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Evaluation of natural enemies released for the biological control of purple loosestrife in Iowa

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Evaluation of natural enemies released for the biological control of purple loosestrife in Iowa

By

Anthony B. Cortilet

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Crop Production and Physiology
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Iowa State University
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This is to certify that the Master’s thesis of

Anthony B. Cortilet

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy
DEDICATION

To Jennifer and Jocie who have helped me to stay focused on my priorities. To Don and Marcia Cortilet for all their love and support throughout the years. To Kathryn and William Kinsel for their inspiration to observe and uphold the beauty of nature. And most importantly, to God for being by my side on a constant basis and helping me to achieve my goals.
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GENERAL INTRODUCTION

Ecosystems throughout the world are being converted rapidly for urban uses and intensified agricultural systems to accommodate the growing human population. Unfortunately, the destruction of native habitats has interrupted the natural succession that organized these ecosystems. Additionally, improvements in transportation over the past 100 years have promoted the introduction of many plant species into new areas where they build up large populations and cause many serious problems for agricultural and natural ecosystems (DeBach 1974, DeBach and Rosen 1991, Van Driesche and Bellows 1996, Westbrooks and Eplee 1996).

Many exotic weed problems exist throughout the world today and there is a need for effective, low cost, and sustainable control methods that will reduce added stresses on native ecosystems (Andres and Goeden 1971). Biological control of weeds, using host-specific phytophagous insects from the exotic weeds native range, has been used successfully throughout the world and shown to be less costly and more environmentally sustaining when compared with herbicide programs (DeBach and Rosen 1991, Van Driesche and Bellows 1996). Additionally, biological control may be the only practical form of weed management in environmentally sensitive areas like wetlands where aquatic organisms are highly susceptible to pesticides. The initial investment for some biological control programs may be high; however, once natural enemies are established, control is long-term and self-sustaining.
Thesis Organization

This thesis is divided into two parts, research examining the plant/insect interactions between purple loosestrife, *Lythrum salicaria* L., and a natural enemy, *Galerucella calmariensis* L., and the mass rearing and release of several natural enemies of purple loosestrife in Iowa. Two papers are included that have been prepared for submission in separate journals. The first paper describes the effects of three larval densities of *G. calmariensis* on the growth of purple loosestrife. The second paper documents the establishment and spread of purple loosestrife natural enemies released in Iowa from 1994 to 1998. The first paper is preceded by a literature review and the second paper is followed by general conclusions, literature citations, acknowledgements, and a brief biographical sketch.

One goal of this research was to demonstrate biological control for an introduced weed species, using purple loosestrife as an example. Many biological control projects in the past have failed to evaluate releases of natural enemies or quantify the relationships between the biological control agent and the host. This type of information is critical to understand plant/herbivore relationships that may lead to increased successes of biological weed control.
LITERATURE REVIEW

Plant Description and Characteristics

Purple loosestrife, *Lythrum salicaria* L. (Myrtilloa: Lythraceae), is a herbaceous European wetland perennial, that was introduced to northeastern North America in the early 1800's (Malecki et al. 1993, Thompson et al. 1987). Purple loosestrife occurs throughout Europe from the 65th parallel south to the Mediterranean basin in North Africa (Blossey 1995b, Thompson et al. 1987). Outside of Europe, its native range includes Japan, northeastern China, southeastern Asia, and India (Thompson et al. 1987).

Mature plants consist of a perennial rootstock producing 20-30 annual stems. The laterally branching rootstock serves as a storage organ providing resources for growth in the early spring and regrowth for cut or damaged stems (Malecki et al. 1993, Edwards et al. 1995, Notzold et al. 1998). Each stem produces several simple, opposite/decussate (sometimes whorled in threes), lanceolate leaves that are sessile (attached directly to the stem). Additionally, stems produce inflorescences (15-30 cm long) with individual flowers that are reddish-purple in color, rotate, and usually contain 12 stamens. Purple loosestrife reproduces sexually and does not produce rhizomes (Thompson et al. 1987, Hight and Drea 1991). Flowers are self-incompatible and insect pollinated by bumble bees (Apidae), honey bees (Apidae), syrphid flies (Syrphidae), Lepidopterans, and small halictid bees (Halictidae) (Hight and Drea 1991, O’Neil and Schmitt 1993, Agren 1996).

The inflorescence makes this plant highly visible during the bloom period, late June-August. Three flower types exist in purple loosestrife populations (long, mid, and short-styled morphs) that differ due to the relative positioning of the stigma and anthers within the

Seeds mature six to eight weeks after flowering and are primarily spread by water (Agren 1996, Edwards et al. 1995, Thompson et al. 1987). Purple loosestrife seeds have high germination rates at the soil surface and can remain dormant in the seed bank for several years (Welling and Becker 1990). Furthermore, seed dormancy has been directly related to moisture content and soil depth. Additionally, Thompson et al. (1987) suggested that seed longevity in the soil is at least three years with a germination percentage of 80% when seed is exposed to optimal growing conditions.

Variation exists for seed production between the three flower morphs. For example, long-styled morphs of Swedish purple loosestrife populations produced 13-20% fewer seeds per fruit and 26-20% fewer seeds per plant than the short-styled morph (Agren and Ericson 1996). However, O’Neil (1992) found that the short-styled morph produced fewer seeds than either the mid or long-styled morphs in North American purple loosestrife populations.

Purple loosestrife typically reaches heights of 1-2 meters and forms dense stands of vegetation in moist-soil habitats associated with floodplains, marshes, stream edges, and drainage ditches (Rawinski and Malecki 1984, Thompson et al. 1987). However, plants can be found growing in a range of water conditions and water availability causes differences in anatomical features of purple loosestrife (Stevens et al. 1997). Young shoots and seedlings begin to sprout in late April or early May. Maximum height is reached late in July. After stems die in mid-September, they continue to stand erect throughout the winter and into the
following spring. Thus, during the growing season, stands of *L. salicaria* are a mixture of new growth and old stems.

In North America, purple loosestrife outcompetes native vegetation resulting in large monotypic stands that displace native plants (Malecki et al. 1993, Thompson et al. 1987). This occurs due to lack of natural enemies in the introduced range, a large storage of overwintered carbon reserves in the rootstock allowing the plant to grow rapidly early in the growing season, production of large amounts of seed per plant, and tolerance to a wide range of ecological conditions (Malecki et al. 1993, Edwards et al. 1995, Thompson et al. 1987). Additionally, increased human landscape disturbance along streams, wetlands, and roads has increased the spread of purple loosestrife throughout North America (Malecki et al. 1993, Edwards et al. 1995, Thompson et al. 1987).

Several species of phytophagous insects have been collected in surveys of the fauna associated with purple loosestrife plants in North America (Batra et al. 1986, Diehl et al. 1997, Hight 1990). In most cases, these insects are incidental feeders that have little or no impact on stand density. However, Diehl et al. (1997) found several native insect species in southern Canada that regularly feed on purple loosestrife and may limit plant growth slightly. In contrast, European populations are suppressed by phytophagous insects, plant pathogens and competition from plant species occupying the same habitats (Blossey et al. 1994a, Blossey et al. 1994b, Blossey 1995b, Edwards et al. 1995, Nyvall and Hu 1997, Nyvall 1995, Thompson et al. 1987). Many of these insects are fairly specific to purple loosestrife and limit growth at high densities (Blossey et al. 1994a, Blossey et al. 1994b, Blossey 1995b, Hight et al. 1995).
Purple loosestrife is an early successional plant in its native range and may be the first species to colonize an area following a disturbance that creates a clearing in existing vegetation. It may persist as the dominant vegetation type for up to three years before eventually being replaced by later successional species, such as *Juncus effusus* L. (Edwards et al. 1995). Purple loosestrife is scattered throughout Europe and generally occupies a specific niche in mixed species stands (Thompson et al. 1987). On occasion, purple loosestrife may form dense monotypic stands in areas of disturbance, but these European habitats eventually become mixed species stands within a few years. Thompson et al. (1987) observed a monospecific stand of purple loosestrife growing along the Weir Wood Reservoir in southeastern England after the vegetation had been cut in 1981-82. By August 1984, the purple loosestrife was replaced by willows, *Salix* spp., that previously inhabited the site.

Purple loosestrife plants invading North American sites also require disturbance for establishment, but once established these plants usually become the dominant vegetation for 20 years or more (Edwards et al. 1995, Thompson et al. 1987). Additionally, Edwards et al. (1995) states that North American varieties of purple loosestrife differ from their European ancestors in that they are multi-stemmed and grow considerably taller. Thus, purple loosestrife grows more vigorously in North America than in Europe.

**History of Purple Loosestrife in North America**

Purple loosestrife seed was likely introduced into North America as a contaminate in European ship ballast, livestock bedding, wool, and through importation of ornamentals (Malecki et al. 1993, Thompson et al. 1987). Problems first arose in the 1930's when purple loosestrife established in the St. Lawrence River basin and began spreading aggressively...
throughout the United States (Thompson et al. 1987). Purple loosestrife spread rapidly westward and has been responsible for the degradation of many wetland habitats in the northeastern and northcentral United States (Stuckey 1980, Malecki et al. 1993, Thompson et al. 1987). Since 1940, it has been spreading at an estimated mean rate of 645 km\(^2\) annually (Thompson 1991).

Historically, purple loosestrife was used in ornamental gardens and as a forage plant for bees (Malecki et al. 1993, Pellet 1977). Early Europeans considered the plants to have medicinal value for treating such illnesses as diarrhea and dysentery (Malecki et al. 1993, Stuckey 1980). Wildlife managers became concerned in the 1950’s that several native plant species, cattail (\textit{Typha} spp.), rushes (\textit{Juncus} spp.), and sedges (\textit{Carex} spp.), were being replaced by purple loosestrife (Rawinski and Malecki 1984). Furthermore, purple loosestrife has been a factor contributing to the extirpation of Long’s bulrush (\textit{Scirpus longii}) and has diminished the habitat for the endangered bog turtle (\textit{Clemmys muhlenbergi}) in the northeastern United States (Malecki and Rawinski 1985, Malecki et al. 1993, Thompson et al. 1987). Purple loosestrife continues to diminish the quality of existing wetlands for many species of wildlife that depend on these unique habitats for survival and threatens habitats in the four major waterfowl flyways in the North America (Thompson 1991).

Methods to control purple loosestrife have centered on physical removal, water level manipulation, mowing, and chemical control (Malecki and Rawinski 1985, Malecki et al. 1993, Thompson et al. 1987). These methods have had some degree of success on small isolated stands of purple loosestrife; however, they are costly, environmentally disruptive, and require repeated action (Malecki and Rawinski 1985, Malecki et al 1993). Management
of purple loosestrife stands in North America using chemical and mechanical methods and losses acquired through the devaluation of natural habitats through loss of biological diversity, have been estimated to cost several million dollars annually (Hight and Drea 1991, Thompson 1991).

**Biological Control**

Plants and animals become pests when they invade areas separated from antagonists, i.e., natural enemies, that limit their population growth. However, introducing natural enemies into a pest's new range may reduce its population to non-pest status (Hight and Drea 1991). Van Driesche and Bellows (1996) define biological control as the use of a parasitoid, predator, pathogen, antagonist, or competitor population to suppress a pest population. Biological control is a population level process in which natural enemies prevent pest populations from increasing and expanding their distribution (Debach and Rosen 1991, Van Driesche and Bellows 1996). In turn, the abundance of the target organism (acting as a host) often influences the abundance of the natural enemies.

