant

(Mooers 1986). However, we found only limited support in our study for the proposition that these factors favor the establishment of the two biological control agents of spotted knapweed discussed here. Our findings can be used to construct a hypothesis about good release sites for these two insects, and can also indicate several factors that might previously have been considered important, for which we found no evidence of importance.

For *A. zoegana*, we found that continuous stands of spotted knapweed were the best infestation type to release the moth. Within sites having continuous spotted knapweed patches, sites should be chosen with loamy soils and even aged forests surrounding the release area. It is a common practice to place this insect in roadside strips of spotted knapweed. We found, however, that *A. zoegana* does not establish as well in such areas. Releases of more than 200 adults of *A. zoegana* per site does not seem to lead to a better rate of establishment than smaller releases (100 to 200), and repeated releases at a site over several years do not seem to improve establishment of this insect. It appears that a release of 100 to 200 insects once at a site would be the most effective choice. The density of the plant at the site and the infestation size do not appear to have an effect on establishment. The site aspect, i.e., warm exposures, degree of bare soil, amount of precipitation, topographic position, amount of canopy cover, habitat type, disturbance factors or land use type do not seem important in the establishment of this insect, so should not be considered as important in choosing sites for release.

For *C. achates*, the failure of our data to function in multifactor analyses was most likely due to the small number (45) of release sites available. Considering only the results of single factor analyses, the variables most likely to be predictive of

establishment for *C. achates* were infestation size, infestation type, knapweed density, number of years of releases, aspect, elevation, forest structure and land use. The number of years in which releases were made was also significantly correlated to establishment.

Considering the single factor analyse, we found evidence that continuous stands of spotted knapweed might be the best infestation type of the plant in which to make releases. The establishment of the weevil also appears to be highest at larger sites (> 2 hectares) and at sites with elevations of 910 to 1515 meters. We found no evidence that use of larger releases (> 200) or releasing the weevil at sites with any particular knapweed density affected the rate of establishment. We found that for the weevil, as for the moth, releasing the weevil along roadside strips of spotted knapweed was less effective. The degree of bare soil, amount of precipitation, topographic type, amount of canopy cover, habitat type, disturbance factors or land use do not seem important factors to consider in choosing release sites for this species. Many of the site characteristics analyzed in this study were shown to be of no importance for establishment; however, these negative conclusions might have been influenced by the more limited set of study sites available for the species. Reconsideration of these relationships in the future, once additional releases have been made, might be useful. Our findings should help land managers know which criteria to be concerned with when releasing these biological control agents.

References

Anon.1991.Timber Management Data Handbook. United States Forest Service. Missoula, Montana. R1-FSH 2409.21h.

Clark, S.E., Van Driesche, R.G., Sturdevant, N., Kegley, S. in press. Comparative efficacy of adult and larval sampling for detection of two root boring insects, *Agapeta zoegana* (Lepidoptera:Cochylidae) and *Cyphocleonus achates* (Coleoptera:Curculionidae), released for biological control of spotted knapweed. Florida Entomology.

Cruttwell McFayden, R.E., and Fletcher A. 1991. Climate modeling and the biological control of weeds: - one view. *Plant Protection Quarterly* 6: 14-15.

Fay, P.K., and Davis, E.S.1988. Testing herbicides for spotted knapweed (*Centaurea maculosa* Lam.) control in alfalfa. *Resource Progress Report WesternWeed Science : The Society* p.152.

French, R.A., and Lacey, J.R. 1983. Knapweed : Its cause, effect and spread in Montana. Montana Cooperative Extension Service, Montana State University, Bozeman Circular 307: 1-13.

Hight, S.D., Blossey, B., Laing, J., and Declerck-Floate, R. 1995. Establishment of insect biological control agents from Europe against *Lythrum salicaria* in North America. *Biological Control* 24: 967-977.

Hosmer, D.W., and Lemeshow, S. 1989. *Applied Logistic Regression.* Wiley-Interscience Publication, New York.

Julien, M.H. 1992. *Biological Control of Weeds : A World Catalogue of Agents and their Target Weeds*. 3rd Edition. Canberra, Australia.

Kennett, G.A., Lacey, J.R., Butt, C.A., Olson-Rutz, K.M., and Haferkamp, M.R. 1992. Effects of defoliation, shading and competition on spotted knapweed and bluebunch wheatgrass. *Journal of Range Management* 45: 363-369.

Lacey, C.A., Lacey, J.R., Fay, P.K., Story, J.M., and Zamora, D.L. 1992. Controlling knapweed on Montana rangeland. Montana Cooperative Extension Service, Montana State University, Bozeman. Circular 311: 1-17.

Lass, L.W., and Callihan, R.H. 1992. Spotted knapweed control in a non-crop site. *Resource Progress Report Western Society Weed Science ; The Society* : I-25.

Lemmon, P.E. 1956. A spherical densitometer for estimating forest overstory density. *Forest Science* 2: 314-320.

Lindquist, J.L., Maxwell, B.D., and Weaver, T. 1996. Potential for controlling the spread of *Centaurea maculosa* with grass competition. *Great Basin Naturalist* 56: 267-271.

Maxwell, J.F., Drinkwater. R., Clark, D., and Hall, J.W. 1992. Effect of grazing, spraying and seeding on knapweed in British Columbia. *Journal of Range Management* 45: 180-182.

McCaffrey, J.P., and Callihan, R.H. 1988. Compatibility of Picloram and 2,4-D with *Urophora affinis* and *U. quadrifasciata* (Diptera:Tephritidae) for spotted knapweed control. *Environmental Entomology* 17: 785-788.

McClay, A.S. 1996. Biological control in a cold climate: temperature responses and climate adaptation of weed biological agents. *Proceedings of the IX International Symposium on Biological Control of Weeds*. Stellenbosch, South Africa. University of Cape Town. pp. 377-383.

Memmott, J., Fowler, S.V., and Hill, R.L. 1998. The effect of release size on the probability of establishment of biological control agents: gorse thrips (*Sericothrips staphylinus*) released against gorse (*Ulex europaeus*) in New Zealand. *Biocontrol Science and Technology* 8: 103-115.

Mooers, G.A. 1986. Relationship of critical environmental factors to the success of spotted knapweed (*Centaurea maculosa* L.) in western Montana. Master of Science thesis, Department of Forestry. University of Montana, Missoula, Montana.

Muller, H., Schroeder, D., and Gassman, A. 1988. *Agapeta zoegana* (L.)(Lepidoptera: Cochylidae), a suitable prospect for biological control of spotted and diffuse knapweed, *Centaurea maculosa* Monnet De La Marck and *Centaurea diffusa* Monnet De La Marck (Compositae) in North America. *The Canadian Entomologist* 120: 109-124.

Myers, J.D., and Harris, P. 1980. Distribution of *Urophora* galls in flower heads of diffuse and spotted knapweed in British Columbia. *Journal of Applied Ecology* 17: 359-367.

Neuenschwander, P. 1984. Observations on the biology of the two species of fruit flies (Diptera: Tephritidae), and their competition with a moth larva. *Israel Journal of Entomology* 18: 95-97.

Nimlos, T. 1992. *Introduction to Soils*. University of Montana: Missoula, Montana.

Pfister, R.D., Bernard, L.K., Arno, S.F., and Presby, R.C. 1977. Forest habitat types of Montana. USDA Forest Service General Technical Report INT-34.

Rees, N.E., Quimby, P.C. Jr., Piper, G.L., Coombs, E.M., Turner, C.E., Spencer, N.R., and Knutson, L.V. 1996. *Biological Control of Weeds in the West*. Western Society of Weed Science. Bozeman, Montana.

SAS Institute. 1996. SAS/STAT User's Guide : Version 6, fourth edition, Volume 1 and 2 SAS Institute Inc., Cary, North Carolina.

Spears, B.M., Rose, S.T., and Belles, W.S. 1980. Effect of canopy cover, seeding depth, and soil moisture on emergence of *Centaurea maculosa* and *C. diffusa*. *Weed Research* 20: 87-90.

Story, J.M. 1984. Collection and redistribution of *Urophora affinis* and *U. quadrifasciata* for biological control of spotted knapweed. Montana Coop. Ext. Service. Circular 308. 9 pp.

Story, J.M. 1985. First report of the dispersal into Montana of *Urophora quadrifasciata* (Diptera:Tephritidae), a fly released in Canada for biological control of spotted and diffuse knapweed. *The Canadian Entomologist* 245: 1061-1062.

Story, J.M., and Norwierski, R.M. 1984. Increase and dispersal of *Urophora affinis* (Diptera:Tephritidae) on spotted knapweed in western Montana. *Environmental Entomologist* 13: 1151-1156.

Story, J.M., and Norwierski, R.M. 1985. Population increase of the seed head fly in western Montana. *Montana Ag Research,* pp. 8-10.

Story, J.M., Nowierski, R.M., and Boggs, K.W. 1987. Distribution of *Urophora affinis* and *U. quadrifasciata*, two flies introduced for biological control of spotted knapweed (*Centaurea maculosa*) in Montana. *Weed Science* 35: 145-148.

Story, J.M., Boggs, K.W., and Graham, D.R. 1989. Effects of nitrogen fertilization on spotted knapweed and competing vegetation in western Montana. *Journal of Range Management* 42: 222-225.

Story, J.M., Boggs, K.W., Good, W.R., Harris, P., and Norwierski, R.M. 1991. *Metzneria paucipunctella* Zeller (Lepidoptera:Gelechilidae), a moth introduced against spotted knapweed: its feeding strategy and impact on two introduced *Urophora* spp. (Diptera:Tephritidae). *The Canadian Entomologist* 123: 1001-1007.

Story, J.M., Good, W.R., and White, L.J. 1994. Propagation of *Agapeta zoegana* L. (Lepidoptera:Cochylidae) for biological control of spotted knapweed : procedures and costs. *Biological Control* 4: 145-148.

Story, J.M., White, L.J., and Good, W.R. 1996. Propagation of *Cyphocleonus achates* (Fahraeus) (Coleoptera : Curculionidae) for the biological control of spotted knapweed: procedures and costs. *Biological Control* 7: 167-171.

Van Driesche, R.G. 1993. Methods for field colonization of new biological control agents. In: Bellows, T.S., Jr. and Van Driesche, R.G. (Eds.) *Proceedings: Thomas Say Publications in Entomology: Steps in Classical Arthropod Biological Control*. Entomological Society of America. Lanham, Maryland. pp. 67-86.

Xanthopoulos, G. 1987. Guidelines for burning spotted knapweed infestations for fire reduction in western Montana. *Symposium and Workshop on Protecting People and Homes from Wildfire in the Interior West* October 6-8, pp. 195-198.

Table 1.1 Percentage of sites in each category for the three spotted knapweed stand variables at which each species of the root feeding biological control agents were detected as established.

Agapeta zoegana	Categories of individual variables and % of sites with insect establishment (number of sites in category)				χ^2	df	*p*
Infestation Size[a]	< 1/2 ha 48% (20/42)	1/2 to 2 ha 64% (14/22)	2 1/2 to 4 ha 63% (5/8)	> 4 ha 79% (11/14)	1.68	1	0.19
Infestation Type	Patchy 52% (20/38)	Linear 36% (4/11)	Continuous 68% (25/37)		8.77	2	0.01
Infestation Density (plants[b]/ 0.09 m^2)	0 to 5 44% (4/9)	6 to 10 53% (19/36)	11 to 20 66% (21/32)	> 20 55% (5/9)	0.25	3	0.97

Cyphocleonus achates	Categories of individual variables and % of sites with insect establishment (number of sites in category)				χ^2	df	*p*
Infestation Size[a]	< 1/2 ha 14% (3/22)	1/2 to 2 ha 23% (3/13)	2 1/2 to 4 ha 100% (4/4)	> 4 ha 50% (3/6)	3.57	1	0.06
Infestation Type	Patchy 0% (0/9)	Linear 30% (3/10)	Continuous 42% (11/26)		7.33	2	0.04
Infestation Density (plants[b]/ 0.09 m^2)	0 to 5 14% (1/7)	6 to 10 50% (7/14)	11 to 20 32% (6/19)	> 20 20% (1/5)	5.34	3	0.14

[a] Chi-Square values for the variable are Wald Chi-Squares from logistic regression analyses, appropriate for continuous variables

[b] post-rosette plants, each with one stem

Table 1.2 Percentage of sites in each category for the site characteristic variables at which *Agapeta zoegana* was established.

