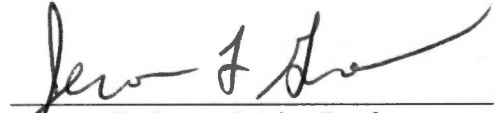


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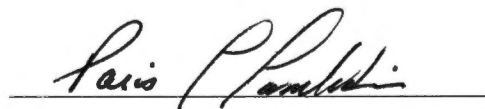
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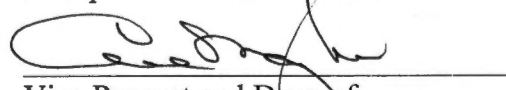
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**BIOLOGY, SEASONALITY, AND IMPACT OF *ALTICA LITIGATA* FALL
ON PURPLE LOOSESTRIFE, *LYTHRUM SALICARIA* L.,
IN NORTHEASTERN TENNESSEE**

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Debra P. Hoyme
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AG-VET-MED.

Thesis

2002

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DEDICATION

This thesis is dedicated to my beloved husband, Craig Hoyme, who inspires, supports, and encourages me to reach higher in order to achieve my goals.

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ABSTRACT

Purple loosestrife, *Lythrum salicaria* L., an exotic, wetland perennial, forms large, monotypic stands throughout many temperate regions of the U.S. and Canada. This invasive plant replaces native vegetation, degrades wildlife habitats, and obstructs waterways. A large population of a native, herbivorous insect, *Altica litigata* Fall, was found feeding on purple loosestrife in upper eastern Tennessee, and both larvae and adults fed on foliage and caused plant damage. Thus, a two-year research project was initiated in 2000 to assess biology, seasonality, and impact of this North American flea beetle, *A. litigata*, on seed production, plant height, and leaf consumption of purple loosestrife in northeastern Tennessee.

The study site was located in Unicoi County, Tennessee, at a wetland along the banks of South Indian Creek where a small infestation of purple loosestrife exists, and was monitored two times weekly from April to September. The site was divided into three subplots, and adult flea beetle densities were determined weekly by sweep-net sampling. Life stage information was obtained from laboratory and field observations and direct sampling. To assess impact of adult feeding on numbers of seed capsules, seeds/capsule, and plant height, plants were placed into four damage rating categories: 1) none, 2) minor, 3) moderate, and 4) major. Ten additional plants, extensively damaged by larvae, also were evaluated. Plant height, number of seed capsules/lateral, number and lengths of seed-bearing laterals, and number of seeds/capsule were measured. In the laboratory, leaf consumption by adults was quantitated using a leaf area meter. Adults (0, 2, 4, or 6 male or female) were fed foliage, which was measured and replaced every 2

days. Estimated numbers of beetles required for defoliation of purple loosestrife were determined.

All life stages of *A. litigata* were found in association with *L. salicaria*. Eggs and adults were first found on 18 May, and larvae were first documented as on 8 June, but had been present in the field for several weeks. Developmental times for eggs, larvae (three instars), and pupae were 3-5 days, 13-16 days, and 7-10 days, respectively. In the field, larval feeding was extensive; skeletonized foliage appeared "burned" and reduced plant viability and flower maturity. Larval damage caused large reductions in seed production (the larval-damaged plants produced 3,003 seeds/plant stem, compared to the non-damaged plants that produced 67,258 seeds/plant stem). The larval-damaged plants averaged only 52 seed capsules/plant stem, while the non-damaged plants were at least 10x greater, averaging 685 seed capsules/plant stem. The extensive larval damage resulted in a 20% reduction in plant stem height when compared to the non-damaged plants (with mean plant heights of 144 and 179 cm for larval-damaged and non-damaged plant stems, respectively). Adult females consumed ca. 2x more leaf area than males (ca. 0.60 cm² and 0.32 cm², respectively). Based on these results, 39 male or 20 female beetles would consume an entire leaf (with an average leaf area of 12 cm²) in a two-day period. Combined leaf consumption by adults and larvae during their entire lifetime could cause significant damage to purple loosestrife.

Synchronization of larval *A. litigata* and the host flower budding stage of *L. salicaria* is a positive attribute of this potential native biological control agent. *Altica litigata* herbivory had a negative impact on seed production and plant height. This work will be the first to document biology, seasonality, and impact of *A. litigata* on purple

loosestrife. This incidence of *A. litigata* also represents a new state record. A better understanding of the biology, life history, and impact of *A. litigata* on *L. salicaria* may lead to its use as a biological control agent. As a native species its use should have less negative environmental consequences than an introduced species. Further research is needed to investigate the biological control potential of *A. litigata* on purple loosestrife.

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CHAPTER 1

LITERATURE REVIEW

Purple loosestrife, *Lythrum salicaria* L., is a wetland herbaceous perennial that is native to Europe and Asia (Thompson 1991). This immigrant plant probably reached the east coast of North America, in the early 1800s, as a contaminant in ship ballast and as seeds embedded in imported wool (Thompson et al. 1987, Cox 1999). In a distribution summary of *L. salicaria*, in 1824, Torrey provided the earliest record of purple loosestrife in North America to be noted in Pursh's *Flora Americae Septentrionalis* of 1814 (Stuckey 1980).

Purple loosestrife was reported as native to North America until the 1889 sixth edition of Gray's *Manual* noted the species as a non-indigenous member of the flora naturalized from Europe (Stuckey 1980). With the building of the canals and the development of waterborne commerce to the Great Lakes, *L. salicaria* spread into the interior of North America, establishing along recently disturbed or stressed habitats (Thompson et al. 1987). Before the 1930s, most botanists considered purple loosestrife to be a marsh plant of minor importance and probably an attractive addition to native wetland vegetation.

In the 1930s, this perennial began its invasive explosion, and the small initial colonies became vast monocultures in many wetlands all across North America (Stuckey 1980, Thompson et al. 1987, Cox 1999). This invasive exotic species continued its spread inward and westward along areas of irrigated farming and drainage ditches next to

interstate highways. The rate of expansion could perhaps be contributed to the overall increase in extent of habitat disturbance and the aggressiveness of the weed. Purple loosestrife has spread throughout North America, but is most widespread in the northeastern United States and adjacent Canada (Thompson et al. 1987). As of 1996, *L. salicaria* was found in all the contiguous states, except Florida, and in all the Canadian provinces (Blossey 2002).

Although purple loosestrife arrived as an immigrant, it also was purposefully introduced as a valued medicinal herb and as an ornamental, contributing to its spread (Thompson et al. 1987). Early escapes of *L. salicaria* could have come from herb gardens that were established with seeds brought from Europe. Due to the intimate settings of herb gardens and the decline of interest in herbals with the rise of modern medicine, escapes from herb gardens probably contributed little to the spread of *L. salicaria*. However, the popularity of purple loosestrife as a horticultural plant and efforts by beekeepers to “naturalize” the plant have been important sources of regional and local spread in the expanding range of purple loosestrife. Purple loosestrife has been widely sold as an ornamental, including many cultivars that were presumed to be sterile. Studies conducted in Minnesota have shown that some of these sterile cultivars produce viable seeds even when self pollinated (Welling and Becker 1992). Consequently, supposedly “safe” cultivars can be expected to have contributed to the spread of this species. Beekeepers have promoted purple loosestrife as a good source of pollen and nectar for bees (Bunch 1977, Marshall 1992). In the Midwest an apicultural supplier offered the seed of purple loosestrife for sale, and instructions for “naturalizing” the plant

have been published, which have added to the recent westward extension of this species (Thompson et al. 1987).

A Greek medical man in Nero's Roman army called the plant lytron, which is Greek for blood (Thompson et al. 1987, Western Aquatic Plant Management Society 2002). So, possibly due to knowledge of its styptic usefulness or because the magenta spike resembled a blood gore withdrawn from a wound, the generic name *Lythrum* was derived. Similarity of leaves of purple loosestrife and willow (*Salix* spp.) resulted in the species name *salicaria*. Herbal folk medicines made from the flowering branches were used as tonics and styptics, while medicines made from leaves and roots were used to treat ailments that included dysentery, internal and external bleeding, and healing of wounds (Thompson et al. 1987, Western Aquatic Plant Management Society 2002).

Lythrum salicaria is a variable species that belongs to the order Myrtales and the family Lythraceae which includes 22 genera. *Lythrum* is the type genus for the loosestrife family (Thompson et al. 1987). Twelve species of *Lythrum* are recognized in the continental United States, and three of these are non-indigenous: Grass poly, *L. hyssopifolia* L., European wand loosestrife, *L. virgatum* L., and *L. salicaria*. *L. hyssopifolia* is an annual that occurs in disturbed wetlands and waste places. *L. virgatum* can be distinguished from *L. salicaria* because it is glabrous throughout, the leaves narrow at the base, and flowers are solitary or merely paired. Winged loosestrife, *L. alatum* Pursh, is a native species that most closely resembles purple loosestrife. However, this plant is smaller in size (average of 0.6 m), has alternate leaves, and has more widely spaced flowers (Southeast Exotic Pest Plant Council 2002).

Purple loosestrife, which can grow 1-3 m tall, can easily be sighted when in bloom, for its showy magenta flower spikes often tower above other wetland plants (Thompson et al. 1987). Mature plants have 30 to 50 stout erect annual shoots that rise from a common rootstock to make a wide-topped crown. Lateral growth of the root crown can produce peripheral stems, but the maximum diameter obtained by the root crown is about 0.5 m. The pubescent stems are square and distinctly four-sided often appearing to be woody at the base. However, stems vary and may be smooth or have as many as six sides. Leaves are downy and without teeth. They are arranged opposite each other and alternate along the stalk at 90° angles or less often in whorls of three (Mullin 1998). The lanceolate (sometimes narrowly oblong) leaves grow up to 10 cm long and 1.5 cm wide being cordate or sometimes rounded at the base. Leaves are variable and those exposed to lower light levels will show an increase in leaf area and a decrease in fine pubescence (Thompson et al. 1987).

The magenta flowers of *L. salicaria* (occasionally pink or white) have five to six petals forming a terminal bracted spike (0.5 cm to 0.9 m long) (Mullin 1998). Blooms have eight to ten stamens, and the greenish calyx tube has hirsute lobes along with bracts. The species has three forms that are distinguished based on the length of the style (Hermann et al. 1999). The tri-morphic flowers have the stigma positioned at one of three levels (short, mid, or long) with the two whorls of stamens positioned reciprocally at the other two levels. Recent research has quantified stigma polymorphism in tristylous *L. salicaria* using both a scanning electron microscope and a light scope to obtain measurements, and future research will quantitatively analyze pollen polymorphism (Hermann et al. 1999). The importance of such studies is to further understand the role of

heterostylous self-incompatibility systems of this species (Proctor et al. 1996). In the wild where the population is exposed to a minimal amount of disturbance, evidence shows that the ratio of the three forms is 1:1:1, which suggests the importance of sexual reproduction in *L. salicaria* (Thompson et al. 1987). The flowers of purple loosestrife are insect-pollinated and cross-pollination prevails. Purple loosestrife cultivars may self-pollinate and produce viable seeds; but this type of mating in *L. salicaria* produces seeds that germinate at much lower rates (Welling and Becker 1992). In most areas purple loosestrife blooms from late June to early September (Thompson et al. 1987).

At 27 days after anthesis, the first viable (germinable) seeds are present (McCaughey and Stephenson 2000). A mature plant can produce more than 2.5 million seeds per growing season, forming enormous seed banks (Welling and Becker 1992). The fruit is an oblong egg-shaped capsule with two valves containing on average 90 to 120 (mature plants and young plants, respectively) minute seeds (0.06 mg) (Thompson et al. 1987). Mature capsules (0.32 to 0.48 cm long) are brown, and seeds (ca. 1 mm) are light tan and almond shaped. Seed dispersal occurs from late summer through the winter predominantly by seeds carried in moving water. Seeds also may adhere to wildlife or be transported by off-road vehicles or motor boats (Thompson et al. 1987, Malecki et al. 1993, Mullin 1998). *L. salicaria* seeds germinate across a broad range of environmental conditions and at temperatures from 14°C to 20°C, but will not germinate below 14° C (Thompson et al. 1987, Welling and Becker 1990). Fresh seeds exceed 90% viability, while seeds after three years of submergence are 80% viable (Thompson et al. 1987, Mullin 1998).

Purple loosestrife is most successful on slightly acidic or neutral soils; however, it can adapt to a wide range of habitats (Thompson et al. 1987). It responds to rising water levels by the growth of aerenchyma (lacunate phellem tissue) in submerged stem and root tissue, to soil nutrient deficiencies by increasing the root to shoot ratio, and to trampling, cutting or crushing of stems with root and shoot buds at the site of damage. Stems that become buried under soil can produce new shoots from buds on the buried stems (Brown and Wickstrom 1997). A lack of agreement and confusion about the vegetative propagation by various plant parts and lateral spread of established *L. salicaria* plants exist. A recent study has assessed the potential of vegetative propagation from stem and root sections and the tissues involved in lateral spread of *L. salicaria* (Stevens et al. 1997). Researchers concluded that lateral spread and perennation of purple loosestrife is aided by lateral shoot meristems in the leaf axils and not shoots developing on roots. Some current control measures suggest that all root and shoot pieces must be removed to prevent re-establishment of the plant. However, their results indicate that all shoot pieces should be removed, but it is not necessary to remove the entire root system to prevent re-establishment.

The crown is the enlarged woody area in the transition region between the root and the stem (Thompson et al. 1987). Shoots emerge from the crown in late April or early May. Extensive carbohydrate reserves in the large woody crown and roots help this species combat stress (Katovich et al. 1999). Seasonal fluctuation of the carbohydrates, predominantly starch and sucrose, in roots and crowns of *L. salicaria* has been determined in field studies of three wetland habitats in Minnesota (Katovich et al. 1998). Carbohydrate levels follow seasonal trends with content decreasing following shoot

emergence until flower bud formation, which marks seasonal lows. Levels of starch and sugars increase during flowering, and this trend continues through to plant senescence in late September. Knowledge of the seasonal fluctuations of carbohydrates provides information to optimize the implementation of control strategies, such as the use of systemic herbicides and biological control agents. Fall applications of a phloem-mobile systemic herbicide, such as glyphosate, would correspond with periods of carbohydrate assimilate transport to roots and crowns of purple loosestrife plants. Fall applications of glyphosate to late flowering plants have been reported to more effectively kill purple loosestrife than mid-flower applications. Understanding the fluctuations of carbohydrate levels could direct managers to time periods when biological control agents would best interfere with production and translocation of carbohydrates to roots and crowns.

This distinctive botanical species was spreading in 1985 at a rate of 190,000 hectares per year impacting wetlands, marshes, pastures, and riparian meadows of North America (Thompson et al. 1987, Cox 1999). Even though few quantitative measurement studies had documented ecological disturbance, Thompson et al. (1987) stated that “the replacement of a native wetland plant community by a monospecific stand of an exotic weed does not need a refined assessment to demonstrate that a local ecological disaster has occurred.” It is believed that *L. salicaria* poses a serious threat to the species richness of wetland flora and fauna by aggressively forming extensive monocultures.

While monotypic stands frequently occur in North America, only small scattered populations of purple loosestrife occur in its native range (Blossey 1995a). Cross-continental transplant experiments to explain the increased vigor of non-indigenous *L. salicaria* have determined that certain genotypes show increased vigor regardless of

growing location (Willis and Blossey 1999). Many of these monoculture stands, sometimes thousands of hectares, displace native vegetation, such as cattails and bulrushes, and threaten the biotic integrity of wetland communities by altering the natural food and cover essential to many wetland wildlife species. In some cases, these large plant stands threaten endangered species, such as the dwarf spikerush, *Eleocharis parvula* (Roemer and J. A. Schultes) Link ex Bluff and Nees and Schauer, in New York, local bulrush, *Scirpus longii* Fernald, in Massachusetts, and the bog turtle, *Clemmys muhlenbergi* Schoepf, in the northeastern United States (Thompson et al. 1987). The hypothesis that dominant stands of *L. salicaria* impacts wildlife negatively has been substantiated (Rawinski and Malecki 1984 and Whitt et al. 1999). Muskrats, *Ondatra zibethicus* L., cannot use purple loosestrife roots for food or shelter and songbirds do not eat the tiny seed (Mullin 1998).

Not all studies have substantiated harmful impacts of purple loosestrife on wildlife communities. A study performed in wetlands near Moses Lake, Washington, to determine if purple loosestrife infestation alters aquatic invertebrate communities is an example (Gardner et al. 2001). They compared the abundance and size distribution of aquatic invertebrates found in purple loosestrife populations to those within stands of broad-leaved cattail, *Typha latifolia* L., and hardstem bulrush, *Scirpus acutus* Bigelow. Results indicate that monotypic stands of *L. salicaria* are not lacking in aquatic invertebrates; but because the study was conducted in a mixed vegetation wetland at an intermediate stage of purple loosestrife infestation, the findings may not be representative of more severe invasions of *L. salicaria* (Gardner et al. 2001).

The environmental impact imposed by *L. salicaria* can be better understood and outcomes more accurately predicted if the competition capabilities of *L. salicaria* are known. A four-year field competition experiment involving *L. salicaria* and narrow-leaved, *T. angustifolia* L., was conducted by placing the two competitors in a modified replacement series experiment with four starting densities (64, 36, 16, and 1 plants/quadrat) and four relative proportions of each species (i.e., 0.25, 0.50, 0.75, or 1.00) (Mal et al. 1997). Overall rate of ramet production was compared. During the first year, *T. angustifolia* gained an advantage in the mixtures, and the second and third year output ratio suggested the two species could coexist. However, the experiment demonstrated the temporal development in competitive behavior of purple loosestrife and found that in experimental field plots it could displace *T. angustifolia* over a four-year period. Results indicate that short-term experiments should be interpreted with care, if they are to be used to predict the long-term outcome of competition in nature.

Infestations of *L. salicaria* are typically associated with wetland disturbance, but upon becoming established purple loosestrife is an effective competitor (Thompson et al. 1987). The height of *L. salicaria*, prolific seed production capabilities, and phenotypic plasticity collectively provide a competitive advantage. The effects of shading on competition between purple loosestrife and broad-leaved cattail were examined using a replacement series design in a greenhouse experiment (Weihe and Neely 1997). Results were consistent with previous findings (Gaudet and Keddy 1988) in that purple loosestrife is less affected by interspecific competition than by competition with itself. In any planting ratio, *T. latifolia* plants were outcompeted by *L. salicaria*. Competition seemed more severe in the unshaded treatment as demonstrated by the substantial decline

in both above and below ground biomass with the presence of even a single purple loosestrife competitor, but shade provided no refuge for *T. latifolia* from competition with *L. salicaria*. An output ratio analysis of biomass suggested local extinction of *T. latifolia* during competition with purple loosestrife regardless of initial densities.

Lythrum salicaria has showy large inflorescences containing short-tubed flowers that produce prolific quantities of nectar that are easily accessed by most insect visitors (Grabas and Lavery 1999). Thus, *L. salicaria* may have strong effects on co-existing plants requiring pollination by insects. Purple loosestrife could compete directly for pollinators thereby lowering the frequency of visits to these plants or alternatively, it could encourage the pollination of some sympatric species by increasing the concentration of pollinators in a local area. Grabas and Lavery (1999) experimentally manipulated *L. salicaria* in field study plots containing specialist or generalist pollinated target species. Their goal was to investigate the effect of purple loosestrife on the pollination and reproductive success of co-flowering plant species (spotted touch-me-not, *Impatiens capensis* Meerburch.; boneset, *Eupatorium perfoliatum* L.; and Joe-Pye-weed, *Eupatorium maculatum* L.). Pollinator visits, heterospecific and conspecific stigmatic pollen loads, fruit and seed set were measured and compared among experimental plots of low, medium, and high densities of purple loosestrife. Data indicated that *L. salicaria* can have a negative impact on the reproductive success of *I. capensis* (30% fewer fruit set) and *E. perfoliatum* (19% reduction in seed production) in some years at some sites. This study provided the first empirical evidence of impact of *L. salicaria* on the reproductive success of native wetland plants in the field.

Conflicting interpretations about the negative impacts of *L. salicaria* and its effect on other plant species were noted by Farnsworth and Ellis (2001). Anderson (1995) and Treberg and Husband (1999) found little evidence for the negative impacts of *L. salicaria* on plant species. A review of impact studies of *L. salicaria* and vegetative compositions from 1984 through 1999 was undertaken by Farnsworth and Ellis (2001). Their studies suggest that “conflicting interpretations of impact can result if inconsistent ecological metrics measuring diversity, density, and other features are used from site to site in defining the relative dominance of the species.”

Lythrum salicaria impacts agriculture by causing loss of wetland pastures and wild hay meadows (Thompson et al. 1987). Young shoots of purple loosestrife are palatable to livestock [also to white-tailed deer, *Odocoileus virginianus* (Boddaert)]; but mature plants are much less frequently chosen as food and therefore can outcompete native forages that are heavily clipped as favorite foods. Since mature purple loosestrife plants are not favored as food, they have a growth advantage over other clipped plants and become a successful wetland pasture invader. Western wild hay harvests are important food sources for the growth and survival of over-wintering yearlings and the health and success of the spring calf crop. *L. salicaria* has invaded wild hay meadows at sites in the Northwest, Midwest, West, and Pacific Northwest and has contributed to reductions in the harvest of wild hay. Purple loosestrife invades drier sites and concern is increasing as the plant becomes more common on agricultural land. Purple loosestrife is not a threat to cultivated crop lands, but is causing concern in areas where wild rice grows.

Wetlands are one of the most biologically diverse ecosystems and many species of plants and animals depend upon healthy wetlands for their survival. Of the estimated 85 million hectares of wetlands that once existed in the contiguous states, only 42 million hectares remain today (Stap and Sibley 2002). Many of the wetlands that continue to exist are further threatened by invasive plants like purple loosestrife, which is a serious problem for private, state, and federal range and wildlife managers. According to former Secretary of the Interior Bruce Babbitt (1998), costs to manage purple loosestrife exceed \$45 million per year in the United States.

Purple loosestrife plants produce millions of seeds and can propagate vegetatively from stem cuttings, which enables it to spread easily producing a real management challenge (Thompson et al. 1987, Stevens et al. 1997b, McCaughey and Stephenson 2000). Welling and Becker (1990) found ca. 410,000 purple loosestrife seeds/m² in the top 5.0 cm of soil in stands of emergent vegetation in wetlands studied in Minnesota. An established stand of *L. salicaria* may be eliminated by managers, but with minor disturbance of the soil, the stand may re-establish through recruitment from the enormous seed bank (Welling and Becker 1990). A minority of established plants can remain dormant above ground for a year and then resume growth the next season; control measures must consider this plant characteristic (Thompson et al. 1987). Various control strategies (hand-pulling, water level manipulation, mowing or cutting, burning and herbicide application) have been employed to eliminate purple loosestrife from wetlands in North America (Malecki and Rawinski 1985, Thompson et al. 1987, Hight and Drea 1991, Nyvall and Hu 1997). Most of these methods are only effective where the plant occurs in small localized stands and can be intensively managed (Thompson et al. 1987,

Hight and Drea 1991). Extensive carbohydrate reserves in the large woody crown and roots help this species combat stress induced by control methods, such as cutting, burning, or foliar herbicides (Malecki et al. 1993, Katovich et al. 1999). Considering the strengths of this aggressive and competitive plant; it is no wonder that many of the control strategies applied have not been effective (Hight and Drea 1991).

