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PREDATOR EFFECTS ON *GALERUCELLA CALMARIENSIS* L. (COLEOPTERA:CHRYSOMELIDAE), CLASSICAL BIOLOGICAL CONTROL AGENT OF *LYTHRUM SALICARIA* L. (MYRTALES:LYTHRACEAE)

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ABSTRACT

PREDATOR EFFECTS ON *GALERUCELLA CALMARIENSIS* L. (COLEOPTERA: CHRYSOMELIDAE), CLASSICAL BIOLOGICAL CONTROL AGENT OF *LYTHRUM SALICARIA* L. (MYRTALES: LYTHRACEAE)

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The leaf-feeding beetle *Galerucella calmariensis* is being released in North America for the control of purple loosestrife, *Lythrum salicaria*, an invasive plant that forms dense stands in wetlands. Neonate *Galerucella calmariensis* were evaluated for behavioral mechanisms of predator avoidance. Larvae oriented upward on stems and moved inside shoot tips where 77% were concealed after 1 h. Neonate survival was higher in tips than on leaves in the presence of *Coleomegilla maculata*. Higher larval density reduced residence time in shoot tips and predation by *C. maculata* was greater at higher larval densities.

Predation tests on *G. calmariensis* life stages in laboratory tests and in a field experiment revealed higher predation rates in the lab than in the field. *Coleomegilla maculata*, *Coccinella septempunctata*, *Harmonia axyridis*, *Podisus maculiventris*, *Forficula auricularia*, and *Pterostichus melanarius* were present in field experiment blocks. The presence of predators in stands of *L. salicaria* can affect *G. calmariensis*, however, observations indicate that low predator abundance and the presence of aphid prey may have been responsible for low predation in the field.

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CHAPTER ONE

History, biology and control of *Lythrum salicaria* L. in North America

Introduction

The past decade has seen a greatly increased awareness of the impact of exotic invasive species on US ecosystems. For example, in 1993 the U.S. Congress Office of Technology Assessment published a study that declared invasive species a serious threat to native species and natural areas. More recently, in February 1999, an executive order signed by the President of the United States recognized the need for increased action on invasive species management and prevention. This order authorized the creation of a federal council to devise a management plan by August 2000. The concern over invasive species is the result of our increasing knowledge about the mounting economic and environmental costs associated with agricultural and natural systems degradation and human health concerns.

Exotic introductions have historically been the result of human migrations, exploration, travel and trade, and more recently due to increasing global interconnectivity. As an example, prior to settlement by humans, Hawaii was colonized by a vascular plant or metazoan at the rate of one species every 50,000 years (Ewel et al. 1999). In the fourth century, after settlement by Polynesians, 3-

4 species were colonizing the island chain every 100 years. Within the last ten years, the rate of colonization has increased to over twenty species per year. The United States is now home to over 30,000 non-indigenous species of all taxa and many of these species are pests that cause an estimated $122 billion in losses annually (Pimentel et al. 1999). These species include the introduction by immigrants of food crops such as potatoes and corn as well as cattle and other livestock. However, they also include pest species such as human and plant pathogens, insect and plant pests, and vertebrate pests such as rats.

Environmental concern over invasive species stems from observed negative effects on native communities. Invasive species have been reported to change ecological characteristics including primary productivity, decomposition rates, hydrology, geomorphology, nutrient cycling and natural disturbance cycles, thus linking invasive species to global environmental change (Dukes and Mooney 1999, Parker and Reichard 1998, Vitousek et al. 1996). In a recent review of competition between native and invasive species, Parker and Reichard (1998) found that the most often cited factor leading to interspecific competition was disturbance. Other key contributors were reproductive factors such as prolific seeding and rapid, efficient seedling establishment. Investigating the prediction that biotic resistance (disturbance regimes and species richness) determines the impact of invasive species, Parker and Reichard (1998) found that experiments aimed at addressing this prediction were inconclusive. The lack of empirical studies regarding invasive species activity and the propensity for using anecdotal

observations are problems in studies of invasive species biology and biological invasions (Ehler 1998, Gordon 1998, Parker and Reichard 1998, White and Schwarz 1998).

The damage that invasive species cause can translate into significant economic, human, and environmental losses annually. For example, the estimated economic cost of cleaning intake pipes clogged by zebra mussels may reach $3.1 billion over ten years (Vitousek et al. 1996). Among invertebrates, the gypsy moth (*Lymantria dispar* L.) has resulted in $11 million annually in control efforts by the U.S. Forest Service (Pimentel et al 1999, Forbush and Fernald 1896). There are about 5,000 non-indigenous plant species residing in natural areas of the United States and these are spreading at the rate of about 700,000 ha each year. Among these, purple loosestrife or *Lythrum salicaria* L. (Myrtales: Lythraceae), invades wetland habitats causing an annual estimated cost of $45 million (Pimentel et al. 1999).

Lythrum salicaria in North America

Lythrum salicaria is a flowering wetland perennial which entered North America in the early 1800's from Europe and Asia (Malecki et al. 1993, Hight and Drea 1991, Thompson et al. 1987, Stuckey 1980). It is believed to have been transported inadvertently as seeds or other plant parts in ships' ballast. It was also intentionally imported as a medicinal herb and as a bee forage plant. Prior to 1900, herbarium records indicate *L. salicaria* was present only in the northeastern

and Atlantic coast of the United States and Canada, although a specimen was recorded in Manitoba prior to 1900 (Stuckey 1980). The plant slowly became established along the New England seaboard and then spread westward along natural and constructed inland waterways. The spread of *L. salicaria* across the United States followed the path of westward expansion. It spread into Michigan via ports in Detroit, Bay City, Saginaw, Port Huron, Saugatuck and Muskegon. By 1940, it had spread to Oregon and by 1980 sightings were recorded in 29 of the 48 contiguous United States and all of the Canadian Provinces. *Lythrum salicaria* was not identified as a pest until the 1930's when it increasingly infested floodplain meadows used for cattle grazing lands along the St. Lawrence River (Thompson et al. 1987). The relatively rapid spread of *L. salicaria* across the United States prompted scrutiny of the plant's effects on invaded wetlands.

Biology of *Lythrum salicaria* in North America

A mature *L. salicaria* plant has an emergent root crown from which twenty to fifty 2.0-3.0 m tall stems may arise. Each stem supports one or more inflorescences of bright purple flowers which bloom from late June to early September. *Lythrum salicaria* has several traits that make it an effective competitor in North American wetland systems. It is highly fecund; sexual reproduction may yield 2.5 million seeds in a mature plant (Malecki et al. 1993) and it is capable of vegetative reproduction (Thompson et al. 1987). Ungerminated seeds can float while germinated seeds sink to the substrate and

establish as seedlings. Seeds may be dispersed by wind and water or transported by animals or machinery, allowing *L. salicaria* to infest new locations.

Once established, *L. salicaria* can become the dominant component of the wetland. In infested wetlands, up to 410,000 seeds may be found in a 5-cm deep by 1-m^2 area (Welling and Becker 1990). These vast seed banks enable rapid recruitment in response to disturbance. In contrast to its native range, in North America *L. salicaria* is reported to out-compete wetland associates, reducing densities and eliminating populations of cattail (*Typha* spp.), sedge (*Carex* spp.), bulrush (*Scirpus* spp.), horsetail (*Equisetum fluviatile*), and other wetland species (Thompson et al. 1987). This reduction in native plant species reportedly results in reduced habitat for wetland fauna. Thompson et al. (1987) observed that muskrats in a mixed stand of *Typha latifolia* (cat-tail) and *L. salicaria* heavily cut and used *T. latifolia*, but only partially cut *L. salicaria*. Cut *L. salicaria* stems were not consumed and floated away, therefore, they may propagate new plants. Thompson et al. (1987) also observed that birds were negatively impacted by invasion of *L. salicaria* which rendered black tern nesting sites unusable and reduced habitat of canvasback ducks. Moreover, the eastern bog turtle *Clemmys muhlenbergii* prefers open marsh and wet fen habitat, which are susceptible to invasion by *L. salicaria*, particularly following human disturbance (Thompson et al. 1987). The resulting tall, dense monotypic stands of *L. salicaria* may further jeopardize populations of this already rare turtle species. In contrast, several researchers have found that native fauna can exploit *L. salicaria*. Anderson (1995)

reviewed 71 articles on *L. salicaria* and found evidence of animal use of *L. salicaria* in 34 of them. Consumption of young shoots by white-tail deer and cotton-tail rabbit was observed, as was cutting of stems by muskrat. Six species of birds including American coots, pied-bill grebes, black-crowned night herons, American goldfinches, gray catbirds and red-winged catbirds were observed utilizing *L. salicaria* (Whitt et al. 1999). Red-winged catbirds selected *L. salicaria*-infested habitat over habitat containing predominantly *T. latifolia*, however, marsh wrens preferred *T. latifolia* habitat. *Rana pipiens* (northern leopard frog) prefers to spawn in stands of *Phalaris arundinacea* (reed canary grass), however, they will spawn in *L. salicaria* as well (Gilbert et al. 1994). These studies indicate that while not part of our native floara, some organisms may be able to exploit wetlands infested with *L. salicaria*. In Europe, *L. salicaria* responds strongly to disturbance, but in subsequent years loses the brief competitive advantage and a more stable plant community returns (Blossey and Kamil 1996). There have been mixed reports about the effects of *L. salicaria* on native plants in North America. Gaudet and Keddy (1995) conducted a study of the relative competitive performances of 44 herbaceous wetland plant species. *Lythrum salicaria* was used as a phytometer against which 44 test species were evaluated. *Lythrum salicaria* was the most competitive plant, reducing the biomass (dry weight) of its nearest conspecifics by up to 96%. A study looking at competition intensity in high versus low standing-crop biomass wetlands showed that high standing-crop wetlands had higher competition intensity (Twolan-Strutt

and Keddy 1996). Weihe and Neely (1997) looked at the effects of shade on competition between *L. salicaria* and *Typha latifolia* in pot studies. In this study, *L. salicaria* out-competed *T. latifolia* leading to localized extinction regardless of the initial densities of either plant. However, in a field study, Treberg and Husband (1999) found no evidence for competitive displacement of native plants by *L. salicaria*. Native plants were found co-existing in a site where *L. salicaria* had been established for at least twelve years.

Other studies have shown that *L. salicaria* can have an effect on the base of wetland food webs (Grout et al 1997). Comparisons of the mean decay rates of *L. salicaria* and a native sedge in a river estuary showed significant differences in litter decay between the two species. *Lythrum salicaria* decomposed rapidly, providing an autumn release of nutrients. By contrast, sedge decomposed slowly, releasing nutrients in the winter and the following spring during periods when detritivores are prevalent. Grout et al. (1997) concluded that dense monotypic stands of *L. salicaria* may have serious implications on the stability of river estuary food webs.

What properties make *L. salicaria* invasive? According to the optimal defense theory (Coley et al. 1985), a plant introduced into a location other than its home range is released from natural enemies and/or is presented with a more favorable environment in which to reside (Blossey and Notzold 1995, Lerdau 1995, Herms and Mattson 1994). Thus, pre-existing tradeoffs in allocation of resources to maintenance, growth, reproduction, and defense shift when released

from these limitations. Following optimal defense theory, the evolution of increased competitive ability (EICA) hypothesis predicts that the absence of those selection pressures results in a more vigorous plant population in the invaded range (Blossey and Notzold 1995). A comparison of one *L. salicaria* population each from the United States and Europe showed that the US population grew nearly 48 cm taller on average and contained nearly 40 g more dry bio-mass than the European population.