Classical biological control methods seek to establish a low stable host equilibrium using natural enemies from the pest's area of origin. These natural enemies typically are host-specific, synchronous with the pest, increase in density when the pest species does, persist when the pest is at a low equilibrium density, and have a high searching ability (Murdoch et al. 1985). Success in biological control is dependent on the establishment of natural enemies and the degree of homeostasis in the pest/host relationship (Dennill and Hokkanen 1990). Biological control methods have been successfully used to control both
animal and plant pest populations throughout the world (Van Driesche and Bellows 1996, Debach and Rosen 1991, Strand and Obrycki 1996).

**Biological Control of Weeds**

Classical biological weed control introduces phytophagous natural enemies from the host plants (weed) origin to reduce the population of the weed and allow more desirable vegetation to flourish (McEvoy et al. 1991). Releases of imported insects for biological weed control represents some of the most impressive, large-scale ecological field experiments ever conducted (Crawley 1989). The first successful program was the control of prickly pear cacti, *Opuntia* spp., in Australia and other countries by a moth, *Cactoblastis cactorum* (Buckingham 1984, Crawley 1989, Huffaker 1958, Huffaker 1959). Since then, biological control methods have been implemented for many species of noxious weeds including: musk thistle (*Cardus nutans*), St. Johnswort (*Hypericum perforatum* L.), leafy spurge (*Euphorbia esula*), Canada thistle (*Cirsium arvense*), and the aquatic weed water-hyacinth (*Eichhormia crassipes* (Mart)) (Burdon and Marshall 1981, Center and Durden 1981, Huffaker 1959).

Exotic natural enemies considered for biological weed control should be able to substantially damage the host, cause damage during a vulnerable phase of the host’s life cycle, and interrupt the reproductive ability of the plant population (Hight and Drea 1991). Buckingham (1984) discussed two phases that biological weed control projects in the United States must undertake prior to the release of exotic natural enemies. First, a foreign phase that involves exploration for natural enemies in the plant’s native range. A second or domestic phase, involves state agencies and the United States Department of Agriculture
Animal and Plant Health Inspection Service (USDA/APHIS) that regulate the importation, quarantine, and release of natural enemies into the United States. The domestic phase evaluates the risks and benefits associated with natural enemies collected during the foreign phase. Additionally, these procedures establish the host-specificity of the natural enemy prior to release on the target plant in the United States.

Host specificity testing is performed when screening potential biological control agents to minimize risks to desirable fauna and flora within the release area (Blossey 1995a, Huffaker 1959, McFadyen 1998, Strand and Obrycki 1996). Host specificity screening is the most important step that a potential biological control agent has to pass prior to introduction (Blossey 1995a). The greater the host specificity, the lower probability that desirable fauna and flora will be affected by the introduction of a natural enemy. However, there is always a low probability that an introduced natural enemy will adapt to another host or that the natural enemy may indirectly affect nontarget organisms (Strand and Obrycki 1996, McFadyen 1998). Therefore, in addition to host specificity testing, both the benefits and risks of natural enemy introductions must be evaluated prior to releases.

Insect natural enemies have been used for many years to biologically suppress weed populations. A benefit of using host specific natural enemies for weed management is that they are self-perpetuating and, in many cases, they can spread throughout a weed infestation without further inputs following the initial release (Wapshere 1989). Historically, 60% of natural enemy releases result in establishment. More research involving the biology, demographics, and ecological interactions of the host plant and its antagonist is needed to improve establishment rates of natural enemies that could potentially limit the population.
growth of a weed species (Crawley 1989). However, establishment of a natural enemy does not mean success for biological weed control, i.e., some natural enemies have little or no effects on the growth and spread of the weed.

**Biological Control of Purple Loosestrife in the United States**

In 1987, a biological control program to manage purple loosestrife in the United States was initiated by the U.S. Fish and Wildlife Service, in association with the USDA Agricultural Research Service (ARS). Interestingly, an attempt was made to start a management program for purple loosestrife in 1960. However, the proposed project was not initiated due to a lack of information justifying the cost of such a program and limited regional interest in the plant (Thompson 1991). Because purple loosestrife is an introduced perennial species, attacked by a series of biotic agents in its area of origin, restricted to a specific and stable habitat, found typically in continuous distributions, and isolated taxonomically from economically valuable plants, it is considered an excellent candidate for biological control (Hight and Drea 1991).

From 1986 to 1991, insect species that attack purple loosestrife were collected throughout Europe and evaluated for host-specificity (Blossey 1995a, Blossey 1995b, Malecki et al. 1993, Hight et al. 1995). Forty-four plant species belonging to 16 families were used in the specificity tests (Blossey 1995a, Blossey 1995b, Blossey et al. 1994a, Blossey et al. 1994b, Hight and Drea 1991). Four categories of test plants were used: species in the family Lythraceae, species in closely related families of Lythraceae, wetland species commonly associated with wildlife, and important agricultural crops (Hight and Drea 1991).
One hundred twenty insect species were associated with purple loosestrife in Europe, but only nine were considered to be host-specific (Blossey 1995b, Hight and Drea 1991, Malecki et al. 1993). These nine host-specific insects were ranked to estimate their effectiveness as biological control agents of purple loosestrife and five species were approved for biological control releases in North America (Blossey et al. 1994a, Blossey et al. 1994b, Blossey 1995a, Blossey 1995b, Malecki et al. 1993, Hight et al. 1995).

The objective of the North American purple loosestrife biological control program was to mass-rear and release these five natural enemies throughout North America. Currently, several states (e.g., New York, Minnesota, Iowa, Illinois) have their own biological control programs for purple loosestrife and individual rearing centers for the mass production and distribution of natural enemies.

**Natural Enemies of Purple Loosestrife**

The natural enemies approved for release on purple loosestrife in North America include: two species of leaf-feeding beetles, one flower-feeding weevil, one seed-feeding weevil, and a root-mining weevil (Table 1). The leaf-feeding beetles, *Galerucella calmariensis* L. and *G. pusilla* Duftshmidt (Coleoptera: Chrysomelidae), feed on the leaves and growing tips of purple loosestrife plants. Both species are similar in appearance and life history traits, however, they are reproductively isolated (Manguin et al. 1993). High densities of *G. calmariensis* and *G. pusilla* cause defoliation and stunting of purple loosestrife plants (Blossey and Shat 1997, Blossey et al. 1994a, Hight et al. 1995, Malecki et al. 1993).
Table 1. European natural enemies of purple loosestrife.

<table>
<thead>
<tr>
<th>Natural Enemy</th>
<th>Order</th>
<th>Family</th>
<th>Feeding Habits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galerucella calmariensis</td>
<td>Coleoptera</td>
<td>Chrysomelida</td>
<td>Leaves, Flowers</td>
</tr>
<tr>
<td>Galerucella pusilla</td>
<td>Coleoptera</td>
<td>Chrysomelida</td>
<td>Leaves, Flowers</td>
</tr>
<tr>
<td>Hylobius transversovittatus</td>
<td>Coleoptera</td>
<td>Curculionida</td>
<td>Leaves, Stems, Roots</td>
</tr>
<tr>
<td>Nanophyes marmoratus</td>
<td>Coleoptera</td>
<td>Curculionida</td>
<td>Flowers</td>
</tr>
<tr>
<td>Nanophyes brevis</td>
<td>Coleoptera</td>
<td>Curculionida</td>
<td>Seeds</td>
</tr>
<tr>
<td>Myzus lythri</td>
<td>Homoptera</td>
<td>Aphididae</td>
<td>Leaves, Flowers</td>
</tr>
</tbody>
</table>

Hylobius transversovittatus Goeze (Coleoptera: Curculionidae) is a root-mining weevil that causes mortality to purple loosestrife plants by feeding on the rootstock (Blossey et al. 1994 b, Notzold et al. 1998, Malecki et al. 1993). Adults oviposit into purple loosestrife stems in the late summer. Larvae hatch, mine the pith of the stem, and overwinter in the rootstock emerging as adults 1-2 years later (Blossey et al. 1994a, Malecki et al. 1993).

A flower feeding weevil, Nanophyes marmoratus Goeze (Coleoptera: Curculionidae), and a seed feeding weevil, N. brevis Boheman (Coleoptera: Curculionidae), are small weevils that reduce the seed production of purple loosestrife plants (Blossey 1995b, Malecki et al. 1993). Nanophyes marmoratus is the most common insect associated with purple loosestrife in Europe (Blossey 1995b).

In addition to these five natural enemies, an aphid species of European origin, Myzus lythri (Schrank) (Homoptera: Aphididae), that was accidentally introduced to North America in the early 1900’s, has been observed to reduce the growth of purple loosestrife plants (Voegtlin 1995). Myzus lythri feeds on the secondary hosts, Lythrum spp. (purple loosestrife) and Epilobium spp., from early spring until autumn and overwinters on its primary host,
mahaleb cherry, *Prunus mahaleb*. Mahaleb cherry is a European cultivar that was introduced to North America for horticultural purposes. At high densities, *M. lythri* was observed to cause severe leaf damage, decreased root growth, and delayed blooming of purple loosestrife plants (Voegtlin 1995). Crawley (1989) suggested that species such as aphids can have important effects on community dynamics by reducing the competitive ability of target plants.

Biological control of purple loosestrife in the United States has focused on the mass rearing and release of the two leaf-feeding beetles and three weevil species. In 1992, *G. calmariensis*, *G. pusilla*, and *H. transversovittatus* were approved for release into several states (Hight et al. 1995). These species have reproduced successfully in the field and have passed the most critical phase for establishment in North America (Hight et al. 1995). The broad geographic distribution of *H. transversovittatus* and both *Galerucella* species and their dramatic effect on purple loosestrife were the primary reasons for selecting these species for the initial phase of natural enemy introductions into the United States (Malecki et al. 1993). Starting in 1996, *N. marmoratus* was released into several states. Currently, *N. brevis* is being detained from releases into North America due to complications with its quarantine (Blossey, personal communication, 1997). Illinois and Iowa have rearing and release programs for *M. lythri*, in addition to *Galerucella*, *Hylobius*, and *Nanophyes* species.

Multiple phytophagous species could have a greater effect on the target plant than a single species alone (Malecki et al. 1993). For example, both *Galerucella* species and *M. lythri* feed on leaves and growing tips of individual stems, thus reducing leaf area and preventing flowering. The flower and seed feeding weevils, *Nanophyes* spp., feed on the
flowers and seeds, thus preventing flowering and reducing seed production. The root-mining weevil, *H. transversovittatus*, feeds extensively on the stems and roots of purple loosestrife plants damaging the main storage organ, eventually killing the plant. All six natural enemies of purple loosestrife stress the plant and compete for available food resources, possibly intensifying the level of stress on the plant in individual ecosystems (Blossey 1995b, Hight et al. 1995, Malecki et al. 1993). Malecki et al. (1993) suggest that control will be better in mixed plant communities where interspecific plant competition for space and nutrients is greater. Furthermore, Blossey (1995b) and Malecki et al. (1993) predict that through multiple species releases, the present population of purple loosestrife in North America will be reduced significantly. However, noticeable reductions at any given site may take several years depending on the establishment and population growth of the released natural enemies (Hight et al. 1995, Malecki et al. 1993).

**Biology of *Galerucella* Species**

In northern and central Europe, *Galerucella calmariensis* and *G. pusilla* were identified as potential biological control agents of purple loosestrife (Blossey et al. 1994a, Hight et al. 1995, Hight and Drea 1991, Malecki et al. 1993, Manguin et al. 1993). Chromosome analysis, electrophoresis data, and behavioral studies demonstrated their reproductive isolation (Blossey et al. 1994a, Manguin et al. 1993).