Lythrum salicaria is a good candidate for biological control (Thompson et al. 1987, Hight 1989). It has similar attributes of other aquatic weeds, like alligator weed [*Alternanthera philoxeroides* (Martius) Grisebach] and water hyacinth [*Eichhornia crassipes* (Martius) Solms-Laubach] that have been successfully suppressed by biological control agents. These attributes include: (1) introduced perennial; (2) controlled by effective biotic agents in native region; (3) occupies and maintains a steady population; (4) more or less continuous distribution; (5) few taxonomically-related species of economic value; and (6) plant not suppressed by native natural enemies in present location. In 1980, purple loosestrife was approved as a candidate for biological control by an interagency committee (Thompson 1991). The goal is to reduce the pest population density to an acceptable environmental level, not eradication. This method could provide long-term management by restoring the self-regulatory potential of the insect-plant interaction (Hight and Drea 1991). Ideally the pest species and the control agents would oscillate within acceptable limits (Thompson et al. 1987).

An International Institute of Biological Control (IIBC) research project for control of *L. salicaria* was completed within seven years from start to final screening reports, which included environmental assessments for six control agents, at a total cost of \$543,000 (Goeden 1993). The first surveys in Europe during 1986-1987 were initiated

and funded by the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) through the Beneficial Insects Introduction Laboratory, Beltsville, Maryland, and the next five years were supported by the United States Department of the Interior (USDI) and the United States Fish and Wildlife Service (USFWS) through Cornell University, Ithaca, New York. The initial success of the interagency effort led to the formation of a scientific advisory group with representation from Canada, and other federal agencies and universities in the United States. Additional funding during the last two years was provided by Washington State Department of Agriculture and Wildlife. The first three insect agents were released in eight states and one Canadian province in 1992 during the seventh year of the project (Goeden 1993, Hight et al. 1995).

A detailed review of *L. salicaria* including its taxonomy, distribution, biology, ecology, economic value, and control in North America was compiled by Thompson and colleagues (1987). Concurrently, scientists in Europe were performing detailed investigations for potential biological control agents and investigating their life history, distribution, host specificity (additional host plant tests were conducted at the Virginia Polytechnic Institute and State University under quarantine conditions), and impact (Batra et al. 1986, Hight and Drea 1991, Malecki et al. 1993, Blossey et al. 1994a,b).

Of the 120 species of phytophagous insects associated with *L. salicaria* in Europe, only 14 were considered host-specific to the target plant (Batra et al. 1986). Six were eventually selected as promising agents based on their impact on the target weed in the field, their feeding niche on purple loosestrife, and their distribution (Malecki et al. 1993). Only one of these insects, a gall midge (*Bayeriola salicariae* Kieffer), which attacks leaf and flower buds did not pass the test for approval for introduction. Five insect species

were approved for field release in North America. Three host-specific species (two leaf beetles, *Galerucella calamariensis* L. and *G. pusilla* Duftschmidt, and one root weevil, *Hylobius transversovittatus* Goeze) were approved for field introduction in 1992 by the USDA-Animal and Plant Health Inspection Service (APHIS) and by the Plant Protection Division of the Food, Production and Inspection Branch, Agriculture Canada (Blossey et al. 1994a,b, Hight et al. 1995). In 1994, two seed weevils, *Nanophyes marmoratus* Goeze and *N. brevis* Boheman, were approved for introduction (Blossey 1995a, Blossey 2002). Releases of these biological control agents have been made in 30 states and six Canadian provinces (Cox 1999).

A goal of the biological control program of purple loosestrife was to contribute information that could improve the success of biological weed control (Blossey 1995a). An overview of research involving the biological control agents of purple loosestrife is presented below.

The two leaf-feeding beetles, *G. calamariensis* and *G. pusilla*, severely damage *L. salicaria* in Europe (Blossey et al. 1994a). Young larvae feed on developing leaf and flower buds, while later instars feed on any part of the plant. At high beetle densities whole populations of purple loosestrife can be defoliated, which can prevent flowering and seed production (Blossey et al. 1994a). The feeding by these beetles can result in shoot height reduction or elimination of purple loosestrife plants (Blossey and Schat 1997). Both species of *Galerucella* share the same life history features (Blossey 1995b). These two species live sympatrically in the same ecological niche on their host plant, and research has indicated that both species have identical competitive abilities.

Predation of eggs of the two-leaf feeding beetles has been studied (Herzig 1999, Wiebe and Obrycki 2002, Cortilet 1998). Caged (in a greenhouse) *G. californiensis* and *G. pusilla* oviposit 56% of all egg masses on plant leaves (Lindgren 1997). Predation level on eggs is 50% higher of the native leaf-feeding beetle, *G. nymphaeae* (L.), than on *G. californiensis* eggs by the multicolored Asian lady beetle, *Harmonia axyridis* Pallas, and the pink spotted lady beetle, *Coleomegilla maculata* (DeGeer), observed on purple loosestrife in New York (Herzig 1999). Herzig (1999) suggests that predation should not prevent *G. californiensis* from maintaining viable populations in North America. *G. pusilla* eggs are suitable prey for common lacewing, *Chrysoperla carnea* (Stephens), and *C. maculata* (Wiebe and Obrycki 2002). Larvae of *C. carnea* and immature and adults of *C. maculata* feed on *Galerucella* eggs and larvae found on purple loosestrife in Iowa fields (Cortilet 1998, Wiebe and Obrycki 2002). These predators could effect establishment of *G. pusilla* (Wiebe and Obrycki 2002).

Original host specificity screening tests discovered that adult *Galerucella* spp. oviposited only on plants within the genus *Lythrum* (Blossey et al. 1994a). Native winged loosestrife experienced reduced oviposition and larval development in caged experiments, but never while the plants were exposed in field tests. These two *Galerucella* species were approved because tests showed that the benefits of an introduction outweigh potential environmental impact (Blossey et al. 1994a). Additional evaluations of *G. californiensis* on wild and ornamental plant species have been conducted by Kaufman and Landis (2000). These tests confirmed that normal feeding, oviposition and development of *G. californiensis* are confined to *L. salicaria*. However, greenhouse studies and field observations indicate that transient feeding by teneral adults may occur

on some nontarget species under choice conditions (Kaufman and Landis 2000).

Feeding by *G. californiensis* has been observed to feed on swamp loosestrife, *Decondon verticillatus* (L.), and winged loosestrife at the Royal Botanical Garden in Burlington, Ontario (Corrigan et al. 1998). It is believed that this feeding represents a short-term “spill-over” effect, and non-target plants should not experience long-term effects.

Experimental releases of *G. californiensis* and *G. pusilla* were made to improve and evaluate procedures for their use as biological control agents of *L. salicaria* (Hight et al. 1995). The importance of climatic preadaptation, number of individuals released, and containment of release were assessed. The two leaf-feeding beetles became established at all release sites regardless of their origin or release method. It was determined that higher survival occurred in cages with releases of 600 beetles compared with releases of 200 beetles, and the reason remains unclear. Establishment was not affected by the amount of litter, number of standing dead stems, or host-plant density. However, in areas of continuous flooding or where beetles had been exposed to the entomophagous fungus *Beauveria bassiana* (Balsamo) Vuillemin, beetle establishment was reduced. Overall, *G. californiensis* and *G. pusilla* successfully established in a varied range of climatic conditions (Hight et al. 1995).

The potential of the two leaf-feeding beetles to establish in an area approaching the northern limit of *L. salicaria* was studied from 1995 through 1998, at wetlands near North Bay, Ontario, Canada (Dech and Nosko 2002). *G. californiensis* successfully established, while *G. pusilla* failed to reproduce at all in 1998. Small population sizes of *Galerucella* spp. produced low feeding pressure at the sites studied in North Bay, Ontario. McAvoy et al. (1997) reported a similar situation at release sites in Virginia

three years after release, where populations of *Galerucella* spp. had become established; but the small populations had caused minimal impact on host plants. Successful introductions of *Galerucella* spp. in southern Ontario have resulted in large (95%) reductions in the flowering frequency and biomass of *L. salicaria* within five years after release at approximately 10% of release sites (Corrigan et al. 1998). If the populations of *G. calmeriensis* in the North Bay area continue to grow, then classical biological control may be a viable option for managing *L. salicaria* in Ontario (Dech and Nosko 2002).

The effects of distance and conspecifics on colonization were quantified using experiments involving *G. calmeriensis* (Grevstad and Herzig 1997). A mark, release, and recapture approach was used to determine how rates of colonization of host patches of *L. salicaria* are affected by distance from the patch and the presence of conspecifics at the patch from the source of release. Conspecifics already present at a patch strongly induce others to colonize the patch. Beetles arrived at the site even from the farthest release point of 847 m away. The probability of an individual arriving at the patch declined steeply with release distance (4% of dispersers lost for every 10 m traveled). These factors could influence the spread and establishment of *G. calmeriensis* (Grevstad and Herzig 1997).

Mass rearing techniques in the greenhouse and in field conditions have been developed for *G. calmeriensis* and *G. pusilla* to increase the availability of control agents for distribution (Blossey and Hunt 1999). Blossey and Hunt (1999) evaluated the success of different rearing techniques on the number of beetles produced and the effect of these methods on offspring quality, female fecundity, larval development, and adult survival. Field rearings consistently produced two to five times more offspring with higher

survival than greenhouse rearings. Field rearings were less time consuming and produced superior quality beetles. Continuous greenhouse rearing reduced female fecundity. Beetles produced in the greenhouse and field released in May or June began to overwinter soon after release sometimes without an, or with a short, egg-laying period. Mortality was high with few individuals surviving almost a year of dormancy. Allowing beetles to overwinter improved fecundity and rearing efficiency in both greenhouse and field rearing techniques. In smaller cages where beetle density increased, female fecundity more than doubled, which shows a positive fitness effect of aggregations. Monitoring the quality of offspring released in the field will provide direction for needed improvements in the rearing method (Blossey and Hunt 1999).

Situations where an integrated management approach (herbicides along with biological control) of *L. salicaria* would be beneficial may be identified. To develop this strategy the potential effects of the chosen herbicide must be tested against the biological control agent in use (Lindgren et al. 1998, Lindgren et al. 1999). For example, compatibility of glyphosate or triclopyr amine with *G. californiensis* has been examined. Adult beetle treatments were: a direct contact group, an indirect contact group, and a control group. Neither adult survival nor oviposition was impacted by glyphosate or triclopyr amine treatments. Exposed third-instar larvae showed no difference compared to controls on the number of teneral adults to emerge in any of the herbicide treatments. Results from these studies indicate that *G. californiensis* is compatible with the use of glyphosate or triclopyr amine in an integrated situation (Lindgren et al. 1998, Lindgren et al. 1999).

European studies have outlined the biology of the root-boring weevil *H. transversovittatus* and confirmed that it is highly host specific to purple loosestrife (Blossey et al. 1994b). All other known *Hylobius* species in Europe or North America feed on conifers. Screening tests were conducted at the same time that the two leaf-feeding beetles were assessed (Blossey et al. 1994b). Two native non-target plants, winged loosestrife and swamp loosestrife, were attacked during the host range screening, possibly due to artificial test conditions. Neither swamp loosestrife nor winged loosestrife occur on United States endangered and threatened plant species list; however, swamp loosestrife is the only host plant for the endangered moth, *Papaipema sulphurata* Bird. Results of an environmental evaluation using a survey questionnaire of the potential effects of the release of *H. transversovittatus* as a biological control agent in North America indicated that benefits outweigh any potential negative impact (Blossey et al. 1994b). Root herbivory by *H. transversovittatus* can reduce plant height, reduce biomass of all host plant parts, delay and abridge the flowering period and change the biomass allocation patterns in *L. salicaria* (Notzold et al. 1998). High attack rates and continued herbivore pressure will be necessary before purple loosestrife will succumb to herbivory by *H. transversovittatus* (Notzold et al. 1998).

Adult *H. transversovittatus* for the initial introductions in 1992 were offspring of weevils collected in Europe that were reared on potted plants in a common garden in Germany (Hight et al. 1995). A total of 1,331 adults and 30,830 eggs was released in nine states and two Canadian provinces through 1993, and initial establishment was confirmed (Piper 1996, Blossey et al. 2000). *H. transversovittatus* has continued to be raised on potted plants at Cornell University (Ithaca, New York) and more than 100,000

eggs had been distributed by 1999. Field establishment rates appear rather low.

However, adult beetles are secretive and nocturnal so detection is difficult. Rootstocks were not excavated for examination to prevent interference with population build-up (Blossey et al. 2000).

Recent research conducted at Cornell University for the development of the mass rearing of *H. transversovittatus* was undertaken to increase the availability of adults for release which is crucial to increase field populations of the weevil (Blossey et al. 2000). A successful diet and method have been developed. Rearing larvae on artificial diet reduced development time from one to two years to two to three months. The mass rearing method made it possible to output several hundred adults per week at a cost of about \$3.00 per individual. Large scale releases of mass produced adult *H. transversovittatus* began in spring 2000. Increased availability of these adult beetles should accelerate the biological program against purple loosestrife (Blossey et al. 2000).

The host specificity and biology of *N. marmoratus* and *N. brevis* were studied in Europe (Blossey and Schroeder 1995). They have a wide geographical range and develop exclusively on *L. salicaria*. These weevils feed on reproductive organs of purple loosestrife and, as biological control agents, are expected to reduce the number of seeds added to the seed bank (Blossey and Schroeder 1995). In 1994, *N. marmoratus* was released the United States; however, *N. brevis* has been found to be infested with a nematode and this infection has prevented its introduction (Blossey 2002).

The biological control program for purple loosestrife has kept federal and state agencies involved through annual internal reports and through participation in the decision-making processes (Blossey 2002). Due to the fact that many natural resource

managers have been involved in the biological control of purple loosestrife and have been willing to participate in basic research, it has been possible to implement a scientific approach to the entire program with the intention to improve biological control as a science (Malecki et al. 1993, Blossey 2002). Leadership provided by Cornell University (Biological Control of Non-indigenous Plant Species directed by Bernd Blossey) and their willingness to share research results have provided a cooperative environment that allowed the program to move forward at a fast pace (Blossey 2002).

Adventive pests like purple loosestrife often must be controlled by introduced agents from their native origin, as in the classical biological control program discussed above. However, if sufficiently effective North American natural enemies of purple loosestrife do exist, there would be multiple advantages to the use of a native species for regulation of *L. salicaria*. Little research concerning insect diversity associated with *L. salicaria* has been done in Tennessee. Thus, a research project was initiated in 2000 to evaluate insect diversity on purple loosestrife in northeastern Tennessee, in hopes of finding potential native biological control agents. *L. salicaria* is located in eight counties in Tennessee (Unicoi, Scott, Roane, Clay, Warren, Marion, Sumner, and Decatur) where it poses a potential management problem (Fig.1) (Chester et al. 1997). At an early point into this research, during sampling, high densities of a flea beetle were observed chewing irregularly-shaped holes in the leaves of *L. salicaria*. The flea beetle was identified later as *Altica litigata* Fall, a native chrysomelid of North America (Downie and Arnett 1996). Due to the high numbers of these beetles and the intense feeding damage observed, the research was directed to evaluate the relationship of *A. litigata* to purple loosestrife to assess its potential as a biological control agent. The objectives of the research were to:

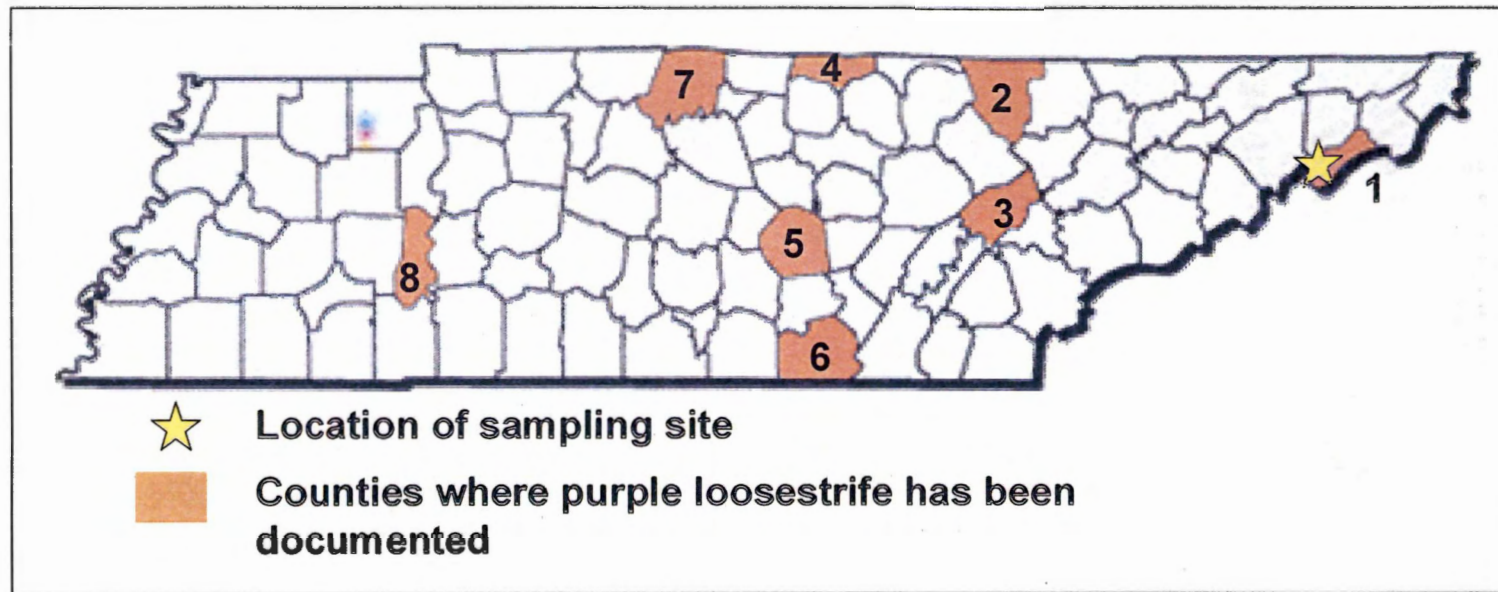


Figure 1. Distribution of purple loosestrife, *Lythrum salicaria*, in Tennessee and location of sampling site. Counties where purple loosestrife has been documented: 1) Unicoi, 2) Scott, 3) Roane, 4) Clay, 5) Warren, 6) Marion, 7) Sumner, and 8) Decatur (University of Tennessee Herbarium 2002).

(1) document biology and seasonal incidence of *A. litigata* on purple loosestrife, (2) assess the impact of herbivory by *A. litigata* on seed production and plant height of purple loosestrife, and (3) to quantitate leaf consumption by adult *A. litigata* on purple loosestrife.

CHAPTER II

BIOLOGY AND INCIDENCE OF *ALTICA LITIGATA* ON PURPLE LOOSESTRIFE, *LYTHRUM SALICARIA*, IN NORTHEASTERN TENNESSEE

i. Introduction

Purple loosestrife is an invasive exotic wetland perennial that forms large monotypic stands across many temperate regions of the United States and Canada. This highly competitive plant replaces native vegetation, degrades wildlife habitats, and obstructs waterways (Thompson et al. 1987). Few studies have assessed the natural enemies associated with *L. salicaria* in its naturalized North American environment. Surveys for natural enemies of purple loosestrife outside of Europe were conducted in the northeastern United States (Hight 1989). From eight collection sites ranging from Massachusetts to Maryland, 59 species of phytophagous insects were collected from purple loosestrife. These included 50 species on foliage, three species on stems, six species on reproductive parts, and no species on roots. None of these species caused appreciable damage to the plant. Insects associated with purple loosestrife in southern Manitoba, Canada were examined, and 41 phytophagous genera were collected, but only 14 were herbivorous on purple loosestrife (Diehl et al. 1997). These herbivores did not reach high enough levels to cause noticeable damage to the large central areas of purple loosestrife present in the survey sites.

A limited study by Snoddy and Oldland (1995) of the Environmental Research Center of the Tennessee Valley Authority of Muscle Shoals, Alabama, reported an *Altica* species (Coleoptera: Chrysomelidae) feeding on a small roadside stand of purple loosestrife in Decatur County, Tennessee. Several adult specimens were sent to the Canadian National Collection of Insects, but a positive identification of the species or its origin was not determined. However, they indicated that this species was taxonomically similar to an *Altica* species that occurs on plants in the primrose family.

An *Altica* species also was found on the leaves of purple loosestrife in New York and Maryland (Hight and Drea 1991). Five *Altica* species are associated with purple loosestrife in Europe [*A. ampelophaga* Guerin, *A. impressicollis* Reiche, *A. lythri* Aube, *A. oleracea* (L.), and *A. palustris* Weise] and one of those, *A. lythri* of northern Italy, was identified as a host specific defoliator of purple loosestrife (Batra et al. 1986).

More than 60 species of *Altica* are known in North America north of Mexico with 26 species occurring in the northeastern United States (Downie and Arnett 1996). The biologies of less than 10% of the North American *Altica* species are known (LeSage 1995), while only those of the most common species, such as the grape flea beetle, *A. chalybea* (Illiger), a common pest of grapes in most all states east of the Rocky Mountains and in Canada, have been recorded (LeSage 1995, Taechenberg and Riedl 1985). *Altica* species are difficult to identify and are greatly in need of revision. LeSage (1995) recently revised the costate species of *Altica* north of Mexico.

Altica litigata Fall, a little known species, was first described by Fall (1910) from specimens collected in Florida and has since been recorded in Indiana, New York, Alabama, Texas, and California (Downie and Arnett 1996). It has been documented to

feed on several plants including water purslane, *Ludwigia palustris* (L.) Elliott var. *americana* (DC.) Fernald Griscom, and evening primrose, *Oenothera* sp. (Family: Onagraceae) (Downie and Arnett 1996). In 1999, *A. litigata* was reported on crepe myrtle, *Lagerstroemia indica* L. (Family: Lythraceae), in North Carolina (Baker and Bambara 1999). Only brief notes about the biology of *A. litigata* can be found such as the fact that the oblong yellow eggs are laid on leaves and that mature larvae pupate in the soil (Barko 2002).