The reported negative effects of *L. salicaria* led to efforts to manage it and to research different methods for controlling it in North America. Flooding, draining, manual removal, mowing, burning and herbicide application were attempted with some success, particularly in newly-established stands, but in older stands seed recruitment led to stabilization of *L. salicaria* populations (Malecki et al. 1993). In light of the ineffectiveness of environmental control, classical biological control was explored as a management option.

Classical Biological Control of *Lythrum salicaria*

Classical biological control (CBC) is the purposeful introduction of a natural enemy of the target pest from the pest's home range with the purpose of reducing pest population levels (adapted from Barbosa and Braxton 1993). The decision to conduct CBC hinges on an analysis of the costs and risks of implementing the method compared to the resulting benefits. The estimated cost of a ten-year program of CBC on *L. salicaria* was initially estimated at about $1 million with an

estimated benefit to cost ratio of 27.0 to 1.0 (Thompson et al. 1987). Some notable examples of CBC of weeds include control of *Hypericum perforatum* L. (St. John's-wort or klamath weed) by the leaf-beetles *Chrysolina quadrigemina* (Suffr.) and *C. hyperici* Forst (Harris 1993) and control of *Alternanthera philoxeroides* (alligatorweed) by three insect species, the flea beetle *Agasicles hygrophila* Selman and Vogt, the moth *Vogtia malloi* Pastrana and a thrip *Amynothrips andersoni* O'Neill (Julien et al. 1995).

While successful control of weeds has been well-documented (McFadyen 1997), reports of negative impacts have also been reported (Louda et al. 1997, McFadyen 1997, Howarth 1991). *Rhinocyllus conicus*, a weevil that feeds on flower heads of thistle, was introduced into North America for control of invasive Eurasian thistles (*Carduus* spp.) such as musk thistle, *Carduus nutans* L. (Louda et al. 1997). While *R. conicus* does control musk thistle, there is increasing evidence of damage to native thistles.

Due to the continued spread and perceived dominance of *L. salicaria* in North America, a program of CBC was explored. Hight (1991) surveyed populations of *L. salicaria* in the United States for associated insect faunas and found 59 species of phytophagous insects present, however, none that had a strong negative impact on the plant. These insects shifted to *L. salicaria* from other hosts and were chiefly generalist herbivores. Surveys for other natural enemies revealed no significant fungal pathogens present from native North American *Lythrum* species (Hight and Drea 1991, Farr et al. 1989). No pathogens were discovered in

purple loosestrife's native range, however, 15 species of oligophagous insects were found closely associated with the plant in Europe (Hight and Drea 1991, Batra et al. 1986, Schroeder and Mendl 1984). Six of these species were selected as the most promising candidates for classical biological control agents. These species included *Hylobius transversovittatus* Goeze, a root mining weevil; *Galerucella calmariensis* L. and *G. pusilla* (Duftschmid), two leaf-feeding beetles; *Nanophyes marmoratus* Goeze and *Nanophyes brevis* Boheman, two flower-feeding weevils; and *Bayeriola salicariae* Kieffer, which attacks flower buds. These insects were studied for several years in their native range to determine their effects on *L. salicaria* (Malecki et al. 1993). Considered the most promising due to the extent of damage they incur on *L. salicaria*, *Hylobius transversovittatus*, *G. calmariensis*, and *G. pusilla* were selected for host-specificity testing at the CAB-International Institute of Biological Control (now CABI-Bioscience). Their life histories, impact on purple loosestrife and ecological interactions were studied (Hight and Drea 1991). Concurrently, USDA-funded quarantine studies were performed at the Virginia Polytechnic Institute. Screening of these insects on native North American plants was performed across four categories. These categories included 18 spp. of Lythraceae, 11 spp. related to Lythraceae, 14 spp. of N. American wetland associates of purple loosestrife, and 7 key agricultural crops. Two non-target plants, *Decodon verticillatus* L. (swamp loosestrife) and *Lythrum allatum* Pursh (winged loosestrife), both members of the Lythraceae were found to be potential alternate hosts of the three candidate biological control

agents. However, field tests in Europe showed that these plants were minimally attacked in the presence of *L. salicaria* and the insects were approved for releases which began in 1992 in New York (Blossey and Schroeder 1995, Hight et al. 1995, Blossey et al. 1994).

Currently, releases of *L. salicaria* natural enemies are being performed in the United States and Canada as part of an international effort coordinated by Cornell University and the University of Minnesota. In Michigan, releases of 5,000 adult *Galerucella calmariensis* and *G. pusilla* were performed at five sites by the Michigan Department of Natural Resources (MI-DNR) in 1994. In 1997, fourteen releases of 5,000+ adults were conducted by the Purple Loosestrife Project at Michigan State University. Continued redistribution of these beetles by Michigan State University at 13-14 new sites per year has resulted in 40 releases over three years and establishment of reproducing populations of *Galerucella* spp. at most of these sites. Monitoring of these sites and the MI-DNR release sites in the spring and fall of each year shows promising results. At three of the original (1994) sites, complete defoliation of above-ground biomass is evident for over 100 m distance from the center of release. Other release sites have shown increased defoliation on *L. salicaria* indicating a progression in suppression similar to the three 1994 sites. However, continued intense defoliation for several years may be required to achieve suppression. In a field study, complete defoliation had little impact on plant mortality, with no change in root stores of carbohydrates observed after two years of complete defoliation (Katovich et al. 1999).

Galerucella spp. Biology, Ecology and Life History

There are three native species of *Galerucella* in North America (Manguin et al. 1993). *Galerucella nymphaeae* has a broad range of aquatic hosts in the U.S and Europe including water lily (*Nuphar lutea*) and purple loosestrife. *Galerucella stefanssoni* uses cloudberry (*Rubus chamaeomorus*) as a host plant and *G. quebecencis* has as its host marsh-flower (*Potentilla palustris*). *Galerucella calmariensis* and *G. pusilla* range from 3-6 mm in length and are about half as wide as they are long. Coloration is light brown, sometimes with a dark stripe located at the margin of each elytron. Manguin et al. (1993) positively identified adult males by dissection of the adeagus and adult females by comparison of the third tergite. Since the two European beetles are nearly identical morphologically, physiologically and ecologically, they will be treated here as a single entity.

Overwintering adults emerge from soil and litter below old loosestrife plants to feed for several days on new foliage before reproduction commences (Blossey 1995a, Hight et al. 1995, Hight and Drea 1991). After mating, females oviposit in frass-covered masses averaging 5-6 eggs per mass, generally on the stem at leaf axils, but also on leaves and flower buds, particularly later in the growing season (Lindgren 1997, Blossey 1995b). An individual female may oviposit up to 500 eggs from mid-May to mid-July, with oviposition peaking in late May and early June. Progeny mature through three instars before entering the pupal stage. Neonate eclosion occurs in 7-10 days and the larvae move to shoot

tips and feed on developing tissues (Lindgren 1997, Hight et al. 1995). After one or two molts, larvae migrate to leaves to feed. Larvae feed and molt over approximately three weeks before the third instars move down into the soil or litter to pupate. Where water levels are higher, larvae may pupate inside the stem by burrowing into aerenchyma tissue (Hight and Drea 1991). Upon emergence tenerals harden, then feed until they overwinter. This period of adult emergence occurs typically from July to September. New adults may exhibit an abbreviated oviposition period prior to diapause (Hight et al. 1995). The total maturation time from egg to adult is approximately 30-40 days (Haas, M. pers. comm.)

At high population densities, *Galerucella* spp. can cause significant damage to purple loosestrife, being capable of complete defoliation and photosynthetic suppression of the plant as well as rendering it incapable of flowering (Hight and Drea 1991). Neonate feeding in developing shoot tips kills or damages primary and secondary meristems. Subsequent lateral shoot growth results in inflorescences much reduced in size. Larvae produce a windowpane feeding pattern on the leaf, eating the softer tissues and leaving the more lignified material. Larval damage to flower and shoot buds reduces plant growth and inhibits flowering. Adults inflict a shot-hole feeding pattern, eating small (1-2 mm) holes through foliage. Adult and larval leaf damage greatly reduces the photosynthetic capability of purple loosestrife, leading to reduced starch stores in the roots and, ultimately, overwintering mortality. Photosynthetic inhibition results in reduced stem height and root length, both essential to overall plant vigor. The resultant

weakening and/or death of loosestrife plants provides an opportunity for previously suppressed native plant species such as cattails (*Typha latifolia* and *T. angustifolia*), grasses, and sedges to return, particularly in mixed stands of wetland plants (Malecki et al 1993).

The two *Galerucella* species have identical phenologies and overlapping distributions in *L. salicaria's* natural range and both occupy inclusive fundamental niches (Blossey 1995b). The stable coexistence of the two species does not appear to be through division of resources or niche partitioning , however, theoretical modeling of natural enemy (predatory) interactions suggests that mortality may have a role in stabilizing coexistence among niche competitors.

The beetles have the ability to fly between plants or plant clusters and both larvae and adults float, allowing current or wind to move them to other nearby plants (pers. obs). Impact on flooded sites is not thought to be possible using the root-mining weevil *Hylobius transversovittatus* as the larvae are unable to survive in the roots under permanently flooded conditions. Long-term standing water does not appear to be detrimental to *Galerucella's* development (pers. obs.). Malecki et al. (1993) predicted that a combination of natural enemies will be necessary to control *L. salicaria*. They also propose that control will be more effective in mixed vs. monotypic plant communities and that 90% reduction of *L. salicaria* could be expected. Thus, the establishment of permanent stable populations of *Galerucella* on purple loosestrife is predicted to reduce this plant to stable levels in North America. In the 7 years since *Galerucella* spp. have been released here

in North America, observations indicate that most sites require several years (3+) for populations of *Galerucella* spp. beetles to increase before significant impact is observed (Blossey, B. pers. comm., Ragsdale, D. pers. comm.). Other introduced herbivores for weed CBC have shown similar patterns in population growth (McFadyen 1998). During this period of low *Galerucella* spp. density, predators may be an important factor in their establishment and spread. Qualitative field observations of predators attacking *Galerucella* spp. have been reported (Malecki et al., 1993). They found predation occurring on the two species of *Galerucella* by spiders occurring in Europe. Hight et al. (1995) observed that the survival rate of *Galerucella* spp. in field cages was significantly higher than those without cages. Since the densities of native herbivores within field cages were also higher, it was concluded that predators were excluded from the cages, thus, predation was lower. A field study detailing predation on the congener *G. nymphaeae* L. was conducted in North America to try and predict the intensity of predation that could be expected once *G. calmariensis* and *G. pusilla* were released (Nechols et al., 1996). In these studies, Nechols et al. (1996) found that several generalist arthropod predators, including the native ladybeetle *Coleomegilla maculata* DeGeer can have an impact on herbivores. About 33% of *G. nymphaeae* egg masses were attacked from late spring to the end of the summer. The number of eggs per mass consumed changed from about 50% in spring to nearly 90% by the end of the season, although attack was not a function of the number of eggs per mass. Larval and pupal survival was low in open cages vs. control cages. Nechols

et al. (1996) concluded that initially caging the *Galerucella* spp. at the release site may aid in their establishment. Goeden and Louda (1976) reviewed studies citing biological factors inhibiting classical biological control and found that indigenous predators and parasitoids were significant contributors to the ineffectiveness or failure of classical biological control projects on weeds.

