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Effects of Vegetation Cover, Mowing, and Soil Temperature on Development and Survival of Two Root-Feeding Biological Control Insects of Spotted Knapweed

by

Cynthia Snyder

B.S. Iowa State University, Ames, 1990

Presented in partial fulfillment of the requirements for the degree of Master of Science

Department of Ecosystem and Conservation Sciences, University of Montana, Missoula

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Approved by:

Chairperson

Dean, Graduate School

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I evaluated the effect of a single seasonal mowing on soil temperature and developmental and growth rates of two root-feeding biological control agents of spotted knapweed, the moth *Agapeta zoegana* L., and the weevil *Cyphocleonus achates* Fahr. The experiment was conducted at two knapweed-infested sites in Granite County, Montana. At each site, 10 – 6.5 x 2.5 meter plots were established and each subdivided into 4 – 1.0 x 1.5 meter subplots. Releases of both insects into the plots were made in 2000. In the summer of 2000 and again in 2001, half of the plots at each site were mowed to a height of 10 centimeters, half were left unmowed as controls. Ambient temperature at each site, and soil temperature at two depths, 4 millimeters and 40 millimeters, were monitored in a randomly chosen subplot within each plot. In 2001, two larval samples were collected, one in June and the other in July, from separate subplots. Adults were collected from the fourth subplot. No *A. zoegana* larva or adults were collected and establishment was considered to have failed. Of the *C. achates* larvae collected, head capsule widths and total dry weights were determined. Head capsule width was used to determine developmental stage and dry weight was used as an index of growth rate within instars. I found no significant difference in either developmental or growth rates of larvae collected from mowed and control plots. There was also no difference in number of adults emerged or in time of emergence. Soil temperature was significantly higher in mowed plots at the 4 millimeter depth after the second mowing but not at the 40 millimeter depth. This suggests that for an integrated weed management program, mowing would not effectively increase the likelihood of establishment or increase development rate of these biological control agents. In a related study, I evaluated several soil factors to identify characteristics that might be correlated with establishment of either *A. zoegana* or *C. achates*. No correlation was found between establishment of either insect and soil texture, pH, water holding capacity, color, or level of disturbance prior to sampling.
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Literature Review

Invasive weeds in the western United States and Canada

The invasion of exotic weeds is considered one of the greatest threats to natural ecosystems in the western United States and Canada, a close second to, and often associated with, habitat destruction (Duncan 2001). On agricultural lands, weeds are defined by their effect on crop production. In natural systems, they are further defined by their effects on ecosystem composition and function (Randall 1997). Problems commonly associated with exotic weeds include the economic costs of control, reduced quality of agricultural products, aesthetic and property values, and reduced ecosystem function. Other potential ecosystem disruptions caused by the invasion of exotic weeds include the acceleration of soil erosion rates and the alteration of geomorphologic processes as well as biogeochemical and hydrological cycles and fire regimes (Mooney and Drake 1989). Biological invasions threaten many natural systems throughout the world by causing the decline or local extinctions of native species and reductions in genetic diversity, productivity, and buffering of ecological perturbations (Elton 1958, Mooney and Hobbs 2000).

With the arrival of European and Asian immigrants to North America in the 19th century, came an influx of Eurasian plant species. Furthermore, many more exotic plants have been introduced over time through intentional or unintentional introductions. The establishment of many of these exotic plants has been, at least in part, facilitated by continual disturbance caused by agriculture, grazing, and development. However, some of these exotic plants (invasive species) are not dependant upon disturbance for
successful colonization of new habitats (Lodge 1993). The two leading theories regarding the extraordinary success of invasive plants are the lack of natural enemies and allelopathy. In their places of origin, these weeds evolved with suites of natural controls including insect herbivores, plant pathogens, fungi, and other competing plants (Sheley et al. 1999). Without these natural regulating agents, exotic plants with characteristics that predispose them to invasiveness are able to dominate the plant communities where they become established. Allelopathy, the release of phytotoxic chemicals by certain plant species to displace surrounding vegetation, has been proposed as another explanation for invasive plant success (Bais et al. 2003). Each theory, either by itself or in combination, has the effect of lowering the diversity and abundance of native plants (Drake et al. 1989). This greatly alters the biological communities, and often results in reduced biodiversity of both macro- and microfauna, altered nutrient cycling, and reductions in forage for both wild and domestic animals (Sheley et al. 1999, Watson and Renney 1974, French and Lacey 1983, Tilman et al. 1996).

Physical characteristics that predispose exotic plants to invasiveness, allowing them to establish and outcompete native plants for light, water, and nutrients include: short generation time, early maturation, high fecundity, tolerance to a wide range of climatic and edaphic conditions, high photosynthetic rates, long-lived seed, and adaptations for spread by natural agents and humans (Lodge 1993). These characteristics are common among many of North America’s most important exotic weed problems. Consequently it can be inferred that a specie’s invasiveness is due to the combined effects of enhanced resource competition, escape from natural enemies, and the biochemical potential of the plant itself.
Spotted knapweed

Spotted knapweed, *Centaurea biebersteinii* D.C. (alt. *C. maculosa* Lam., *C. stoebe* L. ssp. *micranthos* (Gugler) Hayek) (Asteraceae), is one of the most pervasive rangeland weeds in western North America (Rice 2002). Native to the grassland steppes of Eastern Europe and Asia Minor, it was accidentally introduced to North America near the beginning of the twentieth century, as a contaminant of alfalfa seed (French and Lacey 1983). The first records of spotted knapweed in North America were in Victoria, British Columbia in 1883, and in Montana in 1920. It is currently reported as an important weed species in 15 western states and four Canadian provinces (Sheley et al. 1999) and listed as present in 43 states, including Alaska and Hawaii, and six Canadian provinces (Luken and Thieret 1997). Many western grasslands previously dominated by caespitose grasses such as bluebunch wheatgrass (*Pseudoroegneria spicata* Pursh), rough fescue (*Festuca scabrella* Torr.) and Idaho fescue (*F. idahoensis* Elmer), and drought tolerant shrubs such as big sagebrush (*Artemisia tridentata* Nutt.) (Jacobs and Sheley 1999), and annual and perennial forbs such as balsamroot, sunflower and lupine, have been invaded by this aggressive exotic. Knapweed replaces native bunchgrasses and forbs (Tyser and Key 1988) often forming large-scale, monotypic infestations, which have significant economic and aesthetic impacts to agriculture and wildlands. These impacts include reduced long-term productivity of grazing lands, reductions in biodiversity and ecosystem function, and increased soil erosion (Watson and Renney 1974, Tyser and Key 1988, Lacey et al. 1989).
Spotted knapweed is a deeply tap-rooted perennial that reproduces by seed (Watson and Renney 1974). Seeds are able to germinate in both early spring and fall; seedlings become established wherever adequate moisture and space are available. Knapweed overwinters as a rosette and resumes growth in early April of the following year (Duncan et al. 2001). Each plant produces one to six or more multiply branched stems, culminating with the production of a single flowerhead at the tip of most branches in mid to late summer (Watson and Renney 1974, Duncan et al. 2001). Flowers have pinkish-purple petals and dark tipped bracts that create the spotted appearance that gives the plant its name. Flowers bloom from July through August (Watson and Renney 1974); individual flowers bloom for two to six days withering and the bracts close. Bracts reopen after approximately twenty days allowing the mature seeds to be easily dispersed by physical movements of the plant caused by wind or other disturbances (Duncan et al. 2001).

Spotted knapweed produces as many as 1000 seeds per plant per year (Schirman 1981). These seeds are easily dispersed by water, animals, humans, and vehicles, and are able to germinate under a wide range of environmental conditions, or may remain viable in soil for more than eight years (Davis et al. 1993). High seed output and long seed viability make chemical control of spotted knapweed difficult and expensive because seeds may continue to germinate after herbicides have dissipated (Davis et al. 1993, Griffith and Lacey 1991).

Spotted knapweed is well adapted to a wide range of habitats including, but not limited to, open forests, urban interfaces, and rangelands. Though most common in disturbed sites, disturbance is not necessary for knapweed to establish (Lacey et al. 1990,
Spotted knapweed’s invasiveness can be partly attributed to its prolific production of long-lived seed, ability to germinate earlier in spring relative to native plants, and its ability to overwinter in fall as a rosette, tolerance to a wide range of site conditions, and absence of its natural enemies. Another factor in the displacement of native species may be the production of the phytotoxin (-)-catechin in root exudates, which has been shown to inhibit the germination and growth of native species that come into contact with the root systems of knapweed (Bais et al. 2003).

Successful establishment of knapweed is governed, in part, by climate (i.e. annual precipitation, length of frost-free season, and mean maximum summer temperature), soil type, and elevation. However, no single environmental variable has been found to be an effective predictor of site vulnerability to knapweed invasion (Chicoine et al. 1985). Although spotted knapweed is more successful establishing in drier rather than wetter locations, it is able to establish in a wide range of habitats. For example, spotted knapweed is found in every county in the state of Montana, across all habitat types, in every soil type (Mooers 1986). Disturbance, though not a requirement for establishment, has been suggested as the key factor influencing establishment and distribution (Mooers 1986).

**Spotted knapweed management: herbicides**

Ideally, management of spotted knapweed should restore healthy native plant communities that are resistant to invasions and meet desired land-use objectives such as improving livestock forage, wildlife habitat, or quality recreational opportunities. However, spotted knapweed has proven to be difficult to manage. Although herbicides
can be highly effective, most are relatively broad spectrum in their effects, often removing many desirable native plants along with the target weeds (DeLoach 1997). Also, due to long-lived seed, herbicides must be reapplied periodically often making their use cost prohibitive for large areas of low economic return, such as rangelands. Other limitations to their use include increased weed resistance, the potential for polluting streams, lakes, and groundwater, and undesirable effects on native plants (DeLoach 1997).

Although herbicides such as picloram, clopyralid, and 2,4-D are recommended for the eradication of small patches and new infestations of spotted knapweed, herbicide treatments on large-scale infestations are likely to be most effective, and more long term, when combined with other management methods that enhance the competitive ability of desired plant species (Duncan et al. 2001). Furthermore, land managers are faced with fewer and more expensive herbicides, increased weed resistance, environmental concerns, and water quality issues, making alternate management methods desirable in restoration efforts. Control of spotted knapweed will likely require an integrated approach using a combination of chemical, mechanical, and biological methods.