During sampling in spring 2000, *A. litigata*, a native chrysomelid of North America, was found on purple loosestrife in Unicoi County, Tennessee. Since limited information about *A. litigata* exists, any new information is extremely beneficial and adds to our body of knowledge. Because *A. lythri* of Italy had been designated as a possible biological control agent of purple loosestrife (Batra et al. 1986), this discovery of *A. litigata* on *L. salicaria* heightened our interest in its potential as a biological control agent. Due to the high numbers of these flea beetles and the intense feeding damage observed, research was directed to evaluate the relationship of this chrysomelid to *L. salicaria*. The objectives of this research were to: (1) document biology of *A. litigata* on purple loosestrife and (2) document seasonal incidence and abundance of *A. litigata* on purple loosestrife.

ii. Materials and Methods

Description of Study Area

The study site was located near Highway 23 at exit 15 in Unicoi County, Tennessee, along the banks of South Indian Creek (36° 07.46 N, 82°26.87 W) at 0.6 km from confluence with the Nolichucky River, where a small infestation (0.1- 0.5 ha) of

medium density (50 – 1000 plants) of purple loosestrife exists (Keddy 1992, Ontario Federation of Anglers and Hunters 2001). Unicoi County is nestled among the southern Appalachian Mountains of northeastern Tennessee. Highway 23 was under construction in the early 1990s, and this wetland site was disturbed by the building of a new bridge (engineer Carroll Boone, Tennessee Department of Transportation, personal communication). Biologist Joe McGuiness (National Forest Service of Unicoi County) noted *L. salicaria* as a potential problem in the immediate area in the mid to late 1990s (personal communication). The wetland area collects runoff from the steep mountainous region on one side and water overflowing the banks of South Indian Creek during flooding on the stream bound side. During periods of drought the soil at the site dries.

Noted plants in the vicinity besides *L. salicaria* were: sycamore, *Platanus occidentalis* L., willow, *Salix alba* L., arrow-leaved tearthumb, *Polygonum sagittatum* L., path rush, *Juncus tenuis* Willdenow, broad-leaved cattail, *T. latifolia*, common plantain, *Plantago major* L., curly dock, *Rumex crispus* L., bedstraw, *Galium aparine* L., shallow sedge, *Carex lurida* Wahlenb, fox sedge, *C. vulpinoidea* Michaux, spotted touch-me-not, *I. capensis*, reed canary grass, *Phalaris arundinacea* L., and goldenrod, *Solidago* spp. L. Besides purple loosestrife, other exotic pest species identified were multiflora rose, *Rosa multiflora* Thunberg, and musk thistle, *Carduus nutans* L.

Evaluation of Biology and Seasonality of *A. litigata* on Purple Loosestrife

Life History Studies: *Development in Petri Dishes, Measurements of the Life Stages, Caged Development Study, and Field Observations of Biology.* Life history information of *A. litigata* on purple loosestrife was obtained from observations in the laboratory and field in 2000. Research resources and emphasis were on impact studies in 2000, with a

more detailed life history study planned for 2001. This planned study did not occur because only a few beetles were collected in April 2001. Stands of purple loosestrife also were searched in other areas of Unicoi, Marion, and Sumner counties in 2001, but no populations of *A. litigata* were located. *A. litigata* to be used for preliminary life history studies were collected at the Unicoi site on 7 June 2000, taken to the laboratory, and held in containers with cut stems of purple loosestrife (replenished daily).

Development in Petri Dishes. Leaves with egg masses were removed and placed in petri dishes on 19 June 2000. *A. litigata* were allowed to develop from egg to adult on purple loosestrife leaves (foliage replenished as needed) kept in petri dishes (Fisherbrand sterile polystyrene 100 x 15 mm) with moistened filter paper (Whatman #2, 90 mm circles) in the laboratory [ca. 22° to 25° C, natural lighting, and 16:8 h (light:dark) photoperiod]. Time in days was recorded for eggs ($n = 38$) to hatch. Larvae ($n = 3$) from hatched eggs were kept separately in petri dishes as described above and instar stages were monitored. A single larva was observed on an eight-hour schedule for number of molts with exuvium being removed after each molt. Number of days for each larval stage and the pupal stage until adult emergence was recorded. Of ca. 200 beetles, 37 were parasitized (dead beetles and emerging parasitoid larvae were monitored until parasitoid adults emerged).

Measurements of the Life Stages. Measurements of *A. litigata* in different stages were taken using a stereo microscope. Lengths and width of eggs ($n = 253$), larvae ($n = 20$), and one pupa were measured. Eggs were measured to the nearest 0.02 mm at approximately 1.0 power with a 2x converter. Larval and pupal stages were measured to the nearest 0.05 mm at approximately 2.6 power with a 2x converter, and adult lengths [$n = 24$ and $n=40$ (19 females and 21 males)] were measured to the nearest 0.10 mm.

Caged Development Study. A single pot (10 cm deep by 10.5 cm across) containing three or four *L. salicaria* plants (approximately 20 cm tall) was placed in each of five cages (30.5 x 30.5 x 41 cm Plexiglass cage with screen mesh windows and a cloth sleeve entrance). These plants had been grown in commercial potting soil from seeds collected from senesced plants at the Unicoi site in February 2000. *A. litigata* were placed in the cages on 13 June 2000. Four mating pairs were introduced into each cage and removed in five days, but were returned on 19 June 2000 and removed again on 21 June 2000 once eggs were oviposited on plants in most cages. One cage per week (cage 4 and 5 were searched the 5th week) was examined for the number of eggs, larvae, pupae, or emerging adults.

Field Observations of Biology. Life history information and other biological data were gathered by observing *A. litigata* during direct sampling (method described below) on each collecting date. Information was recorded in a field notebook along with the date, time of day, general weather condition, and location. Soil and litter samples were collected (method described below) and examined to determine overwintering stages.

Assessment of Seasonality and Abundance. To monitor seasonal incidence and abundance of *A. litigata* on purple loosestrife, the plot was measured and divided into three subplots, and each subplot was sampled weekly (sweep and direct samples) from May until early September, 2000 and 2001. Plot size measured 27.4 m by 6.1 m (90 ft by 20 ft), and each subplot was 9.1 m by 6.1 m (30 ft by 20 ft). Estimates of adult and larval insect densities were measured weekly by taking five sweep-net samples per subplot. Sampling methods are described below.

Insect Sampling

Direct Sampling. Direct collections were made weekly from each subplot in 2000 and 2001 from May to September. Twenty minutes were spent in each subplot for a total of 1 hour per sampling date. Samples were collected directly from the foliage, flowers, or stems using netting and handpicking. Collected insects were placed in a Ziploc plastic bag (17.78 cm by 20.32 cm) or vial (3.5 dram) and labeled with the site, subplot number, infested plant part, and sampling date. Collected specimens were placed in an ice chest, taken to the laboratory, and processed.

Leaf Litter and Soil Sampling. In 2001 and 2002, leaf litter and soil samples (2 per subplot) were collected on two dates (6 April and 20 April 2001, and 10 April and 26 2002). Leaf litter samples were taken by removing detritus from the surface of the ground, and then soil samples were collected with a small spade to a depth of 15 cm. Samples were placed into individual Ziploc plastic bags (17.78 cm by 20.32 cm), labeled, and taken to the laboratory. Samples were placed into a white larval tray (23.4 cm by 18.6 cm) in small increments and examined under a stereo microscope until the complete sample was inspected. In addition, on each collecting date, several random litter and soil samples per subplot were visually assessed at the site using a 10x magnifying lens.

Sweep-net Sampling. Five sweep-net samples (38 cm diameter, 82 cm deep) were taken randomly from each subplot weekly during 2000 and 2001 from May to September. Each sweep-net sample consisted of five sweeps, and each sweep traversed an arc of 180° through the purple loosestrife. Insects were transferred into Ziploc plastic freezer bags (17.78 cm by 220.32 cm), labeled with the date, site name, subplot number, placed in an ice chest, and taken to the laboratory for processing.

Processing and Identification of Collected Specimens

Adult beetles were placed on a point before pinning and then labeled with date and location collected. Extra adult beetles and larvae were stored in vials (4 dram) containing 70% ethanol and properly labeled. All diagnostic characteristics of the aedeagus of *Altica* spp. are external (LeSage 1995). Therefore, the median lobe was removed, and the aedeagus was glued ventral side up at the tip of a small point, which was pinned under the specimen. Adult pinned beetles were sent to Alexander Konstantinov (Research Entomologist, Curator of Chrysomelidae, Systematic Entomology Laboratory, USDA-ARS, National Museum of History, Washington, D. C.) and identified as *A. litigata*. Adult specimens were also sent to Laurent LeSage (Agriculture Canada, Biosystematics Research Center) who also identified the beetles as *A. litigata*. However, he did not have a Fall type specimen to confirm his identification. Voucher specimens will be stored in the insect museum at The University of Tennessee.

Data Entry and Analysis

Data were entered into spread sheets using Microsoft Excel® software (Microsoft Corporation 2002). Averages and standard deviations of egg, larva, pupa, and adult measurement data were calculated, percentage of eggs per mass and percentage of eggs with fecal matter also were determined. Adult density data entered consisted of the number of *A. litigata* collected per subplot for each collecting date by sweep-net sampling. Total and average numbers of beetles on each collecting date were calculated to arrive at estimates of adult beetle densities.

iii. Results and Discussion

All life stages of *A. litigata* were found in association with purple loosestrife, and these results provide the first documentation of biology, seasonality, and abundance of *A. litigata* on this invasive plant species. The incidence of this flea beetle on purple loosestrife also represents a new state record.

Life History. Development in Petri Dishes and Measurements of the Life Stages.

Developmental time and average length and width measurements are presented for each life stage of *A. litigata* (Table 1, Fig. 2). The yellow fecal marked eggs are oval to somewhat cylindrical in shape. Fecal matter was found on 90.5% of the eggs, which measured an average of 0.71 mm long and 0.33 mm wide. Eggs were usually oviposited underneath leaves and were found in unorganized masses averaging 6.5 eggs per mass (range 2 to 35). Eggs hatched in 3 to 5 days and young larvae were yellowish in color. Larvae skeletonized leaves as they fed in small groups and caused severe defoliation. In the laboratory three larval instars were observed lasting from 13 to 16 days. Duration of first, second, and third instars were 5, 3, and 8 days, respectively (successfully recorded only one larva, so no conclusions can be made). Larvae darkened (brownish) as they matured and crawled under the moistened filter paper to pupate. Pupae were yellow with rusty red eyes, and the pupal stage lasted 7 to 10 days. Elongate oval, moderately convex, and dark metallic green adult beetles emerged. Adult *A. litigata* were 3.2-4.5 mm long. Of a random sample of 40 adults measured for length, 21 were males and 19 were females, indicating a sex ratio of ca. 1:1. Adults that were captured in the field and kept in the laboratory lived approximately 60 days; however, actual longevity could not be determined because the age of collected beetles was not known. Of ca. 200 adult

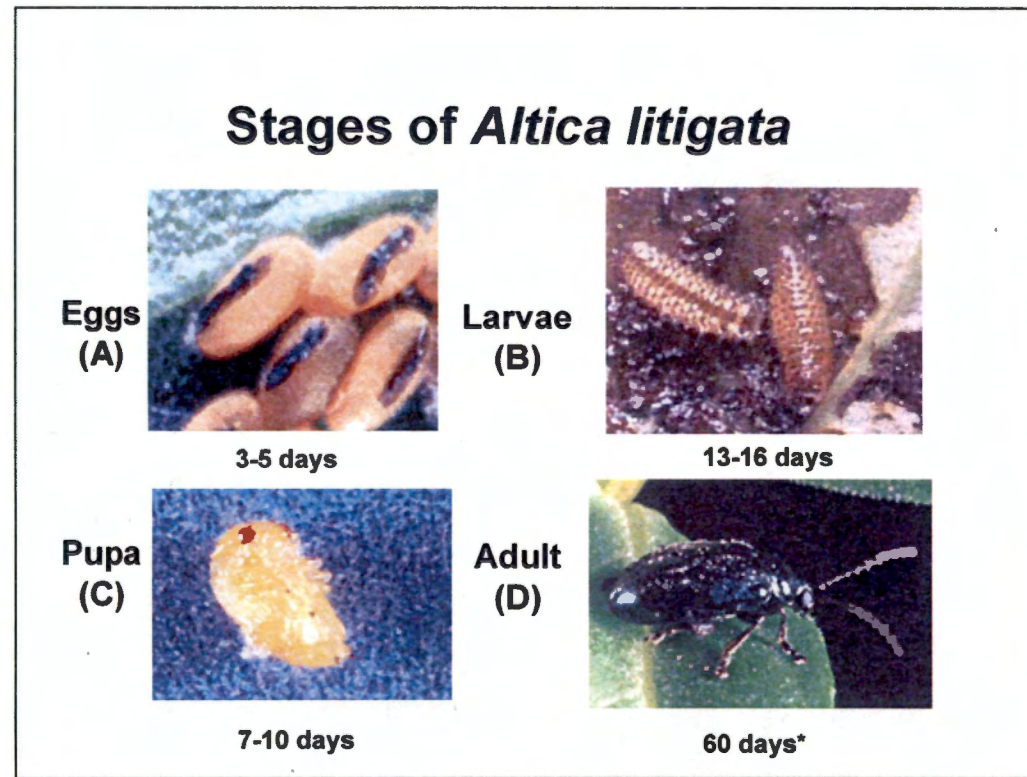
Table 1. Mean measurements of developmental stages of *Altica litigata* from preliminary studies, 2000.

Stage	Number	Length (mm)		Width (mm)	
	n	Mean ± SD	Range	Mean ± SD	Range
Egg	253	0.71 ± 0.06	0.54 - 0.82	0.33 ± 0.03	0.24 - 0.40
Larva	20	4.13 ± 0.66	2.85 - 5.00	1.29 ± 0.16	1.05 - 1.55
Pupa	1	2.80*	*	1.30*	*
Adult	24	3.73 ± 0.29	3.20 - 4.20	**	**
Adult♂	21	3.69 ± 0.25	3.20 – 4.00***		
Adult♀	19	4.05 ± 0.19	3.80 - 4.50***		

*Only one data point.

**Adult width was not measured.

***Of a random sample of 40 adults measured for length, 21 were males and 19 were females, indicating a sex ratio of ca. 1:1.



*Adult beetles captured and observed in the laboratory lived approximately 60 days.

Figure 2. Photographs and durations of the developmental stages of *Altica litigata* [(A) Eggs, (B) Larvae, (C) Pupa, and (D) Adult] found on purple loosestrife, *Lythrum salicaria*, in Unicoi County, Tennessee, 2000.

beetles monitored, ca. 5% of those adults died from parasitoids (five hymenopteran and five dipteran).

Caged Development Study. The life history of *A. litigata* was evaluated in cages. On 26 June 2000, cage 1 was examined (including soil search) and eight eggs were found, but larvae were found in cages 3, 4, and 5. Mating pairs had been removed on 21 June 2000, so all eggs except the eight eggs of cage 1 had hatched within the 3- to 5-day incubation period that was observed in the laboratory petri dish studies. On 3 July 2000 no life stages were found in cage 2 on purple loosestrife plants or in the soil. Mating pairs had behaved abnormally previously in cage 2 moving to the screened window instead of staying on the plants; possibly the cage had been contaminated with something that interfered with mating and oviposition. The other three cages were observed on 3 July 2000, and 49 larvae were present in cage 3 with some larvae crawling on the cage floor probably searching for food, since the plants had been defoliated and turned brown. Larvae also were present in cages 4 and 5. Cage 3 was assessed on 10 July 2000 with three larvae and 19 pupae present. Larvae that had fallen to the bottom of the cage died. On 25 July 2000 cage 4 was examined and 14 adults were present. On that same date cage 5 was searched, and 3 pupae and 19 adults were found. Pupae present were located buried in soil at depths up to 50 mm. Larval and pupal development time approximated that observed in the petri dish study given above.

Field Observations of Biology. Adult beetles aggregated in mass while feeding on purple loosestrife in the field, and the mass would shift together throughout the subplots during the day (Fig. 3). During feeding, adults chewed irregular holes in leaves on the upper

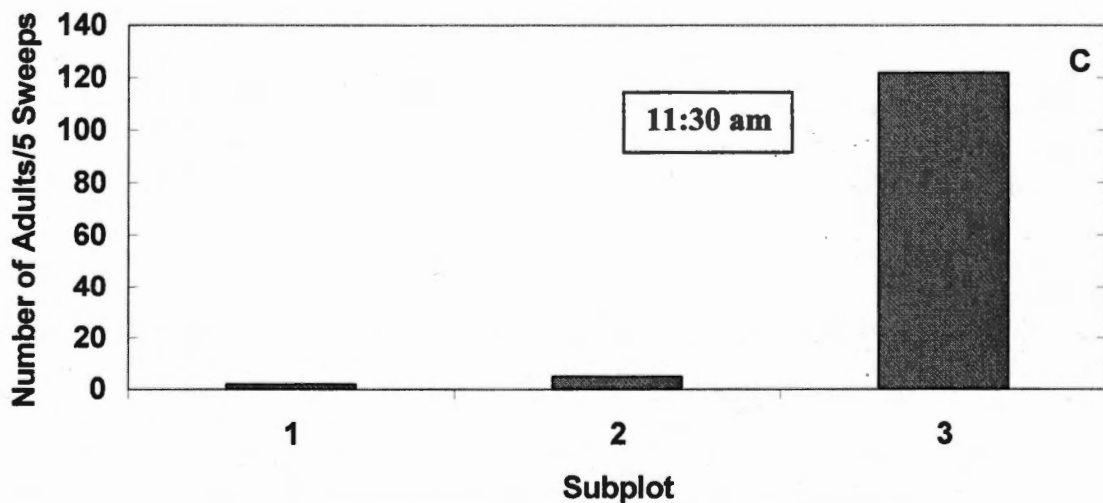
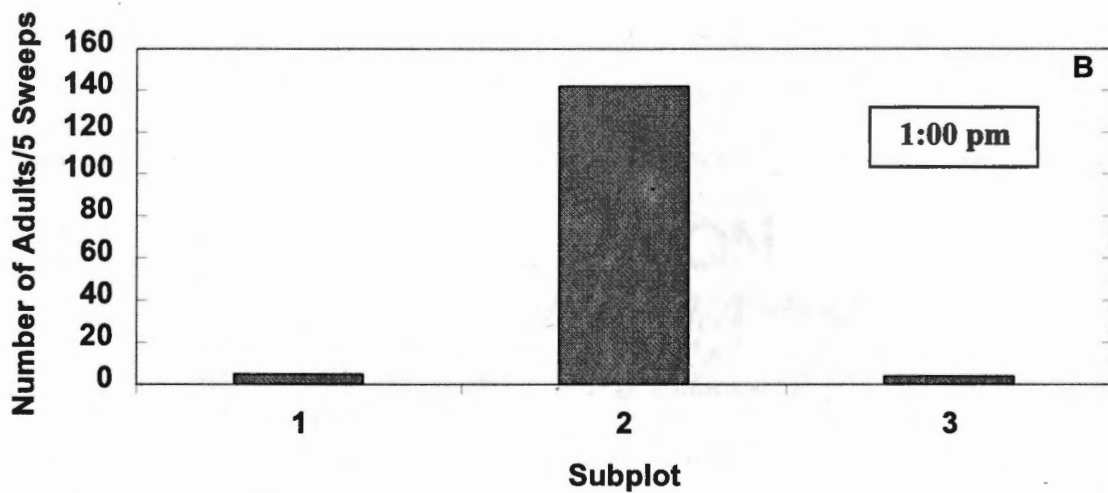
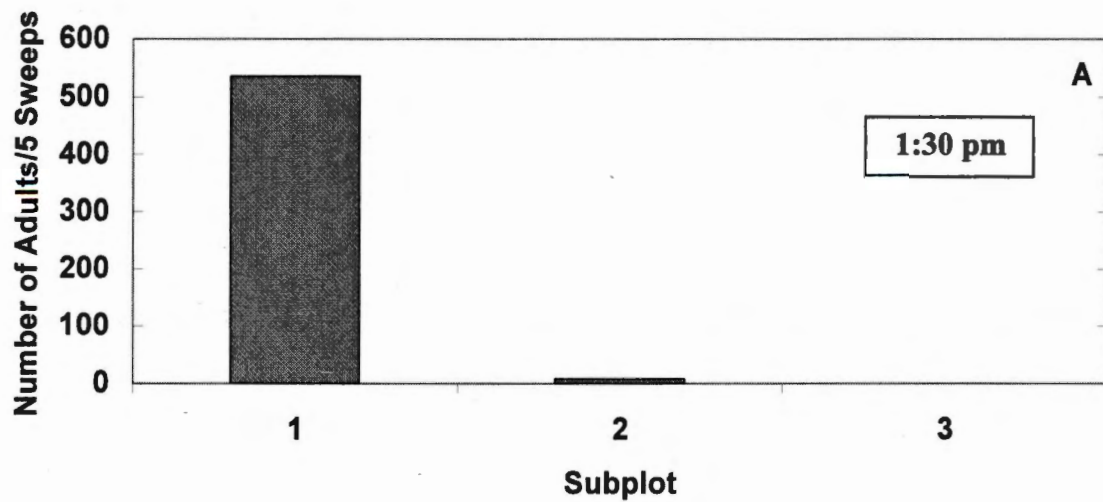


Figure 3. Densities of adult *Altica litigata* in subplots on (A) 25 May, (B) 1 June, (C) 8 June 2000. Note that high densities in specific subplots from date to date show that adults move in masses.

1/3 of the purple loosestrife plant. Where the mass of beetles had moved through, clumps of plants would show signs of heavy feeding damage, while plants in close proximity would not be affected. Adult beetles were observed crawling underneath the leaves of purple loosestrife on hot ($>31^{\circ}\text{C}$) continuously sunny days. Males (3.2-4.0 mm) observed during mating were noted to be smaller than females (3.8-4.5 mm) (Table 1).

A carabid beetle, *Lebia viridis* Say is predaceous on other *Altica* species, such as *A. carduorum* Guerin-Meneville (Peschken 1977, Downie and Arnett 1996), and was collected on several sampling dates in sweep-net and direct samples. This carabid was implicated as the major predator interfering with the establishment of *A. carduorum* for the control of Canada thistle, *Cirsium arvense* (L.) Scopolic (Peschken 1977, Wiebe and Obrycki 2002). Larvae of *L. viridis* are ectoparasitoids of *A. foliaceae* LeConte pupae (Capogreco 1989). Even though predation or parasitism of *A. litigata* by *L. viridis* was not observed, future research would investigate this possibility.

No *A. litigata* at any stage were recovered in the litter or soil site samples taken in early spring, so their overwintering habits are unknown. Laurent LeSage (personal communication) commented, “based on my experience, the biological pattern of *Altica* species of a given locality is identical (or almost), the main difference being only the host-plant.” *A. chalybea* have been reported in Tennessee, and they have only one generation each year and overwinter as adults, so *A. litigata* probably overwinters as an adult and also may be univoltine (Tennessee River Gorge Organization 2001).

Seasonality and Abundance of *A. litigata*. Adult *A. litigata* and eggs were first observed on field populations of purple loosestrife on 18 May 2000, but were probably present the previous week. Larvae were first documented on 8 June 2000, with highest

densities on 13 June 2000 (26/5 sweep-net samples/subplot), and none were collected in sweeps 22 June 2000. Adult densities were high a few weeks with the highest densities (180/5 sweep-net samples/subplot) on 25 May 2000 (Fig. 4). No adults were recovered after 13 June 2000. Adult beetles that were captured and kept in the laboratory lived about two months, so it is unclear why they disappeared from the sampling site. High numbers were present in 2000, and only a few adult *A. litigata* were collected in 2001.