	Categories of individual variables and % of sites with insect establishment (number of sites in category)				χ^2	df	*p*
Aspect	North 50% (5/10)	South 59% (30/51)	East 54% (7/13)	West 42% (5/12)	0.77	3	0.86
Slope[a]	0 - 10 % 62% (31/50)	11 - 30 % 48% (12/25)	30 + % 45% (5/11)		1.70	1	0.19
Elevation[a]	424 - 909 m 57% (20/35)	910 – 1515 m 58% (20/34)	1515 - 1879 m 52% (9/17)		<.001	1	0.99
Bare Soil[a]	0 - 10 % 70% (30/43)	11 - 30 % 50% (15/30)	30 + % 38% (5/13)		0.42	1	0.52
Precipitation[a]	0 to 25 cm 100% (2/2)	26 to 50 cm 64% (27/42)	> 50 cm 43% (18/42)		2.30	1	0.13
Soil Type	Sand 31% (4/13) Sandy Loam 47% (9/19)	Loam 27% (3/11) Silty Clay Loam 47% (9/19)	Clay Loam 100% (6/6) Sandy Clay Loam 100% (6/6)	Silty Loam 75% (9/12)	12.71	6	0.05
Topographic Type	Even Bench 46% (18/39)	Convex 8% (1/13)	Valley Bottom 50% (7/14)	Concave 65% (13/20)	1.70	3	0.64
Site Forest Structure	Even aged 85% (11/13) None 67% (12/18)	Uneven aged 50% (1/2)	Two storied 55% (11/20)	Mosaic 43% (14/33)	8.33	4	0.04
Forest Canopy cover[a]	0 - 9 % 59% (44/75)	10 - 19 % 20% (1/5)	≥ 20 % 50% (3/6)		0.02	1	0.90
Habitat Type	PIPO Series 62% (13/21)	PSME Series 43% (15/35)	ABGR/THPL Series 50% (3/6)	None 71% (17/24)	4.04	3	0.26
Disturbance Factors[b]	Grazing 80% (20/25) Cultivation 0% (0/2)	Logging 53% (10/19) Flood 56% (5/9)	Road 45% (17/38) Construction 43% (3/7)	Fire 44% (4/9)	7.38	6	0.13
Land Use[b]	Grazing 50% (12/24) Wetland 45% (5/11)	Timber 50% (6/12) Developed Recreation 42 % (5/12)	Wildlife 53% (20/38)	Right-of-way 43% (12/28)	4.19	5	0.65

a Chi-Square values for these variables are Wald Chi-Squares from logistic regression analyses, appropriate for continuous variables (Hosmer and Lemeshow 1989)

b For two variables (disturbance factors and land use), totals exceed number of sites (86) because individual sites could be assigned to more than one category

Table 1.3 The proportion of sites (n = number of sites in cell) at which *Agapeta zoegana* successfully established, relative to the stand structure (of the surrounding forest) and the infestation type (of the spotted knapweed infestation).

Categories of infestation type	**Categories of stand structure**			
	Even aged	**Two storied**	**Mosaic**	**None**
Continuous	1.00 (7)	0.63 (8)	0.58 (19)	0.89 (9)
Patchy	0.80 (5)	0.25 (8)	0.22 (9)	0.57 (7)
Linear	0.00 (1)	0.67 (3)	0.33 (6)	0 (1)

Table 1.4 Percentage of sites in each category for the site characteristic variables at which *Cyphocleonus achates* was established.

	Categories of individual variables and % of sites with insect establishment (number of sites in category)				χ^2	df	*P*
Aspect	North 50% (2/4)	South 35% (11/31)	East 13% (1/8)	West 0% (0/2)	4.31	3	0.23
Slope[a]	0 - 10 % 33% (8/24)	11 - 30 % 23% (3/13)	30 + % 38% (3/8)		0.02	1	0.88
Elevation[c]	424 - 909 m 25% (5/20)	910 – 1515 m 54% (7/13)	1515 - 1879 m 17% (2/12)		6.97	2	0.03
Bare Soil[a]	0 - 10 % 43% (9/21)	11 - 30 % 16% (3/19)	30 + % 40% (2/5)		0.03	1	0.86
Precipitation[c]	0 to 50 cm 43% (12/28)	> 50 cm 24% (4/17)			1.18	1	0.28
Soil Type	Sand 50% (3/6) Sandy Loam 7% (1/15)	Loam 0% (0/2) Silty Clay Loam 60% (3/5)	Clay Loam 50% (2/4) Sandy Clay Loam 40% (2/5)	Silty Loam 38% (3/8)	5.98	6	0.43
Topographic Type	Even Bench 38% (3/8)	Convex 42% (5/12)	Valley Bottom 39% (7/18)	Concave 29% (2/7)	0.05	3	0.99
Site Forest Structure	Even aged 44% (4/9) None 33% (3/9)	Uneven aged 100% (1/1)	Two storied 13% (1/8)	Mosaic 39% (7/18)	5.16	4	0.16
Forest Canopy cover[a]	0 - 9 % 38% (16/42)	10 - 19 % 0% (0/3)	≥ 20 % 0% (0/0)		0.65	1	0.42
Habitat Type	PIPO Series 27% (3/11)	PSME Series 33% (5/15)	ABGR/THPL Series 67% (2/3)	None 25% (4/16)	2.65	3	0.45
Disturbance Factors[b]	Grazing 44% (7/16) Cultivation 100% (2/2)	Logging 33% (3/9) Flood 0% (0/2)	Road 31% (4/13) Construction 20% (1/5)	Fire 50% (1/2)	6.51	6	0.37
Land Use[b]	Grazing 42% (5/12) Wetland 14% (1/7)	Timber 33% (2/6) Developed Recreation 55 % (6/11)	Wildlife 31% (4/13)	Right-of-way 21% (3/14)	8.48	5	0.13

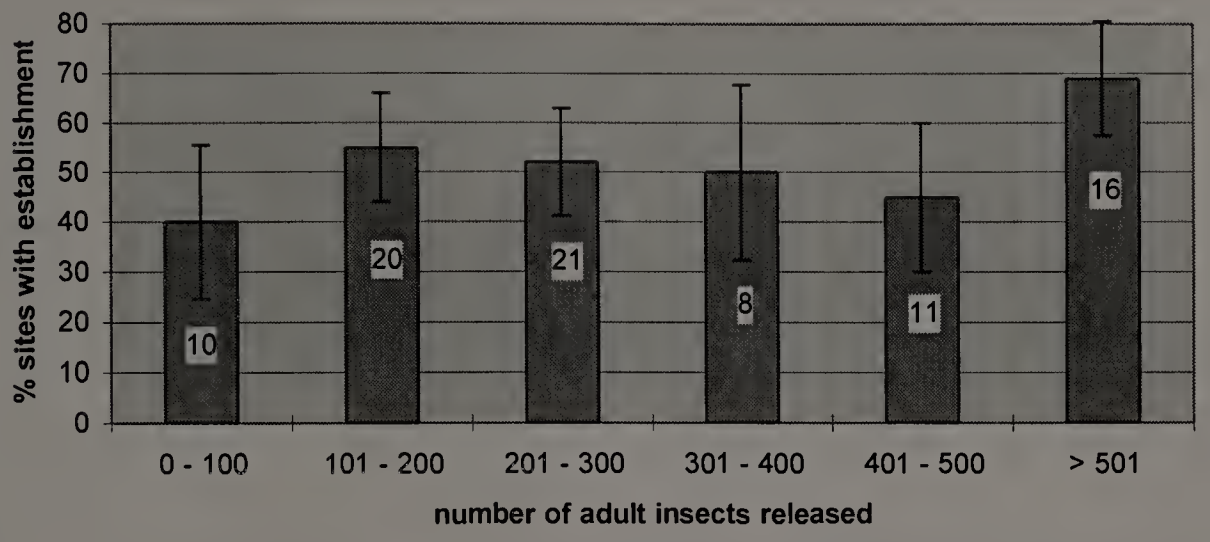
a Chi-Square values for these variables are Wald Chi-Squares from logistic regression analyses, appropriate for continuous variables (Hosmer and Lemeshow 1989)

b For two variables (disturbance factors and land use), totals exceed number of sites (86) because individual sites could be assigned to more than one category

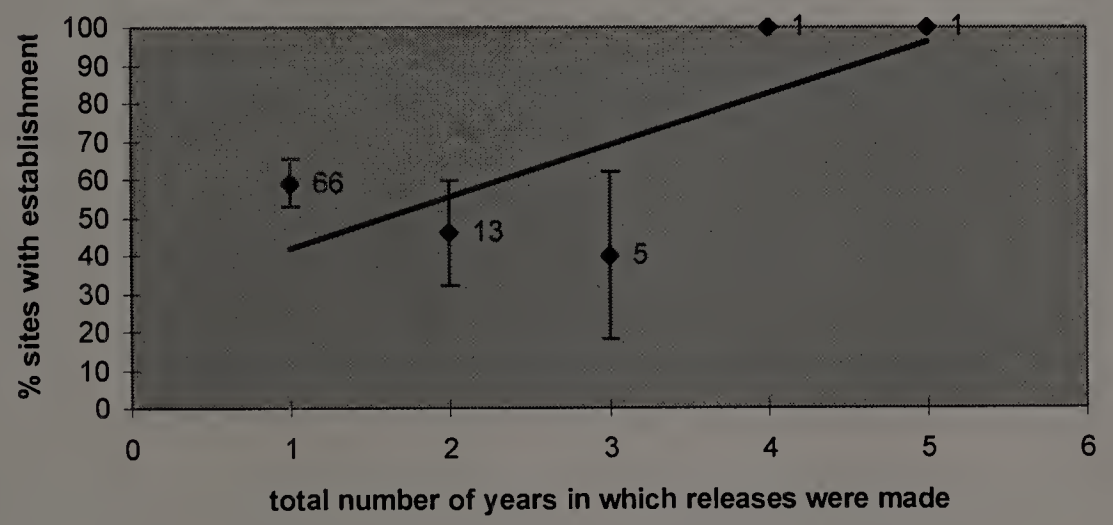
c For elevation and precipitation, the Hosmer and Lemeshow Goodness-of-Fit test indicated a significant departure from the model (Hosmer and Lemeshow 1989)

Figure 1.1 Effect on establishment rate (%) of *Agapeta zoegana* in relation to the number of insects released per site (A); the number of years releases were made at a site (B); and the number of years between the first release and site assessment in this study (C).

(A)



(B)



(C)

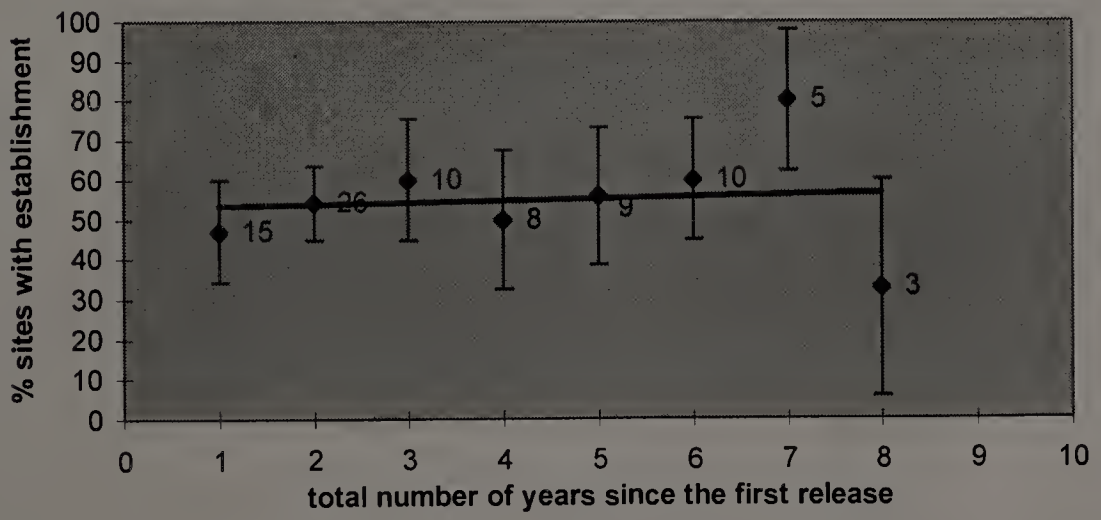
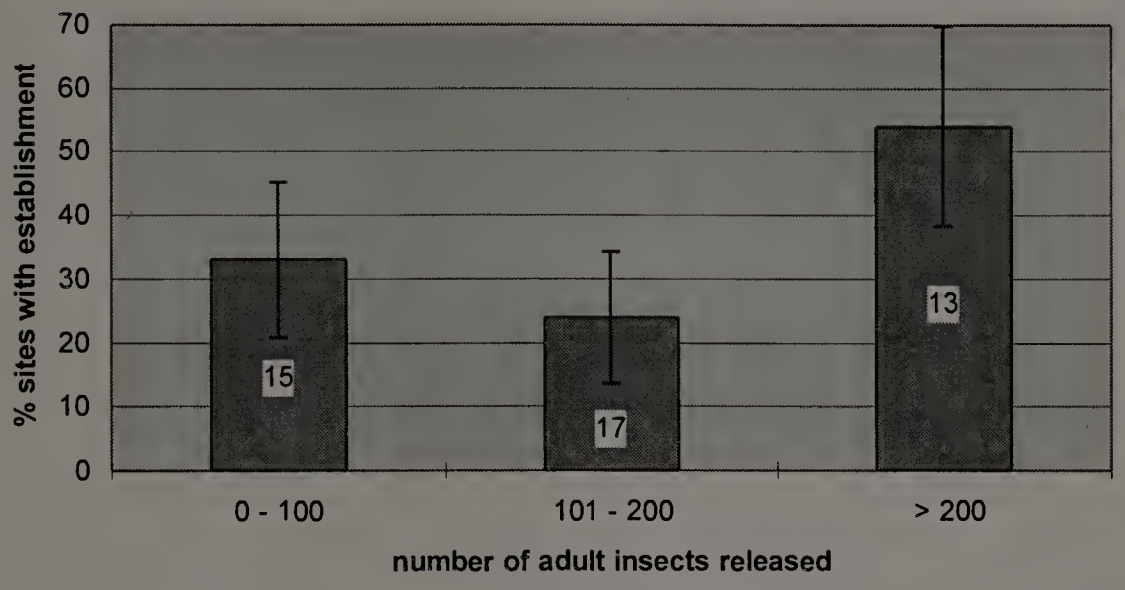
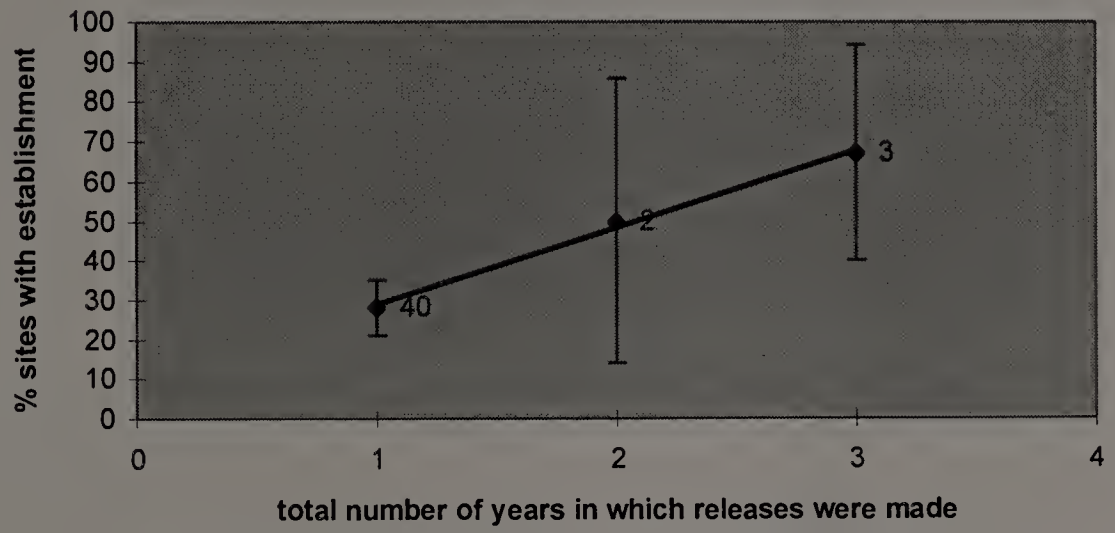


Figure 1.2 Effect on establishment rate (%) of *Cyphocleonus achates* in relation to the number of insects released per site (A); the number of years releases were made at a site (B); and the number of years between the first release and site assessment in this study (C).