The following studies were undertaken to quantify and describe the effects of indigenous predators on the survival, behavior and colonization ability of *Galerucella* spp. beetles. The behavior of neonate *Galerucella calmariensis* was observed in order to determine the lethal and non-lethal effects of predators. In a greenhouse pilot study, we found that predation by *Coleomegilla maculata* was high, however, damage to shoot tips by *Galerucella calmariensis* larvae was evident, suggesting that neonate larvae might avoid predation by feeding in tips (Sebolt and Landis unpub. data). The propensity of neonate *G. calmariensis* larvae to orient to shoot tips was tested in a greenhouse experiment and the effectiveness of those tips as predator refuges tested in a laboratory petri dish bioassay. Since as many as 22 *G. calmariensis* larvae have been observed in shoot tips in the field (pers. obs.), the effects of increased larval density in shoot tips were assessed in a greenhouse experiment.

Quantification of the potential for predation on *G. calmariensis* by arthropod predators has not been previously measured. The occurrence of predator species was determined in field surveys then predation potential tested in the laboratory. The most abundant predators were collected and first tested in

simple petri dish assays to determine which *G. calmariensis* life stages they would consume. Predators that consumed any life stages were then tested in arenas resembling a wetland micro-environment.

The success of *Galerucella* spp. in controlling *L. salicaria* will depend on their ability to colonize new patches unassisted. *Galerucella* spp. disperse and aggregate by finding conspecifics, indicating that initial colonization of new patches results in isolated groups of beetles colonizing at varying densities (Grevstad and Herzig 1997). This scattered distribution of beetles at varying densities in a new patch could result in low density sub-populations that may be susceptible to predation. This prediction was tested in a controlled field experiment in two wetlands.

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CHAPTER TWO

Neonate *Galerucella calmariensis* (Coleoptera: Chrysomelidae) Behavior on Purple Loosestrife (*Lythrum salicaria)* Contributes to Predator Avoidance

Introduction

Many organisms have developed various means to avoid predation including cryptic or aposematic coloration, mimicry, and warning signals (Alcock 1997). Among arthropods, predation can be a significant mortality factor, even leading to local extinctions (Hawkins et al. 1997). Hawkins et al. (1997) found that predation on exotic species was higher than on native prey species.

Insects exhibit a variety of predator avoidance mechanisms. Classic examples of predator deterrents in insects include coloration and warning displays of *Danaus plexippus* L. associated with avoidance learning in avian predators (Brower 1958) and cryptic coloration in moths (Kettlewell 1955). Insects of the family Chrysomelidae exhibit a full range of predator avoidance mechanisms (Begossi and Benson 1988). For instance, the flea beetle *Oedionychis fasciata* will spring away if disturbed. Chemical discharges such as reflex bleeding in roughly handled adults and defensive allomone secretions from larvae responding to tactile stimulation are also evident (Pasteels et al.

1994). Other insects construct refuges as protection from predators, as in the case of some larval Lepidoptera, which construct webs or individual cocoons in which to rest or feed (Larsson et al. 1997). Those insects unable to construct shelter may take advantage of host plant structure to minimize exposure to predators.

Galerucella calmariensis L. and *G. pusilla* (Duftschmidt) are two Eurasian leaf beetles (Coleoptera: Chrysomelidae) imported into North America for biological control of purple loosestrife (*Lythrum salicaria*, L.) (Lythraceae). Following initial release, it often takes 3-5 years for populations of the natural enemies to increase before significant impact on the target weed is observed (Lindgren et al. 1997). During the period of low *Galerucella* density, predators may be an important factor affecting their establishment and spread. In their egg and larval stages both insects are known to be susceptible to attack by several species of arthropod predators including *Coleomegilla maculata* DeGeer (Coleoptera: Coccinelidae), a native ladybeetle (Nechols et al. 1996, Malecki et al. 1993). However, the actual impact that predators may have on the establishment or spread of populations of these natural enemies on purple loosestrife has yet to be quantified.

Galerucella calmariensis adults emerge from overwintering sites in late April or early May, feed and mate for several days. Females may oviposit as many as 500 eggs on stems and leaves of *L. salicaria* in their lifetime (Lindgren 1997, Blossey et al. 1994, Hight and Drea 1991). Eggs hatch in 7-

14 d and larvae disperse to feeding sites within developing shoot tips. They complete their development as 2^{nd}-3^{rd} instars by feeding externally on the stem and leaf tissue. Larval feeding in tips damages meristematic tissues and can result in stunting or suppression of reproductive capacity (Blossey et al. 1994, Hight and Drea 1991). Leaf defoliation by later instars can nearly be complete at very high larval densities (Blossey et al. 1994). Larvae develop through three instars, then move to the litter and soil at the root crown and pupate.

In a preliminary greenhouse experiment (Sebolt and Landis, unpub. data) we determined that *C. maculata* can significantly reduce populations of *G. calmariensis* as previously suggested by Nechols et al. (1996). However, treatments containing both predator and prey still resulted in significant damage to shoot tips despite heavy predation pressure. This suggested that early-instars may gain an advantage by feeding in shoot tips and thus escape predation. Neonates of a closely related chrysomelid, *Galerucella lineola* F., are known to move up the stem of their host *Salix viminalis* L. in search of naturally curled leaves close to the stem tip (Larsson et al. 1997). While Larsson et al. (1997) found that rolled leaves did not protect from predators, they did find that leaf shelters protected neonate larvae from environmental stress. Neonates residing in leaf refuges were more resistant to dessication and grew faster than neonates feeding openly on leaves, indicating that conditions and nutrients in the leaf refuge was optimal for larval development (Larsson et al. 1997, Pelletier 1995, Willmer 1980, 1982).

Because protection from dessication and predation may be important factors governing *G. calmariensis* larval behavior, we predicted that soon after egg hatch, neonates would move up *L. salicaria* stems to shoot tips. Moreover, since tips are structurally complex with tightly packed leaves, we predicted that those larvae choosing to feed concealed inside shoot tips would gain protection from predators as well. We tested these predictions in laboratory and greenhouse experiments. In addition, neonate *G. calmariensis* are constrained to feed on the plant their mother chose for oviposition and at high larval densities, tips may be a limited resource. As many as twenty-two larvae have been observed in one shoot tip in the field (pers. obs.). We predicted that larval density and residence time in the tip are inversely related and that as the shoot tip is destroyed by larval feeding, individuals would be forced to disperse to new feeding sites. We also predicted that at higher densities, larvae would be more susceptible to predators as they disperse or become increasingly exposed due to deterioration of the shoot tip. These predictions were tested in two separate greenhouse experiments.

Materials and Methods

Insects and plants for the studies were from colonies maintained at Michigan State University. Prior to these experiments, adult *G. calmariensis* were overwintered in plastic bags containing moistened paper towels and held at 4.5°C, 4:20 (L:D) h. As needed, beetles were removed and acclimated for

two days at 22°C, 16:8 (L:D) h before being released to feed *ad libidum* on 60 cm tall *L. salicaria* plants. All plants used had been collected in the fall as root-stocks and held at 40°C until planting. Plants were grown in 17.7 l (5 gallon) pots from root crowns planted in soil-less potting media and fertilized with 114 g Osmocote (Mollema and Son, Inc., Grand Rapids, MI)14:14:14 (N:P:K). Pots were enclosed by No-See-Um (Balson Hercules Corporation, New York, New York) sleeve cages over 1 m tall tomato cages. Eggs were collected by moving gravid females to an oviposition cage where they were provided with cut *L. salicaria* stems in an Erlenmeyer flask filled with water. Females were allowed to oviposit for 2 to 8 hours, after which foliage was removed and eggs carefully excised from leaf tissue. Eggs were placed in 60 mm X 15 mm Petri dishes containing filter paper moistened with 0.25 ml dH_2O, sealed with parafilm and held in an incubator at 26°C 16:8 (L:D) h until eclosion. Under these conditions eggs hatched in 3 days. Neonates, defined as larvae less than 4 hours old, were used in all experiments. A sub-sample of neonates was removed and reared through to the adult stage for species identification.

Larval Movement Study.

Experiments were conducted on the campus of Michigan State University in the Center for Integrated Plant Systems greenhouses where temperature conditions ranged from 26-30°C and 60-70% humidity. Daylength in the greenhouse was 16:8 (L:D) h. Only *Lythrum salicaria* plants

having one pair of opposite leaves were used in these studies (i.e. trifoliate forms were excluded from the study). Axilary shoot growth was removed from the stem with forceps so only terminal shoot tips were present prior to introduction of larvae. Using a fine camel hair brush, one larva was placed at the mid-point of each 20-24 cm tall stem (n=39, one larva was killed in transfer) with the long axis of the body oriented perpendicular to the stem. To track location of larvae, internodes were assigned numbers. The portion of the stem from the shoot tip to the first node (excluding its foliage) was assigned a value of five. The stem between the first node (and its foliage) and the next node was assigned a four, continuing to a value of one for the lowest node/internode location on the stem. Any remaining length of stem below the last assigned internode was considered part of that internode and scored a one. The internode on which larvae were placed was recorded as well as the initial choice of direction after one minute. Every ten minutes thereafter, the numerical location (internode), vertical movement up or down in millimeters, and time (nearest minute) to reach the 3rd or 4th layer inside the shoot tip were recorded. Shoot tip layers were designated according to visible pairs of oppositely arranged leaves with the outermost pair at the tip being the first layer, the next inner pair the second layer to the innermost discernible layer which was chiefly the fourth pair of leaves.

The number of larvae moving up, down, or not directed was contrasted within the first minute and at 10 minutes after release. A Chi-Square Test for

Specified Proportions was performed to test the hypothesis that initial choice of direction was equally distributed between up, down and not directed (SAS Institute 1995). A Chi-Square test was used to test the hypothesis that larval locations were equally distributed among the 5 locations on the stem.

Shoot Tip Refuge Study.

The experiment contrasted the survival of *G. calmariensis* neonates feeding on leaves or in shoot tips in the presence and absence of a *C. maculata* adult. Larvae and *L. salicaria* plants were obtained in the same manner as for the larval movement experiment described above. *Coleomegilla maculata* adults were field-collected on the Michigan State University Farms from wheat and corn fields, stored in a plastic bag containing moist paper towel and held in an incubator for a 24-hour starvation period under the conditions described above. Shoot tips and foliage were collected from greenhouse grown *L. salicaria*. The experiment contained 20 replications each of the following treatments: 1) Shoot tip w/1 *G. calmariensis* neonate, 2) Leaf w/ 1 *G. calmariensis* neonate, 3) Shoot tip w/ 1 *G. calmariensis* neonate + 1 *C. maculata* adult, 4) Leaf w/ 1 *G. calmariensis* neonate + 1 *C. maculata* adult. Leaves or shoot tips were attached by their bases to the sides of 60 mm X 15 mm Petri dishes using non-toxic floral clay (FloraCraft Corporation, Ludington, Michigan). This held the plant part off of the dish bottom and provided neonate *G. calmariensis* and adult *C. maculata* access to all surfaces of the leaf or tip. One *G. calmariensis* neonate was placed in each dish and

given one hour to disperse and commence feeding. At this time a single *C. maculata* adult was placed in each of the dishes in treatments three and four. All Petri dishes were sealed with Parafilm™, placed in a completely randomized design on an open tray and held in an incubator (26°C and 16:8 (L:D) h) for 24 h. After 24-h, the dishes were removed to check for neonate and predator survival. Neonates in shoot tips were located by destructive sampling of the shoot tip. A Chi-Square analysis with treatment contrasts was performed in SAS using the General Models Procedure (SAS Institute 1995) to test the hypothesis that mortality between treatments was not significantly different. Abbott's Formula (Finney 1962) was used to adjust the observed mortality in the leaf + predator treatment based on expected non-predatory mortality.