Adult beetles emerge from overwintering in the early spring when purple loosestrife shoots are approximately 20 cm tall. Oviposition begins 1 to 2 weeks following overwintering emergence, peaking in mid-June, and drops off steadily in early July (Blossey et al. 1994a). Eggs are oval and laid in clusters (masses) on the leaves and stems of purple
loosestrife plants. Larvae begin to hatch in late June and feed on developing leaf and flower buds (terminal and apical meristems) until the third instar when they feed on leaves and other plant parts (Blossey et al. 1994a). Larvae drop off the plants and pupate in the soil approximately three weeks following hatch. These pupae emerge as adults in late July and have a short oviposition period prior to overwintering in August (Blossey et al. 1994a).

In Iowa, we observed that established Galerucella adults (i.e. those that overwintered from the previous year) were active from early May to late June. These beetles produced a new generation of adults that were abundant in early July, but appeared to enter dormancy in late July or early August. Additionally, McAvoy et al. (1997) observed that adult Galerucella activity in Virginia decreased near full bloom of purple loosestrife plants, usually at the end of July. This suggests that young purple loosestrife plants are more suitable for Galerucella species than older plants (R. Wiedenmann, Illinois Natural History Survey, personal communication).

Both Galerucella species have been observed to retard shoot growth, defoliate leaves, and prevent flowering (Blossey et al. 1994a, Malecki et al. 1993, McAvoy et al. 1997, Hight et al. 1995). Adult Galerucella feeding causes a shot-hole pattern on leaves; whereas, larval feeding is characterized by long window-paned trails along the leaf surface. Adults oviposit near the leaf axils and growing tips of individual stems, leaves, and flower buds (Blossey et al. 1994a, Lindgren 1997). A single egg mass consists of 8 to 20 individual eggs covered with frass (Blossey et al. 1994a, Hight et al. 1995).

Galerucella calmariensis and G. pusilla have high fecundities and a wide tolerance of habitats making them desirable agents for the biological control of purple loosestrife in North
America (Blossey et al. 1994a, Blossey 1995b, Malecki et al. 1993). A single female can oviposit from 400-600 eggs and larvae complete their development in 3-4 weeks (Blossey 1995b). Therefore, rearing programs for these two species can quickly produce thousands of individuals for field releases.

Although these beetles have high levels of fecundity and tolerate many environments, they are exposed to several species of predatory arthropods in North America. Arthropod predators have been documented to impact the survival of a native Galerucella species, G. nymphaeae, in New York (Nechols et al. 1996). In Iowa, several predators have been observed attacking both Galerucella species and these predators are thought to inflict significant mortality upon released beetle populations (Table 2). Additionally, an insect pathogen, Beauveria bassiana, has also been observed to be a major source of mortality of beetles in rearing and field cages. However, the effect of arthropod predators or insect pathogens on Galerucella species has not been quantified in Iowa.

Table 2. Arthropod predators of Galerucella spp. in Iowa and life stages attacked.*

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Order</th>
<th>Family</th>
<th>Life Stage Attacked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assassin Bug</td>
<td>Hemiptera</td>
<td>Reduviidae</td>
<td>Adults and larvae</td>
</tr>
<tr>
<td>Damsel Bug</td>
<td>Hemiptera</td>
<td>Nabidae</td>
<td>1st and 2nd Instar Larvae</td>
</tr>
<tr>
<td>Brown and Green Lacewings</td>
<td>Neuroptera</td>
<td>Chrysopidae</td>
<td>1st and 2nd Instar Larvae</td>
</tr>
<tr>
<td>Ladybird Beetles</td>
<td>Coleoptera</td>
<td>Coccinellidae</td>
<td>Larvae and Eggs</td>
</tr>
<tr>
<td>Spiders</td>
<td>Araneae</td>
<td>Several</td>
<td>Adults</td>
</tr>
</tbody>
</table>

* Data collected by Cortilet and Obrycki at three northwest Iowa release sites in 1995 and 1996.
Objectives and Rationale for Research

1) To quantify the growth of purple loosestrife in response to larval herbivory by *Galerucella calmariensis*.

Few experiments have examined the interaction between *G. calmariensis* and purple loosestrife. Lack of quantitative measurements prevent a complete understanding of plant/insect relationships that may be crucial for biological control. Understanding the effects of *G. calmariensis* feeding on purple loosestrife growth will allow researchers to develop specific release strategies for this biological control agent.

The purpose of this study was to examine the effects of feeding by three densities of *G. calmariensis* larvae on the growth of purple loosestrife. The null hypothesis was that *G. calmariensis* densities would not effect plant growth. In contrast, the alternative hypothesis stated that increasing larval densities would effect plant growth when compared with plants that were not exposed to larval *G. calmariensis*. To test this hypothesis a field-cage study was performed in 1996 and 1997 at the Department of Entomology Outdoor Field Laboratory, Iowa State University (ISU), Ames, IA.

2) To document the establishment and spread of natural enemies released for the biological control of purple loosestrife in Iowa.

Purple loosestrife is a large threat to the biological diversity and preservation of Iowa prairie-pothole wetlands. Cultural and chemical control programs have not suppressed the growth and spread of this exotic weed. Iowa Department of Natural Resources (IDNR) fisheries and wildlife biologists and several county conservation groups have become very concerned with the spread of purple loosestrife. Therefore, through the support of the IDNR
and U. S. Fish and Wildlife Service, a biological control program for purple loosestrife was started in 1994.

The objective of this biological control program was to establish and document the spread of natural enemy species at purple loosestrife infestations throughout Iowa. An intensive evaluation and recovery program was developed for these natural enemies and has been a key component in determining their establishment and success as biological control agents in Iowa. Information from field evaluations will be used as baseline data for future studies to determine the success of these natural enemy releases and will be critical in the overall assessment of this biological control program.
EFFECTS OF THREE LARVAL DENSITIES OF
GALERUCELLA CALMARIENSIS L. ON THE GROWTH OF
PURPLE LOOSESTRIFE (LYTHRUM SALICARIA L.)

A paper submitted to Weed Science

Anthony B. Cortile, Micheal D. K. Owen, and John J. Obrycki

In 1996 and 1997, experiments were conducted at Iowa State University to study the effects of herbivory by the leaf-feeding beetle Galerucella calmariensis on the growth of purple loosestrife (Lythrum salicaria L.), an invasive wetland weed species. Purple loosestrife rootstocks were obtained from a central Iowa wetland, potted, and covered with cylindrical mesh cages. Individual plants were exposed to four densities (0, 5, 25, and 50) of first instar G. calmariensis larvae. Adults emerging in larval treatments were collected, and survival of larvae was determined. Percentage defoliation and percentage terminal bud damage increased significantly with increasing larval densities in both years. The highest

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density of larvae caused approximately 25% defoliation and damaged more than 20 terminal buds per stem in both years. Percentage bloom and net stem growth of purple loosestrife plants were significantly reduced by larval feeding in 1996 and 1997; however, no differences were found among treatments for net rootstock growth in either year. Survival of larvae was not found to be different for the three larval densities in either year. This study showed that leaf-feeding by *G. calmariensis* larvae negatively affected the above ground growth of purple loosestrife plants.

**Nomenclature:** Purple loosestrife, *Lythrum salicaria* L. LYTSA, purple loosestrife leaf-feeding beetle, *Galerucella calmariensis* L. GALCM.

**Key Words:** Biological control, natural enemy, host specific, herbivory, percentage defoliation, first instar larvae, terminal bud damage, percentage bloom, net stem growth, net rootstock growth, adult emergence.

**Introduction**

Purple loosestrife is an exotic European wetland plant species that was introduced to North America in the early 1800's (Edwards et al. 1995; Malecki et al. 1993; Stuckey 1980; Thompson et al. 1987). Purple loosestrife consists of a perennial rootstock that produces 10-15 annual stems often reaching heights of 2 meters (Malecki et al. 1993; Edwards et al. 1995; Thompson et al. 1987). Once established, purple loosestrife forms dense stands that include dead stalks from the previous year and new growth. In North America, this plant...
outcompetes native vegetation and has little or no benefit for indigenous wildlife species (Malecki et al. 1993; Thompson et al. 1987).

Purple loosestrife reproduces sexually and does not produce rhizomes (Thompson et al. 1987; Hight and Drea 1991). Individual stems produce showy purple inflorescences with individual flowers that are self-incompatible and primarily insect pollinated (Agren 1996; Edwards et al. 1995; Hight and Drea 1991; O’Neil and Schmitt 1993). Mature plants can produce up to 2.5 million seeds annually and have high germination success in moist wetland soils. (Malecki et al. 1993; Thompson et al. 1987; Welling and Becker 1990).

In European ecosystems, purple loosestrife populations are suppressed by plant pathogens, plant competition, and insect herbivores (Blossey 1995b; Batra et. al. 1986; Hight and Drea 1991; Hight et al. 1995; Nyvall 1995; Nyvall and Hu 1997). Unfortunately, in North America these competitive influences are lacking, thus purple loosestrife grows and spreads vigorously, eventually dominating native flora and reducing biotic diversity (Malecki et al. 1993; Hight and Drea 1991; Hight et al. 1995; Thompson 1991; Thompson et al. 1987).

Since its introduction into the United States, purple loosestrife has spread rapidly and displaced many wetland plant and animal species in the northern temperate states (Malecki et al. 1993; Stuckey 1980; Thompson et al. 1987; Thompson 1991). It has been estimated that from 1940 to the present, purple loosestrife has spread at a rate of approximately 645 km$^2$ annually (Thompson et al. 1987). Additionally, purple loosestrife continues to be a threat to nesting habitat in all four of the major North American waterfowl flyways and causes significant economic losses annually through the devaluation of freshwater real estate,
destruction of natural habitats for recreational enjoyment, and negative effects on agriculture (Hight and Drea 1991; Thompson 1991; Thompson et al. 1987).

The threat of purple loosestrife spread and habitat destruction in the United States has concerned wildlife and land managers for the past 30 years. Unfortunately, methods to control purple loosestrife have been largely unsuccessful and centered around cultural methods (physical removal), water level manipulation, fire, mowing, or herbicide applications (Malecki and Rawinski 1985; Malecki et al. 1993; Thompson et al. 1987). Although these methods have short-term success on small isolated stands, they are costly, require additional long-term maintenance, and herbicides are non-selective and not practical on large infestations (Malecki and Rawinski 1985; Malecki et al. 1993).

Biological control, using host-specific natural enemies that suppress the plant’s populations in Europe, has become the primary focus of purple loosestrife management in North America. Five European phytophagous insect species are host specific to purple loosestrife and were approved for biological control in North America (Blossey 1995a; Blossey 1995b; Blossey et al. 1994a; Blossey et al. 1994b; Malecki et al. 1993; Hight et al. 1995). One of these insect species, a leaf-feeding beetle, Galerucella calmariensis L (Coleoptera: Chrysomelidae), has been mass reared and released in biological control programs across the United States.

Galerucella calmariensis has been successfully established in the United States since releases began in 1992 (Blossey and Schat 1997; Hight et al. 1995; McAvoy et al. 1997). Adult beetles emerge in the spring from overwintering, females oviposit eggs on leaves, stems, and flower buds of young purple loosestrife plants, larvae hatch approximately two
weeks later, and complete three instars and a pupal stage prior to emerging as adults (Blossey et al. 1994a; Blossey 1995b; Hight et al. 1995; Lindgren 1997; McAvoy et al. 1997). Adult feeding damages the leaves and tender meristems of the stem tips (terminal buds) reducing flower and seed production (Blossey et al. 1994a; Blossey 1995b; Blossey and Schat 1997; McAvoy et al. 1997).