(A)



(B)



(C)

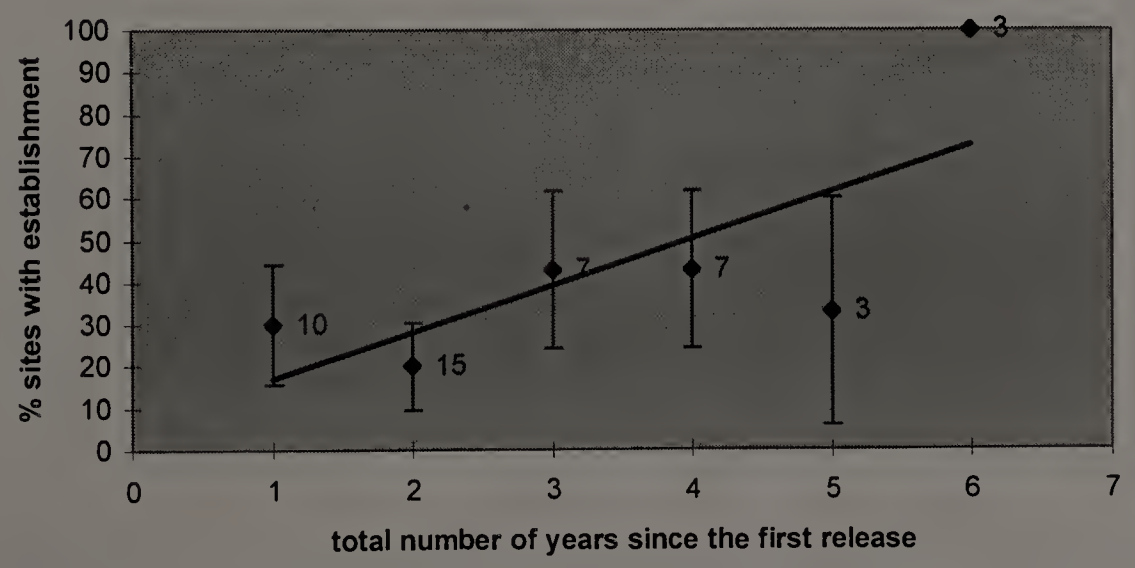
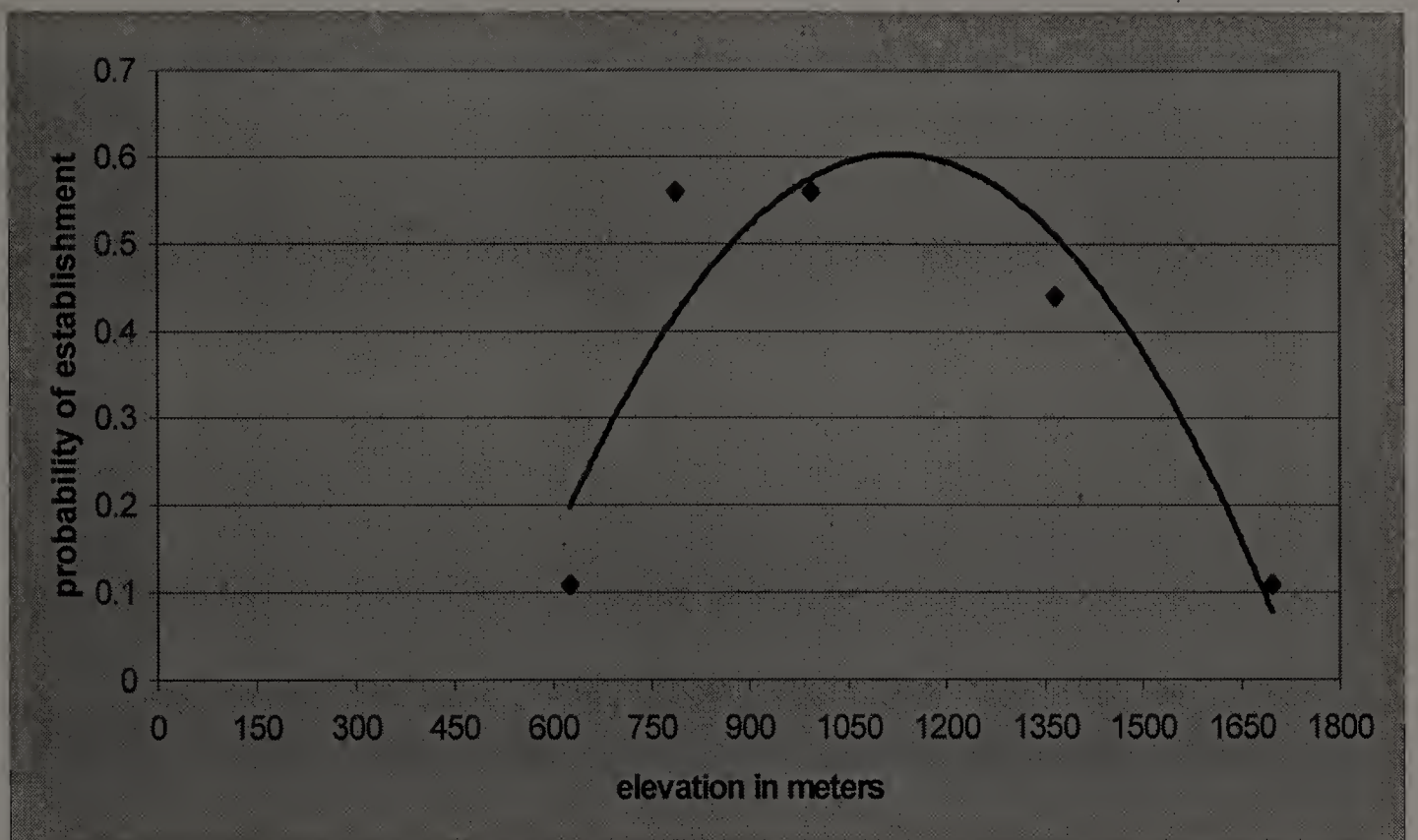


Figure 1.3 Probability of establishment of *Cyphocleonus achates* in relation to elevation at the release sites.



CHAPTER 2

COMPARATIVE EFFICACY OF ADULT AND LARVAL SAMPLING FOR DETECTION OF TWO ROOT BORING INSECTS, *AGAPETA ZOEGANA* (LEPIDOPTERA: COCHYLIDAE) AND *CYPHOCLEONUS ACHATES* (COLEOPTERA: CURCULIONIDAE), RELEASED FOR BIOLOGICAL CONTROL OF SPOTTED KNAPWEED

Introduction

Agapeta zoegana (L.) (Lepidoptera: Cochylidae) and *Cyphocleonus achates* (Fahraeus) (Coleoptera: Curculionidae) are root boring insects that have been released in the United States for the biological control of spotted knapweed, *Centaurea maculosa* (Lamarck) (Asteraceae). *Agapeta zoegana* is Eurasian in origin and is distributed throughout most of Europe (Toth et al. 1985, Muller et al. 1988, Fitzpatrick 1989, Julien 1992, Rees et al.1996). *Cyphocleonus achates,* also of Eurasian origin, is found in eastern and southern Europe and Asia Minor (Julien 1992, Story 1992, Powell et al. 1994, Stinson et al. 1994, Rees et al. 1996, Wikeem and Powell 1999). *Agapeta zoegana* was first introduced into the United States in Montana in 1984 and is now established in Montana, Oregon and Washington. *Cyphocleonus achates* was first introduced into Montana and Washington in 1988 and is now established in Montana, Colorado, Oregon and Washington (Story et al.1991; Julien 1992; Story et al. 1994, 1996, 1997; Rees et al.1996).

Details of the biology of these two species influence the ease and efficacy of sampling to detect larval or adult stages. The moth *A. zoegana* has one generation a year

in the northern United States but can be multivoltine in warmer climates. Eggs are laid singly or in small groups (2-3) on the surface of the stems or leaves of spotted knapweed.. Eggs hatch in seven to ten days, and larvae move to the crown area where they mine the root. Larvae can move to roots of other plants within ten centimeters of an occupied root. Larvae overwinter and pupate in roots. Adults emerge from mid-June to mid-August. They are approximately eleven millimeters in length and have bright yellow wings with distinctive brown markings. The females can mate within twenty-four hours of emergence and can begin laying eggs within two days of emergence. The adults live about eleven days (Muller et al. 1988, Powell et al. 2000, Story et al.1994, Rees et al. 1996).

The weevil *C. achates* also has one generation a year, with eggs being laid singly in notches excavated by the female in the root crown, or in the root just below the soil surface. Eggs hatch in ten to twelve days, and larvae move immediately toward the center of the root. Weevils overwinter as second instar larvae. Older larvae (3^{rd} and 4^{th} instars) cause gall-like enlargements in roots. Larvae pupate in roots, and adults emerge from mid-August to mid-September. Adults are fourteen to fifteen mm in length and live eight to fifteen weeks (Story 1992, Powell et al.1994, Stinson et al. 1994, Wikeem and Powell 1999).

Because both larvae and adults are associated with spotted knapweed plants for extended periods, either stage could in principle be used to detect establishment of these species. The objective of this study was to compare the efficiency of adult and larval sampling methods, in terms of both efficiency and cost, for the detection of establishment of these insects. This information can assist land managers in monitoring release sites to detect establishment.

Material and Methods

Experimental Design and Locations of Study Sites

To compare sampling techniques for these spotted knapweed natural enemies, we evaluated three sampling methods: root dissection to detect larvae, visual counts of adults resting on plants and sweep net sampling of adults. The study was conducted at field sites in Montana, Idaho and Washington in 1997 and 1998, where previous releases of these species had been made (see below for details). In each year, approximately half the available release sites were assessed. See Clark et al. (in press) for information on study site locations.

Sampling Methods

In 1997, we dissected root samples to detect larvae and we made visual observations to detect adults of the two species. In 1998, this approach was repeated with the addition of sweep net sampling to detect adults. Larval samples were taken in June or early July when insects were in the larval stage inside the roots. In July, August, or September (depending on the location and elevation of the site) we revisited all sites to look for adult moths or weevils on spotted knapweed plants by walking transects through the knapweed infestations. Sweep net samples in 1998 were taken at the same time as the visual sampling for adults.

In 1997, we examined forty-four sites where *A. zoegana* had been released, and in 1998 we examined forty-two additional sites. For *A. zoegana*, a total of eighty-six sites were examined over the two years, with seventy-six in Montana, seven in Idaho and three in Washington. In 1997, we also examined twenty-three sites where *C. achates* had been released, and in 1998 we examined twenty-two additional *C. achates* sites.

For *C. achates,* forty-five sites were examined over the two year study, thirty-five sites in Montana, nine in Idaho and one in Washington. More *A. zoegana* release sites were available to study due to the longer period, fifteen years, that this insect has been available for release compared to only ten years for the weevil and to the fact that the weevil has been more difficult to rear in large numbers (Story et al. 1994, 1996).

Larval Sampling. Larvae of both species were sampled by dissecting a fixed number of spotted knapweed roots from each site. Roots were chosen for dissection by establishing four transects (north, south, east, west) originating at the point of original release of the insect at the site. Samples consisted of single roots and these were collected every 0.90 m from the central release point out to a distance of 11.8 m, for a total of fifty-two roots per site. Roots were collected in June and early July before emergence of overwintering larvae. Each root was dissected and the number and species of all recovered larvae recorded. The diameters of all sample roots were measured with calipers two cm below the root collar, and signs of rot or insect boring (by *A. zoegana* and *C. achates*) were noted. To measure how long the sampling process took, we recorded the times needed to dig and dissect each of 1040 roots (20 sites with 52 roots per site).

Visual Sampling for Adult Moths. Adults of *A. zoegana* seen resting on plants were counted by an observer while walking along each of six 50-meter transects at each site. For each moth sighted, we recorded the distance along the transect and distance out (at 90 degrees) from the transect line. The six 50-meter transects were established at the major compass directions (north, south, east, west, northwest, northeast, southwest, southeast) from the insect release point. Transect distances were measured with a surveyor's wheel. To measure how long the sampling process took, we recorded the time

needed to walk each of six transects at twenty sites (120 transects total) and to record our observations on moth presence.