Larval Density Study (No Predators)

Five potted *L. salicaria* were grown in the greenhouse until stems reached 24-26 cm in height at which point the four most uniform stems in each pot were selected and randomly assigned to receive one of four larval densities. Neonate *G. calmariensis* were placed on stems at densities of 1, 2, 7 or 16 neonates per stem (n=5 replications/density). Stems were caged with 22 cm diameter sleeve cages pulled over 20 cm diameter wire frames with the bottom of each frame slipped through an outer ring taped to a 50 cm long wooden stake (Figure 1). The cage was tied around the stem which was wrapped with cotton to prevent larval escapes. Stakes were pushed into the soil

approximately 15-20 cm and were adjustable to the growth of stems by pulling them up out of the soil. Larvae were checked twice daily for five days in the morning and afternoon. Larval numbers at the tip, on the stem, and on leaves were recorded. The proportion of larvae exposed on plant surfaces versus concealed inside tips was also recorded. The hypothesis that higher larval density has no effect on residence time in tips was tested with a Chi-Square analysis using the General Models Procedure in SAS (SAS Institute 1995).

Larval Density Study (Predators)

The larval density experiment was repeated using adult *Coleomegilla maculata* as a model predator. One hour after neonates were placed on tips, one *C. maculata* adult was placed in the enclosure with the larvae. However, in order to minimize chances of losing larvae, a white sheet was placed under each stem as they were checked. Any larvae that fell off were counted then placed back on the stem. All other methods and data collection were identical to those described above. The hypothesis that larval density had no effect on predation was tested using a Chi-Square Analysis in the General Models Procedure (SAS Institute 1995). The relationship of the number of larvae exposed in the no predator experiment with the number of larvae found in the predator experiment was determined with a Simple Linear Regression (SAS Institute 1995). It should be noted that the regression analyses were performed on data sets collected from two distinct experiments. The regressions were conducted to compare trends in the number of larvae exposed over time (No

Predator) and the number of larvae found over time (Predator) in the two experiments. Therefore, the results should be viewed with some caution because these were distinct experiments conducted at different times of the year (spring vs. mid-summer).

Results

Larval Movement Study.

Within the first minute following release, 25 out of 39 (64.1%) larvae moved upwards with the remainder not moving in a directed fashion (either motionless or circling the stem horizontally) (Table 1). At ten minutes there was a significant ($\chi^2 = 9.26$, d.f. = 1, $P = 0.002$) upward orientation with over 70% of individuals moving towards the apex of the shoot. A comparison of larval locations in the tip or at other locations 20, 40 and 60 min after release showed that an increasing proportion of larvae entered the tip over time (Table 2). Forty-one percent of the larvae (16/39) were found concealed within shoot tips after twenty minutes with 77% (30/39) in tips after 1 h. Larvae moved a mean ($\pm$ SEM) distance of 88.4 mm $\pm$ 10.6 mm in the one hour observation period. The mean time for a neonate to reach the shoot tip was 49 $\pm$ 37 minutes at an average displacement rate of 2.0 $\pm$ 0.8 mm/min.

Shoot Tip Refuge Study.

Under the conditions tested, both leaf and tip material had wilted by the end of 24 h. In the absence of a predator, survival of neonates on leaves (70%) was significantly less than on shoot tips (100%). In the presence of predators

(*C. maculata*), shoot tips provided neonate *G. calmariensis* significant protection from predation ($\chi^2 = 18.6$, d.f. = 1, $P = 0.0001$). Survivorship of neonates concealed in shoot tips with adult *C. maculata* present was 70% while survivorship of neonates on leaves in the presence of *C. maculata* was only 7.1% (adjusted for control mortality).

Larval Density Study (No Predators)

The density of larvae in the shoot tip affected larval residence time in or on the shoot tip, with increased likelihood of residence at lower densities (Figure 2). Treatments one (1 larva/tip) and two (2 larvae/tip) showed small changes in the number of individuals at the tip over the 5 d, indicating that one or two larvae in a tip have little effect on residence time (Figure 2). Treatment three (7 larvae/tip) showed no effect on residence time until 4-5 d. About 50% of larvae dispersed away from the tip between 3 d and 5 d. In treatment four (16 larvae/tip) an effect on residence was observed and larvae dispersed away from the tip throughout the experiment. Nearly 33% of larvae had left the tip by 3 d, 56% by 4 d and 80% by the end of the experiment compared to 50% dispersal in treatment three. The proportion of larvae found in exposed locations was also affected by density. In treatments one and two very few larvae were found outside of tips (i.e. exposed to predation) throughout the experiment. In treatment three, larvae became increasingly exposed after 3 d, with nearly 70% exposed at 4 d and 85% at 5 d. In treatment four the number

exposed increased steadily over time from 2 d to 5 d with 10%, 40%, 57% and 79% exposed, respectively.

Larval Density Study (Predators)

Increased density lowered survivorship of *G. calmariensis* larvae in the presence of *C. maculata* (Figure 3). In treatment one 20% of larvae was removed over the 5 d period. Larvae in treatment two suffered 55% predation, in treatment three 70%, and in treatment four 90%. In an *a posteriori* regression analysis, there was no significant relationship between the number of larvae exposed and the number removed in treatments one and two (R^2 = 0.0028, P=0.75 and R^2=0.0015, P=0.79). However, significant negative relationships between the number of larvae exposed and the number consumed were observed in treatments three (R^2= 0.20, P=0.001) and four (R^2=0.56, P=0.0001) (Figures 5-6).

Discussion

Neonate *G. calmariensis* are susceptible to predation by a variety of arthropods (Sebolt 2000 – Chapter 3). By rapidly concealing themselves within sheltered feeding sites, *G. calmariensis* find a suitable microclimate and nutritional resource and further benefit by avoiding predation. The movement experiment demonstrated that neonate *G. calmariensis* larvae orient toward and are able to rapidly conceal themselves in shoot tissues. Within ten minutes of placement on a stem, most larvae were moving toward the apex and appeared

to be actively searching for shoot tips. Many larvae were observed moving up the internodes to leaves, travelling along the abaxial leaf surface to the adaxial surface and back to the stem. This pattern would rapidly bring them into contact with any lateral or apical shoot tips. This behavior appears to be an adaptation to the phenology of the plant since lateral shoot tips become available on stems typically by the 3rd-4th week of growth. Although we removed axillary shoots in our experiment, in the field, larvae were found in both lateral and terminal shoot tips (pers. obs.).

We observed that once in the shoot tip area, larvae concealed themselves in the innermost layers of leaf material. Previous research has shown that female *Galerucella* spp. prefer to lay eggs on stems and tips early in the season, then oviposition shifts to leaves as the season progresses (Lindgren 1997). It is likely that oviposition on stems and tips early in the season may be advantageous to neonates, allowing rapid movement into shoot tips. Oviposition later in the season may reflect females placing eggs in suitable locations for immediate feeding since, at higher larval densities, many shoot tips have been damaged or destroyed. Or it may reflect an aversion by females to place eggs in sites with high densities of conspecific competitors. A few larvae were observed feeding on the underside of leaves even when tips were available. Why they chose to do so is unknown, although an immediate need for moisture or nutrients after eclosion seems likely. In other observations which ran for >24 h, larvae initially feeding on leaves were later

found in shoot tips, suggesting that they had moved after having fed for some period of time.

As long as *Galerucella* larvae remain in shoot tips, (generally 1st and 2nd instar) they should be well-protected from large predators such as *C. maculata* adults. However, at high *Galerucella* densities there are likely to be more larvae present than available refuges. If this coincides with the presence of effective predators, the population of *Galerucella* locally could be reduced. Late-instars may be less susceptible to predation since many of the Galerucinae exhibit chemical deterrents (Blum 1994, Pasteels et al. 1994) and show a preference to reside under leaves (Larsson et al., 1997, pers. obs.) where they may be less apparent to predators.

It is evident that higher larval densities negatively impact residence time in the shoot tip. These findings indicate that feeding sites in tips may be a limiting resource when *G. calmariensis* population densities reach levels needed to impact *L. salicaria*. While larval densities of 7/tip did not initially result in increased dispersal, tips were heavily damaged by 3 d, resulting in increased dispersal and higher predation. The steady departure of larvae from tips holding 16 larvae/tip indicates deterioration in the quality of the tip as a resource. In the experiment with predators present, this dispersal resulted in a dramatic increase in the incidence of predation. At low densities of 1-2 larvae/tip, feeding over 5 d did not affect larval residence time or exposure to predation.

These experiments demonstrated that neonate *G. calmariensis* prefer to feed in tips where they may be partially or completely concealed from predators, however, tips are unable to support high larval densities resulting in dispersal and lower survivorship in the presence of predators. In the field as many as 22 larvae per shoot tip have been observed under natural conditions (pers. obs.). These studies indicate that such densities should result in rapid destruction of the tips and force 1^{st}-2^{nd} instars to disperse at a stage of development in which they are vulnerable to predation. Hence, the occurrence of only a limited number of tips in the presence of arthropod predators on a plant may place a constraint on the increase of *G. calmariensis* populations, contributing to the slow control of *L. salicaria*.