Few studies have quantified the growth of purple loosestrife in response to the damage inflicted by natural enemy plant feeding. The first objective of this study was to determine the effects of leaf and stem-tip feeding by larval *G. calmariensis* on purple loosestrife growth. A second objective determined the effect of intraspecific larval interactions on *G. calmariensis* survival and body size.

**Materials and Methods**

*Galerucella calmariensis* Rearing

Rootstocks of purple loosestrife were obtained from the field and planted into fifteen 38 l plastic pots. Sunshine Sterile Soilless Mix® #1 was used for planting in 1996 and Fafard Sterile Growing Mix® #2 in 1997. When the potted plants reached a height of 0.5 m, 1m tall cylindrical sleeve cages, made of No-See-Um® mesh, were placed around tomato cages covering the plants. These rearing cages were maintained in a greenhouse at 25 C (L:D 16:8). Fifty adult *G. calmariensis* were released inside the cages for mating and oviposition. After 5-7 days, these adults were collected from the rearing cages.

Mesh cages were removed from the pots and plants checked three times daily for presence of hatched first instar larvae. Once larvae were observed, they were collected with a fine hair paintbrush and placed into plastic 7 mm dram vials covered with mesh. In 1996,
these vials were held at 5 C for 24 hr prior to releasing the larvae into the experimental cages. This was done to insure that all larvae in a block were released at the same. Larvae that died during storage were replaced with newly collected first instars. In 1997, first instars were released into the experimental cages within two hours of collection from rearing plants.

**Experimental Design**

This study was conducted outside on the Iowa State University campus from late April to August in 1996 and 1997. Seventy-five purple loosestrife rootstocks were collected from Maffitt Reservoir, Des Moines, Iowa, in March 1996 and 1997. Prior to planting, each rootstock was washed and weighed to determine the initial rootstock weight (IRW). Rootstocks were planted into 38 l plastic pots with Sunshine Mix® #1 in 1996 and Fafard Sterile Growing Mix® #2 in 1997. Rootstocks were divided in three blocks (25 plants per block) and blocks were planted approximately ten days apart (Figure 1A). This was done to insure that enough first instar larvae could be collected to infest all the plants within a block at the same time.

In each block, five plastic tubs (0.47 m³) were filled with 0.12 m³ of tap water. Five potted rootstocks were then randomly assigned to a treatment within each tub. Water was added on a daily basis to simulate natural wetland soil conditions within the pots. Each purple loosestrife plant was fertilized weekly with 0.003 g of fertilizer mixed with 1 l of tap water.

The five treatments were: 0 larvae per plant with no cage (uncaged control), 0 larvae per plant with a mesh cage covering (caged control), 5 larvae per plant, 25 larvae per plant,
Figure 1. Experimental setup. 1A) Block (1-3) Layout consisting of 15 tubs (1-15). 1B) Individual tub containing five pots (treatments), A-E.
and 50 larvae per plant. The three larval treatments and caged control were enclosed by covering the cage frames and the top 1/3 of the pots with 0.15 m³ cylindrical mesh cages that were tied to the pots with elastic. The caged control was used to test for any effects that the mesh covering might have on plant growth.

Larval treatment densities were determined from preliminary sampling data of larval Galerucella densities at three northwest Iowa wetlands in 1994 and 1995 (Cortilet and Obrycki, unpublished data). Based on this field data, 5 larvae/cage was a high population density in natural situations (non-caged situations), but caused only minimal damage; whereas, 25 and 50 larvae/cage should cause higher levels of damage to purple loosestrife plants.

First instar G. calmariensis larvae were introduced to the treatments when purple loosestrife plants were approximately 30 cm tall. Prior to larval releases, each pot was standardized to two purple loosestrife stems. This was done to insure that there would be sufficient room for plant growth within the cylindrical mesh cages. Throughout this experiment plants were monitored daily and additional stems emerging from the rootstock were removed immediately to insure that larvae would only feed on the original two stems.

Larvae that completed development to the adult stage, were collected with aspirators immediately following emergence, counted, and frozen. Soil from each pot containing larvae was then sifted using a 1mm soil sieve to collect adults that had not yet emerged and pupa. Plants were then separated by above and below ground biomass, both stems in each pot were labeled, and rootstocks were washed.
Parameter Evaluation

Percentage Defoliation

Leaves were stripped from both purple loosestrife stems in all treatments, counted, and sorted as damaged and undamaged. A leaf was considered damaged if there was visible larval feeding. A leaf area meter was used to determine the leaf area (LA) of the two stems in each treatment.

An arbitrarily selected subsample (20%) of the damaged leaves was pressed flat onto a white sheet of paper, covered with a transparency, and photocopied. The photocopies were then placed under a Delta-T-Area Meter to determine the leaf area of the subsampled damaged leaves (LAD). The areas of larval feeding damage on the photocopies, which appeared white and were not measured by the leaf area meter, were then filled in with a black ink pen. These photocopies were measured under the Delta-T Area Meter a second time to determine the leaf area without the larval feeding damage present (LAW). The first leaf area measurement was subtracted from the second measurement to estimate the total leaf area removed by larval feeding per subsample. This value was then multiplied by five, because the subsample represented 20% of the total damaged leaves per stem, to give an estimate of the total leaf area removed per stem (TLS). The estimate of TLS was then added to the LA, determined previously, giving an estimate of the total leaf area per stem (TLA). The TLA is an estimate of the theoretical leaf area, had no larval feeding occurred. Finally, the LAF was divided by the TLA and multiplied by 100 to give an estimated percentage defoliation per stem, i.e., the estimated leaf area removed by larval feeding in a treatment.
Percentage Damaged Terminal Buds

All damaged and undamaged terminal buds were counted on each stem. Buds were considered damaged if larval feeding on the terminal meristem was extensive enough to prevent the inflorescence from forming. The percentage terminal buds damaged per stem was determined by dividing the number of damaged terminal buds by the total number of terminal buds (damaged and undamaged) and multiplying by 100.

Net Stem Growth

Stem growth during the experiment was determined by subtracting the starting stem length, when larvae were released, from the final stem length at adult emergence. The stem growth was then divided by the starting stem length to give the net stem growth for each treatment. Dividing stem growth by the starting stem length accounted for the variation in length among stems at larval release. Thus, the net stem growth is a relative proportion of the difference in stem length during the experiment from the time larvae were released to when the stems were removed from the pots.

Net Rootstock Growth

At the end of each experiment, rootstocks were separated from the above ground stems, washed, and weighed to give the final rootstock fresh weight. The initial rootstock weight was subtracted from the final rootstock weight to give an estimate of root growth during the experiment. The root growth estimate was then divided by the starting rootstock weight to give the net rootstock growth per treatment. This measurement is a relative proportion of the difference in rootstock weight during the experiment. Variation among
rootstock weights at the beginning of the experiment were accounted for by dividing the root growth by the starting rootstock weight.

**Biomass Dry-weights**

Following the evaluation of the stem and leaf measurements, above ground and below ground biomass dry-weights were measured for each treatment. Plants were placed into a drying oven\(^9\) maintained at 100 C for three days and weighed.

**Larval Survival and Adult *Galerucella* Measurements**

Adults emerging from the soil at the end of the experiment were collected from treatments infested with larvae. Percentage larval survival, i.e., the percentage of larvae surviving to the adult stage in a treatment, was determined by dividing the number of emerged adults by the number of larvae released in a treatment and multiplying by 100.

Adults were placed in a small petri dish and a stereo microscope\(^{10}\), calibrated to mm using an ocular micronmeter, was used to make the following measurements: 1) total body length, measured from the tip of the abdomen to the origin of the frontal suture on the vertex of the head; 2) elytral length, measured from the anterior to posterior edges along the mesal sutural edge; 3) elytral width, the largest distance between the outer-edge of the epipleuron to the mesal sutural edge, and 4) pronotum width, measured at the widest visible region of the pronotum (Figure 2). Finally, adults were dissected and sexed based upon the presence of an aedeagus in males or a spermatheca in females (Manguin et al. 1993).

**Statistical Analysis**

This experiment was designed as a randomized complete block design containing three blocks of time with five treatments that were replicated five times per block. Data were
Figure 2. Measurements of adult *Galerucella calmariensis*: a) total body length; b) pronotum width; c) elytral length; and d) elytral width (picture drawn by Matthew J. Tucker, Iowa State University).
analyzed using analysis of variance procedures. Statistical computing was performed using General Linear Model Procedures (PROC GLM, SAS® Institute Inc., 1985). When significant differences occurred between treatments within years, means were separated using Fisher's least-significant-difference test at the 0.05 level (LSD, SAS® Institute Inc., 1985).

Results and Discussion

Parameter Evaluation

Comparisons of parameters between years yielded significant differences. Therefore, all plant parameters were analyzed by year. There was no cage effect found for any of the parameters measured in this experiment. The uncaged control data was included in the overall analysis for each plant parameter measured (treatment d.f. = 4); however, because there were no significant differences between the uncaged and caged controls, only the caged control data is presented.

Adult Emergence and Larval Survival

Significant differences were observed among larval treatments for larvae that completed development and emerged as adults at the end of the experiment in both years. As expected, adults emerging within a larval treatment increased with increasing larval densities and more larvae survived to the adult stage for all larval treatments in 1996 than in 1997 (Figure 3). However, the percentage larval survival between treatments did not differ within years. When averaged across all treatments, percentage larval survival was 45% in 1996 and 35% in 1997. The effects of the three larval treatments on purple loosestrife growth measured in this study were dependent on these survival percentages.
Figure 3. Adults emerging in three *Galerucella calmariensis* larval densities at the conclusion of the experiment (means separated within years ± SE).
Larval survival was below 50% in both years suggesting that to obtain densities of 50 larvae per plant in the field, adult beetles would have to lay enough eggs following release to compensate for egg and larval mortality. In Iowa, arthropod predators have been observed to decrease the survival of *G. calmariensis* eggs, larvae, and adults. In our study, larvae were protected from predators with mesh cages, but still had a low survival percentage. The larval survival percentages obtained in this experiment coupled with arthropod predation observed in the field may limit the success of *G. calmariensis* as a biological control agent of purple loosestrife. However, the effects of predation on the survival of *G. calmariensis* in the field have not been quantified and are unknown at this time.

**Percentage Leaf Damage and Defoliation**

The number of leaves produced per stem was not found to be different among the control and the three larval treatments in 1996; however, significant differences were observed in 1997 (Tables 1 and 2). No statistical differences were observed among treatments for total leaf area per stem for either year. As expected, damage to leaves increased substantially with increasing larval densities (Tables 1 and 2 and Figure 4). Separation of means showed that the three larval treatments and the control were significantly different from each other (Figure 4). Defoliation caused by larval feeding also increased significantly in both years with increasing larval densities (Figure 5).