Visual Sampling for Adult Weevils. Because weevils are more sedentary than *A. zoegana,* their occurrence could be observed on an area basis, using a 1 m^2 quadrat sampling frame to sample areas along transects. This approach could not be used for the moth because placing the frame in the foliage caused moths to fly away. Four transects were established along any of the major compass directions (north, south, east, west, northwest, northeast, southwest, southeast), where plants were available, moving outward from the insect release point. Quadrat observations were made at points 1.5, 4.5, 7.5 and 10.6 m. At each point, the numbers of weevils and plants within a 1 m^2 sample area (defined by a rigid frame) were recorded. To measure how long this sampling process took, we recorded sampling times for 320 samples (16 plots at 20 sites).

Sweep Net Sampling. Because land managers are familiar with the use of sweep net sampling to detect the presence of insects, we included this procedure in our 1998 evaluations. At each site, six transects were established, and twenty sweeps with a sweep net were taken while walking along each transect. The six transects were established at the major compass directions (north, south, east, west, northwest, northeast, southwest, southeast) from the insect release point. The numbers of adults of *A. zoegana* or *C. achates* collected per twenty sweeps were recorded, and the distance swept along the transect for each sample set was measured. The distance along transects was measured with a surveyor's wheel. To measure how long this sampling process took, for six transects at twenty sites (120 transects total), we measured the time required to take a set of twenty sweeps and examine the collected material for each species.

Relative Index

The relative index of efficiency is the percentage of positive results for a sampling unit (i.e. roots, transects, plots) divided by the time needed for each method, further divided by the percentage positive for the most efficient sampling unit divided by the time needed for its collection. In essence, this is the yield per unit time for each technique, relative to the yield per unit time for the most efficient method. The index will, therefore, vary from zero to one, with one being the value for the most efficient method.

Statistical Analysis

Chi-Square contingency tables were used to assess if the rate of detection of either the moth, *A. zoegana,* or the weevil, *C. achates*, was significantly related to the sampling method used. The exact binomial test was used to determine the p values for comparisons made in Chi-Square tests. Chi-Square contingency tables were also used to test for relationships between rate of infestation and root diameter (SAS Institute 1996).

Results

A summary of the yield and efficacy of the sampling techniques is presented in Table 2.1.

Agapeta zoegana

Visual sampling for adults was more effective than larval sampling for detecting establishment of *A. zoegana*, with 54.8 % of visual sampling efforts being positive for *A. zoegana* adults, but only 43.0 % being positive for larvae in roots ($n = 86$, $\chi^2 = 19.99$, $df = 1$, $p = 0.001$). Sweep net sampling, in contrast, was less effective than larval sampling for *A. zoegana*, with only 38.1 % of sites showing detection for adult moths in sweep net samples, compared to a 43.0 % detection rate for larval sampling ($n = 42$,

$\chi^2 = 11.18$, df = 1, $p = 0.001$). Sweep net sampling (38.1%) was also less effective than visual sampling (54.8 %) of adults (n = 42, $\chi^2 = 7.32$, df = 1, $p = 0.007$) (Table 2.1) (SAS Institute 1996).

Cyphocleonus achates

In contrast to *A. zoegana,* dissecting roots to detect larvae of the weevil *C. achates* was more effective than visual sampling of adults with only 8.9 % of sites being positive for visually detecting adult weevils compared to 35.6 % of sites being positive for detection of weevil larvae (n = 45, $\chi^2 = 7.96$, df = 1, $p = 0.005$) (Table 2.1). Dissection as a sampling method was also more effective than detection of adult weevils in sweep net samples, with only 18.1 % of sites being positive for the weevil using sweep netting, compared to 35.6 % with root dissection (n = 22, $\chi^2 = 8.56$, df = 1, $p = 0.003$). Sweep net sampling was more effective than visual detection of adults with 18.1 % of sites being positive for adults using sweep net sampling and 8.9 % with adult visual sampling (n = 22, $\chi^2 = 22.00$, df = 1, $p = 0.001$) (SAS Institute 1996).

Sampling Effort Times

Root excavation and dissection. The total time to collect and dissect one sample of 52 roots was 130 minutes (± SE = 8.01, n = 20). It took 0.5 minutes (± SE = 2.01, n = 1040) to dig each root and 2.0 minutes (± SE = 8.09, n = 1040) to dissect each root.

Visual Sampling. Average time needed to visually search for *A. zoegana* adults along six 50-meter transects was 30 minutes (± SE = 1.83, n = 120). The average sampling time per transect was 5 minutes (± SE 0.17, n = 720). Sampling time increased as the number of adults found on the transect increased. The average time for visual

sampling of *C. achates* in 16 $1m^2$ quadrats was 44 minutes (± SE = 3.73, n = 20). The average time per quadrat was 2.75 minutes (± SE = 0.61, n = 320).

Sweep Net Sampling. The average time for collection of a set of six sweep net samples (20 sweeps each) for adults of *A. zoegana* and *C. achates* and examination of net contents was 10 minutes (± SE = 0.09, n = 120). Average time needed to collect one set of 20 sweeps was 0.38 minutes (± SE = 0.05, n = 120). Inspection of the sweep net contents for both species from one sample (20 sweeps) took 1.2 minutes (± SE = 0.06, n = 120), depending on the number of the insects present.

Relative Index of Efficiency

Agapeta zoegana. Sweep net sampling was the most efficient sampling method with a relative index of 1.0. Visual sampling was the second most efficient method (0.49) with the larval (dissection) sampling being the least efficient sampling method (0.09).

Cyphocleonus achates. The sweep net sampling method was again the most efficient method with a relative index of 1.0. In contrast to the moth, the dissection method for the weevil was more efficient (0.15) than the visual sampling method (0.11).

Effect of Root Size

Root diameters ranged from 0.2 to 4.1 cm. The smallest infested root was 0.43 cm in diameter. Most roots (75 %) sampled (4458 of 5924) were in the smallest size class (0.2 to 0.9 cm). For both insects, the proportion of roots infested increased with root diameter, being highest (32.4 %) for *A. zoegana* in roots with diameters of 1.7 to 2.3 cm and highest (4.0 %) for *C. achates* in roots of 2.4 to 4.1 cm (Table 2.2). For both *A. zoegana* (n = 5924, $\chi^2 = 3998.6$, df = 3, $p < 0.005$) and *C. achates* (n = 5924, $\chi^2 = 25.2$,

df = 3, $p < 0.005$) the percentage of infested roots was significantly related to root diameter.

Discussion

For *A. zoegana*, visual sampling was the most effective sampling method in detecting the presence of this species at a release site. However, when time required was considered, sweep net sampling was the most effective method per unit time, followed by visual sampling (Table 2.1). Larval sampling was the most time-consuming method.

For *C. achates*, larval sampling was the most effective method to detect establishment at the sites, if time is not considered. However, the yield per unit of sampling time if not considering travel time (travel time was the time it took to get to the study sites) was again greatest for the sweep net method because larval sampling requires much more time than either adult visual sampling or sweep net sampling (Table 2.1).

Larval sampling, though not the most efficient method per unit of sampling time, has some attributes that make it more attractive for use than the time analysis suggests. First, it is not as sensitive to seasonal timing as visual and sweep net sampling because larvae are present in roots longer than adults are present at field sites. Second, it is not as weather sensitive as are the other two methods and can be used on cloudy or even rainy days when visual counts or sweep net sampling might not be feasible. Third, the level of skill needed by a sampler to collect roots is low. The principal defect of root sampling is the amount of time needed to dissect roots. However, this time is spent in the laboratory and dissection can take place when convenient because roots can be stored for up to two months before dissection. This allows for one person to collect roots and another to dissect them. It also allows part of the labor to be deferred if needed or scheduled around

other activities. If root sampling is chosen, it might be more efficient to chose only plants of the size classes where infestation levels are highest (Table 2.2).

The visual counting methods for *A. zoegana* and *C. achates* are more time efficient than root dissection, but have several problems that should be considered in choosing a sampling method. Visual counts are strongly affected by seasonal phenology of the insects and weather on the sample day. They also require relatively skilled observers because both the moth and weevil can be difficult to see and are easily disturbed. Since weather on sample days would be hard to predict if sites were far away or at different altitudes, visual sampling may prove impossible to do in some cases. Time invested in traveling to sites, would therefore be lost, making this method more costly than it might appear.

The sweep net sampling method was the most effective method per unit effort. Even unskilled observers can correctly use a sweep net. However, the moth *A. zoegana* is easily damaged by this method if the observer sweeps too hard. This method, as with the visual count method, is sensitive to seasonal phenology, time of day, and daily weather. Also sweep netting can be difficult to do in dense vegetation.

Finally, the time that it takes to travel to the sampling site needs to be considered in selecting the method. The visual and sweep net sampling methods should be conducted in the middle of the day, whereas the larval sampling can be conducted at any time. Sampling only in the middle of the day can limit the number of sites per day that can be visited to only a few, if there is a large distance between sites. While all three methods can be used effectively, constraints of time and personnel will likely determine which sampling method is best for particular sites or institutions.

References

Clark, S.E., Van Driesche, R.G., Sturdevant, N., Elkinton, J., and Buonaccorsi, J. P. in press. Effects of site characteristics and release history on establishment of *Agapeta zoegana* (Lepidoptera: Cochylidae) and *Cyphocleonus achates* (Coleoptera: Curculionidae), root feeding herbivores attacking spotted knapweed, *Centaurea Maculosa*. *Biological Control.*

Fitzpatrick, S. M. 1989. A potential collection method for *Agapeta zoegana* (Lepidoptera: Cochylidae), a knapweed root feeding moth. *Journal of the Entomological Society of British Columbia* 86: 55-62.

Julien, M.H. 1992. *Biological Control of Weeds*: *A World Catalogue of Agents and their Target Weeds*. 3rd Edition. Canberra, Australia.

Muller, H., Schroeder, D., and Gassmann, A. 1988. *Agapeta zoegana* (L.) (Lepidoptera:Cochylidae) a suitable prospect for biological control of spotted and diffuse knapweed, *Centaurea Maculosa* Monnet De La Marck and *Centaurea diffusa* Monnet De La Marck (Compositae) in North America. *The Canadian Entomologist* 120: 109-124.

Powell, G.W., Sturko, A., Wikeem, B.M., and Harris, P. 1994. Field guide to the biological control of weeds in British Columbia. *British Columbia Ministry of Forests Research Program Land Management Handbook* 27.

Powell, G. W., Wikeem, B. M., and Sturko, A. 2000. Biology of *Agapeta zoegana* (Lepidoptera:Cochylidae), propagated for the biological control of knapweeds (Asteraceae). *The Canadian Entomologist* 132: 223-230.

Rees, N.E., Quimby, P.C. Jr., Piper, G.L., Coombs, E.M., Turner, C.E., Spencer, N.R., and Knutson, L.V. 1996. *Biological Control of Weeds in the West*. Western Society of Weed Science. Bozeman, Montana.

SAS Institute.1996. SAS/Stat Users Guide: Version 6, Fourth Edition, Volume 1 and 2., SAS Institute Inc., Cary.

Stinson, C.S.A., Schroeder, D., and Marquardt, K. 1994. Investigations of *Cyphocleonus achates* (Fahr.) Col., Curculionidae, a potential biological control agent of spotted knapweed (*Centaurea Maculosa* Lam.) and diffuse knapweed (*C. diffusa* Lam.) (Compositae) in North America. *Journal of Applied Entomology* 117: 35-50.

Story, J.M. 1992. Biological control of weeds: selective, economical and safe. *Western Wildlands* 18: 18-23.

Story, J.M., Boggs, K.W., and Good, W.R. 1991. First report of the establishment of *Agapeta zoegana* L. (Lepidoptera: Cochylidae) on spotted knapweed, *Centaurea maculosa* Lamarck, in the United States. *The Canadian Entomologist.* 119: 411-412.

Story, J.M., Good, W.R., and White, L.J. 1994. Propagation of *Agapeta zoegana* L. (Lepidoptera: Cochylidae) for the biological control of spotted knapweed: procedures and cost. *Biological Control* 4: 145-148.

Story, J.M., White, L.J., and Good, W.R. 1996. Propagation of *Cyphocleonus achates* (Fahraeus) (Coleoptera: Curculionidae) for the biological control of spotted knapweed: procedures and cost. *Biological Control* 7: 167-171.

Story, J.M., White, L.J., and Good, W.R. 1997. First report of the establishment of *Cyphocleonus achates* (Fahraeus)(Coleoptera:Curculionidae) on spotted knapweed, *Centaurea Maculosa* Lamarck, in the United States. *The Canadian Entomologist* 129: 373-374.

Toth, M., Guerin, P.M., Buser, H.R., Muller, H., Szocs, G., Sziraki, G., and Arn, H. 1985. Z-11-Tetradecenyl acetate: sex attractant of *Agapeta zoegana* (Lepidoptera: Tortricidae), a potential species for the biological control of knapweed. *The Canadian Entomologist* 117: 1163-1165.

Wikeem, B.M., and Powell, G.W. 1999. Biology of *Cyphocleonus achates* (Coleoptera: Curculionidae), propagated for the biological control of knapweeds (Asteraceae). *The Canadian Entomologist* 131: 243-250.

Table 2.1. Summary of the yield (percentage recovered) of the insects *Agapeta zoegana* and *Cyphocleonus achates* across the three sampling methods: larval sampling (dissection), adult visual sampling method (visual) and sweep net sampling (sweep) in 1997 and 1998.