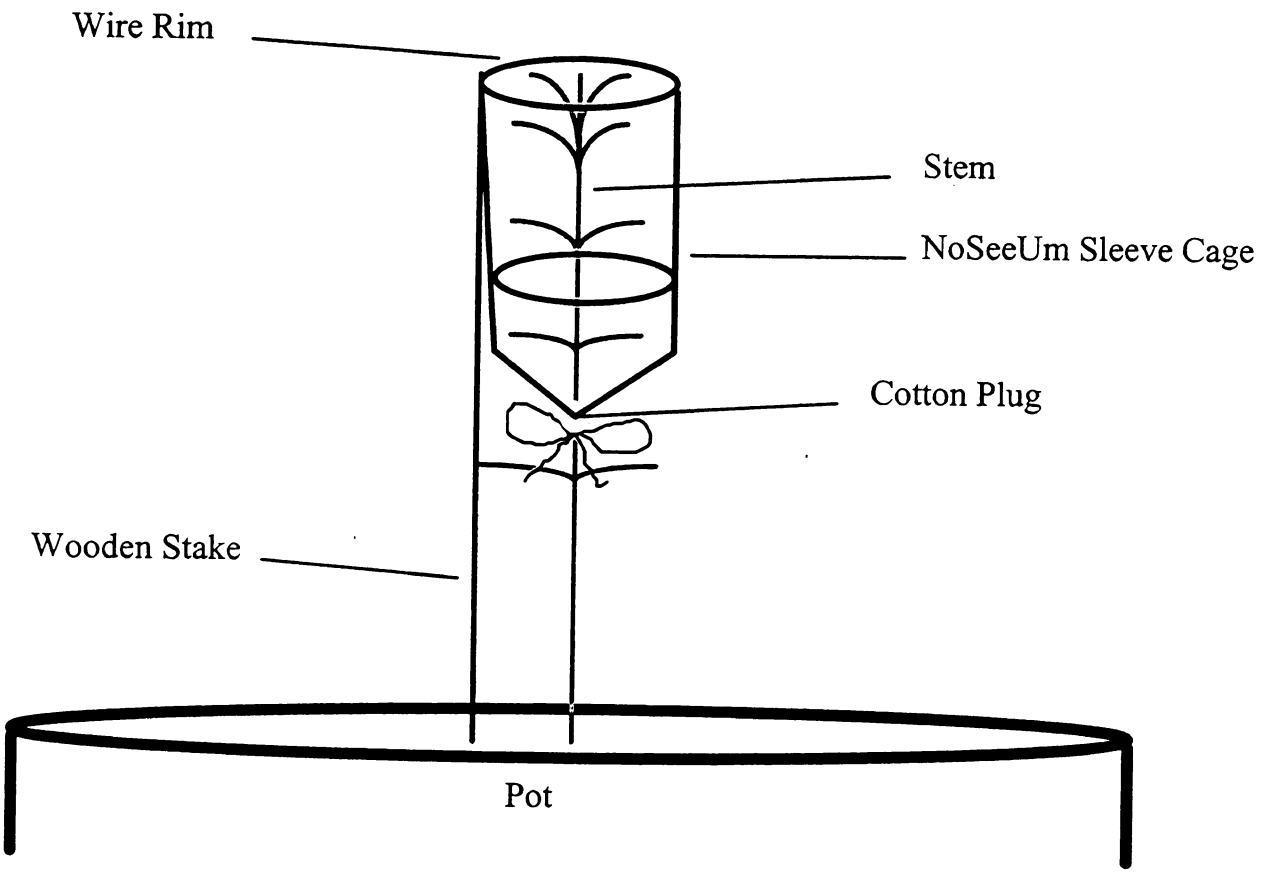


Figure 1. Cage used to contain neonate *G. calmariensis* and adult *C. maculata* in the larval density studies.

Table 1. Initial choice of direction and the direction at 10 min of neonate *Galerucella calmariensis* larvae placed on *Lythrum salicaria* stems (χ^2 Test for Specified Proportions testing the null hypothesis of equal movement of larvae up, down and not directed (n=39, $\alpha = 0.05$).

	0-1 minute	10 minutes
Not Directed	14	10
Up	25	29
P =	0.078	0.002

Table 2. Number of neonate *G. calmariensis* found in *L. salicaria* shoot tips at three time intervals following release on the stem. *P*-values denote comparisons of each time versus time 0 (χ^2, n=30).

Time (Min)	# larvae each location			χ^2	$Pr > \chi$
	In tip	Other	Total		
0	0	39	39		
20	16	23	39	26.4	0.0001
40	25	14	39	46.9	0.0001
60	30	9	39	61.8	0.0001

Table 3. Number of *Galerucella calmariensis* and *Coleomegilla maculata* recovered from Petri dishes after a 24-hour incubation period.

Treatment		24-hour Survivorship			
		G. calmariensis		*C. maculata*	
Plant Part	Predator	#	%	#	%
Shoot Tip	-	20[a]	100	-	-
Leaf	-	14[b]	70	-	-
Shoot Tip	+	14[b]	70	20	100
Leaf	+	1[c]	7.1[1]	20	100

[1] Adjusted for control mortality (Abbot's Formula, Finney, 1962). Treatments were contrasted in PROC GENMOD. Different letters denote statistically significant differences (χ^2=9.4,d.f.=1, P=0.002).

Figure 2. Change in percent larvae at the tip over time in relation to initial larval density in the absence of a predator.

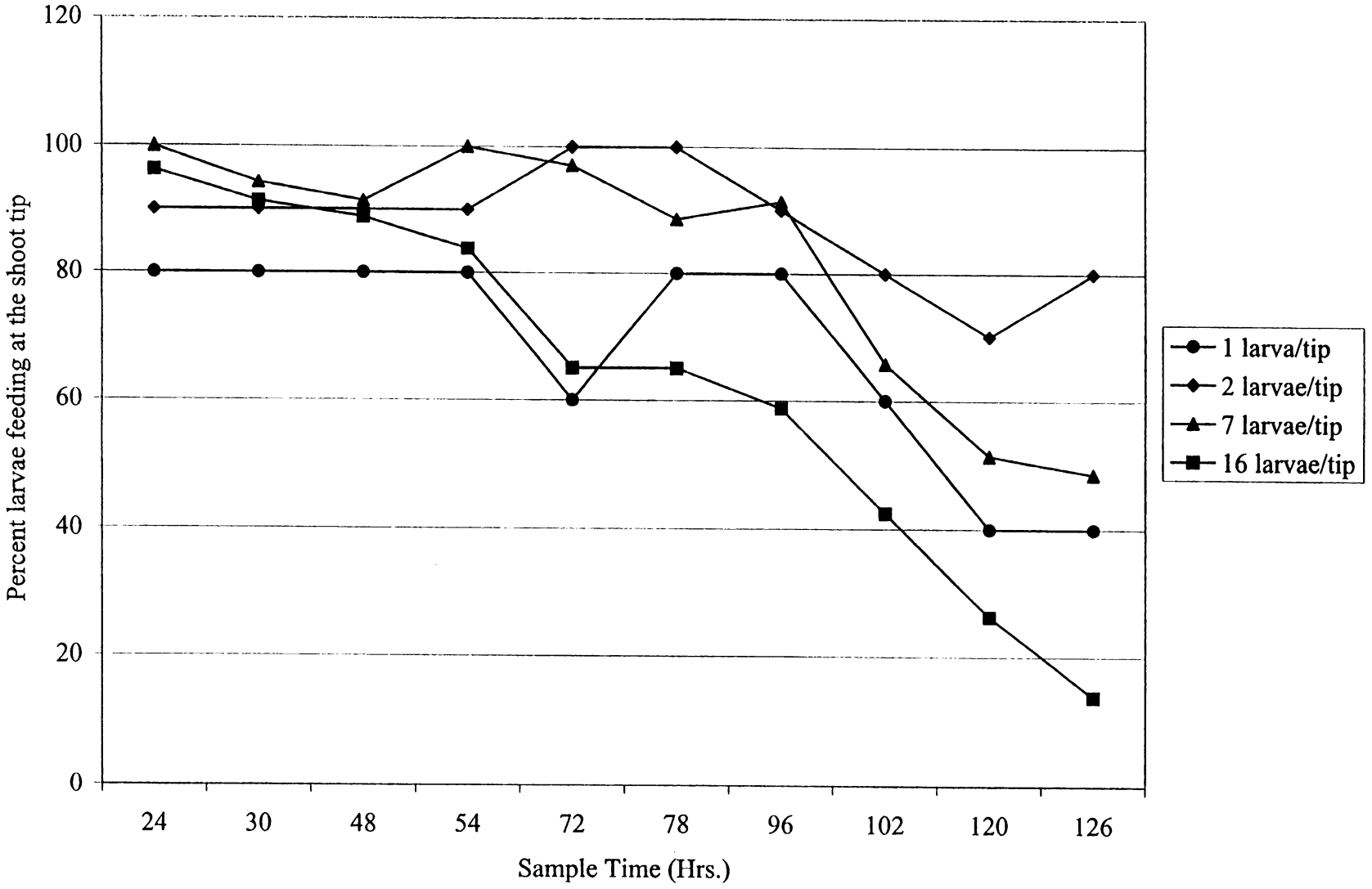

120
100
80
60
40
20
0
Percent larvae feeding at the shoot tip
24
30
48
54
72
78
96
102
120
126
Sample Time (Hrs.)
1 larva/tip
2 larvae/tip
7 larvae/tip
16 larvae/tip

Figure 3. Change in percent larvae exposed over time in relation to initial larval density in the absence of a predator.

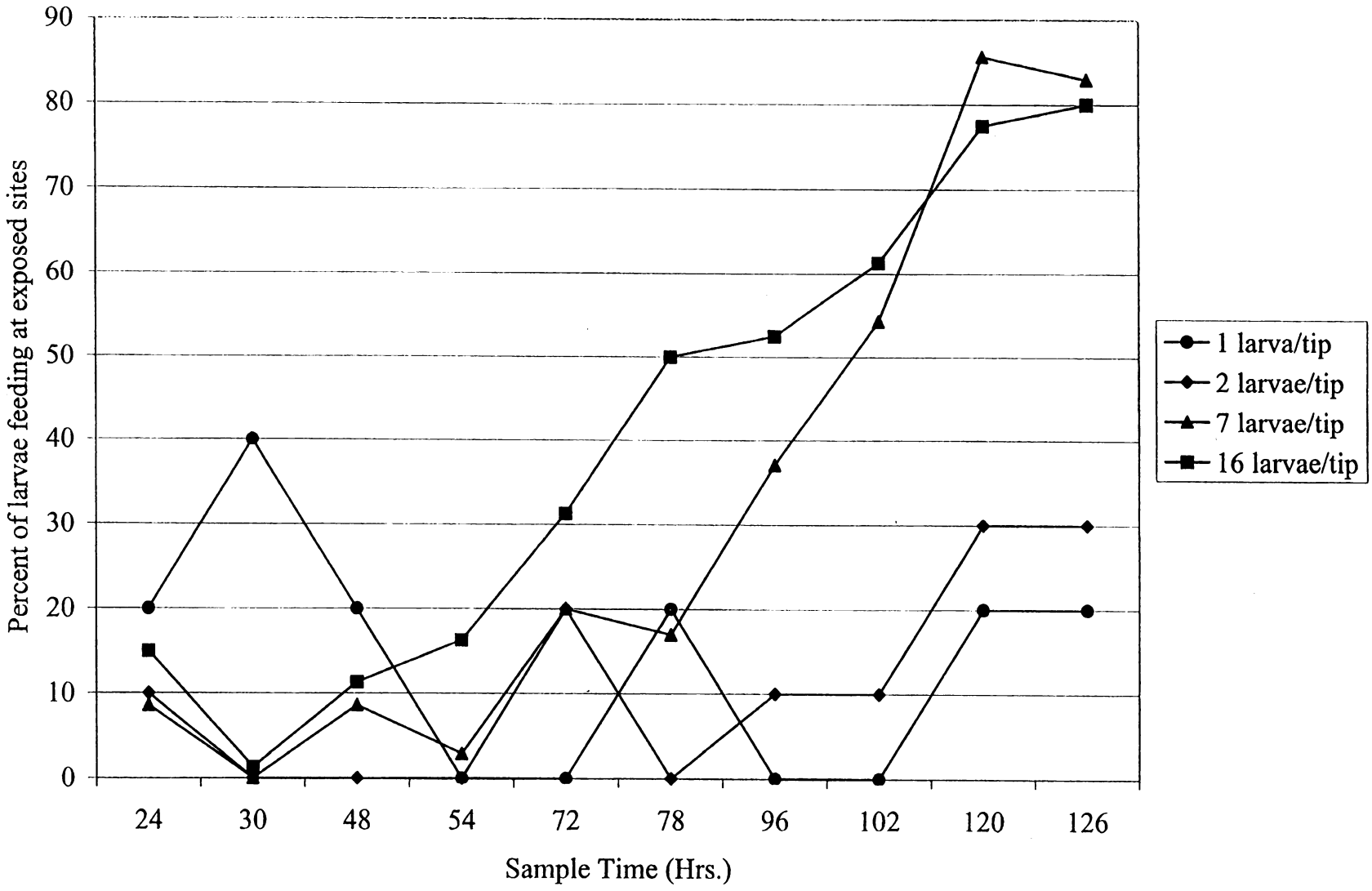

90
80
70
60
50
40
30
20
10
0
Percent of larvae feeding at exposed sites
24
30
48
54
72
78
96
102
120
126
Sample Time (Hrs.)
1 larva/tip
2 larvae/tip
7 larvae/tip
16 larvae/tip

Figure 4. Change in percent live larvae over time in relation to initial density in the presence of a predator.