The highest defoliation percentage in both years was caused by 50 larvae/cage and averaged approximately 25%. In 1996, 50 larvae/cage damaged 70% of the leaves on a stem causing 23% defoliation (Figure 5). However, 50 larvae/cage damaged 40% of the leaves per stem in 1997, but still caused 25% damage (Figure 5). When considering that larvae within
Table 1. Effect of three *Galerucella calmariensis* larval densities on several parameters of purple loosestrife (means ± SE) measured in 1996.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0</th>
<th>5</th>
<th>25</th>
<th>50</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net Root Growth (kg)</td>
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<td>2.95</td>
<td>2.01</td>
<td>1.59</td>
<td>0.89</td>
</tr>
<tr>
<td>Leaves Per Stem</td>
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<td>301.02</td>
<td>289.64</td>
<td>308.11</td>
<td>0.19</td>
</tr>
<tr>
<td>Total Leaf Area (sq. cm)</td>
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<td>905.11</td>
<td>782.45</td>
<td>673.52</td>
<td>1.59</td>
</tr>
<tr>
<td>Terminal Buds Per Stem</td>
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<td>31.93</td>
<td>38.21</td>
<td>37.91</td>
<td>1.11</td>
</tr>
<tr>
<td>Damaged Terminal Buds Per Stem</td>
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<td>5.22</td>
<td>16.73</td>
<td>25.34</td>
<td>79.41</td>
</tr>
<tr>
<td>Starting Plant Height (m)</td>
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<td>0.43</td>
<td>0.44</td>
<td>0.76</td>
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<tr>
<td>Final Plant Height (m)</td>
<td>0.91</td>
<td>0.91</td>
<td>0.82</td>
<td>0.85</td>
<td>2.61 *</td>
</tr>
<tr>
<td>Undamaged Leaves</td>
<td>290.52</td>
<td>192.24</td>
<td>174.61</td>
<td>95.41</td>
<td>9.49 **</td>
</tr>
<tr>
<td>Damaged Leaves</td>
<td>0</td>
<td>27.02</td>
<td>107.47</td>
<td>198.33</td>
<td>69.07 **</td>
</tr>
<tr>
<td>Pre-Fresh Root Weight (kg)</td>
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<td>0.14</td>
<td>0.15</td>
<td>0.15</td>
<td>0.04</td>
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<td>Final-Fresh Root Weight (kg)</td>
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<td>0.42</td>
<td>0.36</td>
<td>0.32</td>
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<tr>
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<td>0.03</td>
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<tr>
<td>Final-Dry Below Ground Biomass (kg)</td>
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<td>0.07</td>
<td>0.06</td>
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* Significant effect, 0.01 < P < 0.05; ** highly significant effect, P < 0.01.
Table 2. Effect of three *Galerucella calmariensis* larval densities on several parameters of purple loosestrife (means ± SE) measured in 1997.

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>50</th>
<th>F-value</th>
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<tbody>
<tr>
<td>Net Root Growth (kg)</td>
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<td>0.19</td>
<td>0.21</td>
<td>0.08</td>
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<td>Leaves Per Stem</td>
<td>281.21</td>
<td>304.81</td>
<td>370.25</td>
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<td>Total Leaf Area (sq. cm)</td>
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<td>1203.42</td>
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<td>Terminal Buds Per Stem</td>
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<td>28.85</td>
<td>37.61</td>
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<tr>
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<td>11.55</td>
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<td>0.28</td>
<td>0.26</td>
<td>8.21 **</td>
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<td>Final Plant Height (m)</td>
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<td>5.61 **</td>
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<td>277.91</td>
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<td>92.37</td>
<td>134.73</td>
<td>39.51 **</td>
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<td>Pre-Fresh Root Weight (kg)</td>
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<td>0.22</td>
<td>0.22</td>
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<tr>
<td>Final-Fresh Root Weight (kg)</td>
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<td>0.25</td>
<td>0.25</td>
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<td>0.21</td>
</tr>
<tr>
<td>Final-Dry Above Ground Biomass (kg)</td>
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<td>0.14</td>
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* Significant effect, 0.01 < P < 0.05; ** highly significant effect, P < 0.01.
Figure 4. Effect of three *Galerucella calmariensis* larval densities on the leaf damage to purple loosestrife plant stems (means separated within years ± SE).
Figure 5. Effect of three *Galerucella calmariensis* larval densities on the defoliation of purple loosestrife plant stems (means separated within years ± SE).
treatments had a 35-45% survival rate, in 1996 and 1997 respectively, populations of beetles released on stands of purple loosestrife would have to increase substantially to cause large amounts of defoliation to plants. In Iowa, observations following releases of *G. calmariensis* suggest that 50 larvae per plant would be unusually high. However, adult feeding also contributes to defoliation of purple loosestrife and would most likely have increased the defoliation percentage in this study if it was measured.

Insect herbivory decreases leaf area and can have dramatic effects on the assimilation of carbon. In addition, feeding damage to leaves can affect the transport of water and nutrients through leaves, thus altering other physiological processes in the plant, and provide avenues for infection by plant pathogens (Salisbury and Ross 1992). Several insect herbivory studies have examined the effects of defoliation on growth and resource allocation in perennial and annual plants. Defoliation by leaf-feeding insects has resulted in reduced leaf biomass, flower and seed production, plant density, and life-time fitness of the plants (Brown et al. 1987; Louda and Potvin 1995; Lee and Bazzaz 1980; Wisdom et al. 1989; Jayanth and Visalakshy 1996; Schierenbeck et al. 1994; Maschinski and Whitham 1989). *Galerucella calmariensis* herbivory on purple loosestrife seedlings has been shown to caused large reductions in biomass allocation to shoots and roots, thus decreasing the overall biomass dry-weight (Blossey and Shat 1997)

**Percentage Terminal Bud Damage**

The number of terminal buds produced per stem was similar among treatments in both years (Tables 1 and 2). Plants averaged 35 buds per stem in 1996 and 1997. However, the percentage terminal buds damaged by *G. calmariensis* larvae was significantly different
for larval treatments in both years and increased with increasing larval densities (Figure 6). In 1996 and 1997, 5 larvae/cage damaged approximately 15% of the terminal buds per stem and 50 larvae/cage reduced potential flowering by more than 50% (Figure 6). This damage to the terminal buds is significant when considering that a single plant may produce 2.5 million seeds annually (Malecki et al. 1993).

Meristem damage to purple loosestrife plants causes reductions in seed production, delays bloom, and reduces plant height (Blossey and Schat 1997; McAvoy et al 1997; Voegtlin 1995). This study showed that increasing larval densities increased the damage to terminal buds (Figure 6). The potential of those buds to produce seeds was severely limited. This experiment ended shortly after larval development and the purple loosestrife plants had little time to overcome the effects of larval feeding on the terminal buds. Therefore, it difficult to determine the number of damaged terminals that may have overcome larval feeding and eventually produced flowers. Field observations in Iowa following Galerucella spp. releases have shown that severely damaged terminal buds will not produce viable flowers and that the stem height is reduced 30 – 40 %. However, in many cases the plant compensates for this damage by producing a pair of stems near the bud damage that produce smaller inflorescences if not fed upon by larvae or adult beetles (Cortilet and Obrycki, unpublished data).

Herbivory studies of parthenium weed, Parthenium hysterophorous have shown that feeding on plant meristems by Mexican bean beetle larvae, Zygogramma bicolorata (Coleoptera: Coccinellidae) suppressed growth and flower production (Jayanth and Visalakshy 1996). Additionally, meristem damage by insect feeding on the perennial herb
Figure 6. Effect of three *Galerucella calmariensis* larval densities on the damage to terminal buds of purple loosestrife plant stems (means separated within years ± SE).
Lathyrus vernus was found to reduce survival, growth, and seed production in the plant (Ehrlen 1995a; Ehrlen 1995b). In our study, damage to terminal buds at higher larval densities indicates that G. calmariensis larval feeding has a strong negative effect on the production of flowers and seeds by purple loosestrife plants (Tables 1 and 2, Figure 6).

**Net Stem and Rootstock Growth**

Significant differences in net stem growth were found among larval treatments in both years (Figure 7). In 1996, differences were observed between the control and the three larval treatments. Stems that were fed upon by larva had approximately 33% decrease in stem growth when compared with the control. However, differences in stem growth did not occur between larval treatments (Figure 7). In 1997 differences were not observed between the control and 5 larvae/cage or between 25 and 50 larvae/cage (Figure 7). There was approximately a 46% decrease in net stem growth for 25 and 50 larvae/cage compared with the control and 5 larvae/cage.

No differences were found between treatments for net rootstock growth in either year (Table 1 and 2). Net rootstock growth averaged 2.2 kg in 1996 and 0.2 kg in 1997. This study was repeated identically in both years, thus no clear explanation exists for the large decrease in net rootstock growth for all treatments in 1997. Based on these results, G. calmariensis larvae appeared to have little or no influence on root growth. Blossey and Shat (1997) showed that the root biomass of purple loosestrife seedlings decreased significantly when plants were fed upon by G. calmariensis. They also suggested that this reduction in root biomass might increase winter mortality or reduce the amount of stored resources available to the plants for growth the following year. In our study, sections of mature
Figure 7. Effect of three Galerucella calmariensis larval densities on the net growth of purple loosestrife plant stems (means separated within years ± SE).
rootstocks were used and growth reductions may not have been as evident than if we had used seedlings, which have less developed root systems.

**Final-Dry Above and Below Ground Weights**

Differences between treatments for final-dry above ground biomass were not observed in 1996; however, small differences were observed in 1997 (Table 2). Differences observed in 1997 for the final-dry above ground biomass were minimal, only differing by a few grams, and not considered biologically important. Treatment means for final-dry below ground biomass were similar in 1996 and 1997 (Tables 1 and 2). Treatments averaged 0.06 kg for root dry weights in both years. Although we found no differences in the dry weights of roots and shoots, Blossey and Schat (1997) showed that *G. calmariensis* significantly reduced the dry biomass of purple loosestrife seedlings. In their study, both shoot and root weight was reduced; although, no significant reductions occurred in the leaf biomass.

**Newly Emerged Adult Beetle Measurements**

Significant differences were found for elytral length of males in 1996 and pronotal width and body length of males in 1997 (Table 3). No other differences were found for newly emerged adult beetle sizes among treatments in either year (Table 3). Mean adult body length (males and females combined) averaged over all treatments for both years was approximately 5 mm. Males and females also had similar elytral lengths, elytral widths, and pronotal widths (Table 3). Therefore, adult *G. calmariensis* body measurements were not affected by larval density. There was no evidence suggesting that larval competition for food caused reductions in adult body size. Based on this data, one can conclude that higher *G.*
Table 3. Effect of three *Galerucella calmariensis* larval densities on newly emerged adult beetle size.

### 1996

<table>
<thead>
<tr>
<th>Measurement (mm)</th>
<th>Larvae Per Cage</th>
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<th>50</th>
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<tr>
<td>Male</td>
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<td>3.3 ± 0.02</td>
<td>3.4 ± 0.01</td>
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<tr>
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### 1997

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<td>4.6 ± 0.02</td>
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</tbody>
</table>

* Significant effect, 0.01 < P < 0.05; ** highly significant effect, P < 0.01.
calmariensis larval densities will not have large intraspecific consequences influencing adult size.

Since G. calmariensis densities were found to have low intraspecific interactions, in terms of adult development and survival, increasing their populations to higher levels, i.e., 50 larvae per plant or greater, at purple loosestrife infestations in North America may be warranted. This increase in population size would support the efforts of Galerucella mass rearing and release programs throughout the United States and Canada and would increase the stress inflicted by these leaf-feeding beetles on purple loosestrife plants.

General Conclusions

Relatively few studies have quantified the effects of insect herbivory on the growth and development of purple loosestrife (Blossey and Schat 1997; Notzold et al. 1998; Voegtlin 1995). Our research has shown that herbivory by G. calmariensis larvae has negative impacts on the growth of purple loosestrife plants. Insect herbivory may cause plants to change resource allocation patterns, where compensatory regrowth following defoliation to leaves, stems, flowers, and roots can complicate the assessment of herbivory on plant growth (Schierenbeck et al. 1994; Tilman 1988). Differences in plant compensatory responses to herbivory can be seen in carbon allocation patterns, growth rates, photosynthetic rates, and reproduction (Schierenbeck et al. 1994; Maschinski and Whitham 1989).