Summary of Yield / Effort Across Methods

	Agapeta zoegana		
	%[a] (#)	Time[b]	Relative Index[c]
Dissection	43.0 (37/86)	130 minutes	0.09
Visual	54.8 (47/86)	30 minutes	0.49
Sweep Netting	38.1 (16/42)	10 minutes	1.0
	Cyphocleonus achates		
	% [a] (#)	Time[b]	Relative Index[c]
Dissection	35.6 (16/45)	130 minutes	0.15
Visual	8.9 (4/45)	44 minutes	0.11
Sweep Netting	18.1 (4/22)	10 minutes	1.0

a % is the percentage of sites at which the method detected the insect to be present (# positive sites/ # all sites examined)

b Time is the total time needed to collect and examine a sample ("sample" for dissection is 52 roots, "sample" for *Agapeta zoegana* visual is 6 transects that are 50 meters in length, "sample" for *Cyphocleonus achates* visual is 16 $1m^2$ plots, "sample" for sweep netting is 6 transects of 20 sweeps)

c Relative index of efficiency is (% positive/ time needed for a method)/ (% positive/ time needed for most efficient method [sweep netting])

Table 2.2. The effect of root diameters of spotted knapweed on levels of infestation by *Agapeta zoegana* and *Cyphocleonus achates*.

Root diameter category (cm)	**Number of roots in all samples combined in category**	**Number (%) of roots infested by**	
		A. zoegana	***C. achates***
0.2 – 0.9	4458	103 (2.3 %)	22 (0.5 %)
1.0 – 1.6	1209	140 (11.6 %)	19 (1.6 %)
1.7 – 2.3	207	67 (32.4 %)	4 (1.9 %)
2.4 – 4.1	50	15 (30.0 %)	2 (4.0 %)
Total	5924	325	47

CHAPTER 3

EFFECT OF ROOT FEEDING INSECTS ON SPOTTED KNAPWEED *(CENTAUREA MACULOSA)* STAND DENSITY

Introduction

Spotted knapweed, *Centaurea maculosa* (Lamarck), is an aggressive biennial or perennial plant that is invading areas of the western United States at a rate of three million hectares per year (Story 1984a). In Montana spotted knapweed is considered the most important weed, infesting at least eleven million hectares (Chicoine et al. 1988). Spotted knapweed reduces the productivity of rangelands and changes the composition of the native plant communities it invades. These changes in vegetation degrade habitats for wildlife (Muller et al. 1988, Maxwell 1992, Lacey et al. 1992, 1994).

Spotted knapweed first invaded the northwestern United States in the early 1900s (Kennett et al. 1992, Lacey et al. 1992, 1994). A native of the Eurasian grasslands, this weed was most likely brought to the United States in contaminated alfalfa seeds (Muller et al.1988, Lacey et al. 1992). Spotted knapweed is highly competitive and will easily establish in disturbed soils (Lacey et al. 1992). It is adapted to a wide range of environmental conditions, at sites ranging in altitude from 576 to 3030 m and 20 to 200 cm of annual precipitation (Lacey et al. 1992). Densest infestations are found on well-drained soils in areas with 25 to 35 cm of precipitation per year. Spotted knapweed is a strong competitor for water and soil nutrients and can suppress seed germination of other species by releasing allelo-chemicals into the soil (Lacey et al. 1992).

In an attempt to suppress spotted knapweed densities through biological control, five Eurasian herbivorous insects have been released in North America and become established (Story 1984b). Two species are root boring insects, the cochylidae moth *Agapeta zoegana* (L.) and the curculionidae weevil *Cyphocleonus achates* (Fahraeus). Three other agents affect spotted knapweed stand densities by reducing seed production. These are the gelechiidae moth *Metzneria paucipaunctella* (Zeller) and two tephritid flies, *Urophora affinis* (Frauenfeld) and *Urophora quadrifasciata* (Meigen) (Story 1984b, Rees et al. 1996).

Although previous studies have suggested reductions in seed production following the establishment of the seed head feeding agents, comparative quantitative measurements of plant density changes are lacking (Story 1984b, 1985; Story et al. 1987, 1991). The goal of this study was to compare the current (1997, 1998) spotted knapweed densities at thirteen sites where either the moth *A. zoegana* or the weevil *C. achates* had been released to the density of spotted knapweed at these sites in 1991, 1992 or 1993 at the time of release.

Material and Methods

Study sites

Spotted knapweed densities were measured at thirteen sites in the Lolo National Forest in Montana in 1991, 1992, or 1993 (Tables 3.1 and 3.2) and releases of either *A. zoegana* (12 sites) or *C. achates* (5 sites) were made. There are no records of either release or presence of the three seed head feeding insects (*U. affinis*, *U. quadrifasciata* and *M. paupcipunctella*) at these sites in the 1991-1993 period, but *U. affinis* is widely distributed throughout the northwestern United States and is likely to have been present

by 1991, 1992 and 1993 (Nowierski et al. 1987, Nowierski and Story 1988, Rees et al. 1996). In both 1997 and 1998, spotted knapweed densities at all thirteen sites were measured to see if densities had changed since the 1991 to 1993 releases. Data on releases of *A. zoegana* and *C. achates*, and on original knapweed densities were taken from Forest Service records.

Estimating Spotted Knapweed Density

Spotted knapweed densities at study sites were measured in July and August when plants were in the flowering stage. Methods used were the same as had been used in 1991, 1992 and 1993. Hoops (0.09 m^2) were placed 3.0, 6.0, 9.0 and 12.1 meters from the plot's center (the original insect release point) aligned in the cardinal directions. Plant height for all plants was recorded. The number of post-rosette plants per plot was counted. The number of seed heads per 0.09 m^2 quadrat was recorded. A total of sixteen sample quadrats was evaluated at each site in each of 1997 and 1998.

Sampling Methods to Determine Natural Enemy Presence at Sites

Two methods were used to detect each insect species at all study sites: root excavation to detect larvae and visual observation to detect adults. Root samples were collected in spring and early summer before emergence of adults, as both species overwinter as immatures in roots. Visual sampling to detect adults was conducted on a subsequent visit later in the summer, at times chosen to be appropriate for each site given its altitude and location (Rees et al. 1996).

Root Sampling for Larvae of Both Species

Four transects were established (north, south, east, west) radiating from the central release point and one plant was excavated every 0.90 m from the central release

point out to a distance of 11.8 meters, for a total of fifty-two roots sampled per site. Roots were collected in June or early July. Roots were held at 14° C in the laboratory until they could be dissected and the species and numbers of all larvae found recorded.

Visual Sampling for *A. zoegana* Adults

Six 50-meter transects were established at various directions (north, south, east, west, northwest, northeast, southwest or southeast) from the insect release point, at each *A. zoegana* release site. An observer walked each transect slowly, scanning the vegetation on either side of the line for adults of *A. zoegana,* which rest on the middle to lower part of the spotted knapweed plant.

Visual Sampling for *C. achates* Adults

Four transects were established at the major compass directions (north, south, east, west, northwest, northeast, southwest or southeast) from the insect release point at each *C. achates* site and observations were made at 1.5, 4.5, 7.5 and 10.6 meters along the transect. The four directions varied depending on the shape of the site and the spotted knapweed infestation. At each observation point, the numbers of weevils and plants in a $1m^2$ quadrat, defined by a metal hoop, were recorded. An observer walked slowly along the transect until reaching a sample point and then placed the hoop on the ground slowly so as not to disturb the weevil. *Cyphocleonus achates* is easily disturbed and will fall to the ground and bury itself in the soil or vegetation. As the hoop was placed over the sample area, the vegetation in the quadrant was observed closely to note any *C. achates* present. The observer would then carefully search the area within the hoop for weevils, examining each plant.

Estimation of Level of Seed Head Predation

To determine if any of three seed head feeding insects (*U. affinis*, *U. quadrifasciata* and *M. paupcipunctella*) were present at each of the thirteen sites, twenty-five seed heads were collected in each of twelve sample quadrats at each site. Sample quadrats were located along transects in four cardinal directions at 1.5, 4.5 and 7.5 meters from the center of the plot. Seed heads were collected in May or early June before the emergence of the adults. The seed heads were held at 14° C in the laboratory until they could be dissected and the species and number of all larvae found recorded (Shorthouse 1988, Story et al. 1992, Harris and Shorthouse 1996, Rees et al. 1996).

Statistical Analysis

Paired t-tests were used to compare plant density in the first period (1991, 1992 and 1993) to each set of data taken in 1997 and 1998. A Chi-Square test was used to determine if change in knapweed density was affected by the presence (in 1997, 1998) of the root-feeding biological control insects. The percent change in knapweed density was also regressed against the number of *A. zoegana* released at each site.

Results

Density of knapweed stems was significantly different between the years of natural enemy release (1991,1992, and 1993) (15.0 stems/ m^2) and the years of reassessment, five to seven years later (1997) (7.7 stems / m^2) ($n = 13$, $t = 3.973$, $df = 12$, $p = 0.002$) and (1998) (6.6 stems / m^2) ($n = 13$, $t = 4.272$, $df = 12$, $p = 0.001$). In contrast, stem densities in 1997 (7.7 stems / m^2) and 1998 (6.6 stems / m^2) were not significantly different ($n = 13$, $t = 1.451$, $df = 12$, $p = 0.173$) (SPSS Inc. 1997).

However, in a Chi-Square analysis, the change in weed plant density (increase or decrease) at specific sites was found to be independent of whether or not root feeding biological control agents had established ($n = 13$, $\chi^2 = 2.00$, $df = 1$, $p = 0.16$). Similarly, the correlation between the change in plant density over the study period and the original number of *A. zoegana* released at a site was not significant (Figure 3.1).

We found the seed head fly *U. affinis* at all thirteen sites in both 1997 and 1998. In 1997, we recovered 1805 of the seed head larvae or pupae from 3900 seed heads that were examined (13 sites with 300 seed heads a site). In 1998, a total of 1459 *U. affinis* larvae or pupae was recovered from 3900 seed heads. *Urophora affinis* was not released at any of the thirteen sites, but is widely distributed throughout the northwestern United States (Nowierski et al. 1987, Nowierski and Story 1988, Rees et al. 1996). We did not detect *M. paucipuntella* or *U. quadrifasciata* at any of the thirteen sites. Average seed head density (across sites) increased from 1997 (29.8±1.91 [SE]) to 1998 (36.5±2.47 [SE]). Seed head density decreased at seven sites, increased at five and did not change at one. No comparison of the seed head density could be made between the 1991, 1992 and 1993 sampling period and the 1997 and 1998 sampling, since no data were recorded for the number of seed heads per m^2.

Discussion

Our results show that the density of spotted knapweed at the thirteen study sites declined from the pre-release period (1991-1993) to the final post-release evaluation in 1998 from 15 plants per m^2 to 6.6. In 1997, 54 % (7/13) of the thirteen sites had one or the other of the root-feeding biological control agents established. In 1998, this increased

to 69% (9/13) (Table 3.2). There was an overall increase in the number of knapweed seed heads per m^2 across all thirteen sites from 1997 to 1998.

We found the seed head feeder *U. affinis* to be established at all thirteen sites even though there are no records of releases of these species at those locations. Also, either *A. zoegana* or *C. achates* was present at all of the thirteen sites with some sites having both species. The presence of *U. affinis* throughout most knapweed infestations by 1991 prevented the establishment of control plots where no biological control agents were present.

Continued monitoring of these sites is needed to determine if knapweed density will continue to decline. Data on seed head density obtained in this study can serve as the basis for comparison in future years to determine if seed density changes occur. Establishment of control plots is an essential part of all biological control projects and agencies involved in such projects should develop policies to ensure such plots are included in all release programs. Because of the lack of such plots in this case, only limited inferences can be drawn about the causes of the decline of knapweed density observed at these release plots.

References

Chicoine,T., Fay, P., and Nielsen, J. 1988. Predicting spotted knapweed migration in Montana. *Montana Ag Research.* Spring, pp. 25-28.

Harris, P., and Shorthouse, J.D. 1996. Effectiveness of gall inducers in weed biological control. *The Canadian Entomologist* 128: 1021-1055.

Kennett, G.A., Lacey, J.R., Butt, C.A., Olson-Rutz, K.M., and Haferkamp, M.R. 1992. Effects of defoliation, shading and competition on spotted knapweed and bluebunch wheatgrass. *Journal of Range Management* 45: 363-369.

Lacey, C.A., Lacey, J.R., Fay, P.K., Story, J.M., and Zamora, D.L. 1992. Controlling knapweed on Montana rangeland. Montana Cooperative Extension Service, Montana State University, Bozeman Circular 307: 1-13.

Lacey, J.R., Olson-Rutz, K.M., Haferkamp, M.R., and Kennett, G.A. 1994. Effects of defoliation and competition on total non-structural carbohydrates of spotted knapweed. *Journal of Range Management* 47: 481-484.

Maxwell, J.F., Drinkwater. R., Clark, D., and Hall, J.W. 1992. Effect of grazing, spraying and seeding on knapweed in British Columbia. *Journal of Range Managment* 45: 180-182.

Muller, H., Schroeder, D., and Gassman, A. 1988. *Agapeta zoegana* (L.)(Lepidoptera: Cochylidae), a suitable prospect for biological control of spotted and diffuse knapweed. *Centaurea maculosa* Monnet De La Marck and *Centaurea diffusa* Monnet De La Marck (Compositae) in North America. *The Canadian Entomologist* 120: 109-124.

Nowierski, R.M., and Story, J.M. 1988. Numerical sampling plans for *Urophora affinis* and *U.quadrifasciata*, two introduced seed head flies on spotted knapweed. *Montana AgResearch* Fall : 17-20.

Nowierski, R.M., Story, J.M., and Lund, R.E. 1987. Two-level numerical sampling plans and optimal subsample size computations for *Urophora affinis* and *Urophora quadrifasciata* (Diptera: Tephritidae) on spotted knapweed. *Environmental Entomology* 16: 933-937.

Rees, N.E., Quimby, P.C. Jr., Piper, G.L., Coombs, E.M., Turner, C.E., Spencer, N.R., and Knutson, L.V. 1996. *Biological Control of Weeds in the West.* Western Society of Weed Science. Bozeman, Montana.