Spotted knapweed management: grazing and mowing

Grazing has appeal as a management strategy in many areas where weed-infested rangelands are already grazed and grazing constitutes a semi-natural process requiring few external chemical or energetic inputs. The feasibility of using livestock to control other invasive weeds such as leafy spurge, *Euphorbia esula* L. has been shown (Lajeunesse et al. 1999); however, it has seldom been successful in controlling spotted
Knapweed (Kennett et al. 1992). Lacey et al. (1994) found that repeated defoliation reduced carbohydrate concentrations and reserves; however, spotted knapweed would need to be selectively grazed throughout the growing season for the method to be successful (Kennett et al. 1992).

Although cattle and sheep will graze spotted knapweed at low to moderate levels, due to reduced palatability of older plants, knapweed is seldom grazed past the early summer (Duncan et al. 2001, Kelsey and Mihalovich 1987, Watson and Renney 1974). Rosettes and first year plants are relatively nutritious and edible, but grow low to the ground making grazing difficult for cattle. Second year and more mature plants are coarse and fibrous which makes them less desirable forage (Watson and Renney 1974). Controlled, repeated, grazing by sheep can reduce the number of one- and two-year-old spotted knapweed plants. However, it has been suggested that grazing be timed when associated grasses are dormant to reduce impacts on these desirable species (Duncan et al. 2001). Because of spotted knapweed's early growth habit, early spring grazing would have minimal impact on the later-emerging native grasses. Jacobs and Sheley (1997) found that short-duration grazing by cattle in early spring reduced the number of seedlings and rosettes, but increased bare ground. Because disturbance, and associated availability of bare ground, are key factors in knapweed establishment, any method that increases bare ground is not recommended (Duncan et al 2001).

Mowing has been suggested as a management tool for spotted knapweed; however, long-term effects of mowing on spotted knapweed are unknown. Watson and Renney (1974) reported that mowing at the flower stage reduced the number of flowering stems and the number of seeds produced. However, clipping experiments have shown
that the plant’s compensatory response is dependent upon environmental conditions (Kennett et al. 1992). Successful control by mowing may be dependant on proper timing. Benefield et al. (1999) found that a single mowing treatment at the bolting stage provides a reduction in seed head production of yellow star thistle (*Centaurea solstitialis* L.), a closely related plant. A similar effect may occur with spotted knapweed. Sheley et al. (1999) found that fall mowing for three consecutive years caused a significant reduction in adult knapweed density and that a single mowing at the late bud growth stage reduced the number of seeds produced but not plant density.

**Spotted knapweed management: biological control**

Biological control is defined as the use of natural enemies to control populations of pests (Van den Bosch and Messenger 1973); biological control of weeds can be further defined as the use of natural enemies, such as insect herbivores, to reduce weed densities to an acceptable level (McFadyen 1998). This approach holds considerable promise for the suppression of many noxious weeds (McFadyen 1998). Advantages of biological control include cost-effectiveness, self-perpetuation, selectivity, and compatibility with many other weed management strategies. Insect herbivory, both above- and below-ground, has been shown, in some cases, to significantly impact plant species composition, vegetation cover and structure, and the direction and rate of plant succession (Brown and Gange 1989).

Unlike herbicides that must be periodically reapplied, biological control is intended to provide long-term regulation of target weeds. However, limitations do exist. Biological control of weeds is often relatively slow to show impacts, rarely results in
eradication, and may be ineffective unless integrated with other management strategies (Duncan et al. 2001). Also, although biological control agents are intensively screened to reduce risks to non-target plants (McFadyen 1998), unexpected indirect effects on other non-target organisms have been documented (DeLoach 1997, Howarth 1991).

Success of biological control programs depend in part, on the effective distribution and establishment of the control agents. Thirteen herbivorous insect species have been released in the western United States for the control of spotted knapweed. These organisms attack the roots, flowerheads, and/or foliage with varying levels of success. The first biological control agents to be released against spotted knapweed in North America were three flowerhead flies (*Urophora* spp.) released in Canada between 1970 and 1973 (Müller et al. 1989). Although these three insects have successfully established, and seed predation can be very heavy (60-80%), no measurable decrease in knapweed density has been attributed to them (Story 2000). Another seed head feeder, a weevil (*Larinus obtusus* L.), is also well established and spreading (Story 2000). The larvae of the weevil consume 100% of the seed in infested flowerheads, and adults cause additional damage by feeding on knapweed foliage. Four other flowerhead feeders have been released but establishment has been poor, possibly due to unsuitable climate and/or competition with other flowerhead feeders (Story 1997, 2000). Five root-feeding insects have also been released. Of the five, only two have become well established, a moth, *Agapeta zoegana* L., and a weevil, *Cyphocleonus achates* Fahr. (Story 2000).

Given that, on average, more than 50% of the net primary productivity of a plant is allocated to below ground tissues (Campbell 1993, Andersen 1987); below-ground herbivores may have important effects on plant fitness and community structure.
(Andersen 1987, Brown and Gange 1989, 1990). The plant root system has many functions including water and nutrient absorption and anchorage, as well as serving as the site for the synthesis of many products involved in growth and development, and the storage of metabolites and photosynthate (Andersen 1987, Campbell 1993). Thus damage to, or removal of, part of the underground system may disrupt physiological processes and be reflected in a variety of responses by the plant (Chapin et al. 1990, Nötzold et al. 1998). Significant reductions in agricultural yield and stand die-off attributed to root-feeding insects provide evidence of their ability to affect plant growth and survival (Brown and Gange 1990, Strong et al. 1995).

A distinct temporal niche separation in development and primary feeding activity occurs between the root- and flowerhead-feeding insects. The root-feeding insects develop and feed primarily in spring and autumn when nutrients are most abundant in root tissues (Andersen 1987, Mueller et al. 1989, Chapin et al. 1990). Plant nutrients are transported to the plant reproductive structures in summer, making them available to seed- and flowerhead-feeding insects (Müller et al. 1989). Though seed-feeding insects have been shown to dramatically reduce seed production, root-feeding insects may be especially desirable for biological control for a number of reasons. Because they are concealed under the soil surface, root feeding insects are protected from many common predators such as birds and spiders. Therefore, larvae are more likely to survive during primary feeding stages. Root herbivores can have a strong impact on plant growth and resource allocation by directly interfering with physiological processes. Removal of root tissues and increase in root exudation caused by feeding may also create secondary
effects such as increased susceptibility to disease and drought that further impact plant

Successful biological control programs demonstrate that insect herbivores can
reduce target plant populations (McFadyen 1998). However, the impact of a biological
control agent on its target plant may differ among habitats. The ability of root-feeding
insects to establish, and their impact on the target plant, are greatly affected by soil
factors such as temperature, moisture, and nutrient status (Crawley 1986, Müller-Scharer

It is important to consider the plant as an interactive component of any biological
control program. By manipulating plant morphological attributes it may be possible to
increase the efficacy of biological control insects (Cortesero et al. 2000). Plants possess
attributes, which increase their tolerance of damage, including the ability to mobilize
reserves of carbohydrates and proteins to facilitate regrowth and plasticity in the
distribution of photosynthate between roots and shoots (Crawley 1986). Kennett et al.
(1992) found that foliage regrowth after clipping occurs at the expense of root growth.
However, both above- and below-ground biomass may be adversely affected by
defoliation. It has been found that compensatory root growth occurs in response to
feeding by *A. zoegana* and *C. achates*. (Steinger and Müller-Scharer 1992, Callaway et
al. 1999). Simulated root herbivory experiments have shown that plants commonly
exhibit some level of compensatory root growth with a corresponding reduction in shoot
growth (Andersen 1987). This response is further influenced by the level of available
nitrogen in the soil (Müller-Scharer 1991). Root herbivores are also capable of altering
water, nutrient, and carbon budgets of their hosts (Gange and Brown 1989).
Agapeta zoegana and Cyphocleonus achates as biological control agents of spotted knapweed

Two root-feeding insects have become established in many areas of western North America as biological control agents of spotted knapweed, the moth, Agapeta zoegana L. (Lepidoptera: Cochylidae) and the weevil, Cyphocleonus achates Fahr. (Coleoptera: Curculionidae) (Müller et al. 1989). Both insects originate from the same regions of Europe and Asia as spotted knapweed. Both insects feed as larvae in the taproot of spotted knapweed, but differ in the part of the root they use and in the timing of their main feeding activity. A. zoegana larvae primarily mine the cortical tissue of the taproot in autumn, and from spring to early summer (Müller et al. 1988). Although single A. zoegana larva may have little short-term impact on the growth of spotted knapweed (Callaway et al. 1999), feeding by multiple larvae over the long term has been shown to reduce above-ground biomass and the plant’s ability to produce seed (Story et al. 2000). C. achates larvae feed primarily in the central vascular tissue from spring through summer causing a gall-like enlargement of the taproot (Stinson et al. 1994). Feeding by C. achates larvae reduce spotted knapweed growth mainly by reducing shoot growth (Stinson et al. 1994). When occurring together, the effect of the two insects is additive. However, the impact of these insects on the host plant can be strongly affected by soil moisture and nutrient status (Müller-Scharer 1991) as destruction of xylem vessels may increase resistance to the flow of water and nutrients, such as nitrogen, to the shoot.

Because these insects each utilize a different portion of the root (Steinger and Müller-Scharer 1992), they are believed to be compatible with, rather than antagonistic
to, one another. The two insects are often released at the same sites in North America. However, very little is known regarding the effects of North American site characteristics, including vegetation cover and soil temperature, on the ability of the two insects to establish and impact spotted knapweed in its new environment. Knowledge of which characteristics best support establishment and increase impact would be valuable in developing guidelines to optimize release efforts and when incorporating these insects into integrated weed management programs.