Flooding may have affected survival of pupae in the soil and subsequent adult emergence. Sweep-net samples collected on 13 June 2000 yielded 53 larvae from subplot 1, 26 larvae in subplot 2, and none in subplot 3. The site was heavily flooded on 29 June 2000. Many beetles would have been in the pupal stage and may not have survived. In 2001 the site experienced drought conditions in spring followed by periodic flooding throughout the summer. These conditions may have interfered with adult emergence. The fluctuation in population densities of *A. litigata* from year to year suggest several possibilities, such as: the immediate habitat was not conducive for colonization by the beetle; the insect is nomadic; it prefers other hosts plants; or the life cycle is similar to that of the cereal leaf beetle, *Oulema melanopus* (L.), which shifts host plants (from small grains to grasses) for part of the cycle (Herbert and VanDuyn 1999).

iv. Summary

Biology, seasonality, and abundance of *A. litigata* on purple loosestrife in northeastern Tennessee were evaluated during 2000 and 2001. All life stages of the flea beetle were found in association with purple loosestrife. This study provided the first documentation of biology, seasonality, and abundance of *A. litigata* on purple loosestrife.

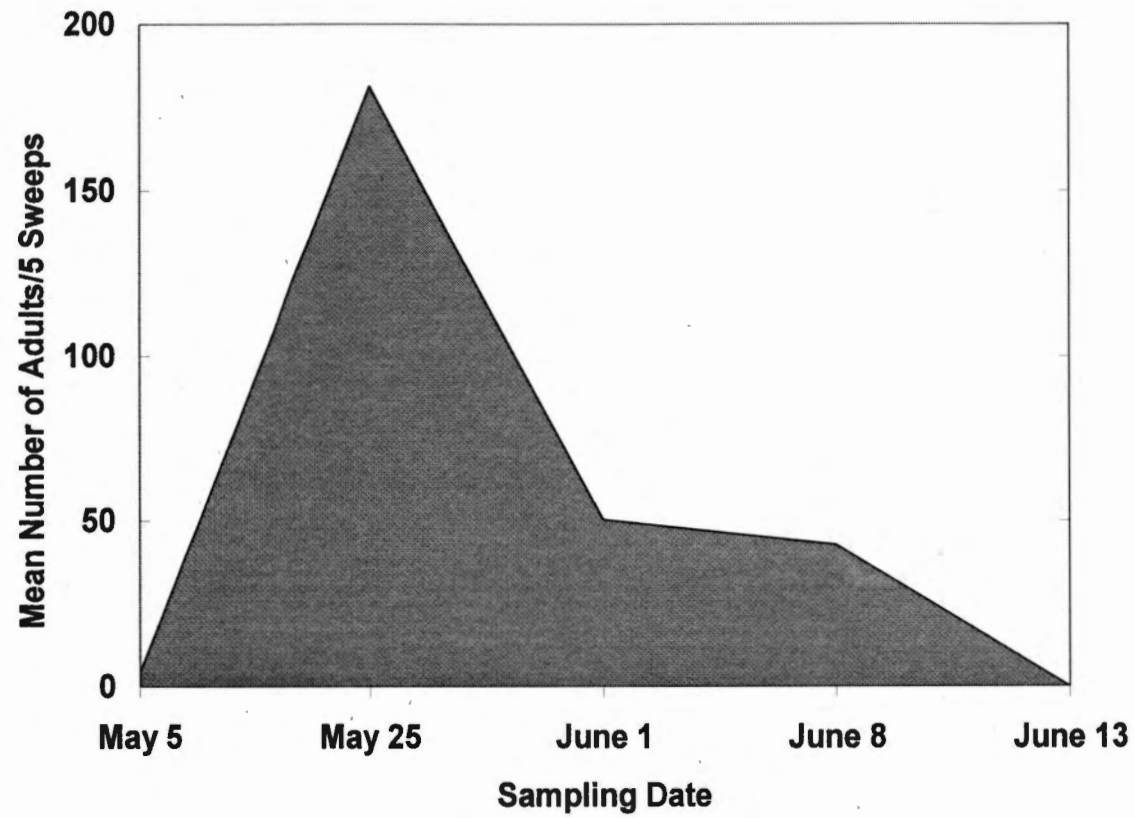


Figure 4. Average densities of adult *Altica litigata* on purple loosestrife, *Lythrum salicaria*, in northeastern Tennessee, 2000.

The incidence of this flea beetle on purple loosestrife also represents a new state record for Tennessee. Preliminary findings of the life history of *A. litigata* were given along with notes of behavior and other biological findings.

Flea beetle densities were high in 2000, and only a few were found in 2001. These results raise some interesting ecological questions about *A. litigata* that need to be addressed. What other hosts does it prefer?; Where did it go when it left the site?; and How does it survive the winter?

Because there is limited information about *A. litigata*, any new fact is extremely beneficial and adds to our body of knowledge. A better understanding of the biology and life history of *A. litigata* on purple loosestrife may lead to its use as a biological control agent. Since it is a native species there would be tremendous advantage if we could use it for control of an exotic species. For example, a native species should have less negative environmental consequences than an introduced species when used as a biological control agent. In addition, the expense normally involved with exploration and quarantine of an introduced biological control agent would not be a factor when using a native species.

It is important to understand any natural enemies of a potential biological control agent. *A. litigata* was parasitized by both hymenopteran and dipteran parasitoids, and *L. viridis* poses a potential threat to *A. litigata*. The relationship of these insects to *A. litigata* needs to be more fully explored in future research.

CHAPTER III

IMPACT OF HERBIVORY BY *ALTICA LITIGATA* ON SEED PRODUCTION AND PLANT HEIGHT OF PURPLE LOOSESTRIFE, *LYTHRUM SALICARIA*

i. Introduction

Altica litigata is an insect herbivore that has been observed to feed on purple loosestrife in northeastern Tennessee. Insect herbivores may influence plant growth, population dynamics, and plant performance in diverse ways, including: reduced flowering and reduced number of seeds produced; reduced seed survival after dispersal and reduced seedling survival; loss of plant biomass from defoliation; reduced growth and reproduction; reduced competitiveness with other plants; reduced root reserves and increased susceptibility to disease and abiotic stress; and increased mortality of established plants (Van Driesche and Bellows 1996). Most plants are able to compensate for low attack levels (Crawley 1989). Frequently, however, effects of herbivores are considered detrimental. Understanding the relationship of insect herbivores associated with purple loosestrife is of particular interest.

The exotic invasive purple loosestrife forms dense monospecific stands in disturbed wetland ecosystems, which displace valuable native wetland species (Thompson et al. 1987). Seed dispersal is the major source of spread of this weed. *L. salicaria* is prolific in seed production, averaging 2.7 million seeds per mature plant (3-5 years old). Extensive seed banks are formed by these plants, and studies have shown that seedlings of *L. salicaria* recruit more successfully than native species (Welling and

Becker 1990). Indirect effects of feeding by insect herbivores, which lead to reduced plant vigor and survival, can in turn affect flowering and seed output of *L. salicaria* (Blossey and Schroeder 1995). The current control strategy for *L. salicaria* focuses on classical biological control which may provide long-term, economical, environmental friendly and sustainable management of this invasive weed (Blossey and Schat 1997).

Previous studies have not revealed any native natural enemies associated with *L. salicaria* which cause any appreciable damage in its naturalized home of North America (Hight 1989). A native North American flea beetle, *A. litigata*, feeds by leaf defoliation on *L. salicaria* in Unicoi County, Tennessee. It is of interest to learn if leaf consumption by *A. litigata* will negatively affect plant performance. The potential of *A. litigata* as a biological control agent merits investigation. The objectives of this research were to assess the impact of herbivory by *A. litigata* on seed production and plant height of *L. salicaria*.

ii. Materials and Methods

Descriptions of the study area and sampling methods were described in general in Chapter II. The techniques used for evaluating the impact of adult and larval leaf defoliation by *A. litigata* on seed production and plant height of purple loosestrife are outlined below.

Evaluation of Impact of Herbivory on Seed Production and Plant Height

Impact of Leaf Consumption on Seed Production and Plant Stem Height, 2000. To assess the impact of leaf consumption on the number of seed capsules and seeds per capsule produced per plant stem and on plant height, plants were evaluated and placed into damage rating categories. Individual plant stems were evaluated on 7 June 2000 and

placed into four damage categories based on the amount of leaf defoliation caused by adults: 1) no damage, 2) minor damage, 3) moderate damage, and 4) major damage. On 21 June 2000 additional plant stems with extensive damage caused by larval feeding by *A. litigata* were included as a 5th damage category. All plants were evaluated by the same person, and a visual damage rating guide was made by photographing an example of a leaf representative for each damage category (Fig. 5). Ten plant stems were selected and tagged for each damage category in the same subplot (subplot 1, 9.1 m by 6.1 m) and subjected to similar environmental conditions. Plant stems were measured weekly from 7 June through 1 September 2000, and important biological information was documented, such as: damage by other herbivores, anthesis of flower buds, presence of seed capsules, and damage by abiotic factors. Plant heights were measured once every two weeks in August. On 1 September 2000, the plant shoots were harvested and taken to the laboratory. Recorded data included the number of capsules per plant stem, the average number of seeds per capsule per plant stem, the number and lengths of seed-bearing laterals, the average number of seeds per plant stem and plant height. Ten randomly selected unopened capsules were chosen from each stem that produced capsules, and the number of seeds per capsule was counted. The average number of seeds was multiplied times the total number of capsules per plant stem to arrive at an average number of seeds per plant stem.

Percentage of Herbivory by Adult A. litigata, 2000. To assess percentages of herbivory caused by adult *A. litigata* within the plot, a one square meter frame (1.27 cm diameter PVC pipe) was tossed randomly into each subplot per evaluation date (on 31 May 2000 and 12 June 2000), and all stems within the frame were evaluated and categorized into

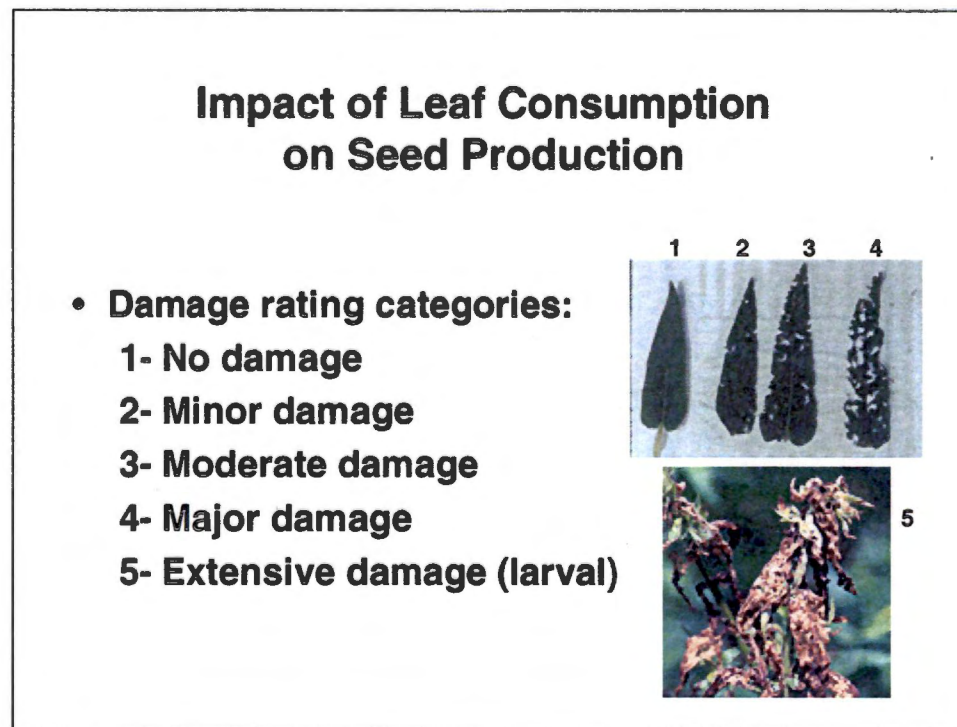


Figure 5. Damage rating categories used to measure impact of herbivory by *Altica litigata* on purple loosestrife, *Lythrum salicaria*. Categories 1-4 were caused by adults and category 5 by larvae. Purple loosestrife leaves photographed for the visual damage rating guide were from the South Indian Creek Site, Unicoi County, Tennessee, 2000.

one of the four adult damage categories and counted. Percentages of herbivory per damage category per assessment date were determined by totaling shoots per category from the three subplots and dividing by the total number of shoots.

Additional Plant Performance Study of 20 Tagged Plants, 2000 and 2001. Twenty plant stems were randomly selected and tagged within the plot on 18 May 2000 to be measured weekly until 1 September 2000. Six plants were tagged in the shadeless subplot 1, nine plants in the partially shaded subplot 2, and five plants in shady subplot 3. All plants could not be measured 29 June 2000 due to heavy flooding. The measurements of 20 July 2000 were not included because several plants were not measured by the staff substitute. Only two measurements were recorded from August until 1 September 2000. A plot map was drawn to show the location of the 20 plant stems throughout the three subplots for future reference in locating the plants (Fig. 6). Biological notes were made weekly which included: damage by herbivores, anthesis of flower buds, presence of seed capsules, and damage by biotic and abiotic factors. Plants were harvested 1 September 2000 and taken to the laboratory. Recorded data included the number of capsules per plant stem, the average number of seeds per capsule per plant stem, the number and lengths of seed-bearing laterals, the average number of seeds per plant stem, and plant height. This study was repeated in 2001; plant stems were selected and weekly measurements began on 5 May 2001. Plant stems were harvested on 11 September 2001, and data were collected.

Estimates of Plant Stem Density 2000, 2001 and 2002. To assess plant stem density of the plot, a one square meter frame (1.27 cm diameter PVC pipe) was tossed randomly into each subplot per date (31 May 2000, 12 June 2000, 28 June 2001 and 30 May 2002).

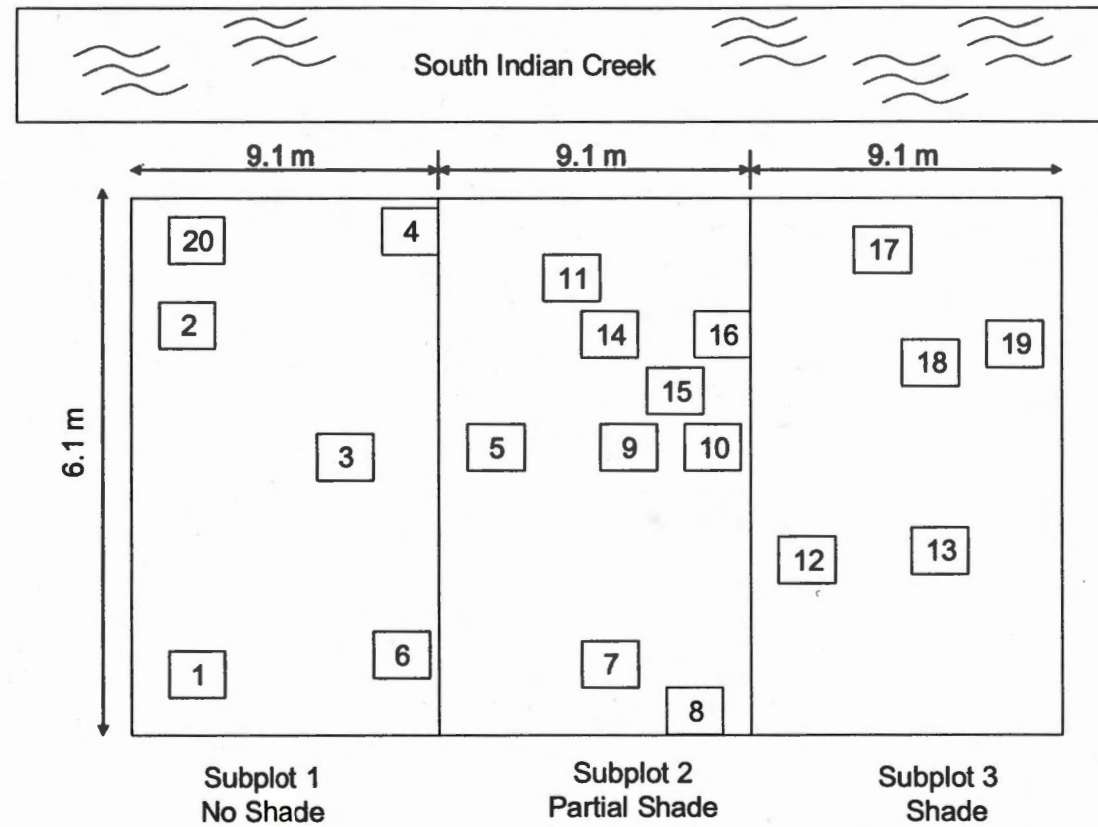


Figure 6. Map of 20 tagged purple loosestrife, *Lythrum salicaria*, stems showing their location within each subplot.

All plant stems were counted within the square meter. Average stem density (stems/square meter) was derived by averaging stem densities of the three subplots. An estimate of total plant stems for the plot was calculated by multiplying the square area of the plot times the average stems per square meter.

Database Entry and Analysis

Data were entered into spread sheets using Microsoft Excel® software (Microsoft Corporation 2002). The number of capsules per plant stem, the average number of seeds per capsule, the number and lengths of seed-bearing laterals, and plant heights from each subplot on each sampling date were entered and tabulated. Once entered data were analyzed by one way analysis of variance F test ($p=0.05$). The Tukey Kramer procedure was applied for multiple comparisons and standard error was calculated (Levine et al. 2001). Data concerning capsules and seeds were square-root transformed to assume normal probability plots. Plant height data were untransformed. Data entered for incidence of herbivory by adult *A. litigata* consisted of the number of shoots per square meter per damage category per subplot, on each sampling date and percentage of feeding damage per category was calculated. Data entered for plant stem density were the number of shoots per square meter per subplot per assessment.

iii. Results and Discussion

Results demonstrate that leaf defoliation by *A. litigata* negatively affects plant performance in the areas of seed production and plant height of *L. salicaria*.

Impact of Leaf Consumption on Seed Production and Plant Stem Height, 2000. Leaf defoliation of *L. salicaria* by *A. litigata* impacted the mean number of capsules produced per plant stem according to damage categories as follows: (1) no damage produced 685

capsules, (2) minor damage produced 607 capsules, (3) moderate damage produced 697 capsules, (4) major damage produced 724 capsules, and (5) extensive damage produced 52 capsules. Extensive damage was caused by larvae, while the other levels of damage were inflicted by adults. The extensive larval feeding was significantly ($p < 0.05$) different from all other adult damage categories and resulted in a 92% reduction in the number of capsules per plant stem (Fig. 7). Large carbohydrate reserves in the woody crown and roots help *L. salicaria* combat stress (Katovich et al. 1999). It appears that the levels of damage caused by adults were not severe enough to exhaust the large carbohydrate reserves and resulted in less of an impact on plant performance, than the more severe larval herbivore damage. The average number of seeds per capsule for each of the five categories (no damage, minor damage, moderate damage, major damage, and extensive damage) of leaf defoliation was 89, 69, 66, 74, and 56, respectively (Fig. 8). The major and moderate damage categories numerically produced more capsules per stem than the no damage category, but they did not produce more seeds per capsule.

The mean number and lengths of seed-bearing laterals per damage categories were: (1) no damage-10 laterals, 16 cm long, (2) minor damage-10 laterals, 15 cm long, (3) moderate damage-14 laterals, 17 cm long, (4) major damage-12 laterals, 14 cm long, and (5) extensive damage-two laterals, 10 cm long (Table 2). The number of seed-bearing laterals was significantly different ($p < 0.05$) among damage categories: (1) extensive damage showed an 80% reduction compared to no damage; (2) extensive damage resulted in 79% reduction compared to minor damage; and (3) extensive damage

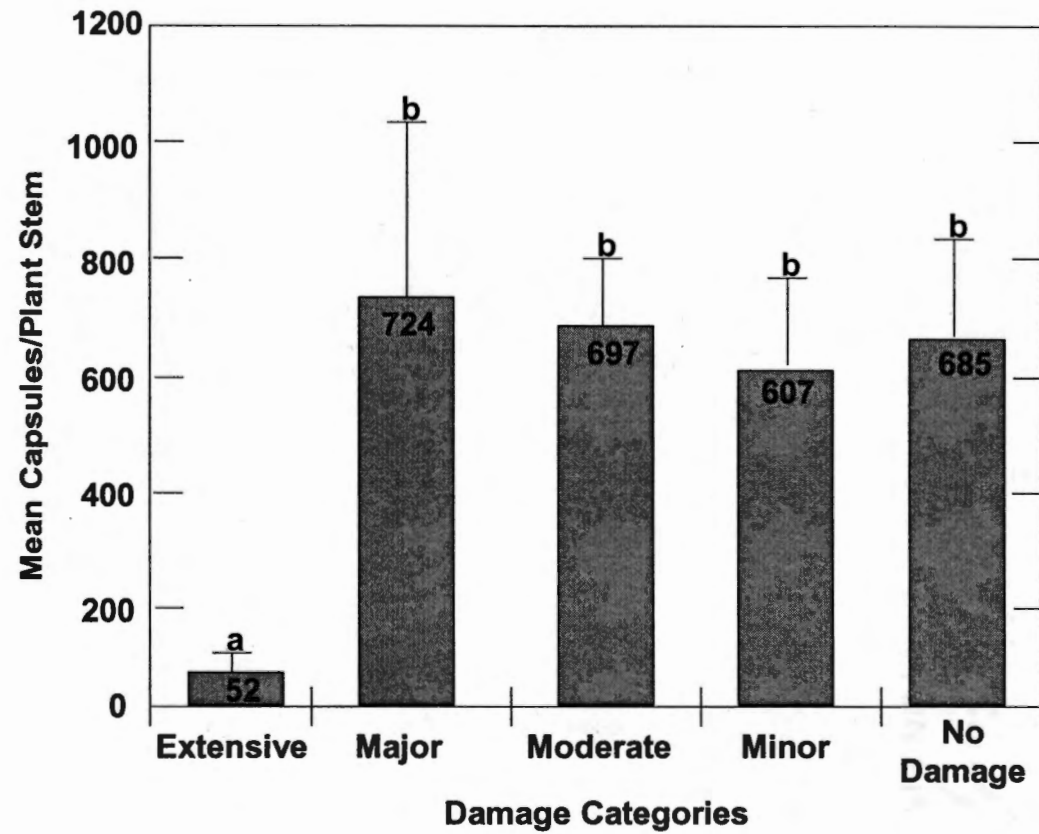


Figure 7. Mean $\pm$ SE number of capsules per plant stem per damage rating category of purple loosestrife, *Lythrum salicaria*, 2000. Means with different letters are statistically different ($p < 0.05$).