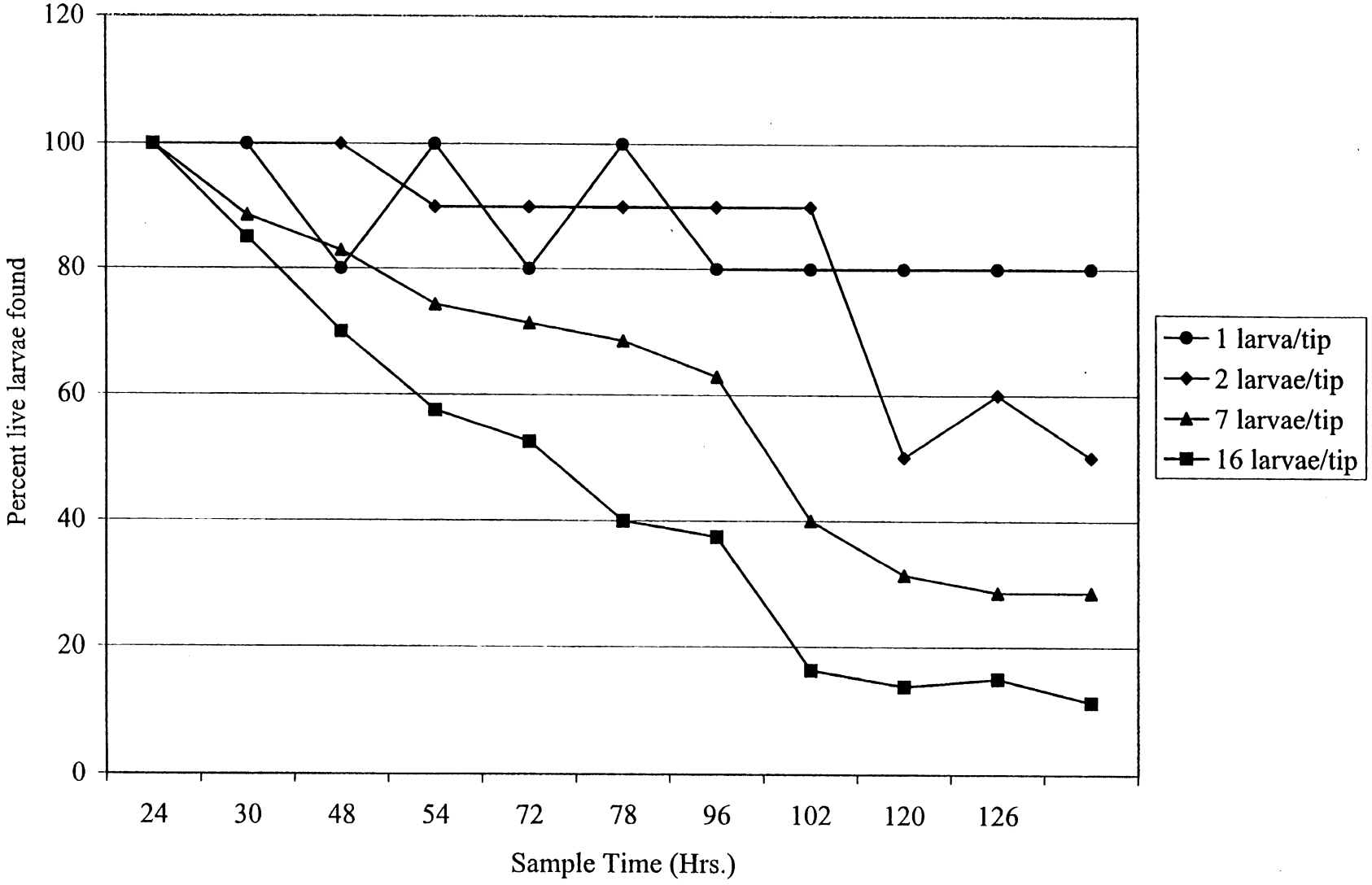

120
100
80
60
40
20
0
Percent live larvae found
24
30
48
54
72
78
96
102
120
126
Sample Time (Hrs.)
1 larva/tip
2 larvae/tip
7 larvae/tip
16 larvae/tip

Figure 5. Linear regression of larvae surviving vs. larvae exposed in predator and no predator trials. Initial larval density was 7 larvae per tip in both studies.

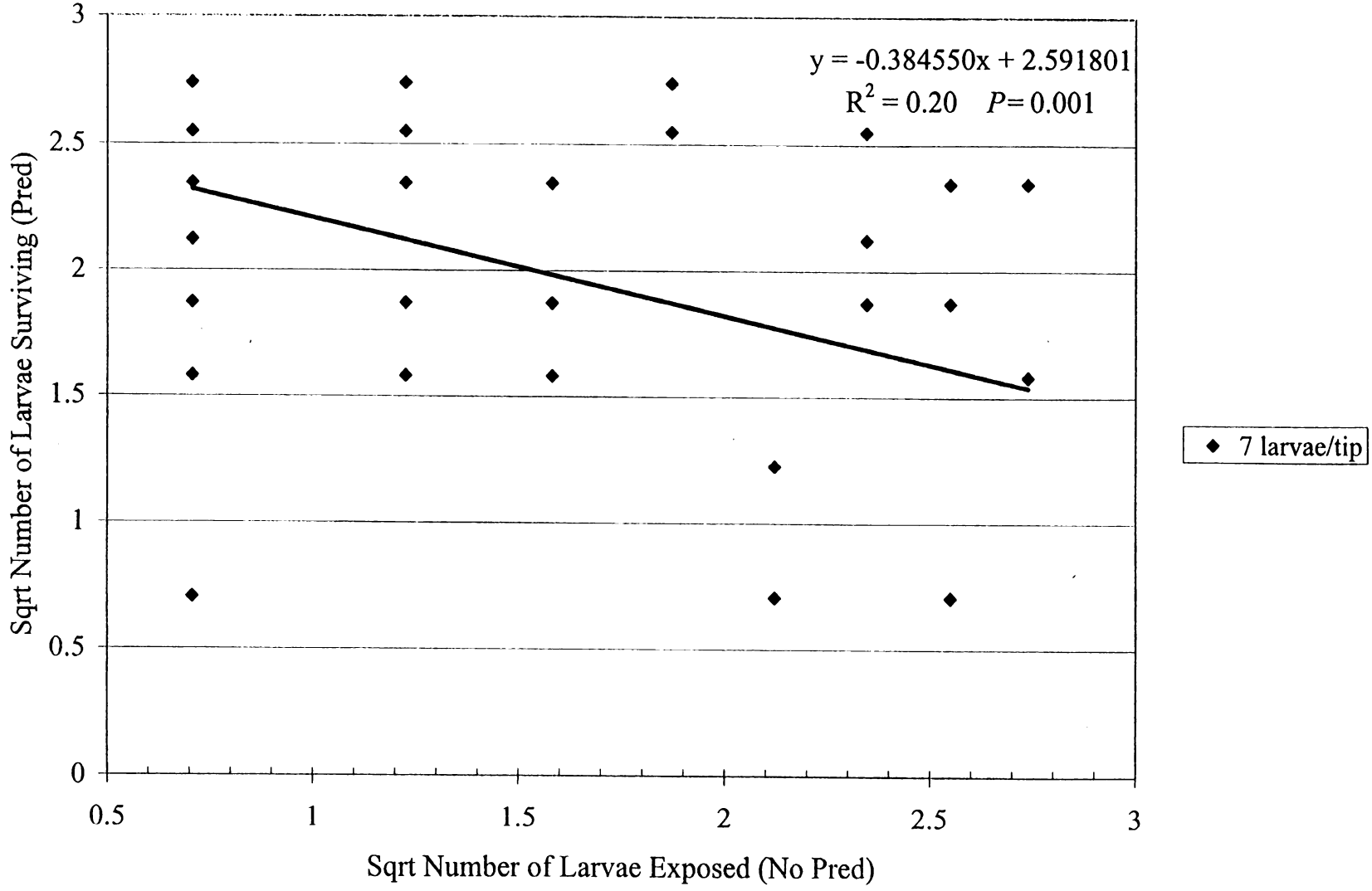
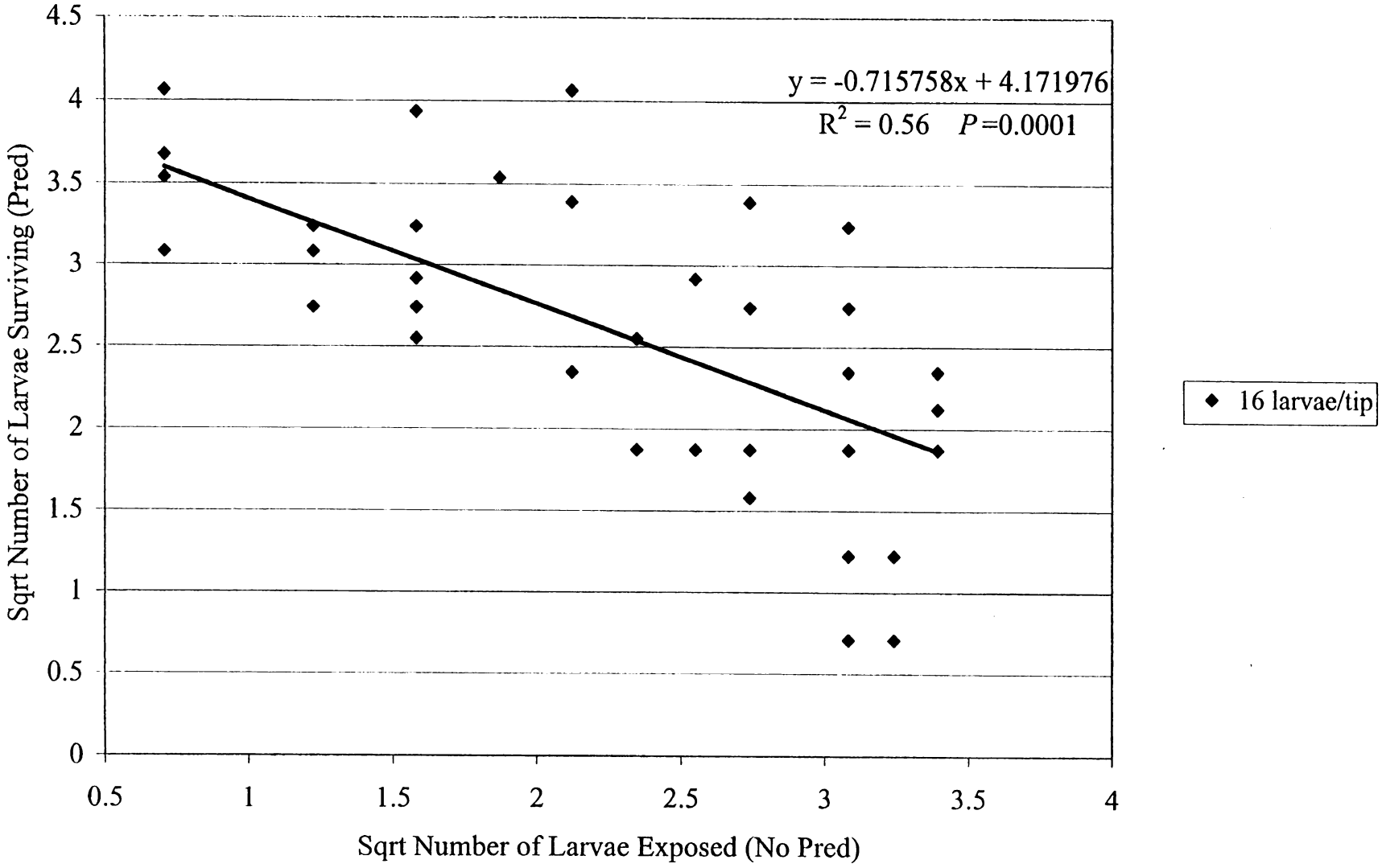