*Agapeta zoegana*

*Agapeta zoegana* is native to Europe and portions of Asia where it is associated with undisturbed grassland sites (Müller et al. 1988). It was introduced to North America for the biological control of knapweeds in Canada in 1982 and 1983 (Stinson et al. 1994). It was first released in the United States in Montana in 1984 and is now well established at many sites (Story et al. 1991). Although facultatively multivoltine in Europe, it is univoltine in North America (Story et al. 1991). Story et al. (2000) suggests that the difference in voltinism may be due, in part, to differences in host plants in Europe and North America. In North America, spotted knapweed is tetraploid and appears to be a distinct species (*C. biebersteinii*) from the diploid native of Europe and Asia (*C. maculosa*) (Stinson et al. 1994). Although recent molecular studies support the existence of two species, many in North America still refer to spotted knapweed as *C. maculosa*.

Similar univoltine life histories for *A. zoegana* have been described for North America and Europe (Story et al. 2000 and Müller et al. 1988). Adult emergence takes place over an eight week period between mid-June and mid-August. Females usually
mate within 24 hours of emergence when temperatures reach 18-30°C and do not require a host plant to be present. Oviposition occurs in the 48 hours following mating with each female laying 150-400 eggs laid singly or in groups of two to three on the surface or in crevices of lower stems and leaves of knapweed and adjacent plants (Müller et al. 1988, Story et al. 2000). While Müller et al. (1988) found the moth preferred rosettes as oviposition sites in Europe, Story et al. (2000) suggests that in North America, the moth preferentially lays eggs on older knapweed plants.

Eggs hatch 7-10 days following oviposition and the young larvae immediately mine into the epidermal tissue of the root crown (Müller et al. 1988). The larvae show no tendency to avoid plants that are already attacked by conspecifics and multiple larvae are often found in a single root (Story et al. 2000). Root diameter appears to have a greater influence on the number of larvae found per root than does plant stage (Story et al. 2000).

*Anisopteragia zoegeana* undergoes six larval instars. The first instar larvae mine into and overwinter within the root. Successive instars mine progressively downward in open, irregular, sometimes spiral mines that are lined with a whitish web spun by the larvae (Müller et al. 1988). *A. zoegeana* pupate in early June within the mines; the pupal stage lasts approximately two weeks.

*Cyphocleonus achates*

*Cyphocleonus achates* is native to southern and southeastern Europe and Asia Minor (Stinson et al. 1994). It was first released in North America for biological control of spotted and diffuse knapweeds in British Columbia in 1987 (Stinson et al. 1994) and in
Montana in 1988 (Story et al. 1997). It is now well established at many sites throughout the northwest.

*C. achates* is univoltine in both Europe and North America. Adult weevils emerge between mid-July and the end of September. Early emerging adults begin mating within one-to-two weeks while later emerging adults may mate soon after emergence (Powell et al. 2000). The females lay eggs singly, attaching them to the root crown of knapweed plants over a ten week period. Estimated average fecundity is approximately 45 eggs per female. Eggs are laid continually between mid-August and mid-November (Stinson et al. 1994) with adults feeding on knapweed foliage throughout the oviposition period. Adults generally live 8-15 weeks, with death generally occurring with the first hard freeze in autumn (Stinson et al. 1994).

Eggs hatch in 10-12 days and the young larvae immediately excavate into the root crown to feed. Larvae feed on, and overwinter in, the root cortex (Stinson et al. 1994, Wikeem et al. 1999). They primarily overwinter as second instars. There are a total of four instars prior to pupation. During the third and fourth instars, conspicuous galls develop in the root at the site of mining. Pupation occurs within the gall in early to midsummer. The pupal stage lasts for approximately two weeks (Stinson et al. 1994).

Field observations in Europe suggest that *C. achates* is associated with early seral habitats and disturbed sites (Stinson et al. 1994). In North America, little is known regarding site characteristics conducive to establishment. However, it is known that a major factor influencing establishment is the availability of plants that have roots large enough to sustain larvae (Stinson et al. 1994). Other factors include release density
Temperature and the cycles of insects

The success of integrated weed management programs incorporating biological control depends on the strength of the interactions between the insect herbivores and the host plant. The duration and timing of these interactions can be important. Insects are poikilothermic, meaning their body temperature varies with the temperature of their environment. As poikilotherms, insect activities are governed by endogenous rhythms, which are synchronized by seasonal changes in temperature (Higley et al. 1986, Clarke 1967). Reviewing the literature for over 300 insect species, Gilbert and Raworth (1996) found that developmental rate increases almost linearly with temperature over a range of mean daily temperatures of 9 - 30° C, tapering off at higher mean daily temperatures. A necessary distinction has been made between developmental rate, (i.e. the reciprocal of the time required to complete the egg, larval, or pupal stages), and growth rate (i.e. the larva’s rate of relative weight increase {[(log(final weight/initial weight))/developmental time]}). Although both are independently genetically variable, environmental changes impact growth and developmental rates in similar ways (Gilbert and Raworth 1996).

In general, insects are selected for slow development and fast growth in the spring and for fast development in the summer, resulting in the following five effects (Gilbert and Raworth 1996).

1) Growth and developmental rates follow a linear increase with temperature.
2) Genetic variability in developmental rate is reduced at high temperatures.

3) Genetic variability in developmental rate is reduced at low temperatures.

4) Development rate is slow at the time of emergence after diapause.

5) Growth rate is slow at low temperatures, but development rate is even slower.

As a result of this pattern, adult insect size is an exponential function of the ratio of growth rate to developmental rate. Therefore, fast growth rate in conjunction with slow development in spring maximizes adult size and fecundity, which is positively associated with adult size in many insects. Fast developmental rate in summer minimizes exposure of relatively defenseless larvae to predation. Predators generally become more efficient during the higher temperatures of late summer and food quality for herbivores has generally decreased by this time (Gilbert and Raworth 1996, Howe 1967).

The average rates of development and growth are restricted by an insect’s basal metabolic rate which is adapted, as a function of temperature, to the species native range. Although temperature affects developmental rates in a linear manner, for each species there is a temperature threshold below which no development occurs, and a threshold above which development and activity ceases (Higley et al. 1986, Gilbert and Raworth 1996).

Degree-days, an estimate of heat accumulation above a given threshold, is widely accepted as one of the best methods for measuring and predicting the effect of temperature on biological processes, including development rates of insects and
interactions between insects and host plant (Baskerville and Emin 1969, Pruess 1983, Higley et al. 1986). The degree-day method makes two basic assumptions: (1) that the response of the insect to temperature is linear and constant over the growth period (Allen 1976, Stinner et al. 1974, Allsopp and Butler 1987), and (2) that the threshold value used is biologically meaningful for the organism (Gilbert and Raworth 1996).

Soil temperature

When investigating below ground communities, including root-feeding insect populations, the spatial and temporal variation of the soil microclimate must be considered (Davidoff et al. 1986, Pierson and Wight 1991). Soil climate is defined as the combination of annual and diurnal cycles of temperature and moisture within the soil developed in relation to the external atmospheric climate, soil physical characteristics, specific site characteristics, and vegetation cover (Shulgin 1978). Soil climate differs from atmospheric climate by exhibiting large variations over space and time due to the nonuniformity of its composition. The magnitude of both spatial and temporal variations may be large enough to exert strong influences on the establishment of organisms inhabiting plant roots near the soil surface (Pierson and Wight 1991).

Solar radiation is the primary source of energy to heat soil (Brady and Weil 1999, Shulgin 1978). This energy is absorbed by the soil surface and transformed into thermal energy, which is transferred via thermal conduction through the surface layers into the subsurface soil. However, not all available solar energy reaches the soil surface. Site characteristics that have a significant influence on the incidence of solar radiation, and consequently soil temperature, include slope and aspect (Brady and Weil 1999); the more
perpendicular the angle of incidence, the more energy available for absorption. Albedo is that fraction of solar energy that is reflected by a surface. Dark-colored and rough surfaces tend to have lower albedos than light-colored smooth surfaces (Brady and Weil 1999). However, this does not necessarily imply that dark-colored soil is always warmer. Organic matter often imparts a dark color to soil, which increases absorption, but organic matter also reduces heat transfer efficiency within a soil (Hillel 1998). Soil temperature is ultimately dependent on the net amount of solar radiation absorbed.

The temperature of a soil is dependent on the net amount of heat energy the soil absorbs, the heat required to bring about a given change in the temperature of the soil, and the energy required for driving processes such as evaporation, which are constantly occurring at or near the soil surface. The thermal conductivity of a soil depends on the soil’s physical properties of texture, percent coarse fragments, porosity, and soil moisture. Thermal conductivity of soil mineral particles is approximately 100 times that of soil air and 24 times that of soil water (Shulgin 1978). Porosity of soil acts as an insulator; however, thermal conductivity increases as pores are filled with water until field capacity is surpassed and the thermal conductivity reaches that of water. Because water has a higher specific heat than soil dry particles, it requires more energy to increase in temperature and has the effect of cooling surfaces that it evaporates from. Thus, a dry soil is more easily heated than a wet one. It is the specific heat of a soil that controls the degree to which that soil heats in the spring (Brady and Weil 1999, Hillel 1998).

Solar radiation that reaches the soil surface slowly penetrates the profile by conduction. Heat exchange processes take place from the surface to the subsurface and vice versa. During a 24 hour cycle, the course of heat exchange follows the course of
solar radiation with significant variation during the day but less at night (Pierson and Wight 1991). During the day, the soil surface is heated to its maximum temperature with heating diminishing with depth. During the night, the soil surface cools and cooling diminishes with depth. Thermal conduction and change in soil temperature are slow processes with a lag between the surface and subsoil (Davidoff et al. 1986, Brady and Weil 1999). Generally, the vertical temperature gradient of soil shows the following regularities: 1) the period of temperature fluctuation remains constant for a given site, 2) the amplitude of variation diminishes with depth, and 3) the time of occurrence of maximum and minimum temperatures relative to ambient temperature is delayed with depth (Shulgin 1978).

The presence of a large spatial variability in temperature within the first 10 cm of soil can be important to the survival of plants and insects (Davidoff et al. 1986, Pierson and Wight 1991). The growth rate of most plants is more sensitive to soil temperature than to air temperature. Root functions affected by temperature include nutrient and water uptake and growth (Brady and Weil 1999).