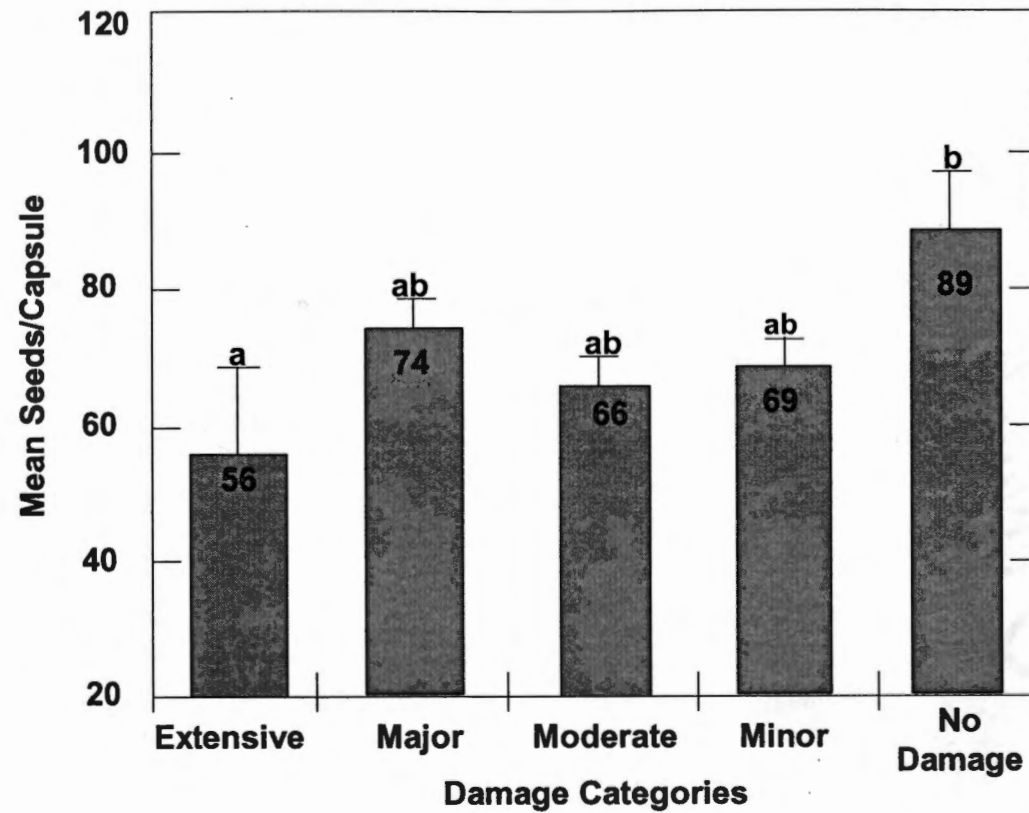


Figure 8. Mean $\pm$ SE number of seeds per capsule per damage rating category of purple loosestrife, *Lythrum salicaria*, 2000. Means with different letters are statistically different ($p < 0.05$).

Table 2. Mean number and lengths of seed-bearing laterals from purple loosestrife, *Lythrum salicaria*, stems, 2000.

Herbivory Damage Categories	*Mean Number	**Mean Lengths (cm)
	Seed-Bearing Laterals ($\pm$ SD)	Seed-Bearing Laterals ($\pm$ SD)
No	10.3 $\pm$ 5.0 ^b	16.0 $\pm$ 4.0 ^a
Minor	10.2 $\pm$ 7.6 ^b	14.8 $\pm$ 4.5 ^a
Moderate	13.5 $\pm$ 8.8 ^b	17.2 $\pm$ 6.5 ^a
Major	12.3 $\pm$ 13.4 ^{ab}	13.9 $\pm$ 6.9 ^a
Extensive	2.2 $\pm$ 3.7 ^a	10.3 $\pm$ 5.5 ^a

*F-value = 2.98. Means of number of seed-bearing laterals with different superscript letters are significantly different ($p < 0.05$).

**F-value = 1.43. Means of lengths of seed-bearing laterals were statistically similar.

resulted in an 84% reduction compared to moderate damage (Fig. 9). No significant difference was seen among the number of seed-bearing laterals between extensive and major categories. Also, no significant difference among any of the categories when comparing seed-bearing lateral lengths was found (Fig. 10). An increase in the extent of herbivory by *A. litigata* (extensive and major categories) showed an impact on plant performance of *L. salicaria* by a decrease in the number of seed-bearing laterals.

Leaf defoliation of *L. salicaria* by *A. litigata* greatly impacted the mean number of seeds produced per plant stem (Fig. 11). The extensive damage category was significantly ($p < 0.05$) different from all others and resulted in a 94% reduction in seeds produced per plant stem. Major, moderate, and minor damage categories numerically resulted in reduced seed production compared to the no damage category. The extensive larval feeding had a significantly ($p < 0.05$) greater impact on plants compared to adult feeding.

Adult *A. litigata* were present in the field and fed on leaves of purple loosestrife before flower budding began. Beginning anthesis of flowers occurred on two plant stems (no damage category) in subplot 1 on 7 June 2000. On 19 June 2000 when extensive larval damage was present, 32% of the 50 tagged damage category plants were in the flower bud stage. Larvae skeletonized the foliage and fed on the flower buds of some plants, giving the plants a rusty brown appearance. Synchronization of larval *A. litigata* and the host flower budding stage is a positive attribute towards the potential of this natural enemy as a biological control agent. Fifty percent of the extensive larval-damaged plants never produced flowers, therefore, no seeds, while 100% of the no damage, 90% of the minor damage, 100% of the moderate damage, and 70% of the major

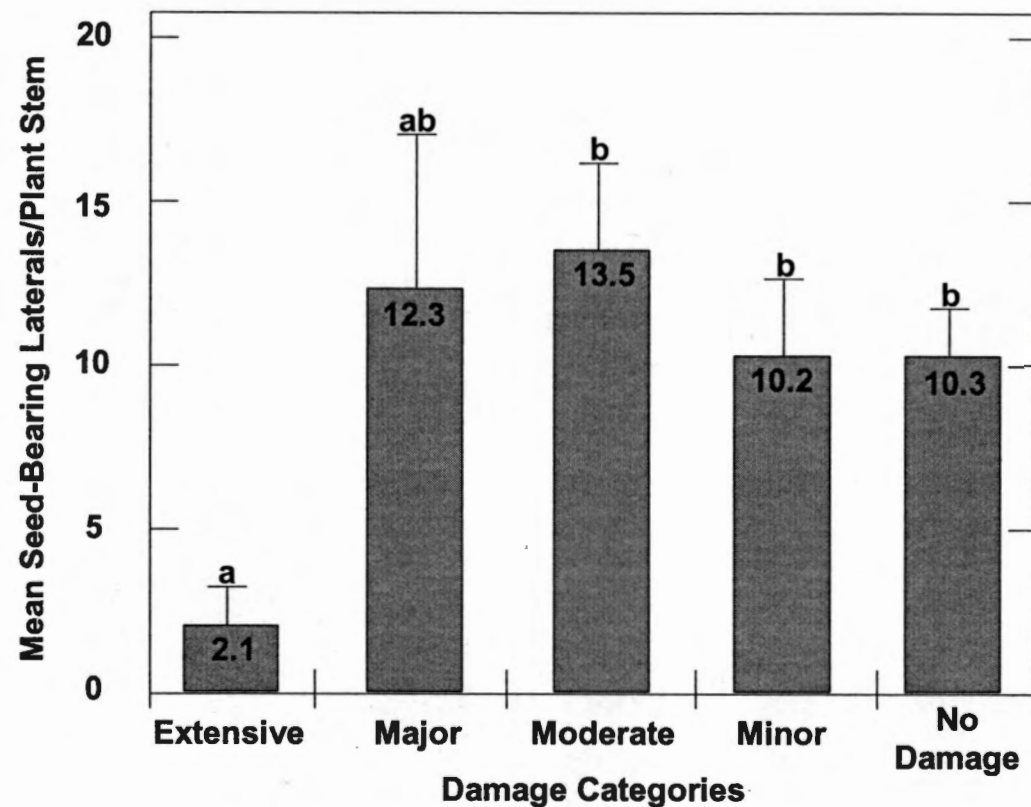


Figure 9. Mean $\pm$ SE number of seed-bearing laterals per plant stem per damage category of purple loosestrife, *Lythrum salicaria*, 2000. Means with different letters are statistically different ($p < 0.05$).

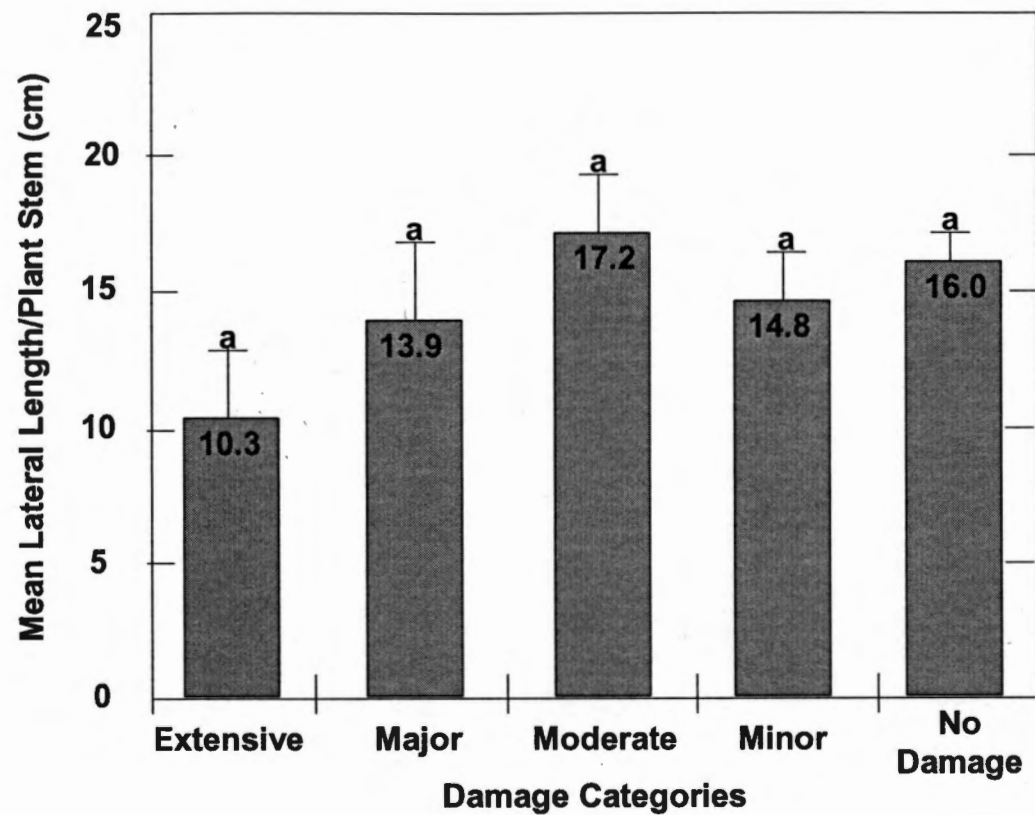


Figure 10. Mean $\pm$ SE seed-bearing lateral lengths per plant stem per damage category of purple loosestrife, *Lythrum salicaria*, 2000. Means with different letters are statistically different ($p < 0.05$).

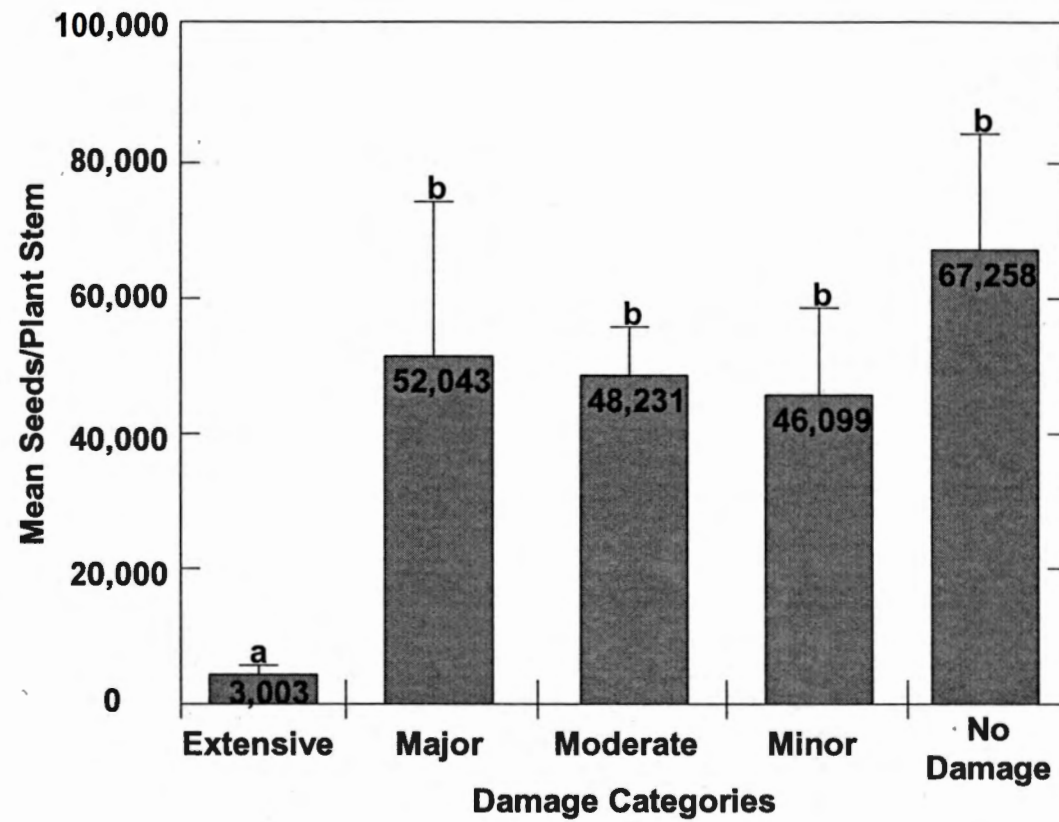


Figure 11. Mean $\pm$ SE number of seeds per plant stem per damage rating category of purple loosestrife, *Lythrum salicaria*, 2000. Means with different letters are statistically different ($p < 0.05$).

damage produced flowers. These results indicate that the beetles caused a decrease in flowering and subsequent seed set. Greatest impact was observed in the two categories of heaviest feeding damage (extensive and major). Reproductive structures are indicators of competitive success; therefore, the impact of the beetles that resulted in reduced flowering also lowered the competitive ability of *L. salicaria* (Weihe and Neely 1997).

By early July, leaf defoliation by *A. litigata* had caused a negative impact on mean plant stem height, and this impact was still obvious at the end of the growing season (Table 3). It is important to note that plant heights taken on the first measurement date (mid June) were not significantly ($p < 0.05$) different among categories. This verifies that all plant groups were statistically similar at the initial measurement, and subsequent difference would have been due to feeding damage (Fig. 12). Significant differences ($p < 0.05$) among categories were first noticed 7 July 2000 when plant heights were significantly shorter in extensive damage category (124 cm) than in no damage (150 cm) or minor damage (148 cm) categories (Fig. 13). The two categories which received no herbivory or the least amount of feeding damage reflected less interference of growth. Plant height differences were apparent until 12 August 2000, when significant differences ($p < 0.05$) were noted only between the extensive and the no damage categories. This trend continued until harvest on 1 September 2000, when plant height in the extensive damaged plants was significantly ($p < 0.05$) lower than those in all other categories (Fig. 14). The extensive larval damage category resulted in a 20% reduction in plant stem height when compared to the no damage category. Open space and height reduction of *L. salicaria* as a result from herbivory by *A. litigata* should offer better access to light for currently suppressed native plant species (Blossey 1995a). Plant stress caused from

Table 3. Mean height of purple loosestrife, *Lythrum salicaria*, stems per damage category per collecting date, 2000.

Sample Date	Damage Category – Mean Plant Height (cm)					
	F-value	No Damage	Minor	Moderate	Major	Extensive
7 June		114	114	99	94	-
13 June		121	127	109	105	-
19 June*	2.20	132 ^a	129 ^a	114 ^a	117 ^a	116 ^a
29 June	1.33	143	136	129	123	115
7 July**	3.78	150 ^b	148 ^b	135 ^{ab}	131 ^{ab}	124 ^a
12 July	3.90	158	147	143	136	123
28 July	3.31	168	161	159	149	135
4 August	3.71	171	166	163	154	137
12 August***	4.17	177 ^b	168 ^b	165 ^b	154 ^b	139 ^a
21 August	4.37	179 ^b	165 ^b	168 ^b	154 ^b	139 ^a
1 September	3.23	179 ^b	168 ^b	169 ^b	155 ^b	144 ^a

*Measurements of extensive damage (caused by larvae) were started on 21 June. Mean plant heights of all damage categories were statistically similar at this early measurement date.

**First statistical differences of plant height means among damage categories were noted on 7 July and this trend continued until 12 August. Mean plant heights of damage categories with different superscript letters are significantly different ($p < 0.05$).

***On 12 August statistical differences were found between the extensive and the no damage categories. This trend continued through 1 September.

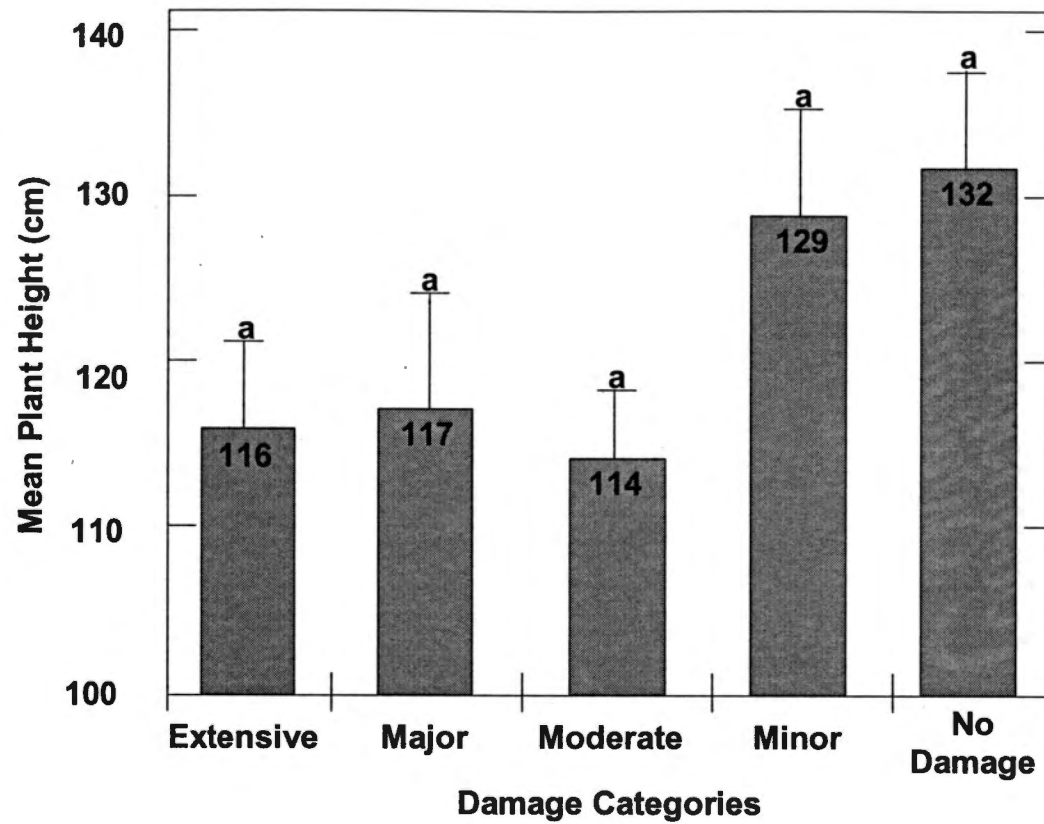


Figure 12. Mean $\pm$ SE plant stem height (mid-June) per damage rating category of purple loosestrife, *Lythrum salicaria*, 2000. Means were not statistically different ($p < 0.05$).

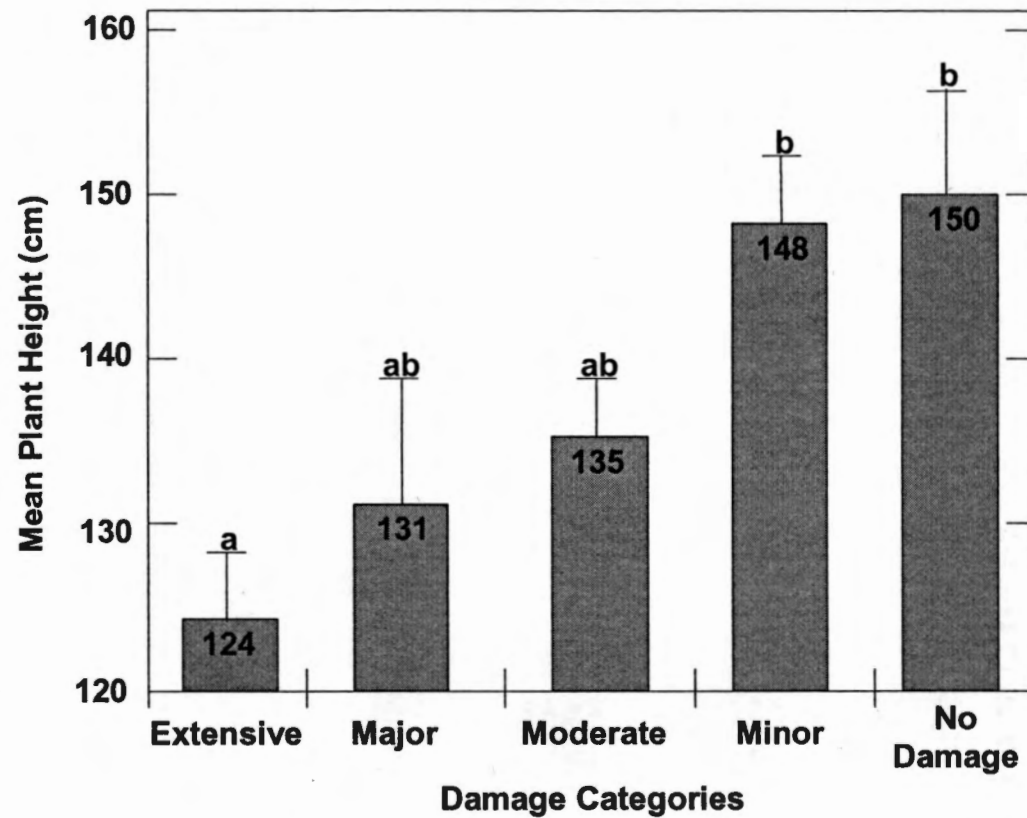


Figure 13. Mean $\pm$ SE plant stem height (7 July) per damage rating category of purple loosestrife, *Lythrum salicaria*, 2000. Means with different letters are statistically different ($p < 0.05$).