Shorthouse, J.D. 1988. Modification of flowerheads of diffuse knapweed by the gall-inducers *Urophora affinis* and *Urophora quadrifasciata* (Diptera: Tephritidae). *Proceedings of the VII International Symposium on Biological Control of Weeds*. Rome, Italy. Laurentian University. pp. 221-228.

SPSS, Inc. Sigma Stat 2.0 for Window's User Manual. 1997. Version 2.0. Chicago, Ill.

Story, J. M. 1984 a. Collection and redistribution of *Urophora affinis and U. quadrifasciata* for the biological control of spotted knapweed. Montana Coop. Extension Service. Circular No. 308. 9 pp.

Story, J.M. 1984 b. Status of biological weed control in Montana, pp.837-842. *Proceedings of the VI International Symposium on Biological Control of Weeds*. Vancouver, Canada, 19-25 August.

Story, J. M. 1985. First report of the dispersal into Montana of *Urophora quadrifasciata* (Diptera: Tephritidae), a fly released in Canada for biological control of spotted and diffuse knapweed. *The Canadian Entomologist* 245: 1061-1062.

Story, J. M., Nowierski, R.M., and Boggs, K. W. 1987. Distribution of *Urophora affinis* and *U. quadrifasciata*, two flies introduced for biological control of spotted knapweed (*Centaurea maculosa*) in Montana. *Weed Science* 35: 145-148.

Story, J. M., Boggs, K.W., Good, W.R., Harris, P., and Nowierski, R. M. 1991. *Metzneria paucipunctella* Zeller (Lepidoptera: Gelechidae), a moth introduced against spotted knapweed: its feeding strategy and impact on two introduced *Urophora* spp. (Diptera: Tephritidae). *The Canadian Entomologist* 123: 1001-1007.

Story, J. M., Boggs, K.W., and Good, W.R. 1992. Voltism and phenological synchrony of *Urophora affinis* and *U. quadrifasciata* (Diptera; Tephritidae), two seed head flies introduced against spotted knapweed in Montana. *Environmental Entomology* 21: 1052-1059.

Table 3.1. Research locations for *Agapeta zoegana* and *Cyphocleonus achates* in the Lolo National Forest in Montana.

Site name	County	Town	Township, Range and Section
Bald Hill	Mineral	Superior	R27W, T17N, Sec. 11
Valley of the Moon	Missoula	Rock Creek	R17W, T11N, Sec. 24
Spring Gulch	Missoula	Missoula	R19W, T14N, Sec. 35
Maclay Flat	Missoula	Missoula	R20W, T13N, Sec. 35
Sawmill Gulch	Missoula	Missoula	R19W, T14N, Sec. 35
Henry Creek	Sanders	Plains	R25W, T20N, Sec.33NW
Weeksville #1	Sanders	Weeksville	R27W, T20N, Sec. 2
Spring Creek Trailhead	Sanders	Weeksville	R27W, T21N, Sec. 5SW
Salmon Lake - 6	Missoula	Seeley Lake	R14W, T15N, Sec. 6
Salmon Lake - 36	Missoula	Seeley Lake	R15W, T16N, Sec. 36
Monture West	Powell	Seeley Lake	R12W, T16N, Sec.19
Swamp Creek	Powell	Seeley Lake	R14W, T17N, Sec. 32
Blue Mountain	Missoula	Missoula	R20W, T12N, Sec. 3

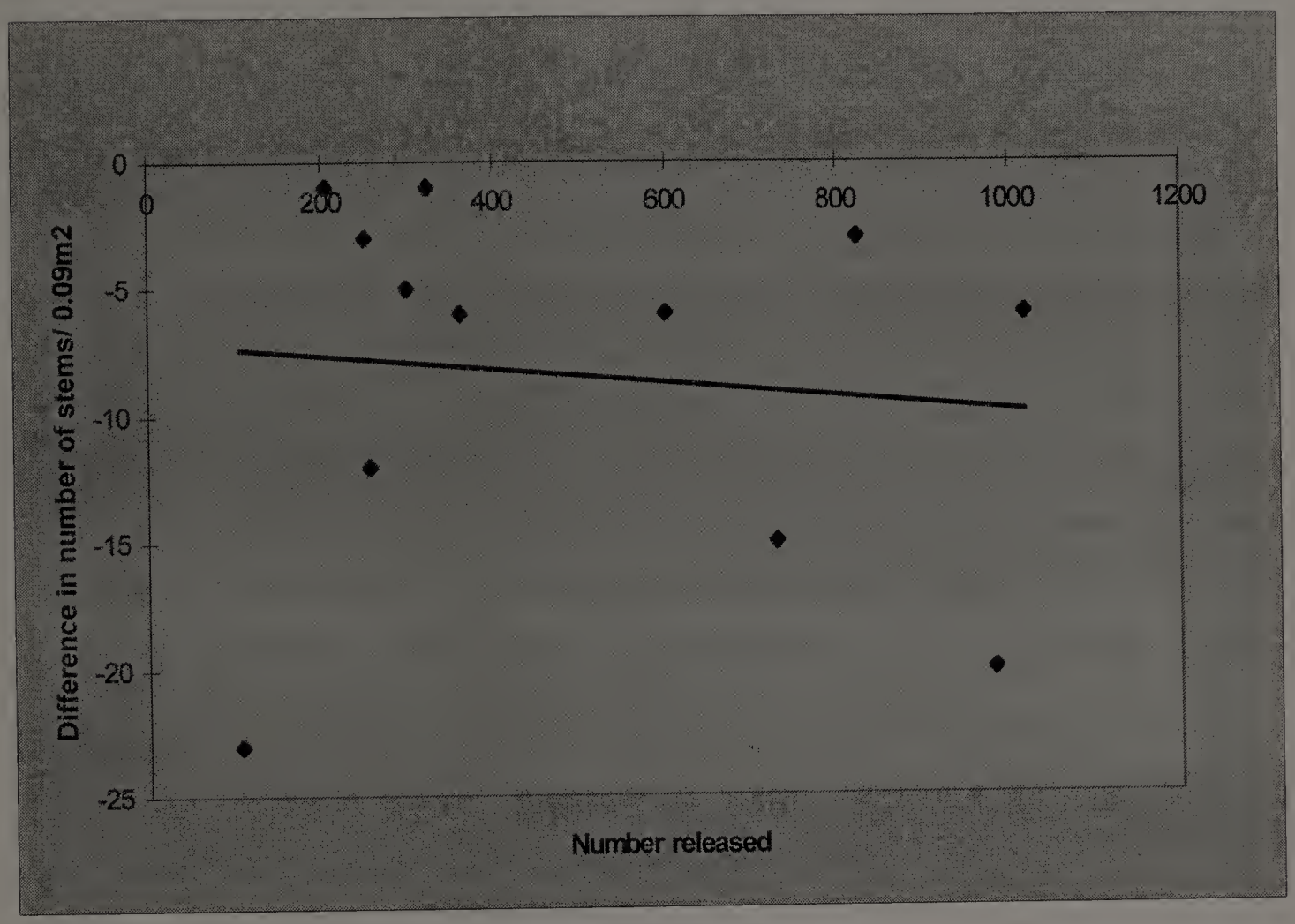
Table 3.2. Comparison of spotted knapweed densities: previously released (1991, 1992, or 1993) and reevaluation (1997 and 1998).

Density Site	Previous Density (stems/sq. meter)[a]	1997 Density (stems/sq. meter) (SE)	1998 Density (stems/sq. meter) (SE)	# of Insects found *C. achates* / *A. zoegana* 1997[b]		# of Insects found *C. achates* / *A.zoegana* 1998[b]		# of insects released *C. achates* / *A. zoegana*	
Blue Mountain	15	1.8 (0.39)	6.7 (1.38)	1	NA	21	NA	200	0
Spring Gulch	25	11.2 (1.63)	10.6 (0.87)	0	4	0	25	250	729
Valley of the Moon	15	12.7 (1.30)	8.9 (0.89)	4	0	2	11	410	1019
Monture West	14	4.9 (0.84)	7.5 (1.91)	NA	1	NA	0	0	362
Swamp Creek	9	3.2 (0.42)	4.4 (0.64)	NA	0	NA	0	0	300
Salmon Lake 6	17	7.8 (1.67)	5.2 (1.01)	NA	4	NA	1	0	256
Salmon lake 36	7	4.5 (1.40)	4.1 (0.26)	NA	2	NA	1	0	252
Sawmill Gulch	25	9.6 (1.53)	3.8 (0.60)	NA	0	NA	5	0	983
Maclay Flat	8	8.7 (1.43)	5.4 (0.58)	6	8	4	11	257	825
Bald Hill	18	16.4 (1.22)	16.7 (1.85)	NA	0	NA	2	0	325
Weeksville #1	28	7.7 (1.96)	4.6 (0.74)	0	0	0	2	200	106
Spring Creek Trailhead	8	3.0 (0.66)	1.5 (0.70)	NA	0	NA	0	0	600
Henry Creek	8	9.1 (1.81)	7.1 (0.97)	NA	0	NA	0	0	207

a The standard errors for the previous density measurements could not be calculated. Only a single measurement was available from records for the density average at a site.

b The number of insects includes the larval stage and the adult stage. NA indicates that particular insect was not released at that site.

Figure 3.1. Difference in the spotted knapweed density between 1991-1993 and 1997- 1998 in relation to the number of *Agapeta zoegana* originally released at each site.



APPENDIX A
RELEASE LOCATONS OF *AGAPETA ZOEGANA* VISITED IN 1997 AND 1998

1997

Site name	State	County	Town	National Forest	Township, Range and Section
Bald Hill	Montana	Mineral	Superior	Lolo	R27W, T17N, Sec. 11
McCormick Creek	Montana	Missoula	Missoula	Lolo	R23W, T16N, Sec. 15
Valley of the Moon	Montana	Missoula	Rock Creek	Lolo	R17W, T11N, Sec. 24
Spring Gulch	Montana	Missoula	Missoula	Lolo	R19W, T14N, Sec. 35
Maclay Flat	Montana	Missoula	Missoula	Lolo	R20W, T13N, Sec. 35
Sawmill Gulch	Montana	Missoula	Missoula	Lolo	R19W, T14N, Sec. 35
Thompson Falls Admin.	Montana	Sanders	Thompson Falls	Lolo	R29W, T49N, Sec. 3
Brushy Gulch	Montana	Sanders	Thompson Falls	Lolo	R31W, T21N, Sec. 19
Henry Creek	Montana	Sanders	Plains	Lolo	R25W, T20N, Sec. 33NW
Weeksville #1	Montana	Sanders	Weeksville	Lolo	R27W, T20N, Sec. 2
Spring Creek Trailhead	Montana	Sanders	Weeksville	Lolo	R27W, T21N, Sec. 25SW
Salmon Lake – 6	Montana	Missoula	Seeley Lake	Lolo	R14W, T15N, Sec. 6
Salmon Lake – 36	Montana	Missoula	Seeley Lake	Lolo	R15W, T16N, Sec. 36
Old Heliport @ Deer Crk.	Montana	Missoula	Seeley Lake	Lolo	R15W, T17N, Sec. 7
Monture West	Montana	Powell	Seeley Lake	Lolo	R12W, T16N, Sec.19
Swamp Creek	Montana	Powell	Seeley Lake	Lolo	R14W, T17N, Sec. 32
Souse Gulch	Montana	Lincoln	Libby	Kootenai	R29W, T31N, Sec. 32
Fairview Work Center	Montana	Lincoln	Libby	Kootenai	R27W, T30N, Sec. 22
Cripple Horse Creek	Montana	Lincoln	Libby	Kootenai	R28W, T31N, Sec. 1
Road 4772B	Montana	Lincoln	Libby	Kootenai	R30W, T28N, Sec. 11
Road 4772E	Montana	Lincoln	Libby	Kootenai	R30W, T28N, Sec. 2
Eastside Road Powerline	Montana	Lincoln	Troy	Kootenai	R34W, T32N, Sec. 15
Koocanusa Bridge	Montana	Lincoln	Eureka	Kootenai	R28W, T35N, Sec. 6
Tobacco River	Montana	Lincoln	Eureka	Kootenai	R27W, T36N, Sec. 8
Rattlebone Right of Way	Montana	Lincoln	Eureka	Kootenai	R25W, T34N, Sec. 26
Strkyker Gravel Pit	Montana	Lincoln	Stryker	Kootenai	R25W, T34N, Sec. 25
Radnor Powerline	Montana	Flathead	Stryker	Kootenai	R24W, T33W, Sec. 8
Canoe Gulch	Montana	Lincoln	Libby	Kootenai	R29W, T30N, Sec. 18
Blue Bell Mine	Montana	Jefferson	Boulder	Deerlodge	R6W, T6N, Sec. 13
Dunkleberg Ridge #2	Montana	Granite	Deerlodge	Deerlodge	R12W, T9N, Sec. 15
Jackson Park #2	Montana	Granite	Deerlodge	Deerlodge	R12W, T9N, Sec. 14
Flint Creek Campgrnd #1	Montana	Granite	Philipsburg	Deerlodge	R14W, T6N, Sec. 36
Flint Creek Campgrnd #2	Montana	Granite	Philipsburg	Deerlodge	R14W, T6N, Sec. 26
Lime Quarry #1	Montana	Granite	Anaconda	Deerlodge	R12W, T5N, Sec. 24
Lime Quarry #2	Montana	Granite	Anaconda	Deerlodge	R12W, T5N, Sec. 24
Horse Pasture	Montana	Broadwater	Townsend	Helena	R7N, T4E, Sec. 29
Confederate Gulch	Montana	Broadwater	Townsend	Helena	R10N, T2E, Sec. 25
Kimber Gulch	Montana	Broadwater	Townsend	Helena	R1W, T8N, Sec. 25
Slim Sam	Montana	Broadwater	Townsend	Helena	R6N, T1W, Sec. 25
Hall Creek	Montana	Broadwater	Townsend	Helena	R7N, T2W, Sec. 36
Cold Springs	Idaho	Clearwater	Orofino	Clearwater	R9E, T39N, Sec. 15
Shanghi Junction	Idaho	Clearwater	Orofino	Clearwater	R7E, T37N, Sec. 6
19 mile	Idaho	Idaho	Riggins	Nez Perce	R8e, T32N, Sec. 29
Wind River	Idaho	Idaho	Lowell	Nez Perce	R4E, T25N, Sec. 3