Vegetation cover serves as a thermo-insulator, absorbing heat and solar radiation and using it for evaporation and the formation of new tissues. Vegetation influences soil temperature by shading the soil surface and reducing heat influx, lowering thermal capacity through evapotranspiration, using heat energy to produce new tissues, blocking radiation at night, and obstructing air flow. During the day, soil shaded by vegetation is cooler, and at night it is warmer, than bare soil. Both diurnal and annual cycles of soil temperature depend on the vegetation cover, its nature, height, and density during the year.
The degree of shading of soil due to vegetation cover and its interaction with site and soil characteristics may be critical elements affecting establishment and efficacy of root-feeding insects released for biological control of weeds. Because these insects develop at a rate governed by the amount of heat they absorb, soil temperature variation can significantly influence the site-specific growth and development of these organisms (Clarke 1967). Shading by vegetation, and the spatial and temporal variability associated with it, causes a great deal of variability in soil temperature at or near the soil surface (Davidoff et al. 1986, Pierson and Wight 1991), although the effect is dampened at lower soil depths (Brady and Weil 1999, Davidoff et al. 1986).

Vegetation insulates the soil surface from incoming solar radiation during the day and from heat loss at night. Lower maximum and higher minimum temperatures are expected in shaded areas than in uninsulated interspace areas between plants. There is also variation in soil temperature caused by the direction of shading; as with site aspect, sunlight is more able to reach the south side of plants creating a slightly warmer and more arid microclimate than is found on the north side (Pierson and Wight 1991). By removing vegetation, such as through mowing, heat absorption by the soil may be increased, consequently increasing the rate at which degree-days are accumulated. This, in turn, may increase the developmental rate and survival of root-feeding insects (Clarke 1967) such as *A. zoegana* and *C. achates*.

**Objectives**

Soil temperature and factors affecting soil temperature are likely to have strong effects on the development rates, reproduction, dispersal, and ultimately establishment
and impact of insects feeding in roots. Shading created by grasses and other vegetation such as spotted knapweed foliage and flower stalks can be expected to decrease soil temperature with increasing density and height and thus may lengthen the development period of the insects. Understanding the effects of shading and temperature on the root-feeding insects of spotted knapweed will be valuable in developing integrated methods of spotted knapweed control.

The overall objective of my research was to investigate which site characteristics best support or can be manipulated to optimize establishment and efficacy of *A. zoegana* and *C. achates* as biological control agents for spotted knapweed. The specific objectives of my project were:

1) Determine whether shading and vegetation cover associated with spotted knapweed affects soil temperature, and consequently, the development and survival rates of *A. zoegana* and *C. achates*.

2) Determine whether mowing enhances development, survival, and establishment of *A. zoegana* and *C. achates*.

3) Determine if particular soil properties are positively associated with establishment of *A. zoegana* and *C. achates*. 
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The effect of mowing on developmental rate, survival, and establishment of *A. zoegana* and *C. achates*

**Introduction**

Spotted knapweed, *Centaurea biebersteinii* D. (alt. *C. stoebe* L. ssp. *micranthos* and *C. maculosa* L.), an invasive perennial of Eurasian origin, was accidentally introduced to North America in 1883 (French and Lacey 1983) and has since spread across much of the continent infesting millions of hectares in 15 western states and four Canadian provinces (Sheley et al. 1999). Spotted knapweed possesses many of the invasive qualities reviewed by Lodge (1993) allowing it to invade both disturbed and undisturbed sites across a wide range of climatic and edaphic conditions (Mooers 1986). Once established, it spreads quickly due to abundant seed production and relatively early growth, enabling it to out-compete many native plants for space, water, and nutrients. Where it becomes established, spotted knapweed commonly displaces native plants with the consequent effects of altering community composition, lowering biodiversity, and altering nutrient cycling (Watson and Renney 1974, Tyser and Key 1988, Lacey et al. 1989). Large-scale infestations often produce extensive near monotypic stands having significant impacts on local economies, not only through the direct costs of management, but also through increased soil erosion, decreased forage production, and reduced property values.

Spotted knapweed has proven to be difficult to manage. Mechanical methods, such as mowing and hand pulling, are labor intensive and their long-term efficacy is as yet unknown. Herbicides, though highly effective, may be impractical or inappropriate in...
many situations. Due to their transient nature, these chemicals must be periodically reapplied (DeLoach 1997) making their cost prohibitive for large areas and areas with low economic returns. Also, social pressures against chemical use in natural systems, based on concern over impacts to nontarget plants and discharge into water systems, are increasing. Biological control is a long-term, alternative management tool that, unlike chemicals that must be periodically reapplied, becomes self-perpetuating once agents are established and has minimal direct effects on nontarget organisms (McFayden 1998).

Two root-feeding insects, a moth, *Agapeta zoegana* L., and a weevil, *Cyphocleonus achates* Fahr., have been released as biological control agents of spotted knapweed in North America (Müller et al. 1989, Müller and Schroeder 1989, Story et al. 1991). Although both are specific to *Centaurea* spp. and feed within the taproot of the plant, each utilizes a different niche within the root (Müller et al. 1988, Stinson et al. 1994, Müller et al. 1989) and, therefore, the two insects are considered compatible with, rather than antagonistic to each other. Early instar *A. zoegana* larvae mine the epidermal tissue of the root crown in the autumn and overwinter in the epidermis. The following spring, late instar larvae mine deeper into the root and feed on the root cortex and endodermis (Müller et al. 1988). *C. achates* larvae feed primarily in the central vascular tissue in the spring and summer following overwintering (Stinson et al. 1994). Both insects depend upon the availability of large roots to support larval development and are often found feeding within the same root (Müller et al. 1989). Both insects are often released at the same sites, therefore, when evaluating establishment and impact, both insects should be assessed together.
Both *A. zoegana* and *C. achates* have been released at various sites throughout the western United States and Canada, and although well established at some sites, have failed to establish at others. Despite extensive biological control efforts, knapweed infestations continue to expand (Müller-Scharer and Schroeder 1993). The lack of consistent establishment and effectiveness may be due, at least in part, to poor adaptation by the insects to conditions at many North American sites. As poikilotherms, insect development and activity rates are temperature dependent. Therefore, since these insects develop below the soil surface, soil temperature, and any site characteristics affecting soil temperature, are likely to have significant impacts on the developmental rates, emergence times, reproduction, dispersal, and ultimately, establishment and impact of these insects.

Shading by spotted knapweed and associated plants can be expected to decrease soil temperature with increasing density (Davidoff et al. 1986). Soil temperatures are often cooler under dense plant canopies than when plants are widely spaced. Such differences in soil temperatures created by vegetation may impact weevil development and emergence (Story et al. 1996). Indeed, Story et al. (1996) found that wide spacing of spotted knapweed plants, which allows more insolation to the soil, to have a strong positive impact on *C. achates* production and emergence. Therefore, soil temperature may be an important factor affecting *A. zoegana* and *C. achates* establishment and may be responsible for differences in establishment observed among sites and different habitat types.

Manipulation of plant density and cover may aid establishment of root-feeding biological control insects by increasing soil temperature (Jacobs et al. 2000). Mowing is one method of manipulating knapweed density that has been suggested as a method for
managing spotted knapweed (Duncan et al. 2001, Sheley et al. 1999). Although ineffective by itself, properly timed, mowing would remove knapweed flower stalks and other vegetation cover thus reducing shading and increasing soil temperatures. This may positively affect insect development rate, survival and establishment. The purpose of this study was to determine whether shading due to vegetation cover, and the partial removal of cover by mowing, influence soil temperature and the subsequent developmental rate, survival, and establishment of A. zoegana and C. achates.

Materials and Methods

Study Sites and Experimental Design

In spring 2000, two study sites were chosen in Granite County, Montana, where knapweed is well established but where A. zoegana and C. achates had not previously been released. Sites without established populations of these two insects were chosen to control for time of release. Both study sites were located in the floodplain of the Clark Fork River between Drummond and the Bearmouth Area with approximately 7 miles separating them. Both sites were at an elevation of about 1100 m with a 0% slope; both were disturbed and had similar knapweed density and height. The first site, Davis Corral, was a seasonal pasture on the north side of the river, grazed annually by yearling steers between mid-July and mid-October. A 40 x 25 m, three strand, barbed wire fence was erected to exclude livestock from the study area at this site. The second site, Jonas Pond, was used as a borrow pit for the construction of I-90 on the south side of the river. It was backfilled, leveled, and seeded to grass in 1972 and subsequently invaded by spotted knapweed. This field was not grazed and, therefore, was not fenced.
To verify that *A. zoegana* and *C. achates* were not present prior to establishing plots, 100 randomly chosen mature knapweed plants were uprooted from each site. The roots of these plants were dissected on site and observed for evidence of larvae. After it was established that these insects were not present, ten 6.5 x 2.5 m plots were established at each site, in areas with relatively uniform knapweed density, for a total of 20 plots. Each plot was further subdivided into four 1.5 x 1.0 m subplots separated and surrounded by a 0.5 m buffer (Figure 1).

One randomly selected subplot within each plot received a gypsum block buried to an average depth of 15 cm to monitor soil moisture in combination with a Delmhorst KS-D1 soil moisture tester (Delmhorst, Towaco, NJ). The same subplots receiving the gypsum blocks also received two thermocouples buried at 4 mm and 40 mm, respectively, to record soil temperature. The thermocouples were connected to a HOBO H8 Outdoor/Industrial data logger (ONSET, Bourne, MA). The data logger recorded soil temperature, for each of the two depths, at 30-minute intervals from July 11, 2000 to October 4, 2001. Ambient temperature was recorded at 30-minute intervals during the same period using a HOBO H8 Temp data logger (ONSET, Bourne, MA) deployed at approximately 1.4 meters above the ground, housed under a radiation shield, at each site.

The remaining three subplots were randomly assigned for one of two larval samples, or for adult sampling. Cumulative degree-days were derived from minimum and maximum data collected by ambient and soil data loggers, using DDAY.EXE (Legg 1994).