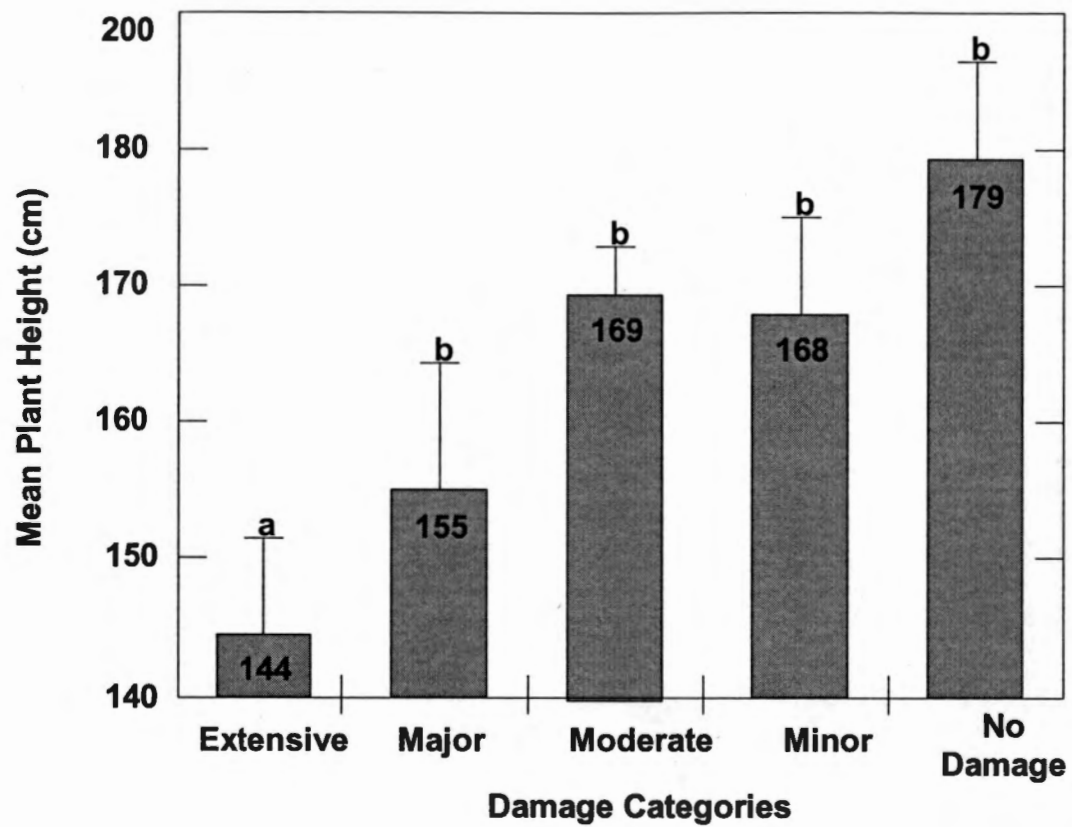


Figure 14. Mean $\pm$ SE plant stem height (1 September) per damage rating category of purple loosestrife, *Lythrum salicaria*, 2000. Means with different letters are statistically different ($p < 0.05$).

defoliation by *A. litigata* resulted in reduced plant stature of *L. salicaria*, which may reduce competitiveness.

Percentage of Herbivory by Adult A. litigata, 2000. Percentages of purple loosestrife stems (according to damage categories) defoliated by adult *A. litigata* per mean square meter on 31 May 2000 were: (1) 6% no damage, (2) 57% minor damage, (3) 27% moderate damage, and (4) 10% major damage; while on 12 June 2000, it was: (1) 0% no damage, (2) 34% minor damage, (3) 39% moderate, and (4) 27% major (Fig. 15). On 31 May 2000, damage on about 63% of the plants was classified as none or only minor damage. However, by 12 June 2000, damage had increased greatly, with damage on about 66% of the plants classified as moderate or major.

Additional Plant Performance Study of 20 Tagged Plants, 2000 and 2001. Data collected on the 20 tagged plant shoots in 2000 are presented in Tables 4-6. Although statistical comparisons between the 20 tagged plants in the different subplots were not possible, by interpreting the collected data some inference comparisons can be made about impacts of *A. litigata* feeding damage in combination with shade on plant performance.

Subplot 3 was shaded, while subplot 1 was unshaded, and subplot 2 received partial shade (Fig. 6). Plant stems in each of these subplots had received minor, moderate, or major leaf defoliation by adult *A. litigata*. From the study discussed above it was determined that these categories of damage did not significantly impact final seed production or plant height. However, when plant stems were exposed to this level of feeding damage in combination with the stress of shading, results suggest that the plants had lower levels of plant performance.

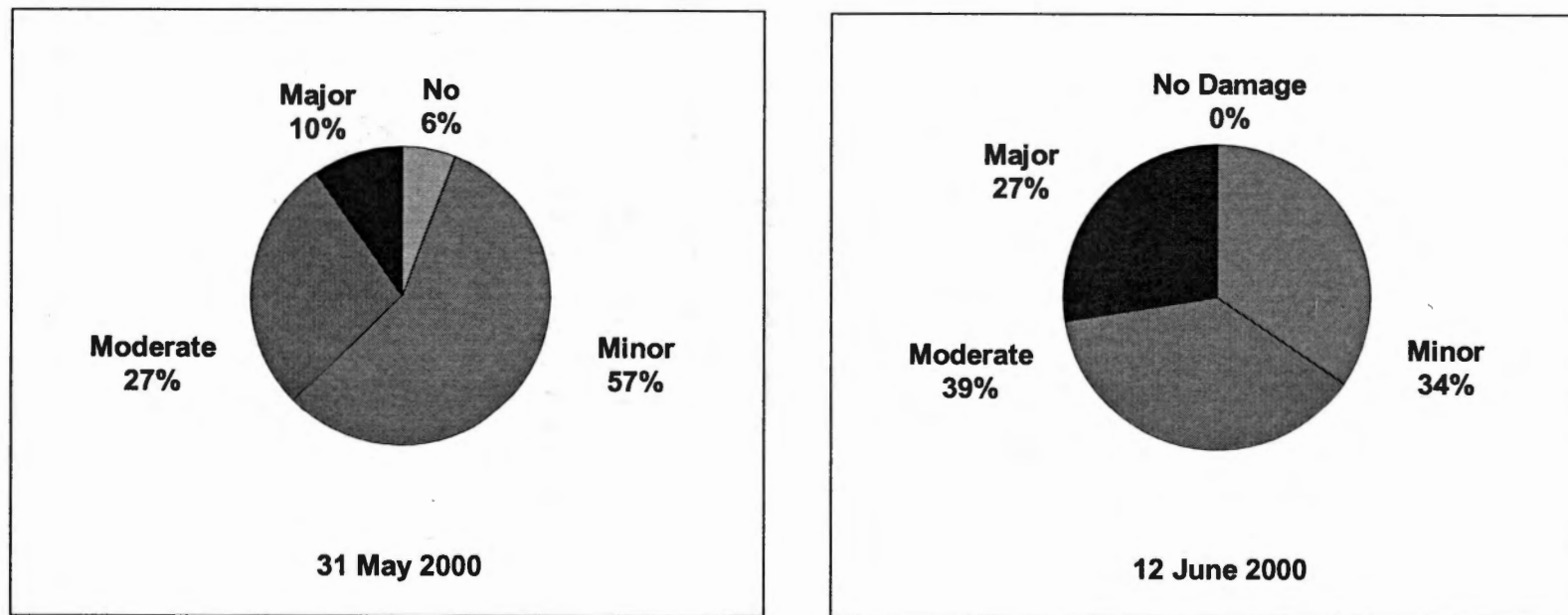


Figure 15. Percentage of plant stems of purple loosestrife, *Lythrum salicaria*, damaged by feeding of adult *Altica litigata* per damage category, 2000.

Table 4. Growth and reproductive characteristics of purple loosestrife, *Lythrum salicaria*, stems for research subplot 1, Unicoi County, 2000.

Parameter	Tagged Stems of Subplot 1 (Stems numbered: 1, 2, 3, 4, 6, and 20)					
	1	2	3	4	6	20
Damage Category	Mi*	Mi	Mi	Mi	Mo	Ma
Flowering	F1	F1	F1	F1	NF	F1
Capsules/ Stem**	3,353	18	3,430	MD	0*	78
Mean Seeds/ Capsule***	94	82	94	MD	0*	60
Mean Seeds/Stem****	315,182	1476	322,420	MD	0*	4,680
Final Height (cm)*****	241	199	244	227	154	171

*Mi = Minor, Mo = Moderate, Ma = Major, F1 = Flowering, NF = Non-flowering, MD = Missing Data, and 0 = Plant died.

**All capsules/stem were counted.

***Mean seeds/capsule were determined by averaging seeds from ten randomly selected capsules/plant stem.

****Mean seeds/stem were determined by multiplying the number of capsules/stem times the mean number of seeds/stem.

*****Mean $\pm$ SE final plant stem height (1 September) of subplot 1 of purple loosestrife stems was 206.0 ± 15.4 cm (n = 6).

Table 5. Growth and reproductive characteristics of purple loosestrife, *Lythrum salicaria*, stems for research subplot 2, Unicoi County, 2000.

Parameter	Tagged Stems of Subplot 2 (Stems numbered: 5, 7, 8, 9, 10, 11, 14, 15, and 16)								
	5	7	8	9	10	11	14	15	16
Damage	Mo*	Mi	Mo	Mi	Mi	Mi	Mi	Mo	Ma
Flowering	Fl	Fl	NF	Fl	Fl	Fl	NF	Fl	NF
Capsules/Stem**	1,059	4,840	0	1,169	204	110	0	17	582
Mean**** Seeds/Capsule	80	83	0	84	70	51	0	44	61
Mean Seeds/Stem****	84,720	401,720	0	98,196	14,280	5,610	0	748	35,502
Final Hgt (cm) *****	231	253	198	215	220	184	190	204	228

*Mi = Minor, Mo = Moderate, Ma = Major, Fl = Flowering, NF = Not Flowering, and Hgt = Height.

**All capsules/stem were counted.

***Mean seeds/capsule were determined by averaging seeds from ten randomly selected capsules/plant stem.

****Mean seeds/stem were determined by multiplying the number of capsules/stem times the mean number of seeds/stem.

*****Mean $\pm$ SE final plant stem height (1 September) of subplot 2 of purple loosestrife stems was 213.7 ± 7.3 cm ($n = 9$).

Table 6. Growth and reproductive characteristics of purple loosestrife, *Lythrum salicaria*, stems for research subplot 3, Unicoi County, 2000.

Tagged Stems of Subplot 3 (Stems numbered: 12, 13, 17, 18, and 19)					
Parameter	12	13	17	18	19
Damage Category	Mo*	Mo	Mo	Mo	Ma
Flowering	NF	NF	NF	NF	NF
Capsules/Stems**	0	0	0	0	0
Mean Seeds/Capsule***	0	0	0	0	0
Mean Seeds/Stem****	0	0	0	0	0
Final Height (cm)*****	175	149	161	173	198

* Mi = Minor, Mo = Moderate, Ma = Major, Fl = Flowering, and NF = Non-flowering.

**All capsules/stem were counted.

***Mean seeds/capsule were determined by averaging seeds from ten randomly selected capsules/plant stem.

****Mean seeds/stem were determined by multiplying the number of capsules/stem times the mean number of seeds/stem.

*****Mean $\pm$ SE final plant stem height (1 September) of subplot 3 of purple loosestrife stems was 171.2 ± 8.2 cm ($n = 5$).

None of the plants in subplot 3 (i.e., shade) produced flowers or seeds, while 83% produced flowers in subplot 1 averaging 128,752 seeds per plant stem. Other researchers have noted the effect of shade on *L. salicaria* resulting in a reduction in flower production (Weihe and Neely 1997). Subplot 2 received a combination of sun and shade. In subplot 2 it was found that 67% of the plants produced flowers with an average of 71,197 seeds per plant stem. Average plant stem heights were shortest in shady subplot 3 (Fig. 16).

These data suggest that the impact of herbivory by *A. litigata* on plant performance of *L. salicaria* in the areas of seed production and plant height is increased when combined with the stress of shading. These impacts indicate that *A. litigata* possibly has some potential as a biological control agent. Biological control attempts to manage the growth of populations of purple loosestrife to acceptable limits across all or much of its range. Reduction of this weed increases the probability that native species which most likely already experience herbivory, can successfully compete and establish in areas that were previously dominated by purple loosestrife (Weihe and Neely 1997). Because the plants in subplot 3 were reduced in plant stature and the seed bank was not replenished, conditions for native plants to compete more successfully against *L. salicaria* are induced (Blossey 1995a).

Data collected in 2001 could not be compared due to environmental conditions. Plants were impacted by deer herbivory, stormy weather that damaged plants, and flooding that covered the plants in mud and stripped their leaves. White-tailed deer, *Odocoileus virginianus* (Boddaert), were seen leaving the plot and their tracks were left in the mud near plants that had been clipped (no trimmings were present, so foraging was

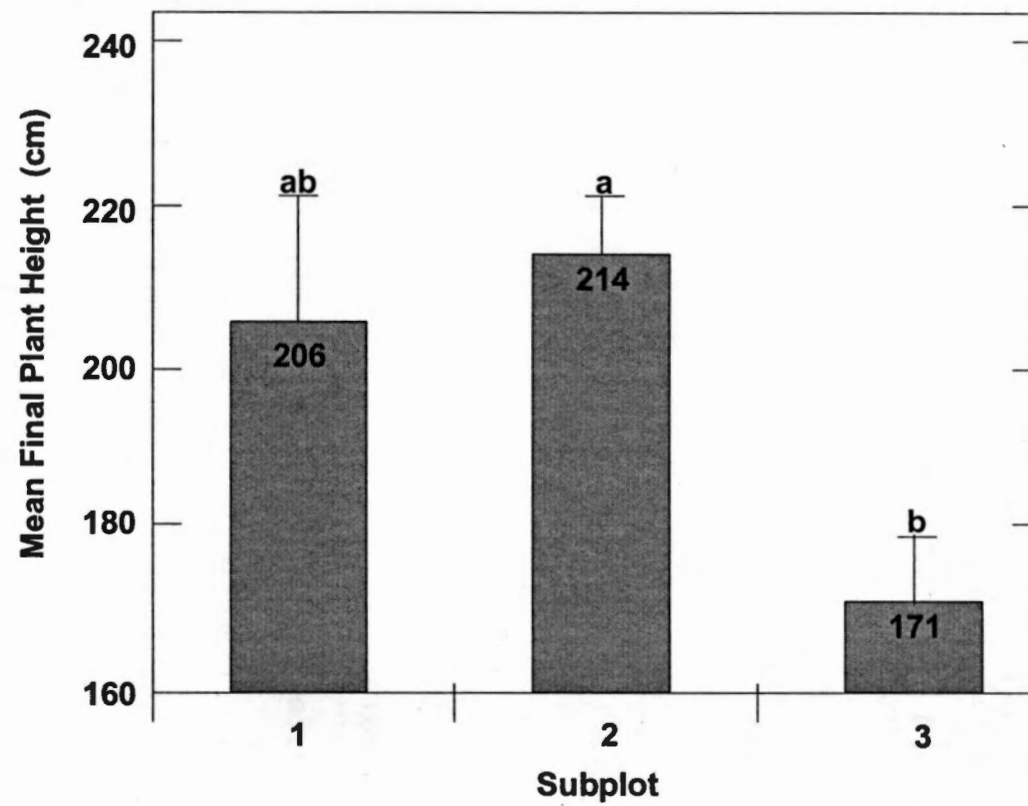


Figure 16. Mean $\pm$ SE final plant stem height (1 September) per subplot of purple loosestrife, *Lythrum salicaria*, 2000.

assumed). Six of the 20 tagged plants were noted as clipped on 14 June 2001 and, by the end of July, an additional five plants, which represented 55% of the tagged stems were clipped. On 7 July 2001 it was noted that stormy weather had caused a limb to fall into subplot 3 breaking some of the plants. Heavy flooding occurred on 2 August 2001 and again on 13 August 2001. The strong flash floods of South Indian Creek stripped the leaves from *L. salicaria*, bent the stems to the ground, covered the plants in mud, and deposited sandy soil of varying degrees in each subplot. The mud deposited at the base of the plants prevented taking accurate height measurements. Tags were washed away and only 10 of the tagged plants could be located. Final data were collected 11 September 2001, and of the remaining plants none produced seed capsules.

Other biological organisms also were observed to impact purple loosestrife. These included feeding damage by the four-lined plant bug, *Poecilocapsus lineatus* (F.), and the Japanese beetle, *Popillia japonica* Newman, and plant competition by arrow-leaved tearthumb, *Polygonum sagittatum* L. Other research has reported that purple loosestrife seedlings cannot compete with *Polygonum* species (Anderson 1995).

Estimates of Plant Stem Density 2000, 2001 and 2002. In 2000, plant stem density of *L. salicaria* averaged 86 stems per square meter. Plant stem densities averaged 83 and 60 stems per square meter in 2001 and 2002, respectively (Table 7). The combination of biotic and abiotic stresses (as discussed above) imposed on *L. salicaria* during this study could have impacted plant performance resulting in the lower stem density found in 2002. In a previous study, shading did not influence density effects of *L. salicaria*; however, a reduction in flower production was noted (Weihe and Neely 1997). In an experimental study, emergence of seedlings from buried seed decreased linearly from 90% at the soil

Table 7. Plant stem densities and mean densities for each subplot of *Lythrum salicaria*, 2000, 2001, and 2002.

Subplot	Plant Stem Density (stems/m ²)			
	31 May 2000	12 June 2000	28 June 2001	30 May 2002
1	121	98	85	106
2	77	68	114	44
3	76	78	51	29
Mean Plot Densities*	91	81	83	60**

*Mean plot densities were the average of the three subplot densities.

**A combination of abiotic and biotic stresses [herbivory by *Altica litigata* in 2000, heavy flooding damage in 2000 and 2001, and varying degrees of sandy soil deposits (preventing seed germination) in 2001] imposed on *L. salicaria* during this study could have impacted plant performances resulting in the lower plot stem density in 2002.

surface to 0% at 2 cm (Welling and Becker 1990). On 13 August 2001, a strong flash flood of South Indian Creek resulted in a large area in subplot 3 receiving soil deposits that amounted up to 36 cm in depth, while subplots 1 and 2 received lesser degrees at 7 cm and 26 cm, respectively. Soil deposits of this amount would interfere with seed germination. For the three-year period, subplot 3 had lower mean stem density than the other two plots (subplot 3 was 22% lower than subplot 2 and 43% lower than subplot 1). Subplot 3 received the stresses of shade, flea beetle herbivory, and the greatest depths of soil deposition from flooding (2001). In spring of 2002, much of subplot 3 was covered with bedstraw, *Galium aparine* L., instead of purple loosestrife. It appears that the competitiveness of *L. salicaria* was lowered due to the combination of stresses, resulting in fewer seeds germinating and therefore a lowered plant density.

iv. Summary

The impact of herbivory on *L. salicaria* by *A. litigata*, a native North American flea beetle, was evaluated in 2000 and 2001. This research demonstrated that leaf defoliation by *A. litigata* negatively affects plant performance in the areas of seed production and plant height of *L. salicaria*. Extensive larval feeding damage was significant and resulted in a 92% reduction in the number of capsules per plant stem. Larvae skeletonized the foliage and fed on the flower buds of some plants, giving the plants a rusty brown appearance. Synchronization of larval *A. litigata* and the host flower budding stage is a positive attribute towards the potential of this natural enemy as a biological control agent. Fifty percent of the extensive larval-damaged plants never produced flowers, therefore, no seeds. Extensive larval feeding had a significantly

greater impact on *L. salicaria* compared to adult flea beetle feeding and resulted in a 94% reduction in seeds produced per plant stem.

Extensive larval feeding damage caused by *A. litigata* resulted in a 20% reduction in plant stem height of purple loosestrife. Plants in shade compared to those without shade showed a greater reduction in seed production and plant height. Reduced plant stature of *L. salicaria* may reduce its competitiveness (Katovich et al. 1999). Open space and height reduction of *L. salicaria* as a result of feeding by *A. litigata* should offer better access to light for currently suppressed native plant species. Data suggest that the impact of herbivory by *A. litigata* on plant performance of purple loosestrife is increased when combined with the stress of shading. A combination of defoliation and winter injury or other stresses are required to induce plant mortality of *L. salicaria* (Katovich et al. 1999).

Negative impacts to *L. salicaria* inflicted through herbivory by *A. litigata* suggest that it has some potential as a biological control agent. However, more research is needed to assess impacts of feeding by *A. litigata* on purple loosestrife. Ideally it would have been beneficial to study the effects of the herbivores on all plant parts and to have designed experiments to study a range of attack levels using both adult and larval *A. litigata* (Blossey and Schat 1997). Future studies would include this type of experimental design.

CHAPTER IV

QUANTIFYING LEAF CONSUMPTION OF PURPLE LOOSESTRIFE, *LYTHRUM SALICARIA*, BY ADULT *ALTICA LITIGATA*

i. Introduction

Purple loosestrife is listed as a noxious weed in at least 20 states, including Tennessee (Southeast Exotic Pest Plant Council 2002, USDA 2002), where governmental agencies have authority to mandate control of *L. salicaria* on all public and private lands. This exotic pest plant (rank 1: severe threat) is established in small, localized populations in several counties in Tennessee where it poses a potential problem (Fig. 1) (Southeast Exotic Pest Plant Council 2001, Chester et al. 1997). Young smaller stands of *L. salicaria* should receive early management before large seed banks accrue (Welling and Becker 1990). Among 44 North American wetland species, *L. salicaria* is ranked as the superior competitor (Gaudet and Keddy 1988), and has been reported to reduce the biomass of native competitors by an average of 60% (Keddy et al. 1994).

Knowledge of the natural enemies of purple loosestrife is needed when developing management strategies for this exotic noxious weed in Tennessee (Van Driesche and Bellows 1996). Objectives of this research were to quantitate leaf consumption capabilities by adult *A. litigata* on purple loosestrife, which will provide a tool for evaluating its attributes as a natural enemy and its usefulness as a potential biological control agent.

ii. Materials and Methods

During 2000, leaf consumption by adult *A. litigata* on purple loosestrife was quantified in a laboratory study. Adult leaf consumption studies were planned for 2000, while larval leaf consumption studies were planned for 2001, due to lack of availability of equipment (which had to be shared with other departments), funding, and technical assistants, during 2000. However, only a few *A. litigata* were collected by direct sampling in 2001, and larval leaf consumption has not been quantified. Purple loosestrife stands in other areas of Unicoi, Marion, and Sumner counties were searched for *A. litigata* in 2001, none was found. *A. litigata* (7 June 2000) and purple loosestrife foliage (19 June 2000) for adult leaf consumption studies were collected from the South Indian Creek site in Unicoi County, Tennessee.

Assessing Leaf Consumption Capabilities of Adult *A. litigata* on Purple Loosestrife
*Laboratory Study for Quantifying Leaf Consumption by Adult *A. litigata*, 2000.* To quantitate leaf consumption by adult *A. litigata*, foliage of purple loosestrife (from greenhouse potted plants and additional cut shoots from site) was measured before and after feeding with a LI-COR Model AI-3000A leaf area meter. Leaf area was measured as the mean of three area determinations to minimize instrument errors. Leaf measurements recorded included area (cm²) and length. Beetles and foliage were placed in petri dishes (Fisherbrand sterile polystyrene 100 x 15 mm) with moistened filter paper (Whatman #2, 90 mm circles), and foliage was measured and replaced every two days from 14 June 2000 through 14 July 2000. Treatments included various densities of male and female *A. litigata*: 0 as a control for male and females; 2, 4, and 6 males; and 2 and 4 females (due to shortage of females). Ten replicates of each treatment were conducted.