y = -0.384550x + 2.591801
R2 = 0.20 P= 0.001
Sqrt Number of Larvae Surviving (Pred)
Sqrt Number of Larvae Exposed (No Pred)
7 larvae/tip
0
0.5
1
1.5
2
2.5
3

Figure 6. Linear regression of larvae vs. larvae exposed in predator and no predator trials. Initial larval density was 7 larvae per tip in both studies.


y = -0.715758x + 4.171976
R2 = 0.56 P=0.0001
Sqrt Number of Larvae Surviving (Pred)
Sqrt Number of Larvae Exposed (No Pred)
16 larvae/tip
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0.5
1
1.5
2
2.5
3
3.5
4

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CHAPTER THREE

Arthropod Predators and *Galerucella calmariensis* (Coleoptera: Chrysomelidae): An assessment of predation potential

Introduction

Natural enemies of herbivorous insects can reduce population size and even induce localized extinctions of herbivore populations (Hawkins et al. 1997). Such impacts could limit the effectiveness of weed biological control by herbivorous insects. Although predators and parasitoids have occasionally been implicated in the failure of weed biological control programs (Stiling 1993, Goeden and Louda 1976), documentation of such interference is scarce or anecdotal (Ehler 1998). Goeden and Louda (1976) reported that *Physonota alutacea*, a chrysomelid released for control of black sage (*Salvia mellifera*) in Spain, failed to establish due to predation by foraging ants. Egg and larval mortality of *Altica carduorum*, a flea beetle released for control of Canada thistle (*Cirsium arvense*), approached 91% in Canada due to the activity of predatory mites. Finally, the failure of the chrysomelids *Chrysolina brunsvicensis* and *C. varians* to establish and the slow establishment of *C. hyperici* introduced into Australia to control St. Johnswort (*Hypericum perforatum*) were attributed to predation and parasitism.

Galerucella calmariensis L. is a leaf-feeding beetle that is currently being widely distributed for control of *Lythrum salicaria* L. (purple loosestrife) in North America (Hight et al. 1995). *Lythrum salicaria* is an exotic invasive perennial from Europe that may displace native wetland vegetation in the United States and Canada (Thompson et al. 1987). Qualitative field observations of predators attacking *G. calmariensis* are limited (Malecki et al. 1993). Field studies detailing predation on the congener *Galerucella nymphaeae* L. and field observations of the potential for predaceous pentatomids to attack *G. calmariensis* and *G. pusilla* larvae have been reported (Diehl et al. 1997, Nechols et al. 1996) and raise the potential for interference to occur.

Success in establishing *Galerucella* spp. in North America and reports of success in control of *L. salicaria* indicate that predator interference can be overcome by large releases of *Galerucella* spp. or by initially excluding predators by caging *Galerucella* spp. in enclosures (Hight et al. 1995). However, the success of *L. salicaria* biological control will ultimately depend on the unassisted spread of these beetles and their ability to successfully colonize new patches. *Galerucella* spp. are known to disperse to *L. salicaria* at distances up to 1,000 m (Grevstad and Herzig 1997). In addition, once within host habitat, they will move around and find conspecifics up to 50 meters away, resulting in local aggregations of adults. Even given these behavioral adaptations, the scattered distribution and potentially low densities in newly

colonized patches may result in sub-populations that may be more susceptible to predation. The successful establishment of these new colonizers will depend on the number of predators present and their attack rate, i.e., predation intensity.

This paper reports the results of predator surveys, laboratory predation tests, and a field study designed to quantify the effects of indigenous predators on establishment of *G. calmariensis*. Field surveys were conducted to record indigenous predators occurring in purple loosestrife stands in which *G. calmariensis* had been released. The most abundant predator species were tested in two levels of laboratory bioassays to determine their attack rates on immature life stages of *G. calmariensis*. A field study was then conducted in two adjacent wetlands that had not previously received *Galerucella* spp. beetles in order to quantify the response of predators to populations of *Galerucella* spp. beetles.

Materials and Methods

Field Surveys for Predators

Field surveys were conducted bi-weekly from 15 May 1998 to 30 June 1998 at two locations: Lake Lansing Park North in Ingham County, MI and at the USDA Avian Disease and Oncology Laboratory on the campus of Michigan State University, Ingham County, MI. At each location three transects (10 m apart) were constructed running from shore into the wetland. Each transect contained three $1m^2$ quadrats at 5, 10, and 15 meters from the

shoreline and a line of three pitfall traps, 12 cm diameter by 16 cm tall cups (Sweetheart Cup Company Inc., Chicago, IL) bisecting each transect at 7.5 m and 12.5 m. Pitfall traps were used for live-trapping of predators, therefore, no killing solution was used. Traps were arranged 1 m apart and checked four times each week; once the day before each collection (to remove rainwater, etc.) and once on each day of collection (occurring bi-weekly). All arthropod predators found in pitfall traps were collected in plastic 3.0 cm diameter X 6.5 cm tall vials and held in a cooler. One-minute timed counts of arthropod predators occurring on foliage or the ground inside the 1 m^2 quadrats were conducted.

Level I Predator Testing

The most abundant predator species found in *L. salicaria* stands were collected in greater numbers at other field locations for testing. Predators were starved and held without food in plastic bags containing moistened paper towel at 24°C, 80% humidity and 16:8 (L:D) h for 24 h. After the starvation period, predators were tested in 60 by 15mm Petri dishes containing filter paper moistened with 0.2-0.3 ml of dH_2O. To prepare for testing egg predation, cut stems were provided to gravid female *G. calmariensis* in an oviposition cage 24 h prior to testing. Ten eggs were carefully excised from cut stems and placed in each Petri dish with one predator. Petri dishes were stored in a sealed plastic container with a moistened paper towel and held under the conditions described above. After 24 h, the number of *G. calmariensis* eggs damaged or

consumed was compared to a matching control containing eggs alone. Similar tests were conducted for other *G. calmariensis* life stages including 1st instars, 2nd-3rd instars and pupae containing *G. calmariensis* (n=5) of the appropriate stage. All life stages were held in Petri dishes in the absence of foliage. After 24 h the number injured or consumed was compared to the control. Mean comparisons (versus appropriate control) were conducted with t-tests (SAS Institute, 1995).

Level II Predator Testing

Predators that preyed on *G. calmariensis* in Level I testing were tested under Level II conditions which were designed to simulate more realistic environmental conditions. Foliar predators were introduced into circular 25.4 by 8.89 cm plastic arenas (Tri State Molded Plastics) each containing a 22-24 cm tall *L. salicaria* stem held in a 22 ml plastic cup covered with a cardboard cap (Fill-Rite Corp., Newark, NJ). The cup was supported by high-porosity soil-less potting mix (Michigan Peat Company) sloped to the lip of the cup down to a depth of 4 cm at the edges. Five late 2nd to early 3rd instars were placed at random on the stem and given 30 min to settle, then the predator was placed on the cup lid at the base of the stem. The entire stem was covered by a cage constructed from a 2 L plastic bottle with the spout end removed. Two 10 by 10 cm squares were cut midway on the bottle and covered with NoSeeUm netting (Balson Hercules Company, New York) to provide ventilation. The open end was embedded ca. 4 cm into the soil to prevent escape of predators.

Arenas were placed in the incubator (described above) for 24 h after which the number of larvae injured or absent was compared to the number of larvae in the control. To prepare for testing pupa predators, 50 g (wet weight from bag) of soil-less potting mix saturated with 75 ml dH_2O to give a total wet weight of 90 g was placed in each arena. Five pupae were placed in slight depressions in the soil about 4 cm apart in a square pattern with one pupa at each corner and one in the center. The soil was covered with a 3 cm layer of sphagnum moss and a predator placed on the sphagnum at the center of the arena. Arenas were placed in the incubator for 24 h, then the number of pupae injured or absent were compared to the control using t-tests (SAS Institute, 1995).

Field Predation Experiment

In late April, 36 *Lythrum salicaria* root crowns were potted in 5-gallon pots and placed in the greenhouse. When plants reached 40-45 cm in height (2^{nd}-3^{rd} week of May), stems were selectively removed to equalize biomass among pots. Two, four or eight *G. calmariensis* gravid females were then placed in each pot (12 pots for each female density) for 24 h. Pots were covered with cages of NoSeeUm netting (Balson Hercules Company, New York). After 24 h, females were removed from pots and the number of egg masses recorded. Egg mass locations were identified using non-toxic typewriter correction fluid, White Out (The Gillette Co., Boston, MA) by placing a dot next to each mass. Pots were assigned one of three treatments according to the number of egg masses present. Undesired egg masses were

removed with a razor blade to achieve desired densities of 2-3 masses (Low), 5-6 masses (Medium) and 11 or 13 masses (High) per plant.