Average snow depth was monitored weekly through the winter of 2000-2001 using a meter stick extended through the snow pack and measuring to the nearest cm at three randomly chosen locations within each plot.
Overall vegetation density was determined by percent frequency. Vegetation was divided into type (i.e. knapweed, and grass and other herbaceous plants) and frequency was determined by placing a 10 cm square grid above each subplot and counting the number of touches of each vegetation type on metal pins extending through the grid vertices to the ground (Brown and Gange 1989). By using touches we get a three dimensional picture of density. Touches to bare ground and duff-covered ground were also counted to determine percent bare soil. Spotted knapweed density was determined as an average number of adult plants (rosette and bolted) per square meter for each plot. Seedlings were not included because they would not contribute to the establishment of either insect. Average height of knapweed within each plot was also determined.

Soil samples were collected from each plot and analyzed to determine particle size distribution, pH, and water holding capacity at -1/3 bar. T-tests were performed to determine differences that would warrant the creation of blocks in the plot design. Since variation in soil characteristics was not significant among plots, a complete randomized design was used to assign mowing or control treatments to plots. Soils at the two sites are classified as floodplain and disturbed.

Half of the plots at each site were randomly chosen to receive the mowing treatment. Vegetation in these plots was clipped with manual hedge shears to an average height of 10 cm on July 20, 2000, with the remaining plots left unmowed as controls. Treatments were made to whole plots, including buffer zones.

On July 31, 2000, 40 *A. zoegana* adults were released into each plot. Although the moths were not sexed, this moth typically has a 1:1 sex ratio (L.J. White pers. com.), and it was assumed that the moths released into plots were approximately half males and...
half females. Because this moth usually mates and lays eggs within 48 hours after emergence, prior to releases each plot was tented with netting. The netting was left in place for 48 hours to prevent escape of the moths prior to oviposition. On August 15, 15 cm tall corrals of aluminum flashing were erected around each plot and 20 *C. achatas* weevils were released into each plot. Again, it was assumed that the insects released had approximately a 1:1 sex ratio (L.J. White pers. com.). *C. achatas* is flightless and aluminum corrals are a common method used to contain them during release. Any vegetation reaching the corrals was clipped to prevent escape of the weevils. The 0.5 m buffer surrounding the subplots was used to control for the potential heating of soil by reflected solar energy from the flashing. Corrals were removed the following spring at snowmelt and replaced later to collect adults (see below).

**Insect Sampling**

**Larval Development**

On June 7, 2001, one subplot randomly chosen from each plot was excavated, using garden spades and hand trowels, to remove all knapweed plants with as much of the root intact as possible. The roots of these plants were dissected for larvae. Any larvae found were placed in labeled vials and preserved in Pampel’s solution (BioQuip Products, Inc., Gardena, CA) which preserves soft-bodied insects without altering shape and size. On July 13, 2001, a second subplot in each plot was sampled in the same manner.

Although the relationship between insect developmental rate and environmental temperature is not a linear function, the degree-day model, which incorporates a linear
function with a break at a developmental zero, defined as the temperature at which all activity and development ceases, is commonly used to describe the relationship (Allsopp et al. 1987). Estimates are most accurate where the actual developmental zero, and thus the actual area under the degree-day curve is known. However, these data are not available for either *C. achates* or *A. zoegana*. It has been suggested that standardized thresholds should be used for reporting degree-days in practical applications (Pruess 1983) and that these thresholds be the same as for existing agronomic applications. A threshold of 0°C was chosen for *C. achates* because that is the threshold used in similar studies of that insect in Montana (Hansen 1996b).

*C. achates* larvae were found in most plots. For *C. achates* larvae from each sampling date, head capsule widths were measured to determine instar stage, and thus developmental rate. Each larva was also oven dried and the dry weights used along with the head capsule measurements to determine differences in growth rates within instars. Only one *A. zoegana* larva was found, therefore, no larva measurements were made for this insect.

**Establishment and Survival to Adult**

The remaining subplot was caged, using tulle fabric stretched onto 1.5 x 1.0 x 1.0 m wooden frames, from June 10 to July 24, 2001, to collect emerging adult *A. zoegana* moths. Cages were monitored daily between 10:00 am and 4:00 pm, the diel period when moths are most active, by searching knapweed flowers, stems, and foliage for 10 minutes per plot. On July 24, several cow-calf pairs were unexpectedly released into the Davis Corral site. Several of the calves breached the fence erected around the
plots and destroyed nearly all of the cages. However, since no moths had emerged by that date, and no larvae had been collected, establishment was considered to have failed and the remaining cages were dismantled. The cages at the Jonas Pond site remained in place until July 30, but again, no moths were captured.

Aluminum flashing corrals were again erected around the same subplot on July 30 in order to collect adult *C. achates*, which emerge later in the season than *A. zoegana*. Corrals were monitored daily between 10:00 am and 4:00 pm when adult weevils are most active, often climbing or perching on knapweed flower stalks. Adult weevils were collected by searching knapweed flowers, foliage, and stems, as well as the ground and other vegetation for 10 minutes per plot. Adult weevils were collected, counted, sexed, weighed, and measured for total body length.

**Statistical Analysis**

Statistical analysis included computation of the sample mean and standard deviation for the head capsule widths and dry weights of the larvae collected on both June 7 and July 13, 2001. Differences between means of insect measurements in mowing and control treatments were analyzed, using two-sample t-tests and One-Way ANOVA ($\alpha = 0.5$). The June 7 sample from Davis Corral was not normally distributed, and could not be normalized using standard transformations. Therefore, a Mann-Whitney Rank Sum test and a Kruskal-Wallis One-Way ANOVA on Ranks were performed on differences in median head capsule width between mowed and control plots for that site. Tests for significant differences in peak date and cumulative emergence of adult
*C. achates* were also analyzed using two-sample t-tests and One-Way ANOVA (SigmaStat version 2.03, SPSS Inc., San Rafael, CA 1995).

**Results**

**Site Characteristics**

Spotted knapweed was the dominant vegetation type at each site with mean densities of 34.76 (sd 3.89) adult plants per square meter at Davis Corral and 37.38 (sd 4.63) adult plants per square meter at Jonas Pond. However, a greater percentage of bare ground is found at Davis Corral than at Jonas Pond; Jonas Pond has greater amounts of duff and grass cover (Table 1). Both sites were on similar soil types with no difference seen in soil characteristics.

ambient temperatures were nearly identical at the two sites. However, soil temperatures exhibited various trends that defined the two sites (Figures 2 and 3) with average temperatures warmer in mowed plots at Davis Corral than at Jonas Pond and warmer in control plots at Jonas Pond than at Davis Corral. The mean number of cumulative degree-days was consistently higher for mowed plots than for control plots at each soil depth, on both sampling dates, at each site (Appendix 2).

At Davis Corral, there was no difference in the mean accumulation of degree-days between mowed and control plots at either the 4 mm or 40 mm depth (T = 42,329.00, df = 206, P = 0.86 at 4 mm and T = 43,807.00, df = 206, P = 0.26 at 40 mm). However, at Jonas Pond, mowed plots showed a significantly greater number of cumulative degree-days than control plots, especially at the 4 mm depth (T = 46,429.00, df = 206, P = 0.03).
Mean snow depth was consistently greater in control vs. mowed plots throughout the winter (Jonas Pond $t = 3.87$, df = 39, $P < .01$, and Davis Corral $t = 2.35$, df = 39, $P = 0.02$) (Figures 4 and 5). However, as snow began to melt in spring, it was observed to melt around clumps of vegetation sticking through the surface of the pack faster than areas without exposed vegetation.

Soil moisture was not different in mowed than control plots, at either site, through the study period ($t = -1.31$, df = 39, $P = 0.20$ at Davis Corral and $t = 1.46$, df = 39, $P = 0.16$ at Jonas Pond).

**Larval Development and Establishment**

No *A. zoegana* larvae were collected from either site on June 7, 2001. From the July 13 sample, only one *A. zoegana* larva was collected. Therefore, establishment for this insect was assumed to have failed at the study sites. Therefore, all results presented pertain only to *C. achates*.

At the first larval sampling date (June 7, 2001), 596 ambient degree-days had accumulated at each site. The mean number of cumulative degree-days for soils at the 4 mm depth at Davis Corral were 975 (sd 139.69) for the control plots and 1007 (sd 122.13) for the mowed plots, the difference of 32 degree-days was not significant ($t = -0.38$, df = 8, $P = 0.71$). At the 40 mm depth, at the same site, the control plots averaged 744 (sd 167.19) degree-days and the mowed averaged 779 (sd 54.13) degree-days, with no difference in cumulative degree-days ($t = -0.45$, df = 8, $P = 0.67$). At Jonas Pond, 4 mm depth, for the same sampling period, the mean number of cumulative degree-days was 742 (sd 111.11) and 943 (sd 132.46) for control and mowed plots.
respectively, with a mean difference of 210 degree-days ($t = -2.59$, $df = 8$, $P = 0.03$). At the 40 mm depth at Jonas Pond, the control plots averaged 645 (sd 107.65) degree-days and the mowed plots averaged 697 (sd 48.15) degree-days, the difference of 53 degree-days was not significant ($t = -0.99$, $df = 8$, $P = 0.35$).

At the second larval sampling date, (July 13, 2001), 1128 ambient degree-days had accumulated at each site. At Davis Corral, at the 4 mm depth, the mean number of cumulative degree-days was 1737 (sd 225.46) at the control plots and 1808 (sd 177.39) at the mowed plots, with no difference in cumulative degree-days ($t = -0.56$, $df = 8$, $P = 0.59$). At the 40 mm depth for the same site, the control plots averaged 1436 (sd 284.77) degree-days and the mowed plots averaged 1488 (sd 71.39) cumulative degree-days, again no difference in cumulative degree-days ($t = -0.39$, $df = 8$, $P = 0.71$). At Jonas Pond, at the 4 mm depth, for the July 13 sampling date, the mean number of cumulative degree-days was 1492 (sd 138.33) and 1850 (sd 202.26) for control and mowed respectively, a difference of 358 degree-days ($t = -3.26$, $df = 8$, $P = 0.01$). At the 40 mm depth at Jonas Pond, the control plots averaged 1344 (sd 184.29) degree-days and the mowed averaged 1453 (sd 70.12) degree-days, again no difference in cumulative degree-days ($t = -1.24$, $df = 8$, $P = 0.25$). Although only significantly at the 4 mm depth at Jonas Pond, mowed plots tended to accumulate degree-days at a faster rate than control plots.