Estimating Numbers of Beetles Required to Defoliate Purple Loosestrife. Mean leaf areas consumed by beetle treatments in a two-day period were derived from the study described above. A mean leaf area of foliage fed to the beetles was calculated by averaging mean areas of leaves supplied for consumption. Leaf area consumed per individual beetle was calculated by dividing the area consumed per two days by the number of beetles per treatment. Mean leaf area per plant stem was taken from thesis work by Cortilet (1998).

The equation below was used to determine estimates of beetles needed to defoliate a single leaf in two days:

$$\left(\frac{\text{Mean Area (cm}^2\text{)}}{\text{Leaf}} \right) \left(\frac{\text{Beetle}}{\text{Mean Area Consumed (cm}^2\text{)}} \right) = \frac{\text{Beetles}}{\text{Leaf}}$$

The following equation was applied to determine estimates of beetles needed to defoliate total leaf area per stem of purple loosestrife:

$$\left(\frac{\text{Mean Leaf Area (cm}^2\text{)}}{\text{Plant Stem}} \right) \left(\frac{\text{Beetle}}{\text{Mean Area Consumed (cm}^2\text{)}} \right) = \frac{\text{Beetles}}{\text{Plant Stem}}$$

Data Entry Analysis

Data were entered into spread sheets using Microsoft Excel® software (Microsoft Corporation 2002). To quantify leaf consumption, the leaf area before and after feeding by each treatment was entered into spreadsheets. Averages and standard errors of leaf area consumed per treatment were calculated. Once entered, data were analyzed by one way and two way analysis of variance F test ($p=0.05$). The Tukey-Kramer procedure was applied for multiple comparisons (Levine et al. 2001). Means of leaf area consumed by treatments for 14 consecutive two-day periods was examined through liner regression.

Equations to derive estimate numbers of beetles needed to defoliate leaf area also were calculated.

iii. Results and Discussion

To better understand leaf consumption capabilities of adult *A. litigata*, means of leaf area consumed per two-day periods by various densities of beetles were quantified and compared. Numbers of beetles required to consume an entire leaf or stem of purple loosestrife are estimated based on information learned from the laboratory study.

Laboratory Study for Quantifying Leaf Consumption by Adult A. litigata, 2000. When the means of leaf area consumed per two-day period by beetle treatments were compared, all means of each treatment were significantly different ($p < 0.05$); except for the treatments of male beetles in the two and four groupings, which were not significantly different ($p < 0.05$) (Fig. 17). Females consumed more leaf area compared to males in the two and four groupings. In fact, females consumed approximately 2x more area than males. Mean leaf area consumed per two-day period by two, four, and six male beetle groupings was 0.76 cm^2 , 1.08 cm^2 , and 1.86 cm^2 , respectively; while means for female two and four treatments were 1.34 cm^2 and 2.10 cm^2 , respectively.

Mean leaf areas consumed by individual beetles per treatment also were compared (Fig. 18). Individual males consumed a similar amount in the two, four, and six beetle groupings, and means of leaf area consumed were not significantly ($p < 0.05$) different among male treatments. Means of leaf area consumed by individual females were significantly ($p < 0.05$) different from each other and all male treatments. Female individuals consumed on average 0.60 cm^2 while male individuals consumed 0.32 cm^2 ;

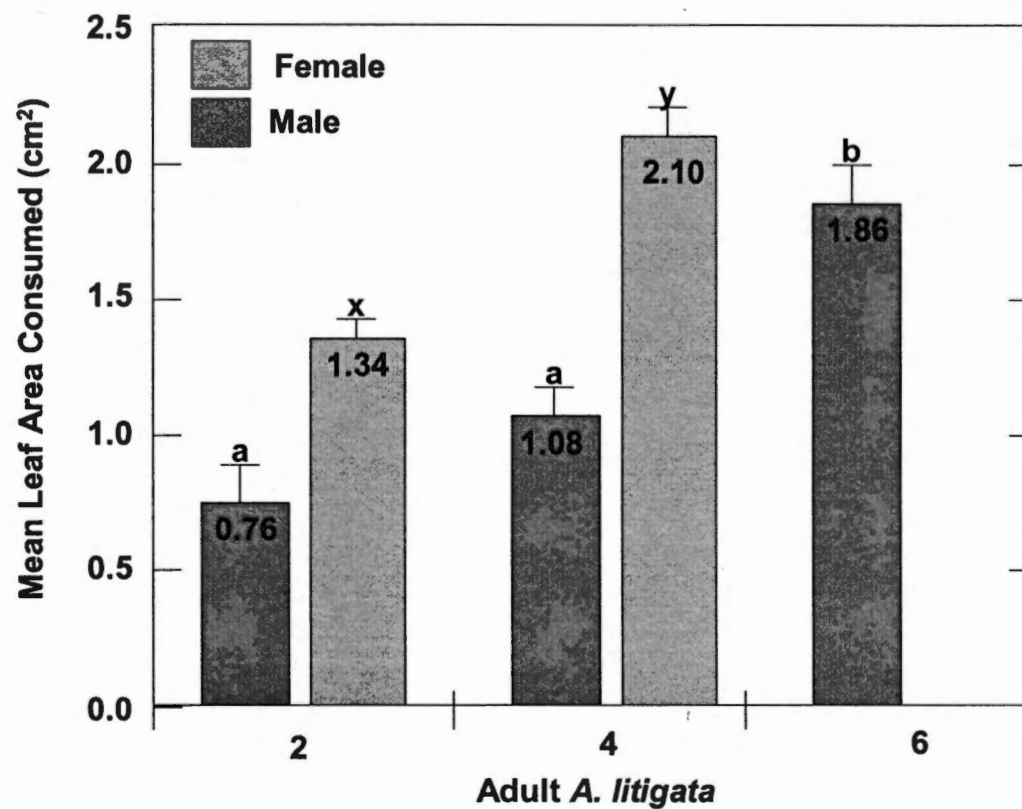


Figure 17. Mean $\pm$ SE leaf areas (cm²) of purple loosestrife, *Lythrum salicaria*, consumed in a two-day period by various densities of flea beetles, *Altica litigata*. Bars with the same letters are not significantly ($p < 0.05$) different.

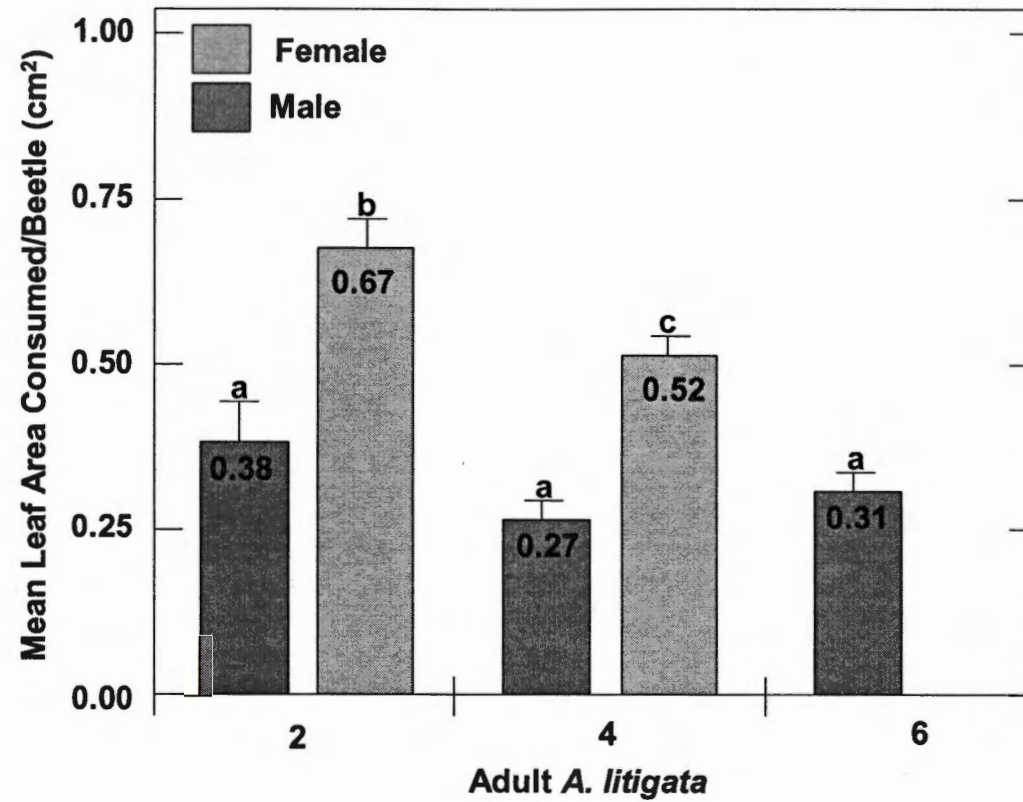


Figure 18. Mean $\pm$ SE leaf areas (cm²) of purple loosestrife, *Lythrum salicaria*, consumed in a two-day period by various densities of individual male and female flea beetles, *Altica litigata*. Bars with the same letters are not significantly ($p < 0.05$) different.

which further illustrates that females consume approximately 2x more leaf area than males in a two-day period. The fact that female *A. litigata* are larger than males and would have had greater energy requirements for egg production could help explain the larger leaf area consumed by females. Females in groups of two consumed significantly ($p < 0.05$) more than females in groups of four which cannot be fully explained by this study, but perhaps crowding had a negative effect on leaf consumption behavior of females. The fact that females consume nearly 2x more leaf area (plus the benefit of reproduction) indicates that it would be beneficial to release higher concentrations of female *A. litigata*, if this insect were being used as a biological control agent of purple loosestrife.

Linear regression of means of leaf area consumed by beetle treatments for 14 consecutive two-day periods showed a general trend by beetles to consume less leaf area as the study progressed (Figs. 19 and 20). Linear equations from a least squares analysis of the means of leaf area consumed by beetle groupings are presented in Table 8. Although the fit for a linear equation is not good, as indicated by the correlation coefficient (R^2) for each group, the linear equation does highlight the trend for *A. litigata* to consume less leaf area as the study progressed. Curvilinear regression of means did not provide a better linear fit. Possibly the decline in leaf area consumed by beetles could be explained by aging factors, because captured adult *A. litigata* lived only about 60 days in the laboratory.

Estimate Numbers of Beetles Needed to Defoliate Purple Loosestrife. Biological control of purple loosestrife seeks to reduce weed populations to acceptable levels by using the negative impact of host specific herbivores to reduce competitive abilities of the plants

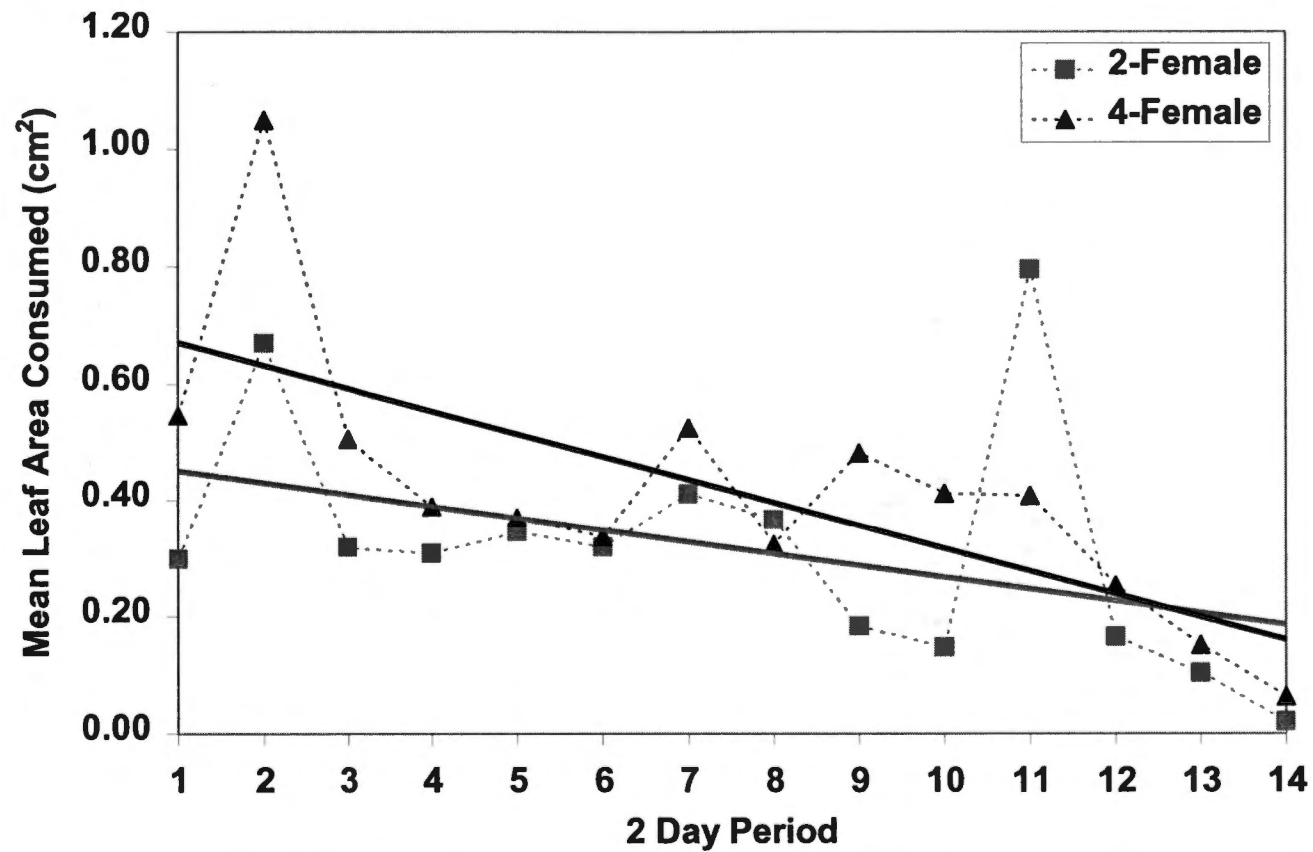


Figure 19. Linear regression and comparisons of mean leaf areas (cm²) of purple loosestrife, *Lythrum salicaria*, consumed by female *Altica litigata* for 14 consecutive two-day periods.

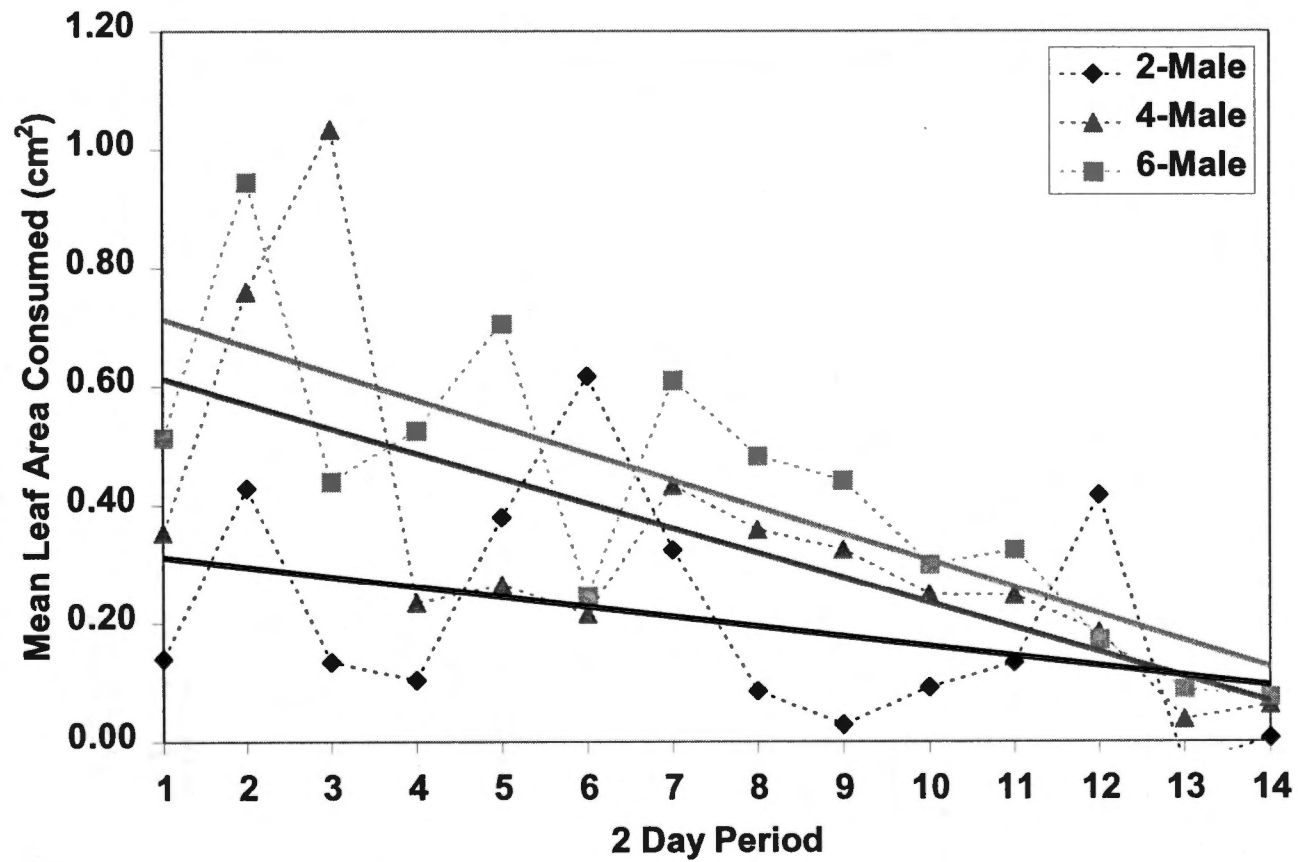


Figure 20. Linear regression and comparisons of mean leaf areas (cm²) of purple loosestrife, *Lythrum salicaria*, consumed by male *Altica litigata* for 14 consecutive two-day periods.

Table 8. Linear equations of leaf area of purple loosestrife, *Lythrum salicaria*, consumed by all groupings of *Altica litigata* (least squares analysis).

Beetle Treatments	Linear Equations	Correlation Coefficient (R^2)
2 Female	$y = -0.0204x + 0.4708^*$	0.1657
4 Female	$y = -0.0392x + 0.7099$	0.5142
2 Male	$y = -0.0167x + 0.3286$	0.1290
4 Male	$y = -0.0420x + 0.6552$	0.4418
6 Male	$y = -0.0451 + 0.7576$	0.6069

* y = leaf area consumed and x = number of beetles.

**Curvilinear regression of means did not provide a better fit.

(Blossey and Schat 1997). If adult *A. litigata* are to be used as biological control agents of purple loosestrife, then understanding their leaf consumption capabilities and knowing how many beetles would be needed to consume the leaf area on purple loosestrife plant stems become a valuable tool for determining how many agents would be needed to regulate the plant (Van Driesche and Bellows 1996). Adult females consumed ca. 2x more leaf area than males (ca. 0.60 cm² and 0.32cm², respectively). Based on these results, ca. 39 male or 20 female beetles would consume an entire leaf (with an average leaf area of 12 cm²) in a two-day period. In an Iowa University study, purple loosestrife stems averaging 0.91 m in height had a total leaf area of 881.91 cm² (Cortilet 1998); ca. 184 males or 99 females could consume that amount of leaf area in 30 days. Studies have shown that more than two years of severe defoliation in combination with other stress factors are required for mortality of mature *L. salicaria* (Katovich et al 1999).

Effects of grazing by a population of *Altica* spp. on two species of *Ludwigia* in the floodplain of the Savannah River, in southwestern South Carolina were evaluated by quantifying herbivory (Scott and Haskins 1987). Results indicated that grazing of *Ludwigia* by *Altica* (adults and larvae) removed large amounts of plant leaf tissue (16% to 43% of each species) during the growing season. Larval leaf consumption by *A. litigata* also should be quantified to further understand the potential of this flea beetle for use as a potential biological control agent. An image analysis method that reliably quantifies feeding damage by leaf beetle larvae of *A. carduorum* Guerin-Meneville and *A. cirsiicola* Ohno on Canada thistle, *Cirsium arvense* (L.) Scopoli, leaf disks has been developed (Kokko et al. 1995). *A. litigata* larvae skeletonize leaves similarly and could be evaluated by this method.

iv. Summary

During 2000, leaf consumption capabilities of adult *A. litigata* on purple loosestrife were quantified in a laboratory study. Means of leaf area consumed per two-day period by various densities of male and female beetles were compared, and numbers of beetles required to consume an entire leaf and stem of purple loosestrife were determined.

Female *A. litigata* consume approximately 2x more leaf area than males in a two-day period. Average leaf area consumed by female individuals was 0.60 cm² while males consumed 0.32 cm² each for the two-day period. Females in groups of two consumed significantly ($p < 0.05$) more leaf area than females in groups of four which cannot be completely explained by this study. The fact that females consume approximately 2x more leaf area than males indicates the importance of releasing higher concentrations of female *A. litigata*, if this insect were to be used as a biological control agent of *L. salicaria*. Additionally, females lay the eggs that perpetuate the population. Beetles exhibited a general trend to consume less leaf area as the study progressed, which perhaps could have been due to aging factors.

Gaining an understanding of the numbers of beetles that are necessary to defoliate purple loosestrife, becomes a valuable tool essential in determining how many agents would be necessary to regulate plant populations (Van Driesche and Bellows 1996). Estimated numbers of beetles needed to consume an entire leaf (with an average leaf area of 12 cm²) in a two-day period were 39 male or 20 female beetles. This information could be applied to determine quantities of beetles needed in field releases. With high probability though, impact of corresponding larvae would reduce total of adults required.

Quantification of leaf consumption of purple loosestrife by adult *A. litigata* and estimated numbers of adult beetles needed for foliage consumption of this exotic weed have provided some groundwork for the evaluation of this potential biological control agent. To further assess this natural enemy for the biological control of *L. salicaria*, larval consumption by this flea beetle should be quantified.

CHAPTER V

CONCLUSIONS

Purple loosestrife is an invasive, wetland perennial that forms large monotypic stands across many temperate regions of the United States and Canada (Cox 1999). This highly competitive plant replaces native vegetation, degrades wildlife habitats, and obstructs waterways (Thompson et al. 1987). Purple loosestrife plants produce millions of seeds and can propagate vegetatively from stem cuttings, which enables it to spread easily becoming a challenge for land managers. Currently, management efforts for *L. salicaria* focus on classical biological control, which may provide long-term, economical, environmentally friendly and sustainable management of this invasive weed (Malecki et al. 1993).

Little research concerning insect diversity associated with *L. salicaria* has been conducted in Tennessee. Thus, a two-year research project was initiated in 2000 to evaluate insect diversity on purple loosestrife in northeastern Tennessee in hopes of finding potential biological control agents. Early into the research a flea beetle, *A. litigata*, was observed in high numbers inflicting intense feeding damage to purple loosestrife. Therefore, research was shifted to evaluate the relationship of *A. litigata* to purple loosestrife.