Herbivory studies of purple loosestrife using insect biological control agents have shown that resource allocation in plants fed upon by insects differs noticeably from plants that are not exposed to herbivory (Blossey and Schat 1997; Notzold et al. 1998; Voegtlin 1995). Feeding by the root-mining weevil, Hylobius transversovittatus (Coleoptera:
Curculionidae), has been observed to reduced shoot weight, plant height, and total dry biomass of purple loosestrife seedlings (Notzold et al. 1998). Additionally, a 50% biomass allocation to root storage and another 30% allocated to shoots following weevil herbivory was observed. *Myzus lythri* (Homoptera: Aphididae), a host-alternating aphid species that feeds on purple loosestrife during the summer, was also shown to decrease seedling biomass and flowering of purple loosestrife seedlings (Voegtlin 1995). Similarly, *G. calmariensis* feeding on purple loosestrife seedlings reduced plant height, root biomass, and flower production (Blossey and Schat 1997).

In contrast to these seedling studies, our experiment examined herbivory by the larval stages of *G. calmariensis* on stems growing from sections of mature purple loosestrife rootstocks. In this study, the effects that larval feeding had on above ground growth were similar to Blossey and Schat (1997). In both years all larval densities caused substantial removal of leaf area through defoliation and terminal bud damage resulting in decreased flowering potential and stem growth. Conversely, no effects of larval feeding were observed for root growth in either year of our study and no clear evidence exists for differences in resource allocation in rootstocks. In 1996, higher larval survival may explain why there was more leaf damage, greater defoliation, higher percentages of terminal bud damage, and lower net stem growth per stem observed than in 1997.

These findings provide more insight into the effects of natural enemies on the growth and spread of purple loosestrife in North America. If *G. calmariensis* alone can cause reductions in biomass and reproduction, the outlook for multiple species releases of purple loosestrife natural enemies seems good. However, we are not aware of any studies that have
documented the effects of multiple natural enemies on purple loosestrife plant performance and the effects of interspecific competition. For example, does a leaf-feeding insect have a synergistic effect on a root-mining weevil like *H. transversovittatus*, or does the feeding by *H. transversovittatus* inhibit the leaf-feeders actions.

The success of biological control for purple loosestrife may depend on increased knowledge of plant/insect interactions and how we manipulate this interaction and exploit the plant’s resource allocation patterns. Solutions to complicated weed problems posed by exotic plants like purple loosestrife will only come from increased research and understanding of how these plant species react to the entire complex of natural enemies, plant pathogens, and competition by other plant species within the communities that they invade.

**Sources of Materials**


2. Sunshine Sterile Soilless Mix® # 1, Sun Gro Horticulture Inc., 15831 NE 8th St. #100, Bellevue, WA 98008.

3. Fafard Sterile Growing Mix® # 2, Conrad Fafard Inc., 711 Silver St., Agawam, MA 01001.

4. No-See-Um® Mesh, Balson-Hercules Group, 545 Pawtucket Ave., Pawtucket, RI 02860.

5. Peters Professional All Purpose Soluble Plant Food (20-20-20) with macronutrients, Spectrum Group, 8494 Chapin Industrial Dr., Overland, MO 63114.

Acknowledgments

We thank the hard work and dedication of Jennifer Barnes, Bryan Clark, Brad Tucker, and Anna Keyte for technical assistance. We would also like to thank Woody Hart, Department of Entomology, ISU, for use and assistance with leaf area measurements, Dave Cox and Kari Jovaag, Department of Statistics, ISU, for statistical assistance, and Matt Tucker, Department of Design, ISU, for a beetle drawing. This research and a graduate assistantship were supported by the Department of Agronomy, Iowa State University. Journal Paper No. XXXXX of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. XXXX.

Literature Cited


BIOLOGICAL CONTROL OF PURPLE LOOSESTRIFE, 

LYTHRUM SALICARIA L., IN IOWA WETLANDS

A paper submitted to Journal of the Kansas Entomological Society

Anthony B. Cortile, John J. Obrycki, and Micheal D. K. Owen

Abstract: Between 1994 and 1998, more than 600,000 natural enemies were released in Iowa for biological control of purple loosestrife, *Lythrum salicaria*. The objectives of this biological control project were to establish natural enemies that would suppress populations of *L. salicaria*. Two species of leaf-feeding beetles, *Galerucella calmariensis* and *G. pusilla* (Coleoptera Chrysomelidae), a flower-feeding weevil, *Nanophyes marmoratus* (Coleoptera: Curculionidae), a root-mining weevil, *Hylobius transversovittatus* (Coleoptera: Curculionidae), and an aphid species, *Myzus lythri* (Homoptera: Aphididae), have been released at *L. salicaria* infested wetlands in Iowa. These natural enemies, with the exception of *H. transversovittatus*, have been observed to severely damage leaves, stems, roots and flowers of *L. salicaria* plants when at high population densities. Both *Galerucella* species have successfully overwintered in Iowa and are increasing in abundance at all release sites.

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Purple loosestrife, *Lythrum salicaria* L. (Myrtilorae: Lythraceae), is a herbaceous European wetland perennial that was introduced to northeastern North America in the early 1800's (Malecki et al., 1993; Thompson et al., 1987). Mature plants consist of a perennial rootstock that produces 20-40 annual stems (Malecki et al., 1993; Edwards et al., 1995; Notzold et al., 1998). The inflorescence makes this plant highly visible during the bloom period from late June to August. A single plant can produce more than 2.5 million seeds annually (Malecki et al., 1993; Thompson et al., 1987). *Lythrum salicaria* typically grows to 1.5 meters and forms dense stands in moist-soil habitats associated with floodplains, marshes, stream edges, and drainage ditches (Rawinski and Malecki, 1984; Thompson et al., 1987).

In North America, *L. salicaria* dominates native vegetation resulting in large monotypic stands that reduce the biological diversity of native ecosystems (Malecki et al., 1993; Thompson et al., 1987). Management of *L. salicaria* in the United States has consisted of physical removal (digging), mowing, water level manipulation, burning, and application of non-specific herbicides (Malecki et al., 1993; Rawinski and Malecki, 1984; Stevens et al., 1997; Thompson et al., 1987; Thompson, 1991). These techniques have been largely unsuccessful, environmentally disruptive, and economically unfeasible.

In Iowa, *L. salicaria* threatens wetlands throughout the state. Several wetlands in northwest Iowa are infested with *L. salicaria* demonstrating noticeable reductions in biological diversity. Surveys completed by J. Harri, Iowa Department of Agriculture and Land Stewardship, R. Pope, Department of Entomology, Iowa State University, and our observations indicate that *L. salicaria* is scattered throughout Iowa. Thirty-three Iowa
counties have documented stands of *L. salicaria* (Figure 1). Attempts by state and county agencies to manage these infestations have been unsuccessful.

In 1994, a biological control program at Iowa State University (ISU) to manage *L. salicaria* was established. The objectives of this biological control program were to mass rear, release, and monitor five natural enemies of *L. salicaria*: two species of leaf-feeding beetles, *Galerucella calmariensis* L. and *G. pusilla* Duftshmidt (Coleoptera: Chrysomelidae); a root-mining weevil, *Hylobius transversovittatus* Goeze (Coleoptera: Curculionidae); a flower-feeding weevil, *Nanophyes marmoratus* Goeze (Coleoptera: Curculionidae); and a host-alternating aphid species, *Myzus lythri* (Schrank) (Homoptera: Aphididae) (Blossey, 1995; Blossey et al., 1994a; Blossey et al., 1994b; Hight et al., 1995; Malecki et al., 1993; Notzold et al., 1998; Voegtlin, 1995). *Myzus lythri* feeds on *L. salicaria* leaves, flowers, and stem tips from early spring until autumn and overwinters on mahaleb cherry, *Prunus mahaleb* (Voegtlin, 1995). This paper summarizes the releases and documents the establishment and spread of these natural enemies.

Materials and Methods

NATURAL ENEMY REARING AND RELEASE: In 1994, *G. calmariensis* and *G. pusilla* were purchased by the Iowa Department of Natural Resources (IDNR) from Dr. Bernd Blossey at Cornell University, Ithaca, New York. These adult beetles were used to start the mass rearing and release program at ISU.
Beetles were reared year round in a greenhouse, 25 ± 3 °C (L:D 16:8), and outside from May to August. Field dug, *L. salicaria* rootstocks were planted into 38 l pots (Lerio Corp., Mobile, AL) filled with Sunshine Sterile Soilless Mix® #1 (Sun Gro Horticulture Inc. Dellevue, Washington). One meter tall cylindrical sleeve cages, made of No-See-Um® mesh (Balson-Hercules Group, Pawtucket, RI), were placed around tomato cages covering the *L. salicaria* plants. When plants reached 0.5 m, 25 adult beetles were released into the cages for mating and oviposition. Approximately four weeks later, newly emerged adults were collected with a pump-driven aspirator in sleeves sewn to the tops of the mesh cages. Adults were counted, stored at 5 °C for up to three days, and taken to the field for release.

In 1998, a mass rearing program began for *N. marmoratus*. Approximately 500 adult weevils were obtained from the United States Department of Agriculture/Animal Plant Health Inspection Service (USDA/APHIS), Mission, Texas Biological Control Laboratory. Rearing procedures for *N. marmoratus* were identical to those used for *Galerucella* species; however, adults were released into rearing cages with plants that had 5-10 blooms per cage.

Field releases of *G. calmariensis* and *G. pusilla*, during 1994 and 1995, were made inside 5.8 m³ field cages to protect the beetles from predation for several weeks following the release. During this period, beetles mated, oviposited, and were eventually released from field cages once their offspring emerged as adults. Starting in 1996, both *Galerucella* species and, in 1998, *N. marmoratus* have been released directly onto *L. salicaria* plants in the field. These releases were made by placing a 5 ft pole (the top painted florescent orange) 1 ft into the soil and releasing beetles at the pole. This pole served as a center of origin to monitor beetle dispersal following a release.
In 1995 and 1996, *H. transversovittatus* eggs were obtained from Cornell University and Dr. Rob Wiedenmann, Illinois Natural History Survey (INHS), University of Illinois, Champaign/Urbana, for stem inoculations at three northwest Iowa wetlands. *Hylobius transversovittatus* eggs were inoculated into stems of *L. salicaria* in late July by cutting three stems/plant in half, drilling into the top end of each stem approximately 5 cm deep with a 0.32 cm drill bit, placing one egg into each chamber with a fine-hair paintbrush, and covering the chambers with modeling clay. A sample of inoculated stems was taken the following September to determine if eggs had hatched and larvae moved down into the rootstock for overwintering.

In 1998, a small colony of *M. lythri* was initiated. Aphids were obtained from Drs. Rob Wiedenmann and David Voegtlin (INHS) and reared for three months on *P. mahaleb* seedlings. In August 1998, these seedlings had high aphid densities and were planted at five *Galerucella* release sites to provide *M. lythri* with an overwintering location. Several *P. mahaleb* seedlings were kept at ISU to provide aphids for future rearing and releases.

Iowa release sites for natural enemies were located at: Black Hawk Wildlife Area (Sac County), Little Storm Lake (Buena Vista County), Sunken Grove Marsh (Pocahontas County), Shade’s Pond (Calhoun County), Boone Forks Wildlife Area (Hamilton County), Maffitt Reservoir (Polk County), Turkey Foot Prairie and Shell Rock River (Worth County), Mississippi River (Allamakee County), and private land in Warren and Madison Counties.
FIELD MONITORING OF *LYTHRUM SALICARIA* AND *GALERUCELLA* SPP.:

From 1995 to 1998, observations were made at all *Galerucella* release sites to determine if the beetles had overwintered. These observations consisted of walking through the release area and detecting eggs, larvae, or adults of both *Galerucella* species. During these overwintering observations, the dispersal of beetles around the 5 ft poles was determined by flagging the areas, furthest from the release origin, where beetles were detected. This perimeter of flags was updated every year by moving them farther out when beetles were discovered away from the original perimeter.