*C. achates* develops through four instars. Stinson (et al. 1994) established that the following ranges of head capsule widths can be used to estimate instar. I used these ranges to estimate instar for *C. achates* larvae removed from roots in the subplots.
L₁ mean head capsule width 0.85 mm ± 0.04 (operative range < 1.1)
L₂ mean head capsule width 1.22 mm ± 0.08 (operative range 1.1 – 1.5)
L₃ mean head capsule width 1.86 mm ± 0.12 (operative range 1.6 – 2.2)
L₄ mean head capsule width 2.46 mm ± 0.11 (operative range > 2.3)

Furthermore, Stinson et al. (1994) determined average fecundity for *C. achates* to be about 45 eggs per female with an average survival rate of 66% under field conditions in Europe. Assuming similar conditions at my sites in Montana, with an average of ten females released per plot, it was expected that approximately 83 insects would be collected per plot.

The first larval sample, taken June 7, 2001, yielded a total of 57 *C. achates* larvae in both treatments from both sites (Table 2). At Davis Corral, 20 larvae were collected from the control plots with head capsule widths ranging from 0.7 to 2.1 mm (\( \bar{X} = 1.63, \) sd 0.40). Using the operative ranges described above, the larval instars represented in the samples ranged from first to third instar, with most in the third instar. From the mowed plots at Davis Corral, 14 larvae were collected with head capsule widths ranging from 1.3 to 2.1 mm (\( \bar{X} = 1.76, \) sd 0.24), representing second and third instars, again with most in the third instar. From Jonas Pond, 19 larvae were collected from the control plots with head capsule widths ranging from 0.7 to 2.3 mm (\( \bar{X} = 1.61, \) sd 0.51), with all four instars present but with most individuals in the third instar. Only four larvae were collected from the Jonas Pond mowed plots. These had head capsule widths ranging from 1.1 to 1.2 mm (\( \bar{X} = 1.18, \) sd 0.05), representing only the second instar.
More larvae were collected from the control plots than the mowed plots at Davis Corral; however, no difference was observed in head capsule widths between treatments \( (T = 266.00, P = 0.47, \text{df} = 14) \) (Figure 6). Jonas Pond, during the same sampling period, also produced fewer total larvae from the mowed than from control plots, again with no significant difference in head capsule widths between larvae collected from mowed and control plots \( (T = 25.00, P = 0.07, \text{df} = 4) \) (Figure 7).

Growth rate, as measured by mean dry weight for each instar, was not significantly different between mowed and control plots at Davis Corral for the June sampling period \( (t = -1.87, P = 0.07, \text{df} = 32) \) (Figure 8). However, larvae collected from the Jonas Pond site, for the same sampling period, had significantly greater growth rates in control than mowed plots \( (T = 21.00, P = 0.03, \text{df} = 4) \) (Figure 9).

The second larval sample, collected July 13, 2001, yielded a total of 34 \( C. \text{achates} \) larvae from both sites. At Davis Corral, ten larvae with head capsule widths ranging from 2.3 to 2.5 mm \( (\bar{x} = 2.39, \text{sd} 0.09) \) were collected from control plots, representing only fourth instars. From the mowed plots at Davis Corral, eight larvae with head capsule widths ranging from 2.2 to 2.6 mm \( (\bar{x} = 2.51, \text{sd} 0.11) \) were collected, representing third and fourth instars. However, a single pupa was also collected from a mowed plot at the Davis Corral site. From Jonas Pond, control plots yielded a total of twelve larvae with head capsule widths ranging from 2.2 to 2.8 mm \( (\bar{x} = 2.51, \text{sd} 0.17) \). Three larvae with head capsule widths ranging from 2.2 to 2.5 mm \( (\bar{x} = 2.33, \text{sd} 0.15) \), were collected from the mowed plots. These ranges indicate that third and fourth instars were present in both treatments at this site.
Comparing head capsule widths of *C. achates* from the Davis Corral site for the July 13 sample, the mean head capsule widths for larvae are not significantly greater in the mowed plots than in the control plots (*T* = 92.50, *P* = 0.15, df = 8) (Figure 10). At Jonas Pond, mean head capsule widths were also not significantly different in the mowed plots compared to the control plots (*t* = 1.59, *P* = 0.14, df = 13) (Figure 11).

Growth rate was significantly increased in the mowed plots of Davis Corral by the second larval sample (*t* = -2.76, *P* = 0.01, df = 17) (Figure 12). The only pupa found was also in a mowed plot at Davis Corral. At Jonas Pond, for the same sampling period, mean dry weights were not different between control and mowed plots (*t* = -0.07, *P* = 0.95, df = 13) (Figure 13).

**Establishment and Survival**

No *A. zoegana* adults emerged in the caged subplots between May 31, 2001 and late-July, when the cages were dismantled, nor were any moths found in the surrounding area. Therefore, this insect was not considered further.

*C. achates* began emerging on August 13, 2001. The first observed adults were removed from a control plot at the Davis Corral site. Peak emergence, defined as the date on which the greatest number of adults was collected, occurred on August 31, 2001 for both mowed and control plots at both sites (Figures 14 and 15). By this date, 2047.60 ambient degree-days had accumulated at both sites. For mowed plots at the Davis Corral site, the mean number of degree-days accumulated at the 4 mm and 40 mm soil depths were 2980.60 (sd 283.08) and 2550.91 (sd 154.71), respectively. For the control plots at the same site, average cumulative degree-days for the two soil depths were 2837.59
(sd 349.94) at 4 mm and 2425.81 (sd 394.65) at 40 mm. At the Jonas Pond site, from the first frost-free day of 2001 to the peak emergence date of August 31, 2001, for the mowed plots, an average of 3140.81 (sd 250.05) degree-days and 2516.89 (sd 89.51) degree-days were accumulated at 4 mm and 40 mm depths, respectively. For the control plots, average cumulative degree-days for the two soil depths were 2641.46 (sd 231.22) at 4 mm and 2292.41 (sd 218.21) at 40 mm.

The last emergence of *C. achates* adults occurred on September 23, 2001. Final counts were 53 adult *C. achates* collected from the mowed plots and 57 adults from control plots at the Davis Corral site, and, at the Jonas Pond site, 18 from the mowed plots and 10 from the control plots. The difference in total adult emergence between mowed and control plots at both sites was not statistically significant (T = 0.22, P = 0.83, df = 14 for Davis Corral, and T = -0.66, P = 0.52, df = 14 Jonas Pond). Expected survival of 83 insects per plot was not achieved. Rather, an average of thirteen insects per plot were collected, 28% of expected establishment.

Discussion

Generally, an insect’s phenology is well-adapted to its native environment and climate. However, when releasing an insect in a biological control program, the insect is removed from its native environment and placed into an exotic one. Ideally, the new environment shares similar climatic and other attributes with the native environment. However, in reality, the new environment is likely to differ greatly from the insect’s native range in many respects. While considerable effort is put into finding appropriate release sites, a perfect fit for the insect is not likely to be achieved.
Based on its European distribution, Stinson et al. (1994) suggested that *C. achates* would be best suited to the warmer, drier areas in western North America; however, more specific bioclimatic guidelines for release were not provided. Releases of this insect have been widespread; however, establishment of, and impact by, *C. achates* has not been consistent. For example, Hansen (1996a) described two areas in Montana with similar latitude and elevation, where this insect has been released. At one site, the insect was well established with anecdotal evidence of impact (Story pers. com.), while at the other site, establishment did not occur. Hansen showed that these differences in establishment were primarily due to differences in the accumulation of degree-days between these sites. The site where establishment occurred had accumulated 2320 degree-days (using a threshold of 0° C) 17 to 20 days earlier than the site with no establishment (Hansen 1996a).

As poikilotherms, the developmental rate of insects is temperature dependent. Because insects develop at a rate governed by temperature, the magnitude of soil temperature variation may significantly influence site specific growth and development, and consequently, establishment of *C. achates* larvae. Calculations used for prediction of below-ground insect growth are strongly dependent on the accurate measurement of soil temperature. However, soil temperature is a function of many soil, vegetation, and atmospheric variables which vary greatly in space and time.

For example, vegetation insulates the soil surface from incoming solar radiation during the day and from heat loss during the night. Thus, the surface soils below vegetation have lower maximum daytime temperatures and higher minimum nighttime temperatures than uninsulated interspace areas between plants (Pierson and Wight 1991).
Increased average soil temperatures were observed in mowed plots at Jonas Pond, especially at the 4 mm depth. However, probably due to the dampening effect associated with depth, this variation in temperature was not evident at the 40 mm depth. However, a similar effect of mowing on soil temperature was not seen at Davis Corral. This may have been due to the greater percentage of bare soil at Davis Corral; although bare soil warms faster during the day, it also cools faster in the evening.

Data collected from the larval samples was inconclusive due primarily to poor establishment resulting in a very small sample size. Based on estimates of fecundity in Stinson et al. (1994), it was anticipated that approximately 53 insects should have been collected per plot given the numbers released. The total sample collected was far less: an average of 3.6 insects per plot in mowed plots at Davis Corral, 6.8 in control plots, and only 1.4 in mowed plots at Jonas Pond, 6.2 in control. These samples, although very small, are highly skewed to the control plots. The higher number of insects collected in control plots may have reflected effects of the mowing treatment on the physiology of the knapweed plants. Removal of photosynthetic can cause a compensatory increase in photosynthetic rate in the remaining above ground plant tissues and the mobilization of nitrogen and carbohydrate stores away from roots to replace the removed tissue (Lambers et al. 1998). This movement of nitrogen and carbohydrates away from the root would reduce the food quality, with potential negative impacts on developing larvae.

In future work looking for effects of temperature on development of these insects, a different study design, possibly using shade-cloth as an alternative to mowing, should be used to control for compensatory responses by the plant. Releases of larger numbers
of insects should be considered or the use of areas where insects are already well
established and numerous to ensure adequate sample size.