1998

Site name	State	County	Town	National Forest	Township, Range and Section
Irvine Bridge	Montana	Gallatin	Bozeman	NA	R4E, T1S, Sec. 9
Jocko	Montana	Sanders	Moiese	Bison Range	R21W, T18N, Sec. 26
Bradshaw's/Stanahan's	Montana	Lake	Moiese	NA	R20W, T18N, Sec. 31
Agency Springs	Montana	Sanders	Moiese	Bison Range	R21W, T18N, Sec. 10
Kicking Horse	Montana	Lake	Ronan	NA	R20W, T20N, Sec. 25
Pablo Dam	Montana	Lake	Ronan	NA	R20W, T22N, Sec. 27
Rourke's	Montana	Missoula	Greenough	NA	R15W, T14N, Sec. 26
National Wildlife Fed.	Montana	Missoula	Missoula	Nat.Wild.Fed.	R19W, T14N, Sec. 28
Reserve Street	Montana	Missoula	Missoula	NA	R19W, T13N, Sec. 5
Cramer Creek I-A	Montana	Missoula	Rock Creek	NA	R16W, T12N, Sec. 35
Cramer Creek I-B	Montana	Missoula	Rock Creek	NA	R16W, T12N, Sec. 35
Cayuse Creek	Montana	Missoula	Missoula	NA	R22W, T15N, Sec. 27
St. Ignatius	Montana	Lake	St. Ignatius	NA	R20W, T18N, Sec. 23
Slide Area	Montana	Beaverhead	Dillon	NA	R10W, T8S, Sec. 36
Pipe Organ	Montana	Beaverhead	Dillon	NA	R10W, T9S, Sec. 11
MRI	Montana	Silverbow	Butte	NA	R7W, T3N, Sec. 18
Montana Tech	Montana	Silverbow	Butte	NA	R8W, T3N, Sec. 14
Copper King	Montana	Silverbow	Butte	NA	R7W, T3N, Sec. 6
Rotary Club	Montana	Silverbow	Butte	NA	R7W, T3N, Sec. 34
Garden Creek	Montana	Lake	Polson	NA	R25W, T21N
Geldrich	Montana	Sanders	Polson	NA	R20W, T20N, Sec. 17
River Corridor	Montana	Lake	Polson	NA	R21W, T20N
Sloan Bridge	Montana	Lake	Polson	NA	R21W, T20N, Sec. 18
Kicking Horse # 1	Montana	Lake	Polson	NA	R19W, T20N, Sec. 1
Deep Creek	Montana	Broadwater	Townsend	Helena	R7N, T5E, Sec. 19
Earthquake Lake	Montana	Madison	W. Yellowstone	Gallatin	R3E, T11S, Sec. 36
Belgian Gulch	Montana	Sanders	Trout Creek	Kootenai	R31W, T25N, Sec. 33
Burnt Bridge	Montana	Sanders	Trout Creek	Kootenai	R31W, T22N, Sec. 4
Marten Creek	Montana	Sanders	Trout Creek	Kootenai	R33W, T25N, Sec. 26
Heliport @ Ranger Station	Montana	Sanders	Trout Creek	Kootenai	R31W, T24N, Sec. 6
Blacktail Creek	Montana	Sanders	Trout Creek	Kootenai	R32W, T25N, Sec. 28
E and W fork Elk Creek Confluence	Montana	Sanders	Trout Creek	Kootenai	R34W, T26N, Sec. 17
Bull River	Montana	Sanders	Trout Creek	Kootenai	R33W, T28N, Sec. 16
Barron Creek	Montana	Lincoln	Libby	Kootenai	R31W, T31N, Sec. 27
Sheldon flats	Montana	Lincoln	Libby	Kootenai	R29W, T32N, Sec. 22
Hensley Hill	Montana	Lincoln	Troy	Kootenai	R32W, T36N, Sec. 35
Bluff Creek	Idaho	Shoshone	Avery	IPNF	R7E, T44N, Sec. 23
Bottle Creek	Idaho	Shoshone	Avery	IPNF	R31W, T17N, Sec. 20
Farragut Residence	Idaho	Kootenai	Athol	NA	R2W, T53N, Sec. 8
BP Station	Washington	Spokane	Spokane	NA	R42E, T25N, Sec. 28
Moab	Washington	Spokane	Spokane	NA	R45E, T26N, Sec. 25
Liberty Lake #2	Washington	Spokane	Spokane	NA	R45E, T25N, Sec. 22

APPENDIX B
RELEASE LOCATIONS OF *CYPHOCLEONUS ACHATES* VISITED IN 1997AND 1998

1997

Site name	State	County	Town	National Forest	Township, Range and Section
Valley of the moon	Montana	Missoula	Rock Creek	Lolo	R17W, T11N, Sec. 24
Spring Gulch	Montana	Missoula	Missoula	Lolo	R19W, T14N, Sec. 35
Maclay Flat	Montana	Missoula	Missoula	Lolo	R20W, T13N, Sec 35
Blue Mountain	Montana	Missoula	Missoula	Lolo	R20W, T12N, Sec. 3
Weeksville #1	Montana	Sanders	Weeksville	Lolo	R27W, T20N, Sec. 2
Souse Gulch	Montana	Lincoln	Libby	Kootenai	R29W, T31N, Sec. 32
Cripple Horse Creek	Montana	Lincoln	Libby	Kootenai	R28W, T31N, Sec. 1
Tobacco River	Montana	Lincoln	Eureka	Kootenai	R27W, T36N, Sec. 8
Old Boulder Road	Montana	Lincoln	Eureka	Kootenai	R28W, T35N, Sec. 6
Blue Bell Mine	Montana	Jefferson	Boulder	Deerlodge	R6W, T6N, Sec. 13
Dunkleberg Ridge #1	Montana	Granite	Deerlodge	Deerlodge	R12W, T9N, Sec. 15
Jackson Park #1	Montana	Granite	Deerlodge	Deerlodge	R12W, T9N, Sec. 14
Flint Creek Campgrnd #3	Montana	Granite	Philipsburg	Deeerlodge	R14W, T6N, Sec. 26
Pburg Admin. Pasture	Montana	Granite	Philipsburg	Deerlodge	R14W, T7N, Sec. 36
Horse Pasture	Montana	Broadwater	Townsend	Helena	R7N, T4E, Sec. 29
Confederate Gulch	Montana	Broadwater	Townsend	Helena	R10N, T2E, Sec. 25
Slim Sam	Montana	Broadwater	Townsend	Helena	R6N, T1W, Sec. 25
Kimber Gulch	Montana	Broadwater	Townsend	Helena	R1W, T8n, Sec. 25
Bat Hill	Idaho	Idaho	Lowell	Clearwater	R10E, T34N, Sec. 6
Zodiac Cut Bank	Idaho	Clearwater	Orofino	Clearwater	R7E, T40N, Sec. 5
Bungalow	Idaho	Clearwater	Orofino	Clearwater	R8E, T38N, Sec. 18
19 Mile	Idaho	Idaho	Riggins	Nez Perce	R8E, T32N, Sec. 29
Wind River	Idaho	Idaho	Lowell	Nez Perce	R4E, T25N, Sec. 3

1998

Site name	State	County	Town	National Forest	Township, Range and Section
Jocko North	Montana	Sanders	Moiese	Bison Range	R21W, T18N, Sec. 26
Jocko	Montana	Sanders	Moiese	Bison Range	R21W, T18N, Sec. 26
Pablo Dam	Montana	Lake	Ronan	NA	R20W, T22N, Sec. 27
Rourke's	Montana	Missoula	Greenough	NA	R15W, T14N, Sec. 26
Cramer Creek I-A	Montana	Missoula	Rock Creek	NA	R16W, T12N, Sec. 35
Cayuse Creek	Montana	Missoula	Missoula	NA	R22W, T15N, Sec. 27
St. Ignatius	Montana	Lake	St. Ignatius	NA	R20W, T18N, Sec.23
Pipe Organ	Montana	Beaverhead	Dillon	NA	R10W, T9S, Sec. 11
MRI	Montana	Silverbow	Butte	NA	R7W, T3N, Sec. 18
Montana Tech	Montana	Silverbow	Butte	NA	R8W, T3N, Sec. 14
Geldrich	Montana	Sanders	Polson	NA	R20W, T20N, Sec. 17
Deep Creek	Montana	Broadwater	Townsend	Helena	R7N, T5E, Sec. 19
Earthquake Lake	Montana	Madison	W. Yellowstone	Gallatin	R3E, T11S, Sec. 36
Belgian Gulch	Montana	Sanders	Trout Creek	Kootenai	R31W, T25N, Sec. 33
E & W fork Elk Crk	Montana	Sanders	Trout Creek	Kootenai	R34W, T26N, Sec. 17
Bull River	Montana	Sanders	Trout Creek	Kootenai	R33W, T28N, Sec. 16
Hensley Hill	Montana	Lincoln	Troy	Kootenai	R32W, T36N, Sec.35
Bluff Creek	Idaho	Shoshone	Avery	IPNF	R7E, T44N, Sec. 23
Bottle Creek	Idaho	Shoshone	Avery	IPNF	R31W, T17N, Sec. 20
Farragut # 2	Idaho	Kootenai	Athol	NA	R2W, T53N, Sec. 8
Farragut V.C.	Idaho	Kootenai	Athol	NA	R2W, T53N, Sec. 8
BP Station	Washington	Spokane	Spokane	NA	R42E, T25N, Sec. 28

APPENDIX C
HABITAT CATEGORIES USED TO CLASSIFY POTENTIAL CLIMAX VEGETATION OF RELEASE SITES, AS TAKEN FROM PFISTER ET AL. 1977

Habitat type	Description of Habitat type (Key components of each layer)
100 PIPO	*Pinus ponderosa* major tree species/ no shrub layer/ no grass layer
130 PIPO/AGSP	*Pinus ponderosa* major tree species/ no shrub layer/ *Agropyron spicatum* as the grass layer
140 PIPO/ FEID	*Pinus ponderosa* major tree species/ no shrub layer/ *Festuca idahoensis* as the grass layer
141 PIPO/ FEID/ FEID	*Pinus ponderosa* / no shrub layer / *Festuca idahoensis* as the grass layer
142 PIPO/ FESC	*Pinus ponderosa* major tree species/ *Festuca scabrella* as the grass layer
171 PIPO/ SYAL/ SYAL	*Pinus ponderosa* major tree species/ *Symphoricarpos albus* as the shrub layer/ *Symphoricarpos albus* as the phase since no grass layer
172 PIPO/ SYAL/ BERE	*Pinus ponderosa*/ *Symphoricarpos albus* as the shrub layer/ *Beberis repens* as the phase since no grass layer
210 PSME/ AGSP	*Pseudotsuga menziesii* major tree species/ no shrub layer/ *Agropyron spicatum* as the grass layer
220 PSME/ FEID	*Pseudotsuga menziesii* major tree species/ no shrub layer/ *Festuca idahoensis* as the grass layer
250 PSME/VACA	*Pseudotsuga menziesii* major tree species/ *Vaccinium caespitosum* as the shrub layer/ no grass layer
260 PSME/ PHMA	*Pseudotsuga meneziesii* major tree species/ *Physocarpus malvaceus* as the shrub layer
262 PSME/PHMA/ CARU	*Pseudotsuga menziesii* major tree species/ *Physocarpus malvaceus* as the shrub layer / *Calamagrostis rubescens* as the grass layer
283 PSME/ XETE	*Pseudotsuga meneziesii* major tree species/ *Xerophyllum tenax* as the grass layer
292 PSME/ CARU	*Pseudotsuga meneziesii* major tree species/ *Calamagrostis rubescens* as the grass layer
310 PSME/ SYAL	*Pseudotsuga menziesii* major tree species/ *Symphoricarpos albus* as the shrub layer/ no grass layer
311 PSME/ SYAL/ AGSP	*Pseudotsuga menziesii* major tree species/ *Symphoricarpos albus* as the shrub layer/ *Agropyron spicatum* as the grass layer
312 PSME/ SYAL/ CARU	*Pseudotsuga menziesii* major tree species/ *Symphoricarpos albus* as the shrub layer/ *Calamagrostis rubescens* as the grass layer
313 PSME/ SYAL/ SYAL	*Pseudotsuga menziesii* major tree species/ *Symphoricarpos albus* as the shrub layer/ *Symphoricarpos albus* as the phase since no grass layer
323 PSME/ CARU/ CARU	*Pseudotsuga menziesii* major tree species/ *Calamagrostis rubescens* as the grass layer
350 PSME/ ARUV	*Pseudotsuga meneziesii* major tree species/ *Arctostaphylus uva-ursi* as the forb layer
360 PSME/ JUCO	*Pseudotsuga meneziesii* major tree species/ *Juniperus communis* as the shrub layer/ no grass layer
524 ABGR/ PHMA	*Abies grandis* major tree species/ *Physocarpus malvaceus* as the shrub layer
530 THPL/CLUN	*Thuja plicata* major tree species/ *Clintonia uniflora* as the forb layer
870 PIAL	*Pinus albicaulis* major tree species/ no shrub layer/ no grass layer
950 PIPO/CARU	*Pinus ponderosa* major tree species/ no shrub layer/ *Calamagrostis rubescens* as the grass layer
NONE	The knapweed release site is not near a forest type

BIBLIOGRAPHY

Anon.1991.Timber Management Data Handbook. United States Forest Service. Missoula, Montana. R1-FSH 2409.21h.