In 1994 and 1995, *L. salicaria* plants were sampled at Black Hawk Wildlife Area, Little Storm Lake Marsh, and Sunken Grove Marsh. Five 1 m² quadrat samples were taken once a week from the beginning of July to early September (8 weeks in 1994 and 10 weeks in 1995). From each 1 m² quadrat sample, estimates of plant density (stems/m²), mean height (average of five randomly selected stems measured per sample), and percentage cover (visual estimate of *L. salicaria* ground cover within the quadrat) were determined for each site.

Additionally, percentage bloom (number of blooms divided by total number of stems) of *L. salicaria* plants inside of field cages containing *Galerucella* species was also estimated at each site in mid-August of both years. These bloom estimates were compared with 1 m² quadrat samples (10) of *L. salicaria* plants taken outside the cages.

In 1998, 120 and twenty ¼ m² quadrats were established along two 60 m transect lines, one arranged east to west and the other north to south, randomly placed in patches of *L. salicaria* at Black Hawk Wildlife Area, Sunken Grove Marsh, and Shade’s Pond. Thirty-two quadrats were randomly sampled within each transect for percentage cover (visual estimate...
of *L. salicaria* ground cover within the quadrat), number of stems, and mean height of *L. salicaria* plants on June 3, 10, and 16. Additionally, counts of *Galerucella* egg masses, larvae, and adults were made during these samples.

In 1995, counts of both species of *Galerucella* were taken weekly throughout the summer (12 weeks) to monitor beetles inside and outside of 5.8 m$^3$ field cages at Black Hawk Wildlife Area, Little Storm Lake Marsh, and Sunken Grove Marsh. Within each field cage, five purple loosestrife stems were randomly selected and the number of eggs, adults, and larvae observed per stem sample was recorded. In addition to field cage samples, five randomly selected 1 m$^2$ quadrat samples were taken outside the cages, the number of eggs, larvae, and adults were recorded for each sample.

Means were calculated for natural enemy and *L. salicaria* data, but were not subjected to statistical analysis. The purpose of data collection was to develop baseline information for individual release sites that will be used in future years as a comparison to evaluate the effects of released natural enemies on growth and spread of *L. salicaria* in Iowa.

Results and Discussion

NATURAL ENEMY RELEASES: From 1994 to 1998, approximately 600,000 adult *G. calmariensis* and *G. pusilla* were released at 11 sites in Iowa (Table 1, Figure 1). Overwintering analysis at all release sites since 1994 demonstrated that both species of *Galerucella* successfully overwintered and continue to disperse throughout the release areas.
Table 1. Number of *Galerucella* spp. adults released for the biological control of purple loosestrife in Iowa.

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<td>26,500</td>
<td>160,000</td>
<td>201,500</td>
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<tr>
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<td>Pocahontas</td>
<td>NE 1/4 Sec. 8 T90N R34W</td>
<td>1,000</td>
<td>3,000</td>
<td>15,000</td>
<td>25,000</td>
<td>30,000</td>
<td>74,000</td>
</tr>
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<td>NW 1/4 Sec. 30 T89N R34W</td>
<td>0</td>
<td>0</td>
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<td>24,000</td>
<td>30,000</td>
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<td>100,000</td>
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<td>20,000</td>
<td>23,000</td>
</tr>
<tr>
<td>Turkey Foot Prairie</td>
<td>Worth</td>
<td>SW 1/4 Sec. 23 T99N R20W</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5,000</td>
<td>1,000</td>
<td>6,000</td>
</tr>
<tr>
<td>Shell Rock River</td>
<td>Worth</td>
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<td>0</td>
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<td>1,000</td>
<td>4,000</td>
</tr>
<tr>
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<td>NW 1/4 Sec. 29 T98N R19W</td>
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<td>0</td>
<td>5,000</td>
<td>2,000</td>
<td>7,000</td>
</tr>
<tr>
<td>Private Pond</td>
<td>Warren</td>
<td>SW 1/4 Sec. 7 T77N R25W</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10,000</td>
<td>10,000</td>
</tr>
<tr>
<td>Private Property</td>
<td>Madison</td>
<td>SW 1/4 Sec. 12 T77N R25W</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>21,000</td>
<td>21,000</td>
</tr>
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</table>

**Year Totals**

<table>
<thead>
<tr>
<th></th>
<th>3,000</th>
<th>10,000</th>
<th>50,000</th>
<th>122,100</th>
<th>410,000</th>
<th>595,100</th>
</tr>
</thead>
</table>
Figure 1. *Lythrum salicaria* and natural enemy distribution in Iowa. Highlighted counties have known established stands of *Lythrum salicaria*. Counties with letters 2-5 indicate the number of natural enemy species released. 5 = *Galerucella calmaniensis*, *G. pusilla*, *Hyllobius transversovittatus*, *Nanophyes marmoratus*, and *Myzus lythri*. 4 = *G. calmaniensis*, *G. pusilla*, *N. marmoratus*, and *M. lythri*. 3 = *G. calmaniensis*, *G. pusilla*, and *N. marmoratus*. 2 = *G. calmaniensis* and *G. pusilla*. Rearing facilities at Iowa State University and Upper Iowa University are represented on the map by the acronyms ISU and UIU respectively.
increasing their population size. In addition to Galerucella spp., H. transversovittatus, N. marmoratus, and M. lythri have also been released in Iowa (Table 2, Figure 1).

In 1995 and 1996, 1,550 H. transversovittatus eggs were inoculated into stems at three northwest Iowa wetlands (Table 2, Figure 1). Lythrum salicaria stem samples taken in the fall of both years, showed that in approximately 60% of stems sampled (n = 40), larvae had completely mined the pith and moved into the rootstock. No adults or larvae have been observed in Iowa following the initial egg inoculations. Plant mortality due to H. transversovittatus has not been observed in Iowa.

In 1998, 3,000 N. marmoratus were reared and released in seven Iowa counties (Table 2, Figure 1). Five to 10 inflorescences were collected from each site following these releases. Larval N. marmoratus were observed to be developing inside of individual flower buds on the inflorescences (1 larva per bud). Larval feeding appears to severely damage the reproductive organs of the flowers, thus preventing seed production. Collection of seed heads from N. marmoratus release sites in September 1998 showed that these weevils damaged from 70 to 80% (n = 12) of the individual flowers on an inflorescence.

An estimated 5,000 M. lythri were transplanted with P. mahaleb seedlings into L. salicaria infestations in 1998 (Table 2, Figure 1). Following these releases, aphid numbers increased at release sites and they were feeding on L. salicaria leaves and flowers. Additionally, aphid densities continued to increase on the transplanted P. mahaleb seedlings.
Table 2. Releases of *Hylobius transversovittatus*, *Nanophyes marmoratus*, and *Myzus lythri* in Iowa, 1995 to 1998.

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hylobius transversovittatus</em></td>
<td>Black Hawk Wildlife Area</td>
<td>Sac</td>
<td>550</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>650</td>
</tr>
<tr>
<td></td>
<td>Little Storm Lake Marsh</td>
<td>Buena Vista</td>
<td>200</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>Sunken Grove Marsh</td>
<td>Pocahontas</td>
<td>500</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>600</td>
</tr>
<tr>
<td></td>
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<td>1,250</td>
<td>300</td>
<td>0</td>
<td>0</td>
<td>1,550</td>
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<tr>
<td><em>Nanophyes marmoratus</em></td>
<td>Little Storm Lake Marsh</td>
<td>Buena Vista</td>
<td>400</td>
<td>400</td>
<td></td>
<td></td>
<td>800</td>
</tr>
<tr>
<td></td>
<td>Sunken Grove Marsh</td>
<td>Pocahontas</td>
<td>400</td>
<td>400</td>
<td></td>
<td></td>
<td>800</td>
</tr>
<tr>
<td></td>
<td>Shade's Pond</td>
<td>Calhoun</td>
<td>400</td>
<td>400</td>
<td></td>
<td></td>
<td>800</td>
</tr>
<tr>
<td></td>
<td>Boone Forks Wildlife Area</td>
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<td>600</td>
<td></td>
<td></td>
<td>1,200</td>
</tr>
<tr>
<td></td>
<td>Malfitt Reservoir</td>
<td>Polk</td>
<td>600</td>
<td>600</td>
<td></td>
<td></td>
<td>1,200</td>
</tr>
<tr>
<td></td>
<td>Three Miles East of Cummings, IA</td>
<td>Warren</td>
<td>200</td>
<td>200</td>
<td></td>
<td></td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>Five Miles East of Cummings, IA</td>
<td>Madison</td>
<td>400</td>
<td>400</td>
<td></td>
<td></td>
<td>800</td>
</tr>
<tr>
<td></td>
<td><strong>Yr. total</strong></td>
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<td>3,000</td>
<td>3,000</td>
<td></td>
<td></td>
<td>6,000</td>
</tr>
<tr>
<td><em>Myzus lythri</em></td>
<td>Black Hawk Wildlife Area</td>
<td>Sac</td>
<td>500</td>
<td>500</td>
<td></td>
<td></td>
<td>1,000</td>
</tr>
<tr>
<td></td>
<td>Little Storm Lake Marsh</td>
<td>Buena Vista</td>
<td>500</td>
<td>500</td>
<td></td>
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<tr>
<td></td>
<td>Boone Forks Wildlife Area</td>
<td>Hamilton</td>
<td>500</td>
<td>500</td>
<td></td>
<td></td>
<td>1,000</td>
</tr>
<tr>
<td></td>
<td>Shade's Pond</td>
<td>Calhoun</td>
<td>500</td>
<td>500</td>
<td></td>
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<td>1,000</td>
</tr>
<tr>
<td></td>
<td>Sunken Grove Marsh</td>
<td>Pocahontas</td>
<td>500</td>
<td>500</td>
<td></td>
<td></td>
<td>1,000</td>
</tr>
<tr>
<td></td>
<td><strong>Yr. total</strong></td>
<td></td>
<td>2,500</td>
<td>2,500</td>
<td></td>
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<td>5,000</td>
</tr>
</tbody>
</table>
GALERUCELLA AND LYTHRUM SALICARIA SAMPLES: Stem samples taken from 5.8 m$^2$ field cages in 1994 and 1995 showed that *G. calmariensis* and *G. pusilla* defoliated purple loosestrife plants when densities of 250 to 300 beetles were released inside. Feeding damage to terminal and apical meristems of *L. salicaria* plants by adults and larvae was estimated to reduce the bloom of stems inside field cages by 75 to 100%. Estimates of bloom for *L. salicaria* plants outside of cages were 90% in 1994 and 95% in 1995. Additionally, the height of *L. salicaria* plants within field cages was reduced by 50% compared to plants outside cages.