In this study, most results were insignificant and inconclusive. Only larval dry
weight, an indicator of growth rate within developmental stages, was significantly greater
in the mowed than control plots at Davis Corral at the later larval sample date, this should
be viewed cautiously as it may be indicative of a type II error. Although results from
this study were inconclusive, trends in the data suggest that manipulating vegetation to
alter soil temperature does not greatly affect developmental rates and survival of
*C. achates*. Results were inconclusive because although an effect of mowing on soil
temperature was observed at Jonas Pond, it was not associated with an effect on the
insect, and although no effect of mowing on soil temperature was seen at Davis Corral, a
possible effect on insect development was suggested.

Peak emergence of *C. achates* is predicted to occur when 2320 degree-days have
accumulated from the first frost-free day of spring; for Granite County this should occur
between August 14 and September 2 (Hansen 1996a). Peak emergence of *C. achates* in
this study, occurred within that period, but was not significantly different between sites
or between mowed and control plots within each site. Hansen (1996a) also suggested
that releases would be more successful in areas where the mean number of days between
the accumulation of 2320 degree-days and the date of first frost was a minimum of 21-28
days. This amount of time would allow adequate heating of the below-ground
environment where the larvae are developing. The emergence of the first *C. achates* at
each site occurred in both mowed and control plots on the same day; however, emergence
began at Davis Corral a week prior to Jonas Pond. This may be due to the increased
accumulation of degree-days associated with the increased amount of bare ground at Davis Corral.

The results of this study were inconclusive. Mowing appears to have only minimal effects on soil temperature at knapweed infested sites, and may have no-to-minimal effects on the developmental and growth rates of *C. achates*. Further study is needed to determine if reducing plant density, thereby increasing the accumulation of degree-days, and possibly root size, would increase the developmental and growth rates of *C. achates*, especially on marginal sites. Further study would also be required to see if by increasing the developmental and growth rates, we would see an increase in fecundity in the newly established adult population and an associated increase in impact to the host plant.

Because *A. zoegana* is established in nearby areas, it was assumed that conditions were conducive to the establishment of this insect at the study sites. The lack of *A. zoegana* establishment may have been due to a number of factors occurring at the time of release. A continuing drought may have affected the quality of oviposition sites by prematurely drying vegetation, which would also reduce the quality of available food for the adults. Extremely smoky conditions, accompanied by ash falls, produced by the nearby Ryan Gulch Fire at the time of releases, may also have affected adult flight, mate finding, oviposition, or the survival of eggs and early instar larvae. Furthermore, the numbers of insects I released was small due to low availability of the insects that summer. Higher release numbers may have greatly increased establishment.
Acknowledgements: Thanks to J. Story and L. White of the Western Agriculture Research Station, Corvallis, MT, for providing the insects for initial releases and for expert advice. I also thank Dr. Nancy Sturdevant of the US Forest Service for generously providing support in developing the study plan with Dr. Diana Six and myself. Funding was partially provided by the U.S. Forest Service.

Literature Cited


Figure 1. Plot design used at the Davis Corral and Jonas Pond sites in Montana.
Table 1. Vegetation data collected from Davis Corral and Jonas Pond (MT) sites prior to mowing in 2000 and 2001.

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*a* = number of adult (rosette and bolted) spotted knapweed plants per square meter

*b* = cow manure

53
Figure 2. Average soil temperatures (°C) for 4mm and 40mm at Davis Corral.

Figure 3. Average soil temperatures (°C) for 4mm and 40mm at Jonas Pond.
Figure 4. Comparison of average snow depth of mowed and control plots at Davis Corral.

Figure 5. Comparison of average snow depth of mowed and control plots at Jonas Pond.
Table 2. Number of *C. achates* larvae collected from Davis Corral and Jonas Pond (MT), June 7 and July 13, 2001 with associated range of head capsule widths (HCW) in millimeters and dry weights (DW) in grams.

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Figure 6. Mean head capsule width of *C. achates* larvae collected from Davis Corral, MT, June 7, 2001.
Figure 7. Mean head capsule width of *C. achates* larvae collected from Jonas Pond, MT, June 7, 2001.
Figure 8. Mean dry weight of C. achates larvae collected from Davis Corral, MT, June 7, 2001.
Figure 9. Mean dry weight of *C. achates* larvae collected from Jonas Pond, MT, June 7, 2001.
Figure 10. Mean head capsule width of *C. achates* larvae collected from Davis Corral, MT, July 13, 2001.
Figure 11. Mean head capsule width of *C. achates* larvae collected from Jonas Pond, MT, July 13, 2001.
Figure 12. Mean dry weight of *C. achates* larvae collected from Davis Corral, MT, July 13, 2001.
Figure 13. Mean dry weight of *C. achates* larvae collected from Jonas Pond, MT, July 13, 2001.
Figure 14. Cumulative emergence of *C. achates* adults at Davis Corral, MT, 2001.
Figure 15. Cumulative emergence of *C. achates* adults at Jonas Pond, MT, 2001.
The effect of soil characteristics on establishment of \textit{Agapeta zoegana} and \textit{Cyphocleonus achates}

\textbf{Introduction}

Spotted knapweed, \textit{Centaurea biebersteinii} D. (alt. \textit{C. maculosa} L. and \textit{C. stoebe} L. ssp. \textit{micranthos}), a deeply taprooted, short-lived perennial of the aster family, native to regions of south central Europe and Asia Minor, is one of the most pervasive weeds in western North America (Duncan et al. 2001). Spotted knapweed reproduces by seeds, which are able to germinate throughout spring and fall, and persist for many years in the soil (Davis et al. 1993, Schirman 1981). Young plants overwinter as rosettes and resume growth in early spring (Watson and Renney 1974).

Although often associated with disturbed sites, disturbance is not a requirement for establishment of spotted knapweed (Mooers 1986, Lacey et al. 1990, Tyser and Key 1988). Once established, it is able to compete vigorously with native vegetation due to its abundant seed production, high seed viability, ability of seeds to germinate in the fall with young plants overwintering as rosettes, early spring growth, and absence of native enemies (Watson and Renney 1974, French and Lacey 1983). Large infestations alter community composition, lower biodiversity, alter nutrient cycling, and impact management objectives (Tyser and Key 1988, Duncan et al. 2001).

Knapweed may be effectively controlled with herbicides, environmental concerns of periodic reapplication, accessibility of affected areas, and the economic return from the ecosystems affected, often limit chemical use. Biological weed control, the use of natural enemies, such as insect herbivores, to reduce the population of a weed species, is a
potentially self-perpetuating, environmentally safe alternative tool for management of spotted knapweed. A root feeding moth, *Agapeta zoegana* L., and a root-feeding weevil, *Cyphocleonus achates* Fahr., have been released in the western United States and Canada with varying levels of establishment (Muller-Scharer and Schroeder 1993, Story et al. 1991, Story et al. 1997). Due to their Eurasian distribution, it has been suggested that these insects would do well as biological control agents in warmer-drier temperate areas of northwestern North America (Stinson et al. 1994). Little is known regarding other specific site and climatic factors for establishment of these insects, although it is thought that root size may be important (Story et al. 1991, Story et al. 1997).

Successful plant establishment and growth are governed by edaphic and climatic characteristics including soil type, elevation, annual precipitation, potential evapotranspiration, and length of frost-free season (Chicoine et al. 1985). However, no single variable effectively predicts site vulnerability to knapweed infestation (Mooers 1986). This invasive species is adapted to a wide range conditions and occurs at many sites ranging in elevation from 576 to 3030 meters and precipitation from 20 to 200 centimeters annually (Mooers 1986).

Several factors influence the probability that a newly released biological control agent will establish. Van Dreische (1993) reviewed the effects of number released, frequency of release, genetic diversity of the population released, climatic adaptability, ability to meet nutritional and reproductive requirements at the release site, and synchronicity of the phenologies of the host and agent. Clark et al. (2001) investigated the effect of specific site characteristics, such as knapweed density, patch size and continuity, slope, aspect, elevation, land use, vegetation type, and soil type on the
establishment of *A. zoegana* and *C. achates*. They found that soil type, defined in this case as texture, may be an important factor in the establishment of these insects. Establishment was defined by the ability to collect either larvae and/or adults through field sampling five to ten years after release (Clarke et al. 2001).

Soil texture describes the relative proportions of different sizes of mineral particles in a soil (Brady and Weil 1999). However, texture alone does not fully describe the environment within which plant roots and associated root-feeding insects develop. Other factors such as porosity, organic matter, coarse material, pH, moisture content, and temperature are also important factors affecting the quality of the soil as habitat for plants and other organisms.

Soil climate can be defined as the combination of annual and diurnal cycles of physical properties, such as temperature and moisture, within the soil developed in relation to atmospheric climate, soil physical properties, and vegetation (Shulgin 1978). As with atmospheric climate, soil climate is characterized by diurnal and annual trends; however, soil climate differs from atmospheric climate by exhibiting greater variations in time and space due to the soil's non-uniform composition. Soil climate also differs from atmospheric climate because it is directly affected by the nature of the vegetation cover (Shulgin 1978).

Several factors that influence soil climate and establishment of root-feeding biological control agents were chosen for evaluation. The purpose of this study was to assess the individual effects of specific soil physical properties: particle size distribution, textural class, percent coarse fragments greater than two millimeters, water holding capacity, and pH, on the ability of *A. zoegana* and *C. achates* to establish.
Materials and Methods

Soil Analysis

Throughout the summer of 1998, soil samples were collected at 58 diverse sites throughout Montana, Idaho, and eastern Washington. Sites were chosen where knapweed was the primary vegetation and where A. zoegana and/or C. achates had been released one or more times in the previous decade. Soil samples were removed using a hand trowel to a depth of approximately 20 cm, sieved to a size ≤ 2 mm, and air dried. Samples were brought to the laboratory where they were stored individually in Whirl-Pak bags (M-Tech Diagnostics Ltd. Cheshire, UK). At the same time as sample collection, percent coarse material (> 2.00 mm), color as determined by a Munsell Color Chart (GretagMacbeth LLC. New Windsor, NY), and depth of organic layer were also determined.