Clark, S.E., Van Driesche, R.G., Sturdevant, N., Elkinton, J., and Buonaccorsi, J. P. in press. Effects of site characteristics and release history on establishment of *Agapeta zoegana* (Lepidoptera: Cochylidae) and *Cyphocleonus achates* (Coleoptera: Curculionidae), root feeding herbivores attacking spotted knapweed, *Centaurea Maculosa*. *Biological Control.*

Clark, S.E., Van Driesche, R.G., Sturdevant, N., Kegley, S. in press. Comparative efficacy of adult and larval sampling for detection of two root boring insects, *Agapeta zoegana* (Lepidoptera:Cochylidae) and *Cyphocleonus achates* (Coleoptera:Curculionidae), released for biological control of spotted knapweed. Florida Entomology.

Chicoine,T., Fay, P., and Nielsen, J. 1988. Predicting spotted knapweed migration in Montana. *Montana Ag Research.* Spring, pp. 25-28.

Cruttwell McFayden, R.E., and Fletcher A. 1991. Climate modeling and the biological control of weeds: - one view. *Plant Protection Quarterly* 6: 14-15.

Fay, P.K., and Davis, E.S.1988. Testing herbicides for spotted knapweed (*Centaurea maculosa* Lam.) control in alfalfa. *Resource Progress Report WesternWeed Science : The Society* p.152.

Fitzpatrick, S. M. 1989. A potential collection method for *Agapeta zoegana* (Lepidoptera: Cochylidae), a knapweed root feeding moth. *Journal of the Entomological Society of British Columbia* 86: 55-62.

French, R.A., and Lacey, J.R. 1983. Knapweed : Its cause, effect and spread in Montana. Montana Cooperative Extension Service, Montana State University, Bozeman Circular 307: 1-13.

Harris, P., and Shorthouse, J.D. 1996. Effectiveness of gall inducers in weed biological control. *The Canadian Entomologist* 128: 1021-1055.

Hight, S.D., Blossey, B., Laing, J., and Declerck-Floate, R. 1995. Establishment of insect biological control agents from Europe against *Lythrum salicaria* in North America. *Biological Control* 24: 967-977.

Hosmer, D.W., and Lemeshow, S. 1989. *Applied Logistic Regression.* Wiley-Interscience Publication, New York.

Julien, M.H. 1992. *Biological Control of Weeds : A World Catalogue of Agents and their Target Weeds*. 3rd Edition. Canberra, Australia.

Kennett, G.A., Lacey, J.R., Butt, C.A., Olson-Rutz, K.M., and Haferkamp, M.R. 1992. Effects of defoliation, shading and competition on spotted knapweed and bluebunch wheatgrass. *Journal of Range Management* 45: 363-369.

Lacey, C.A., Lacey, J.R., Fay, P.K., Story, J.M., and Zamora, D.L. 1992. Controlling knapweed on Montana rangeland. Montana Cooperative Extension Service, Montana State University, Bozeman Circular 307: 1-13.

Lacey, C.A., Lacey, J.R., Fay, P.K., Story, J.M., and Zamora, D.L. 1992. Controlling knapweed on Montana rangeland. Montana Cooperative Extension Service, Montana State University, Bozeman. Circular 311: 1-17.

Lacey, J.R., Olson-Rutz, K.M., Haferkamp, M.R., and Kennett, G.A. 1994. Effects of defoliation and competition on total non-structural carbohydrates of spotted knapweed. *Journal of Range Management* 47: 481-484.

Lass, L.W., and Callihan, R.H. 1992. Spotted knapweed control in a non-crop site. *Resource Progress Report Western Society Weed Science ; The Society* : I-25.

Lemmon, P.E. 1956. A spherical densitometer for estimating forest overstory density. *Forest Science* 2: 314-320.

Lindquist, J.L., Maxwell, B.D., and Weaver, T. 1996. Potential for controlling the spread of *Centaurea maculosa* with grass competition. *Great Basin Naturalist* 56: 267-271.

Maxwell, J.F., Drinkwater. R., Clark, D., and Hall, J.W. 1992. Effect of grazing, spraying and seeding on knapweed in British Columbia. *Journal of Range Management* 45: 180-182.

McCaffrey, J.P., and Callihan, R.H. 1988. Compatibility of Picloram and 2,4-D with *Urophora affinis* and *U. quadrifasciata* (Diptera:Tephritidae) for spotted knapweed control. *Environmental Entomology* 17: 785-788.

McClay, A.S. 1996. Biological control in a cold climate: temperature responses and climate adaptation of weed biological agents. *Proceedings of the IX International Symposium on Biological Control of Weeds*. Stellenbosch, South Africa. University of Cape Town. pp. 377-383.

Memmott, J., Fowler, S.V., and Hill, R.L. 1998. The effect of release size on the probability of establishment of biological control agents: gorse thrips (*Sericothrips staphylinus*) released against gorse (*Ulex europaeus*) in New Zealand. *Biocontrol Science and Technology* 8: 103-115.

Mooers, G.A. 1986. Relationship of critical environmental factors to the success of spotted knapweed (*Centaurea maculosa* L.) in western Montana. Master of Science thesis, Department of Forestry. University of Montana, Missoula, Montana.

Muller, H., Schroeder, D., and Gassman, A. 1988. *Agapeta zoegana* (L.)(Lepidoptera: Cochylidae), a suitable prospect for biological control of spotted and diffuse knapweed, *Centaurea maculosa* Monnet De La Marck and *Centaurea diffusa* Monnet De La Marck (Compositae) in North America. *The Canadian Entomologist* 120: 109-124.

Myers, J.D., and Harris, P. 1980. Distribution of *Urophora* galls in flower heads of diffuse and spotted knapweed in British Columbia. *Journal of Applied Ecology* 17: 359-367.

Neuenschwander, P. 1984. Observations on the biology of the two species of fruit flies (Diptera: Tephritidae), and their competition with a moth larva. *Israel Journal of Entomology* 18: 95-97.

Nimlos, T. 1992. *Introduction to Soils*. University of Montana: Missoula, Montana.

Nowierski, R.M., and Story, J.M. 1988. Numerical sampling plans for *Urophora affinis* and *U.quadrifasciata*, two introduced seed head flies on spotted knapweed. *Montana AgResearch* Fall : 17-20.

Nowierski, R.M., Story, J.M., and Lund, R.E. 1987. Two-level numerical sampling plans and optimal subsample size computations for *Urophora affinis* and *Urophora quadrifasciata* (Diptera: Tephritidae) on spotted knapweed. *Environmental Entomology* 16: 933-937.

Pfister, R.D., Bernard, L.K., Arno, S.F., and Presby, R.C. 1977. Forest habitat types of Montana. USDA Forest Service General Technical Report INT-34.

Powell, G.W., Sturko, A., Wikeem, B.M., and Harris, P. 1994. Field guide to the biological control of weeds in British Columbia. *British Columbia Ministry of Forests Research Program Land Management Handbook* 27.

Powell, G. W., Wikeem, B. M., and Sturko, A. 2000. Biology of *Agapeta zoegana* (Lepidoptera:Cochylidae), propagated for the biological control of knapweeds (Asteraceae). *The Canadian Entomologist* 132: 223-230.

Rees, N.E., Quimby, P.C. Jr., Piper, G.L., Coombs, E.M., Turner, C.E., Spencer, N.R., and Knutson, L.V. 1996. *Biological Control of Weeds in the West*. Western Society of Weed Science. Bozeman, Montana.

SAS Institute. 1996. SAS/STAT User's Guide : Version 6, fourth edition, Volume 1 and 2 SAS Institute Inc., Cary, North Carolina.

Shorthouse, J.D. 1988. Modification of flowerheads of diffuse knapweed by the gall-inducers *Urophora affinis* and *Urophora quadrifasciata* (Diptera: Tephritidae). *Proceedings of the VII International Symposium on Biological Control of Weeds*. Rome, Italy. Laurentian University. pp. 221-228.

Spears, B.M., Rose, S.T., and Belles, W.S. 1980. Effect of canopy cover, seeding depth, and soil moisture on emergence of *Centaurea maculosa* and *C. diffusa*. *Weed Research* 20: 87-90.

SPSS, Inc. Sigma Stat 2.0 for Window's User Manual. 1997. Version 2.0. Chicago, Ill.

Stinson, C.S.A., Schroeder, D., and Marquardt, K. 1994. Investigations of *Cyphocleonus achates* (Fahr.) Col., Curculionidae, a potential biological control agent of spotted knapweed (*Centaurea Maculosa* Lam.) and diffuse knapweed (*C. diffusa* Lam.) (Compositae) in North America. *Journal of Applied Entomology* 117: 35-50.

Story, J.M. 1984. Collection and redistribution of *Urophora affinis* and *U. quadrifasciata* for biological control of spotted knapweed. Montana Coop. Ext. Service. Circular 308. 9 pp.

Story, J. M. 1984 a. Collection and redistribution of *Urophora affinis and U. quadrifasciata* for the biological control of spotted knapweed. Montana Coop. Extension Service. Circular No. 308. 9 pp.

Story, J.M. 1984 b. Status of biological weed control in Montana, pp.837-842. *Proceedings of the VI International Symposium on Biological Control of Weeds*. Vancouver, Canada, 19-25 August.

Story, J.M. 1985. First report of the dispersal into Montana of *Urophora quadrifasciata* (Diptera:Tephritidae), a fly released in Canada for biological control of spotted and diffuse knapweed. *The Canadian Entomologist* 245: 1061-1062.

Story, J.M., and Norwierski, R.M. 1984. Increase and dispersal of *Urophora affinis* (Diptera:Tephritidae) on spotted knapweed in western Montana. *Environmental Entomologist* 13: 1151-1156.

Story, J.M., and Norwierski, R.M. 1985. Population increase of the seed head fly in western Montana. *Montana Ag Research,* pp. 8-10.

Story, J.M., Nowierski, R.M., and Boggs, K.W. 1987. Distribution of *Urophora affinis* and *U. quadrifasciata*, two flies introduced for biological control of spotted knapweed (*Centaurea maculosa*) in Montana. *Weed Science* 35: 145-148.

Story, J.M., Boggs, K.W., and Graham, D.R. 1989. Effects of nitrogen fertilization on spotted knapweed and competing vegetation in western Montana. *Journal of Range Management* 42: 222-225.

Story, J.M., Boggs, K.W., and Good, W.R. 1991. First report of the establishment of *Agapeta zoegana* L. (Lepidoptera: Cochylidae) on spotted knapweed, *Centaurea maculosa* Lamarck, in the United States. *The Canadian Entomologist.* 119: 411-412.

Story, J. M., Boggs, K.W., Good, W.R., Harris, P., and Nowierski, R. M. 1991. *Metzneria paucipunctella* Zeller (Lepidoptera: Gelechidae), a moth introduced against spotted knapweed: its feeding strategy and impact on two introduced *Urophora* spp. (Diptera: Tephritidae). *The Canadian Entomologist* 123: 1001-1007.

Story, J.M., Boggs, K.W., Good, W.R., Harris, P., and Norwierski, R.M. 1991. *Metzneria paucipunctella* Zeller (Lepidoptera:Gelechilidae), a moth introduced against spotted knapweed: its feeding strategy and impact on two introduced *Urophora* spp. (Diptera:Tephritidae). *The Canadian Entomologist* 123: 1001-1007.

Story, J.M. 1992. Biological control of weeds: selective, economical and safe. *Western Wildlands* 18: 18-23.

Story, J. M., Boggs, K.W., and Good, W.R. 1992. Voltism and phenological synchrony Of *Urophora affinis* and *U. quadrifasciata* (Diptera; Tephritidae), two seed head Flies introduced against spotted knapweed in Montana. *Environmental Entomology* 21: 1052-1059.

Story, J.M., Good, W.R., and White, L.J. 1994. Propagation of *Agapeta zoegana* L. (Lepidoptera:Cochylidae) for biological control of spotted knapweed : procedures and costs. *Biological Control* 4: 145-148.

Story, J.M., White, L.J., and Good, W.R. 1996. Propagation of *Cyphocleonus achates* (Fahraeus) (Coleoptera : Curculionidae) for the biological control of spotted knapweed: procedures and costs. *Biological Control* 7: 167-171.

Story, J.M., White, L.J., and Good, W.R. 1997. First report of the establishment of *Cyphocleonus achates* (Fahraeus)(Coleoptera:Curculionidae) on spotted knapweed, *Centaurea Maculosa* Lamarck, in the United States. *The Canadian Entomologist* 129: 373-374.

Toth, M., Guerin, P.M., Buser, H.R., Muller, H., Szocs, G., Sziraki, G., and Arn, H. 1985. Z-11-Tetradecenyl acetate: sex attractant of *Agapeta zoegana* (Lepidoptera: Tortricidae), a potential species for the biological control of knapweed. *The Canadian Entomologist* 117: 1163-1165.

Van Driesche, R.G. 1993. Methods for field colonization of new biological control agents. In: Bellows, T.S., Jr. and Van Driesche, R.G. (Eds.) *Proceedings: Thomas Say Publications in Entomology: Steps in Classical Arthropod Biological Control*. Entomological Society of America. Lanham, Maryland. pp. 67-86.

Wikeem, B.M., and Powell, G.W. 1999. Biology of *Cyphocleonus achates* (Coleoptera: Curculionidae), propagated for the biological control of knapweeds (Asteraceae). *The Canadian Entomologist* 131: 243-250.

Xanthopoulos, G. 1987. Guidelines for burning spotted knapweed infestations for fire reduction in western Montana. *Symposium and Workshop on Protecting People and Homes from Wildfire in the Interior West* October 6-8, pp. 195-198.