In 1994 and 1995, *L. salicaria* stem densities, average height, and percentage cover estimates were fairly consistent for sites across years (Table 3). Sunken Grove Marsh had the highest density, mean plant height, and percentage cover of the three sites (Table 3). Data from ¼ m$^2$ samples of *L. salicaria* plants taken at Black Hawk Marsh, Shade’s Pond, and Sunken Grove Marsh is shown in Table 4. Black Hawk Marsh had the highest *L. salicaria* stem density and percentage cover of the three sites (Table 4). Sunken Grove Marsh had the tallest plants and Shade’s Pond had the lowest stem density per ¼ m$^2$ (Table 4). In 1998, percentage cover and mean plant height of *L. salicaria* plants sampled in ¼ m$^2$ quadrats at Black Hawk Marsh, Shade’s Pond, and Sunken Grove Marsh (Table 4) were lower than 1 m$^2$ samples taken in 1994 and 1995 (Table 3). However, these differences may be explained by the timing of the samples. In 1994 and 1995, samples were taken from July through September when purple loosestrife plants were fully grown. In 1998, samples were taken in early June when *L. salicaria* was still growing; thus, percentage cover and plant height were lower.
Table 3. Mean height, stem density, and percentage cover (means ± SE) of *Lythrum salicaria* at three IDNR wetlands in 1994 and 1995.

<table>
<thead>
<tr>
<th>Year</th>
<th>Site</th>
<th>Stem Density (stems/m²)</th>
<th>Mean Height (meters)</th>
<th>Percentage Cover</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994</td>
<td>Little Storm Lake</td>
<td>27.3 ± 5.2</td>
<td>2.0 ± 0.8</td>
<td>67.0 ± 2.3 %</td>
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<tr>
<td></td>
<td>Black Hawk Marsh</td>
<td>31.5 ± 3.0</td>
<td>1.4 ± 0.2</td>
<td>60.0 ± 3.1 %</td>
</tr>
<tr>
<td></td>
<td>Sunken Grove Marsh</td>
<td>37.1 ± 5.1</td>
<td>2.5 ± 0.5</td>
<td>70.0 ± 5.7 %</td>
</tr>
<tr>
<td>1995</td>
<td>Little Storm Lake</td>
<td>29.0 ± 5.5</td>
<td>2.0 ± 0.1</td>
<td>66.0 ± 3.4 %</td>
</tr>
<tr>
<td></td>
<td>Black Hawk Marsh</td>
<td>33.2 ± 3.7</td>
<td>1.5 ± 0.4</td>
<td>69.0 ± 7.1 %</td>
</tr>
<tr>
<td></td>
<td>Sunken Grove Marsh</td>
<td>36.1 ± 7.3</td>
<td>2.0 ± 0.5</td>
<td>73.0 ± 4.6 %</td>
</tr>
</tbody>
</table>

*a* Means based on 8 sampling dates in 1994 (n = 40) and 10 sampling dates in 1995 (n = 50).
Table 4. *Lythrum salicaria* characteristics and life stages of *Galerucella* spp. observed in ¼ m² plots at three northwest Iowa wetlands in 1998.

<table>
<thead>
<tr>
<th>Site</th>
<th>n&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Stem Density (Stems/1/4 m²)</th>
<th>Mean Plant Height (m)</th>
<th>Percentage Cover</th>
<th>Adults</th>
<th>Larvae</th>
<th>Egg Masses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black Hawk Marsh&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96</td>
<td>7.5 ± 0.4</td>
<td>1.0 ± 0.1</td>
<td>42.0 ± 4.0 %</td>
<td>0.1 ± 0.1</td>
<td>1.3 ± 0.5</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>Sunken Grove Marsh&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96</td>
<td>4.9 ± 0.7</td>
<td>1.3 ± 0.1</td>
<td>37.0 ± 6.3 %</td>
<td>0.1 ± 0.1</td>
<td>0.2 ± 0.4</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Shade's Pond&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96</td>
<td>5.8 ± 0.6</td>
<td>1.0 ± 0.1</td>
<td>23.0 ± 6.0 %</td>
<td>0.1 ± 0.1</td>
<td>6.0 ± 2.0</td>
<td>10.0 ± 6.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data presented as means (± SE) per ¼ m² samples (n).

<sup>b</sup> Samples combined for June 3, 10, and 16.
In 1994 and 1995, higher numbers of eggs, larvae, and adults for both *Galerucella* species were observed inside field cages than in samples taken outside the cages. Inside field cages, mean numbers of egg masses, larvae, and adults per stem averaged over both years were 126, 137, and 41 respectively (n = 60). Mean numbers of egg masses, larvae, and adults in 1 m$^2$ samples taken outside of field cages were 1, 3, and 4 respectively (n = 60). Field cages offered protection from predators and reduced dispersal, thus allowing beetles to have higher densities than outside cages.

In 1998, samples for *Galerucella* life stages at Sunken Grove and Black Hawk marshes were similar to the samples taken in 1994 and 1995 at those sites (Tables 3 and 4). However, the density of larvae per 1/4 m$^2$ was higher at Black Hawk Marsh in 1998 than in 1994 and 1995. Shades Pond had the highest beetle density observed in all quadrat samples (Table 4) and the most visible defoliation. Additionally, percentage bloom estimates of *L. salicaria* plants at 12 beetle release sites in August 1998 showed that Shade’s Pond had only 5 to 10% bloom while the other 11 sites were at 85 to 90% bloom. This reduction in bloom was attributed to the high numbers of *Galerucella* adults and larvae observed feeding on the stem tips of *L. salicaria* plants at Shade’s Pond throughout the summer.

Releases of the two *Galerucella* beetles have shown promise for reducing *L. salicaria* growth at several sites in Iowa, especially at Shade’s Pond. With the addition of *N. marmoratus* and *M. lythri* at several of the *Galerucella* release sites, we anticipate that added herbivory will suppress *L. salicaria* populations in a shorter time period. Establishment of *H. transversovittatus* in Iowa has not been confirmed. Therefore, the status of this weevil remains a mystery.
Future directions for this biological control program will involve additional rearing, release, and field monitoring of *L. salicaria* natural enemies. Release sites will be evaluated using the baseline data collected in 1994, 1995, and 1998 to determine the effects of these natural enemies on *L. salicaria* in Iowa. If biological control of *L. salicaria* is successful, it will offer an alternative long-term method of weed control to costly, short-term, and potentially environmentally degrading herbicide programs.

**Acknowledgments**

We would like to thank B. Clark, J. Barnes, N. Payne, B. Tucker, L. Gomez, W. Morjan, C. Bogran, and A. Keyte for technical assistance, J. Harri, Iowa Department of Agriculture and Land Stewardship, and R. Pope, Department of Entomology, Iowa State University, for survey information, and Drs. R. Wiedenmann, Illinois Natural History Survey, and B. Blossey, Cornell University, for natural enemy shipments. This project was partially funded by the Iowa Department of Natural Resources, Leopold Center for Sustainable Agriculture, and the USDA National Biological Control Institute. A. B. Cortilet was supported by a research assistantship from the Department of Agronomy, Iowa State University. Journal Paper No. XXXXX of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. XXXX.
Literature Cited


GENERAL CONCLUSIONS

Releases of host-specific natural enemies for the biological control of purple loosestrife in Iowa have shown that these insect herbivores stress this plant when they are at high population densities. Since the beginning of the Iowa State University Purple Loosestrife Biological Control Program, over 600,000 natural enemies have been released in Iowa. These natural enemies include: two leaf-feeding beetles, *Galerucella calmariensis* and *G. pusilla*, a root-mining weevil, *Hylobius transversovittatus*, a flower-feeding weevil, *Nanophyes marmoratus*, and an aphid species, *Myzus lythri*.

Monitoring of both *Galerucella* species in the field has shown that these beetles are established in Iowa. Furthermore, they have been observed to reduce plant height, leaf area, and flower production of purple loosestrife plants. *Nanophyes marmoratus* reproduced in Iowa in 1998 and has demonstrated the ability to damage over 50% of the individual flowers on purple loosestrife inflorescences. *Myzus lythri* population densities were increasing following their release in 1998 and were causing substantial damage to purple loosestrife plants. No sightings have been made for *H. transversovittatus* since egg inoculations in 1995 and 1996. The effects of this weevil on purple loosestrife in Iowa are unknown at this time.

A field cage experiment at ISU has shown that herbivory caused by three larval densities (5, 25, and 50 larvae/plant) of *Galerucella calmariensis* reduces the above ground growth and reproduction potential of purple loosestrife plants. Increasing larval densities caused greater defoliation to purple loosestrife plants and reduced the number of terminal and buds that produced inflorescences. Additionally, larval feeding on purple loosestrife plants reduced the net stem growth. Larval survival and newly emerged adult beetle size were
found to be independent of density, suggesting that intraspecific competition between larvae for space and food resources was minimal.

We expected to see differences in below ground growth by purple loosestrife plants in response to larval feeding. However, herbivory by *G. calmariensis* larvae did not affect the rootstock growth of purple loosestrife. Previous studies have shown significant reductions in root growth of purple loosestrife seedlings when plants were fed upon by insect natural enemies (Blossey and Schat 1997, Notzold et al. 1998, Voegtlin 1995). In contrast to these studies of seedlings, we used mature rootstocks in our research which were not affected by above ground herbivory.

Our research did not detect differences in resource allocations from the roots attributable to increased larval feeding pressure on the plant. We did observe that plants compensated for herbivory. For example, purple loosestrife plants that had high percentages of leaf and stem biomass removed by larval *G. calmariensis* feeding also had similar or higher final dry-weights than the controls, suggesting that these plants were allocating more resources to the stems to compensate for larval biomass removal.

More research is needed to gain a thorough understanding of the effects that natural enemies have on purple loosestrife growth. A major goal for Iowa’s purple loosestrife program is to establish multiple natural enemies at individual sites that will attack and stress the plant from its inflorescence down to the roots. Currently, we have released five species of natural enemies that feed on specific regions of the plant. Several sites have combinations of natural enemies feeding on the purple loosestrife plants collectively. Individually, these natural enemies have shown that they can stress purple loosestrife plants significantly;
however, it has been too early for us to assess the significance of multiple natural enemy releases at these sites. An increased understanding of how multiple insect species interact with one another to lessen the vigor of purple loosestrife growth, in addition to plant competition and disease, will allow us to make better decisions in the future concerning the direction of this biological weed control program.
LITERATURE CITATIONS


Manguin, S., R. White, B. Blossey, and S. D. Hight. 1993. Genetics, taxonomy, and
   ecology of certain species of Galeruella (Coleoptera: Chrysomelidae). Annals of the
   Entomological Society of America. 86: 397-410.

Maschinski, J. and T. G. Whitham. 1989. The continuum of plant responses to
   herbivory: the influence of plant association, nutrient availability, and timing.

   of Galeruella calmariensis (L.) and G. pusilla (Duft.) (Coleoptera: Chrysomelidae)
   on purple loosestrife, Lythrum salicaria L. (Lythraceae), in southwest Virginia.
   Biological Control. 9: 106-111.

McEvoy, P., C. Cox, and E. Coombs. 1991. Successful biological control of ragwort,
   Senecio jacobaea, by introduced insects in Oregon. Ecological Applications. 1: 430-
   442.

   43: 369-93.


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Finally, I would like to thank the Iowa DNR and Leopold Center for Sustainable Agriculture at ISU for their funding and support of this project over the past five years.
BIOGRAPHICAL SKETCH

Anthony B. Cortilet was born January 7, 1971 in Sioux City, Iowa. He received his Bachelor of Science in Fisheries and Wildlife Biology in 1995 and the Master of Science in Crop Production and Physiology in 1998 from Iowa State University. Mr. Cortilet has been a member of GAMMA SIGMA DELTA, the National Honor Society of Agriculture, since 1997. He worked for the Biological Control Laboratory at ISU for five years as a biological technician gaining valuable experience in the biological control of weeds and insects. He also developed and served as the project leader for the Purple Loosestrife Biological Control Program at ISU from 1994 to 1998.