Particle size distribution was determined using the following hydrometer method (Gee and Bauder 1986). Oven dried 50 g soil samples were suspended in 100ml of 5% (NaPO₃)₆ solution allowing 60 seconds for clay dispersal. Samples were blended for 15 minutes and then analytically transferred the suspension to a one liter cylinder and bringing to a volume of 1000 ml with distilled water. The contents were mixed thoroughly by inverting the cylinder and a hydrometer, calibrated to scale (RL) using 100 ml (NaPO₃)₆ in 1 l distilled water solution at 30° C, was placed in the cylinder and read at the end of 40 seconds. This step was repeated three times and the average reading calculated. The suspension was allowed to settle for two hours and the hydrometer was again placed into the cylinder for another reading. The following calculations were made to determine particle size distribution:
(A – RL)/50 x 100 = % silt and clay

100% - % silt and clay = % sand

(D – RL)/50 x 100 = % clay

100 - (% sand + % clay) = % silt

where: A = the average of first readings, D = two hour reading, and RL = scale reading from hydrometer calibration.

Textural class was then determined using a texture triangle (Brady and Weil 1999). These texture classifications were further reduced to sand types, clay types, and loams for statistical analysis.

Soil pH was determined using an Orion pH meter and glass electrode. For this test, ten g of soil from each sample was mixed with 20 ml of 0.01 M CaCl₂ for 30 seconds and then allowed to settle for 5 minutes. Measurement of pH of the liquid solution above soil was made using a digital ionalyzer (Allometrics, Inc. Baton Rouge, LA) three times and the average was calculated.

Water holding capacity was determined using a gravimetric method to estimate water filled pore space. Approximately two tablespoons of soil was mixed with enough distilled water to form a wet paste. Water was then extracted from the paste using a pressure plate extractor to -1/3 bar. Gravimetric moisture content was determined (Gardner, 1986) after oven drying the soil samples at 105°C for 24 h using the following calculation: W = {((weight of wet soil) – (weight of dry soil))/(weight of dry soil)}*100.

Porosity was not determined because pores were destroyed by the way that soil samples were collected and stored. I used color as a key to determine organic matter content because organic matter tends to darken each soil type and mask the colors
derived from iron oxides. I used the second number in the three number color
determination collected in the field to determine the intensity of the color. Organic
matter content may effect establishment due to its relation to soil porosity, nutrition, heat
absorption, and water holding capacity.

**Statistical Analysis**

Because I was looking for a trend in insect establishment, expressed as a binary
variable, based on a soil physical property, multiple logistical regression and Pearson
Chi-squared statistic were developed for the data from each site with insect establishment
as the dependent variable (Sigma Stat 2.03, SPSS Inc. 1995).

**Results**

Percentages of sand, silt, and clay were not strongly correlated with the ability of
either insect to establish (Table 1). The same was true when texture was divided into
three classes: sand types, clay types, and loams. All other factors: pH, water holding
capacity, percent coarse fragments, color hue, and depth of the organic matter on the soil
profile were also not significantly associated with establishment of either insect as
determined by use of a Chi square test (Table 2).

**Discussion**

Clark et al. (2001) had determined that soil type, defined as texture class, had a
significant effect on the establishment of *A. zoegana*; however, none of the soil physical
property variables was shown to be an effective predictor of establishment either
individually, or as a class. The discrepancy between these findings may be partially explained by the fact that Clark et al. (2001) estimated textural class using a qualitative hand texture method. Such methods are inherently inaccurate and subject to human bias. Furthermore, these methods do not correct for organic matter influence on texture, and thus the results may be influenced by the organic matter content of these soils. Percent coarse fragments, depth of organic matter, pH, water holding capacity, and color data were also examined and none had an impact on establishment of either *A. zoegana* or *C. achates*.

Although soil texture and percent coarse fragments cannot be used to effectively predict the ability of these insects to establish, they are strongly associated with porosity which may have a stronger influence on establishment, especially macro porosity. Porosity allows both air and water movement through the soil providing oxygen and moisture to root structures and microorganisms, which make nutrients available to roots. Soil pores associated with macro porosity create the paths of least resistance for root expansion and elongation. Larger roots are required by both insects for larval growth. Therefore, it may be an important soil factor and should be studied further to determine if it impacts establishment of these biological control insects.
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Table 1. Texture class, pH, and water holding capacity at -1/3 bar of soil samples collected from *A. zoegana* and *C. achates* release sites.

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<th>% Silt</th>
<th>% Clay</th>
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Table 1 continued. Texture class, pH, and water holding capacity at -1/3 bar of soil samples collected from *A. zoegana* and *C. achates* release sites.

<table>
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<tr>
<th>A. zoegana Established</th>
<th>C. achates Established</th>
<th>Texture Class</th>
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<th>% Silt</th>
<th>% Clay</th>
<th>pH</th>
<th>Water Holding Cap. at -1/3 bar</th>
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Table 2. Chi-Square values for soil variables based on establishment of biological control insects.

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<th>$P$</th>
<th>df</th>
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<tbody>
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<td></td>
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<tr>
<td>% Sand</td>
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</tr>
<tr>
<td>% Silt</td>
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<td>0.368</td>
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</tr>
<tr>
<td>% Clay</td>
<td>51.47</td>
<td>0.377</td>
<td>51</td>
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<tr>
<td>% Coarse Fragments</td>
<td>52.00</td>
<td>0.358</td>
<td>51</td>
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<tr>
<td>Texture Class</td>
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<td>Depth of O Layer</td>
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<td>Color Hue</td>
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<tr>
<td>pH</td>
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<tr>
<td>Water Holding Capacity</td>
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Conclusions and Applications

Biological control of weeds, the attempt to reduce the population of an invasive exotic plant by introducing natural enemies from its area of origin, has become a widely accepted tool for noxious weed management in North America. While this method has a long and sometimes controversial history, it has been successful controlling many weeds throughout the world (McFayden 1998). Regulation of weed populations may be achieved by using biological control agents to selectively increase mortality, decrease vigor, or prevent reproduction of the target plant. A key and necessary first step in biological control is the establishment of the biological control agent.

Successful establishment of newly released insect herbivores for biological control of weeds is influenced by a number of factors including numbers insects released, time of release, and life stage of the insect, as well as many site factors including, but not limited to, elevation, climate, weed patch size and density, and habitat type (Van Dreische et al. 1993, Clark et al. 2001). While it is accepted that habitat should have important effects on the success of biological control insects ability to establish at a site, few studies are able to identify factors correlated with the probability of establishment (Clark et al. 2001, Hight et al. 1995).

By understanding the relative effects of site characteristics on the insect herbivore, it may be possible to increase the rate of establishment through properly timed manipulation of certain factors. Clark et al. (2001) found soil type to have a strong positive correlation with the establishment of A. zoegana. However, for C. achates, the most important factor was the occurrence of large areas with intermediate densities of
knapweed (Clark et al. 2001). Jacobs et al. (2000) found weevil populations to be highest when spotted knapweed cover was between 30 and 70%, and Story et al. (1996) found that wide spacing between knapweed plants in rearing operations increased establishment.

In this study, I wanted to determine if manipulating vegetation cover by mowing would increase soil temperature and consequently positively affect developmental rate and survival of *Cyphocleonus achates* Fahr. and *Agapeta zoegana* L., two root-feeding biological control insects of spotted knapweed. Results from this study were inconclusive. However, trends in the data suggest that mowing to alter soil temperature does not affect developmental rates and survival of *C. achates*. No data is available for *A. zoegana* due to the insect’s failure to establish at the study sites. Results were inconclusive because although an effect of mowing on temperature was observed at one of the two sites, it was not associated with an effect on the insect. A possible effect on the insect was noticed at the other site where no temperature difference was seen between the mowed and control plots.

I also wanted to determine if particular soil characteristics were correlated with the ability of *C. achates* and *A. zoegana* to establish. Although no soil characteristics were found to have significant effects on the ability of either *A. zoegana* or *C. achates* to establish, there may yet be an effect of soil porosity. Porosity and macroporosity have significant affects on soil temperature and moisture (Brady and Wiel 1999) and may also be indicative of the level of disturbance to a site. Further study is needed to determine if soil porosity may be a factor. It has been suggested that *A. zoegana* may leave one root
and travel to another below-ground (Müller and Gassmann 1988). Soil macropores would be a key factor in the ability of an insect to migrate within the soil profile.
Literature Cited


Appendix 1

Comparison of average daily temperatures recorded by ambient temperature sensors and soil temperature sensors at both soil depths (4 mm and 40 mm) from first frost-free day of spring to the last day of adult *C. achates* emergence at Davis Corral and Jonas Pond, Granite County, Montana, 2001.

Figure 1. Average daily ambient temperatures recorded from first frost-free day of spring to last day of adult *C. achates* emergence at Davis Corral.
Figure 2. Average daily ambient temperatures recorded from first frost-free day of spring to last day of adult *C. achates* emergence at Jonas Pond.
Figure 3. Mean daily soil temperatures at 4 mm and 40 mm depths in mowed plots at Davis Corral Site.

Figure 4. Mean daily soil temperatures at 4 mm and 40 mm depths in control plots at Davis Corral Site.
Figure 5. Mean daily soil temperatures at 4 mm and 40 mm depths in mowed plots at Jonas Pond Site.

Figure 6. Mean daily soil temperatures at 4 mm and 40 mm depths in control plots at Jonas Pond Site.
Appendix 2. Comparison of accumulated degree-days for all sensors, both soil and ambient, at Davis Corral and Jonas Pond, Granite County, Montana from first frost-free day of spring to the last day of adult *C. achates* emergence, 2001.

Figure 1. Ambient cumulative degree-days from first frost-free day of spring to last day of adult *C. achates* emergence, 2001, at Davis Corral.
Figure 2. Ambient cumulative degree-days from first frost-free day of spring to last day of adult C. achates emergence, 2001, at Jonas Pond.
Figure 3. Cumulative degree-days at soil depths of 4 mm and 40 mm at Davis Corral site.
Figure 4. Mean cumulative degree-days at soil depths of 4 mm and 40 mm at Jonas Pond site.