During 2000 and 2001, biology, seasonality and abundance of *A. litigata* on purple loosestrife in northeastern Tennessee were evaluated. Also, the impact of herbivory by *A. litigata* on seed production and plant height of purple loosestrife was

assessed to investigate if *A. litigata* may have potential as a possible biological control agent. Leaf consumption capabilities of adult *A. litigata* on purple loosestrife were quantified to provide another tool for evaluating this natural enemy's attributes as a potential biological control agent for this exotic noxious weed.

All life stages of the flea beetle were found in association with *L. salicaria*. This study provided the first documentation of biology, seasonality and abundance of *A. litigata* on purple loosestrife. The incidence of this flea beetle on purple loosestrife represents a new state record for Tennessee. This research imparts preliminary findings of the life history of *A. litigata* along with notes of behavior and biological observations.

Altica litigata is a little known species, so the biological information learned from our research has greater significance. Yellow fecal marked eggs were laid in small groups on leaf surfaces of purple loosestrife, and the larvae heavily skeletonized leaves. Mature darkened larvae crawled into the soil to pupate. At normal room temperature (ca. 25°C), development times for eggs, larvae (three instars), and pupae were 3-5 days, 13-16 days, and 7-10 days, respectively (sample sizes were not large enough to make any definite conclusions). Adult beetles that were captured and held in the laboratory lived approximately 60 days. Leaf litter and soil samples did not reveal any overwintering stages of the flea beetle. Adult emergence may have possibly been affected by flooding. High numbers of the flea beetle were present in 2000 and only a few *A. litigata* were collected in 2001. This fluctuation in *A. litigata* population densities from year to year suggest several possibilities, such as: the immediate habitat was not conducive for colonization by the beetle; the insect is nomadic; it prefers other hosts plants; or the life cycle is similar to that of the cereal leaf beetle, *Oulema melanopus* (L.), which shifts host

plants (from small grains to grasses) for part of the cycle (Herbert and VanDuyn 1999).

A complete understanding of the life history of *A. litigata* needs to be established through future research before conclusions about its potential as a biological control agent can be made.

This research demonstrates that leaf defoliation by *A. litigata* negatively impacts plant performance (i.e., seed production and plant height) of *L. salicaria*. Adult feeding alone did not significantly ($p < 0.05$) impact plant performance; however, extensive larval feeding damage was significant ($p < 0.05$) and resulted in a 92% reduction in the number of seed capsules per plant stem. Larvae fed on the foliage and the flower buds of some plants. Synchronization of larval *A. litigata* and the host flower budding stage is a positive attribute when evaluating the potential of this natural enemy as a biological control agent. Fifty percent of the extensive larval damaged plants never produced flowers, therefore, no seeds. Extensive larval feeding resulted in a 94% reduction in seeds produced per plant stem. This considerable reduction in seed production imposed by *A. litigata* herbivory adds to the significance of this natural enemy; particularly, since seed dispersal is the major source by which this plant spreads.

Defoliation by *A. litigata* imposed stress that resulted in reduced plant stature of *L. salicaria*, which may reduce competitiveness. The extensive larval herbivory resulted in a 20% reduction in plant stem height. Open space and height reduction of *L. salicaria* as a result of feeding by *A. litigata* should offer better access to light for currently suppressed native plant species.

Leaf consumption capabilities of adult *A. litigata* on purple loosestrife were quantified in a laboratory study. Female *A. litigata* consumed approximately 2x more

leaf area than males in a two-day period, with female individuals consuming 0.60 cm² while male individuals consumed 0.32 cm². Females in groups of two consumed significantly ($p < 0.05$) more than females in groups of four which may have been caused by congestion among female adults. If *A. litigata* were to be used as a biological control agent of *L. salicaria*, it would be beneficial to release higher concentrations of female *A. litigata*; because females reproduce and consume approximately 2x more leaf area than males. Beetles exhibited a general trend to consume less leaf area as the study progressed, which perhaps could have been due to aging factors.

Knowledge of the numbers of beetles necessary to defoliate purple loosestrife is essential information for determining quantities of beetles needed to regulate plant populations (Van Driesche and Bellows 1996). It was determined that approximately 39 male or 20 female individual beetles are needed to consume an entire leaf in a two-day period.

Altica litigata may have potential as a biological control agent of *L. salicaria* due to the negative impact that herbivory had on seed production and plant height. Leaf consumption of purple loosestrife by *A. litigata* occurred briefly from mid May until mid June. Effective biological control agents would need to be present at the site for longer periods of defoliation of purple loosestrife (Katovich et al. 1999). Further research is needed to better understand the biology and impact of *A. litigata* on purple loosestrife.

Flea beetle densities were high in 2000; however, only a few were found in 2001. These varying densities in *A. litigata* populations raises some interesting ecological questions that need to be addressed: What other hosts does it prefer?; Where did it go when it left the site?; Why did it leave the site?; and How does it survive the winter?

Because limited information about *A. litigata* is available, any new biological knowledge is extremely beneficial. A better understanding of the biology, life history, and impact of *A. litigata* on purple loosestrife may lead to its use as a biological control agent. There would be multiple benefits in being able to use a native species for regulation of *L. salicaria*. Advantages of using a native natural enemy for biological control would include: *A. litigata* would have its own natural enemy check and balance system present; there would be no need for concerns about the risks that come with introducing still another non-native organism that may disrupt native diversity; and the expense normally involved with exploration and quarantine of an introduced biological control agent would not be a factor. Further evaluation of this species and its potential as a biological control agent against this exotic wetland plant is needed.

REFERENCES CITED

- Anderson, M. G. 1995. Interactions between *Lythrum salicaria* and native organisms: A critical review. *Environmental Management* 19: 225-231.
- Babbitt, B. 1998. Statement on invasive alien species. Science in Wildland Weed Management Symposium, Denver, Colorado. Tennessee-Exotic Pest Plant Council News 5: 3.
- Baker, J. R. and S. B. Bambara. 1999. Ornamentals and turf: *Altica litigata*. North Carolina Pest News 14: 1.
- Barko, J. W. 2002. Aquatic plant information system online: *Altica litigata*- "Primrose willow flea beetle." U. S. Army Corp of Engineers- Aquatic Plant Control Research Program. <http://www.wes.army.mil/el/aqua/>.
- Batra, S. W. T., D. Schroeder, P. E. Boldt, and W. Mendl. 1986. Insects associated with purple loosestrife (*Lythrum salicaria* L.) in Europe. *Proceedings of the Entomological Society of Washington*. 88: 748-759.
- Blossey, B. 1995a. A comparison of various approaches for evaluating potential biological control agents using insects on *Lythrum salicaria*. *Biological Control* 5: 113-122.
- Blossey, B. 1995b. Coexistence of two leaf-beetles in the same fundamental niche: Distribution, adult phenology, and oviposition. *Oikos* 74: 225-234.
- Blossey, B. 1996. Biological control of weeds in natural areas. Cornell Community Conference on Biological Control (April 11-13, 1996), Ithaca, New York. <http://www.nysaes.cornell.edu/ent/bcconf/talks/blossey.html>.
- Blossey, B. 2002. Biological control of non-indigenous plant species: Purple loosestrife. <http://www.invasiveplants.net/plants/purpleloosestrife.html>.
- Blossey, B. and T. R. Hunt. 1999. Mass rearing methods for *Galerucella californiensis* and *G. pusilla* (Coleoptera: Chrysomelidae), biological control agents of *Lythrum salicaria* (Lythraceae). *Journal of Economic Entomology* 92: 325-334.
- Blossey, B. and M. Schat. 1997. Performance of *Galerucella californiensis* (Coleoptera: Chrysomelidae) on different North American populations of purple loosestrife. *Environmental Entomology* 26: 439-445.

- Blossey, B. and D. Schroeder. 1995. Host specificity of three potential biological weed control agents attacking flowers and seeds of *Lythrum salicaria* (purple Loosestrife). *Biological Control* 5: 47-53.
- Blossey, B., D. Eberts, E. Morrison, and T. R. Hunt. 2000. Mass rearing the weevil *Hylobius transversovittatus* (Coleoptera: Curculionidae), biological control agent of *Lythrum salicaria*, on semiartificial diet. *Journal of Economic Entomology* 93: 1644-1656.
- Blossey, B., D. Schroeder, S. D. Hight, and R. A. Malecki. 1994a. Host specificity and environmental impact of two leaf beetles (*Galerucella californiensis* and *G. pusilla*) for biological control of purple loosestrife (*Lythrum salicaria*). *Weed Science* 42: 134-140.
- Blossey, B., D. Schroeder, S. D. Hight, and R. A. Malecki. 1994b. Host specificity and environmental impact of the weevil *Hylobius transversovittatus*, a biological control agent of purple loosestrife (*Lythrum salicaria*). *Weed Science* 42: 128-133.
- Brown, B. J. and C. E. Wickstrom. 1997. Adventitious root production and survival of purple loosestrife (*Lythrum salicaria*) shoot sections. *Ohio Journal of Science* 97: 2-4.
- Bunch, Q. 1977. Purple loosestrife- A honey plant. *American Bee Journal*, June: 398.
- Capogreco, J. V. 1989. Immature *Lebia viridis* Say (Coleoptera: Carabidae): Bionomics, descriptions, and comparisons to other *Lebia* species. *Coleopterists Bulletin* 43(2): 183-194.
- Chester, E. W., B. E. Wofford, and B. Kral. 1997. Atlas of Tennessee Vascular Plants Volume II. Center for Field Biology, Austin Peay State University, Clarksville, TN, 112 pp.
- Corrigan J. F., D. L. Mackenzie, and L. Simser. 1998. Field observations of non-target feeding by *Galerucella californiensis* (Coleoptera: Chrysomelidae), an introduced biological control agent of purple loosestrife, *Lythrum salicaria* (Lythraceae). *Proceedings of the Entomological Society of Ontario* 129: 99-106.
- Cortilet, A. B. 1998. Evaluation of natural enemies released for the biological control of purple loosestrife. M. S. Thesis. Iowa State University, Ames, IA. 85 pp.
- Cox, G.W. 1999. Alien species in North America and Hawaii: Impacts on natural ecosystems. Washington, DC; Island Press. 384 pp.

- Crawley, M. J. 1989. Insect herbivores and plant population dynamics. *Annual Review of Entomology* 34: 531-564.
- Dech, J. P. and P. Nosko. 2002. Population establishment, dispersal, and impact of *Galerucella pusilla* and *G. californiensis*, introduced to control purple loosestrife in central Ontario. *Biological Control* 23: 228-236.
- Diehl, J. K., N. J. Holliday, C. J. Lindgren, and R. E. Roughley. 1997. Insects associated with purple loosestrife, *Lythrum salicaria* L., in southern Manitoba. *The Canadian Entomologist* 129: 937-948.
- Downie, N. M. and R. H. Arnett Jr. 1996. The Beetles of Northeastern North America. Gainesville; Sandhill Crane Press (2 vols.), 1721 pp.
- Fall, H. C. 1910. Miscellaneous notes and descriptions of North American Coleoptera. Philadelphia, PA. *Transactions of American Entomological Society* 36: 153.
- Farnsworth, E. J. and D. R. Ellis. 2001. Is purple loosestrife (*Lythrum salicaria*) an invasive threat to freshwater wetlands? Conflicting evidence from several ecological metrics. *Wetlands* 21: 199-209.
- Gardner, S. C., C. E. Grue, W. M. Major III, and L. L. Conquest. 2001. Aquatic invertebrate communities associated with purple loosestrife (*Lythrum salicaria*), cattail (*Typha latifolia*), and bulrush (*Scirpus acutus*) in central Washington, USA. *Wetlands* 21: 593-601.
- Gaudet, C. L., and P. A. Keddy. 1988. A comparative approach to predicting competitive ability from plant traits. *Nature* 334: 242-243.
- Goeden, R. D. 1993. Arthropods for suppression of terrestrial weeds. *Proceedings of Beltsville Symposium XVIII*, ARS-USDA, Beltsville, Maryland. American Chemical Society, Washington, DC: 231-237.
- Grabas, G. P. and T. M. Lavery. 1999. The effect of purple loosestrife (*Lythrum salicaria* L.; Lythraceae) on the pollination and reproductive success of sympatric co-flowering wetland plants. *Ecoscience* 6: 230-242.
- Grevstad, F. S. and A. L. Herzig. 1997. Quantifying the effects of distance and conspecifics on colonization: experiments and models using the loosestrife leaf beetle, *Galerucella californiensis*. *Oecologia* 110: 60-68.

- Herbert, D. A. and J. W. VanDuyn. 1999. Cereal leaf beetle biology and management. Virginia Polytechnic Institute and State University, Virginia Cooperative Extension, Publication: 444-350.
- Hermann, B. P., T. K. Mal, R. J. Williams, and N. R. Dollahon. 1999. Quantitative evaluation of stigma polymorphism in a tristylous weed, *Lythrum salicaria* (Lythraceae). *American Journal of Botany* 86: 1121-1129.
- Herzig, A. 1999. Society Meeting of February 24, 1999: Biology of the leaf beetles introduced as biocontrol agents of purple loosestrife. *Entomological News* 110(3): 189-192.
- Hight, S. D. 1989. Available feeding niches in populations of *Lythrum salicaria* (purple loosestrife) in the northeastern United States. *Proceedings of International Symposium VII of Biological Control of Weed*, March 6-11, 1988. Rome, Italy, pp. 269-278.
- Hight, S. D. and J. J. Drea, Jr. 1991. Prospects for a classical biological control project against purple loosestrife (*Lythrum salicaria* L.). *Sixteenth Annual Natural Areas Conference*, October 17-20, 1989, Knoxville, Tennessee.
- Hight, S. D., B. Blossey, J. Laing, and R. Declerck-Floate. 1995. Establishment of insect biological control agents from Europe against *Lythrum salicaria* in North America. *Environmental Entomology* 24: 967-977.
- Katovich, E. J. S., R. L. Becker, and D. W. Ragsdale. 1999. Effect of *Galerucella* spp. on survival of purple loosestrife (*Lythrum salicaria*) roots and crowns. *Weed Science* 47: 360-365.
- Katovich, E. J. S., R. L. Becker, C. C. Sheaffer, and J. L. Halgerson. 1998. Seasonal fluctuations of carbohydrate levels in roots and crowns of purple loosestrife (*Lythrum salicaria*). *Weed Science* 46: 540-544.
- Kaufman, L. N. and D. A. Landis. 2000. Host specificity testing of *Galerucella californiensis* L. (Coleoptera: Chrysomelidae) on wild and ornamental plant species. *Biological Control* 18: 157-164.
- Keddy, C. J. 1992. A universal manual for purple loosestrife control. A publication of the Ontario Federation of Anglers and Hunters. 60pp.
- Keddy, P. A., L. Twolan-Strutt, and I. C. Wisheu. 1994. Competitive effect and response rankings in 20 wetland plants: Are they consistent across three environments? *Journal of Ecology* 82: 635-643.

- Kokko, E. G., R. A. DeClerck-Floate, and F. L. Leggett. 1995. Method to quantify leaf beetle consumption of leaf disks using image analysis. *The Canadian Entomologist* 127: 519-525.
- LeSage, L. 1995. Revision of the costate species of *Altica* Muller of North America North of Mexico (Coleoptera: Chrysomelidae). *The Canadian Entomologist* 127: 295-411.
- Levine, D. M., P.P. Ramsey, and R. K. Smidt. 2001. *Applied statistics for engineers and scientists: Using Microsoft Excel® and Minitab®*. Prentice Hall, Upper Saddle River, New Jersey. 671 pp.
- Lindgren, C. J. 1997. Oviposition site preferences of *Galerucella californiensis* and *G. pusilla*, biological control agents of purple loosestrife (*Lythrum salicaria*). *Weed Technology* 11: 824-827.
- Lindgren, C. J., T. S. Gabor, and H. R. Murkin. 1998. Impact of triclopyr amine on *Galerucella californiensis* L. (Coleoptera: Chrysomelidae) and a step toward integrated management of purple loosestrife, *Lythrum salicaria* L. *Biological Control* 12: 14-19.
- Lindgren, C. J., T. S. Gabor, and H. R. Murkin. 1999. Compatibility of glyphosate with *Galerucella californiensis*; a biological control agent for purple loosestrife (*Lythrum salicaria*). *Journal of Aquatic Plant Management* 37: 44-48.
- Mal, T. K., J. Lovett-Doust, and L. Lovett-Doust. 1997. Time-dependent competitive displacement of *Typha angustifolia* by *Lythrum salicaria*. *Oikos* 79: 26-33.
- Malecki, R. A. and T. J. Rawinski. 1985. New methods for controlling purple loosestrife. *New York Fish and Game Journal* 32: 9-19.
- Malecki, R. A., B. Blossey, S. D. Hight, D. Schroeder, L. T. Kok, and J. R. Coulson. 1993. Biological control of purple loosestrife: A case for using insects as control agents, after rigorous screening, and for integrating release strategies with research. *BioScience* 43: 680-686.
- Marshall, A. 1992. Don't destroy purple loosestrife. *American Bee Journal*, March: 143.
- McAvoy, T. J. and L. T. Kok. 1999. Effects of temperature on eggs, fecundity, and adult longevity of *Hylobius transversovittatus* Goeze (Coleoptera: Curculionidae), a biological control agent of purple loosestrife. *Biological Control* 15: 162-167.

- McCaughey, T. L. and G. R. Stephenson. 2000. From flowering to seed viability in purple loosestrife (*Lythrum salicaria*). *Aquatic Botany* 66: 57-68.
- Microsoft Corporation. 2002. Microsoft Excel® Software (10.2614.2625): Essential editing and formatting techniques.
- Mullin, B. H. 1998. The biology and management of purple loosestrife (*Lythrum salicaria*). *Weed Technology* 12: 397-401.
- Notzold, R., B. Blossey, and E. Newton. 1998. The influence of below ground herbivory and plant competition on growth and biomass allocation of purple loosestrife. *Oecologia* 113: 82-93.
- Nyvall, R. F. and A. Hu. 1997. Laboratory evaluation of indigenous North American fungi for biological control of purple loosestrife. *Biological Control* 8: 37-42.
- Ontario Federation of Anglers and Hunters. 2001. Purple loosestrife: What you should know, what you can do. Ontario Federation of Anglers and Hunters, Peterborough, Ontario, Canada.
- Peschken, D. P. 1977. Biological control of creeping thistle (*Cirsium arvense*): Analysis of the releases of *Altica carduorum* [Col.: Chrysomelidae] in Canada. *Entomophaga* 22: 425-428.
- Piper, G. L. 1996. Biological control of the wetlands weed purple loosestrife (*Lythrum salicaria*) in the Pacific northwestern United States. *Hydrobiologia* 340: 291-294.
- Proctor, M., P. Yeo, and A. Lack. 1996. The natural history of pollination. Timber Press, Portland, Oregon. 479 pp.
- Rawinski, T. J. and R. A. Malecki. 1984. Ecological relationships among purple loosestrife, cattail and wildlife at the Montezuma National Wildlife Refuge. *New York Fish and Game Journal* 31: 81-87.
- Scott, M. L. and J. L. Haskins. 1987. Effects of grazing by chrysomelid beetles on two wetland herbaceous species. *Bulletin of the Torrey Botanical Club* 114 (1): 13-17.
- Snoddy, E. L. and W. K. Oldland. 1995. A new native chrysomelid pest of purple loosestrife in the Tennessee Valley. Environmental Research Center; Tennessee Valley Authority, Muscle Shoals, Alabama: 1-3.

- Southeast Exotic Pest Plant Council. 2001. Invasive exotic pest plants in Tennessee: Report from the Tennessee Exotic Pest Plant Council. Southeast Exotic Pest News 7: 6-9.
- Southeast Exotic Pest Plant Council. 2002. Purple loosestrife. Southeast Exotic Pest Plant Council Publications. <http://www.se-eppc.org/doc.cfm?id=487>.
- Stap D. and D. A. Sibley. 2002. Living on the edge: With wetlands declining and shorebirds in trouble, the question is: How do we reclaim the habitat the birds need to survive? Audubon 104 (2): 56-52.
- Stevens, K. J., R. L. Peterson, and G. R. Stephenson. 1997. Vegetative propagation and the tissues involved in lateral spread of *Lythrum salicaria*. Aquatic Botany 56: 11-24.
- Stuckey, R. L. 1980. Distributional history of *Lythrum salicaria* in North America. Bartonia 47: 3-20.
- Taechenberg, E. F. and H. Riedl. 1985. New York state integrated pest management fact sheet: Grape flea beetle. NY State, Agricultural Experiment Station, Geneva, NY College of Agricultural and Life Sciences, Cornell University, Ithaca, NY. Publication: 102GFSG-IVI.
- Tennessee River Gorge Organization. 2001. Animals species list: Insects. <http://www.trgt.org/Scientific/animal/page3.htm>.
- Thompson, D. Q. 1991. History of purple loosestrife (*Lythrum salicaria* L.) biological control efforts. Natural Areas Journal 11(3): 148-150.
- Thompson, D. Q., R. L. Stuckey, and E. B. Thompson. 1987. Spread, impact, and control of purple loosestrife (*Lythrum salicaria*) in North American wetlands. U. S. Dept. Interior Fish and Wildlife Service. 55 pp.
- Treberg, M. A. and B. C. Husband. 1999. Relationship between the abundance of *Lythrum salicaria* (purple loosestrife) and plant species richness along the Bar River, Canada. Wetlands 19: 118-125.
- United States Department of Agriculture. 2002. Plant profile *Lythrum salicaria*. http://plants.usda.gov/cgi_bin/plant_profile.cgi?symbol=LYSA2.
- University of Tennessee Herbarium. 2002. Vascular: *Lythrum salicaria*. <http://www.bio.utk.edu/botany/herbarium/vascular/vascular.html>.
- Van Driesche, R. G. and T. S. Bellows, Jr. 1996. Biological control. Chapman and Hall, International Thomson Publishing, Florence, KY. 447 pp.

- Weihe, P. E. and R. K. Neely. 1997. The effects of shading on competition between purple loosestrife and broad-leaved cattail. *Aquatic Botany* 59: 127-138.
- Welling, C. H. and R. L. Becker. 1990. Seed bank dynamics of *Lythrum salicaria* L.: Implications for control of this species in North America. *Aquatic Botany* 38: 303-309.
- Welling, C. H. and R. L. Becker. 1992. Life history and taxonomic status of purple loosestrife in Minnesota: Implications for management and regulation of this exotic plant. Minnesota Department of Natural Resources, Special Publication, 146: 1-15.
- Western Aquatic Plant Management Society. 2002. *Lythrum salicaria*, purple loosestrife: A problem aquatic plant in the western United States. <http://www.wapms.org/plants/purpleloosestrife.html>.
- Whitt, M. B., H. H. Prince, and R. R. Cox Jr. 1999. Avian use of purple loosestrife dominated habitat relative to other vegetation types in a Lake Huron wetland complex. *Wilson Bulletin* 111: 105-114.
- Wiebe, A. P. and J. J. Obrycki. 2002. Prey suitability of *Galerucella pusilla* eggs for two generalist predators, *Coleomegilla maculata* and *Chrysoperla carnea*. *Biological Control* 23: 143-148.
- Willis, A. J. and B. Blossey. 1999. Benign environments do not explain the increased vigor of non-indigenous plants: A cross-continental transplant experiment. *Biocontrol Science and Technology* 9: 567-577.

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