Pots were placed in two adjacent wetlands in Jackson County, Michigan that consisted of approximately 65% *L. salicaria*, 10% *Typha latifolia*, and 10% *Carex* spp., 10% *Cornus* spp. and 5% other species. Within each density treatment, half (6) of the pots were randomly assigned in a general randomized block design as caged (control) and the other half uncaged for a total of 12 pots in each block. Blocks were set up a few days apart, with block one established on 20 May, block two on 23 May, and block three on 26 May. In each block, the 12 pots were arranged 15 m apart and 15 m from any edge (woods, road, or neighboring blocks) with treatments randomly assigned to blocks, two treatment replications were present per block (Figure 1). Pots were buried until their rims were four inches above the water/soil level. Four 1 m^2 quadrats were placed in each block along two transects running between the three rows of pots (Figure 1) for sampling insects present in each block. Forty-eight h after removing cages, the number of egg masses missing or damaged was recorded and continued daily until neonate eclosion. Larval counts were conducted twice weekly until larvae reached the third instar, then pots were covered, removed from the wetlands, and brought to the lab where adult emergence was recorded over the next three weeks until emrgence was complete. The presence of predaceous arthropods in each block was estimated at each sample date by recording their presence on each test plant and by

recording numbers present in eight 1 m^2 quadrats in each block. The proportion of egg masses missing in each treatment in contrast to control treatments was analyzed with a Chi-Square (SAS Institute 1995). Mortality was adjusted by using Probit Analysis (Finney 1962). The proportion of larvae missing was also tested using a Chi-Square with treatment contrasts to test for differences in number missing by treatment and compared to controls. Previously reported mean egg mass size of 5-6 eggs/mass was used as the basis to estimate larval numbers (Lindgren 1997, Blossey 1995). The total number of eggs set out (based on 5 eggs per mass) was compared to the total number of adults recovered in the pots after removal from the field.

Results

Field Surveys

The ladybeetles *Coleomegilla maculata* DeGeer, *Coccinella septempunctata* L., and *Harmonia axyridis* L. were very abundant predators (>10 per nine quadrats during a sampling period) observed in the two Ingham County field sites (Table 1). Abundant predators (5-10 per nine quadrats during a sampling period) were the predaceous stink bug *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae), the earwig *Forficula auricularia* L. (Dermaptera: Forficulidae), and the ground beetle *Pterostichus melanarius* Illiger (Coleoptera: Carabidae). Terrestrial and foliar arachnids were present (<5 per nine quadrats during a sampling period), although collection and identification of these species proved difficult, therefore, they were not tested.

Level I Predator Testing

High levels of egg predation by *Coleomegilla maculata* and *F. auricularia* were observed on eggs in the Level I tests (Table 2). *Coleomegilla maculata* consumed 75% of eggs and *F. auricularia* 67% of eggs present. *Coleomegilla maculata* removed 85% and *F. auricularia* 100% of 1st instars. Late-instar predation by *C. maculata* accounted for 54% and *F. auricularia* 85% of late-instars. Only slight feeding damage to eggs by *C. septempunctata* and *H. axyridis* was noted and was confined to minor exterior damage to the chorion. *Coccinella septempunctata* crushed but did not consume 1st instars, causing 90% mortality, but did not prey on any other life stage tested. *Harmonia axyridis* consumed 100% of 1st instars and 60% of 2nd-3rd instars. *Podisus maculiventris* consumed only on 3rd instars, accounting for 92% predation. *Forficula auricularia* attacked 51% of *G. calmariensis* pupae, but did not prey on mature adults used in these tests, however, attacks on teneral adults were observed. *Pterostichus melanarius* consumed 100% of 1st instars and 76% of pupae.

Level II Predator Testing

With the exception of *C. septempunctata*, predator species that preyed on *G. calmariensis* in Level I testing also preyed on them under Level II conditions, where they were forced to search a larger and more realistic environment to encounter prey items. *Coleomegilla maculata*, *H. axyridis*, *F. auricularia*, *P. maculiventris,* and *P. melanarius* all effectively encountered

and consumed 2nd-3rd instars feeding on leaf tissue (Table 3). Late-instar predation by *C. maculata* reached 52%, *H. axyridis* 70% and *P. maculiventris* 70%. *Forficula auricularia* consumed 40% of 2nd-3rd instars and *P. melanarius* encountered and consumed 66% of 2nd-3rd instars and 54% of pupae. In the pupae test with *F. auricularia*, 60% were consumed, however, recovery of pupae in the control was poor, resulting in a lack of significance.

Field Experiment

Aphids (n=352) and ants (n=175) were the two very abundant (>10 per nine quadrats during a sampling time) insect species present in the two Jackson County sites (Table 4). Generalist predators occurred at low densities (<5 per nine quadrats during a sampling time) and were primarily coccinelids, *C. maculata* (n=9), *H. axyridis* (n=2), and *C. septempunctata* (n=3). Two other herbivores observed were *Poecilocapsus lineatus* (Hemiptera: Miridae) (n=39) and *Lymantria dispar* L. (Lepidoptera: Lymantriidae) (n=10).

Egg removal was low in this field experiment (Table 5). There was significant difference in the percent of eggs removed among egg density treatments in comparison to their controls. In treatment one 11.6% of eggs were removed compared to 0% removal in the control and in treatment two 11.4% removal was observed compared to 1.4% in the control. In treatment three (11 or 13 masses/pot) egg removal was only 1.3% in open pots and 0% in the control.

Adult emergence counts, compared to the estimated number of eggs set out, indicated significant differences between low and medium density and their controls. There was no significant difference between the low density and its control, however, medium and high densities were both significantly different from their controls. In all treatments, more adults were recovered in control pots. The highest recovery in open cages occurred at low (21.1%) and high (20.3%) densities, while only 12.2% of *G. calmariensis* were recovered at medium densities. Recovery in the controls ranged from 5% higher to 38% higher when compared to recovery in open pots. Although blocks were placed in the field several days apart, there was no evidence indicating that predation differed among blocks.

Discussion

The Level I and II predator tests demonstrated that several common generalist arthropod predators feed on *G. calmariensis* immature life stages. These predators readily consumed *G. calmariensis* in the lab, however, in the field the impact of predation was very low. The relatively low predator abundance probably contributed to this effect. In addition, the high predation rates observed on *G. calmariensis* in these laboratory studies may be a result of behavioral changes induced by confinement. (Luck et al. 1988). Nechols et al. (1996) reported that *C. maculata* and other predators were responsible for 20% of *Galerucella nymphaeae* L. egg predation during the spring and early summer in central New York state. Although predator numbers were not

reported by Nechols et al. (1996) their observations indicate that *C. maculata* numbers were low in the New York study sites as well.

Studies of *P. maculiventris* attack rates on the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chysomelidae) and the Mexican bean beetle, *Epilachna varivestis* Mulsant (Coleoptera: Chrysomelidae) in lab versus field trials described similar results (O'Neil 1989, Wiedenmann and O'Neil 1991, O'Neil 1997). The above studies concluded that laboratory analyses of functional responses did not predict attack rates in the field because prey densities in the laboratory studies were artificially high and handling increased with increasing prey density. In the field, where attack rates were very low, handling time was also low.

Others have pointed out that laboratory studies can artificially increase predator efficiency due to simplified architecture or the absence of interference by other predators (Wells and McPherson 1999). Finally, aphids may have served as an alternative or preferred prey for these generalist predators, resulting in reduced predation on *G. calmariensis*. The effects of alternate prey presence on predators is documented in other work (Wells and McPherson 1999, Feng et al. 1992). The presence of alternate prey can reduce the efficiency of generalist predators by increasing handling time or causing predators to move to locations holding preferred prey.

As suggested by Grevstad and Herzig (1997), the successful colonization of new patches by *Galerucella* may depend on their ability to

locate their conspecifics. In addition, *Galerucella* must aggregate in sufficient numbers at "hot spots" to overcome the effects of predators. Ultimately, colonization may depend on the response of predators present in newly colonized patches to the presence of an abundant prey source. This experiment showed that predators occurred at low densities and did not prey heavily on *G. calmariensis* at low densities. For predation to affect the stability of *Galerucella* populations in the field, it is likely that predator abundance will need to be greater than what was encountered during this experiment.

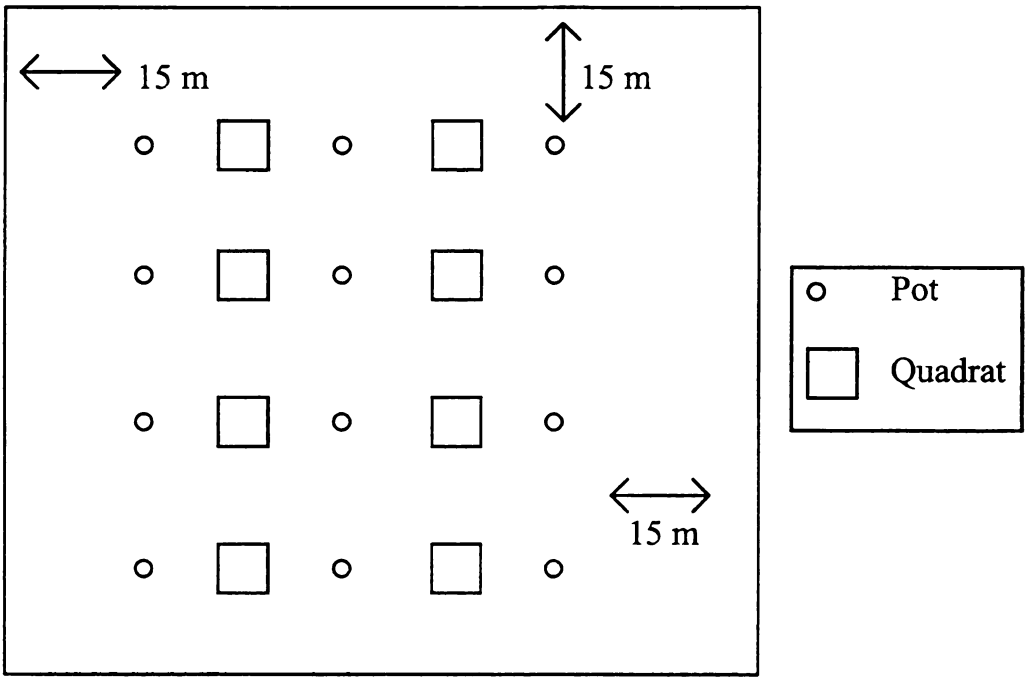

Figure 1. Organization of blocks in the field predation experiment with pots arranged 15 m apart and 15 m from any wetland, forest, or road edge.

Table 1. Predators found during field surveys at Lake Lansing Park-North, Ingham County, MI in 1998.

Order:Family	Predator	Abundance[1]
Coleoptera: Coccinelidae	*Harmonia axyridis*	Abundant
Coleoptera: Coccinelidae	*Coleomegilla maculata*	Abundant
Heteroptera: Pentatomidae	*Podisus maculiventris*	Abundant
Coleoptera: Coccinelidae	*Coccinella septempunctata*	Abundant
Dermaptera: Forficulidae	*Forficula auricularia*	Present
Coleoptera: Carabidae	*Pterostichus melanarius*	Very Abundant
Arachnida	Terrestrial arachnids	Very Abundant
Arachnida	Foliar arachnids	Abundant

[1] Present = Observed <5 present per nine quadrats during a sampling period.

Abundant = Observed 5-10 per nine quadrats during a sampling period.

Very Abundant = Observed >10 per nine quadrats during a sampling period.

Table 2. Results of Level I predator tests showing percent of individuals injured/absent compared to recovery in a control (t-test) in each life stage tested.

Species	Percent Injured/Absent									
	Egg	Control	1st Instar	Control	2nd-3rd Instar	Control	Pupae	Control	Adult	Control
Coccinella septempunctata	5	0	40	4	10	0	-	-	-	-
Coleomegilla maculata	75***	0	85***	0	54***	2	-	-	-	-
Forficula auricularia	67***	0	100***	0	85***	0	51***	0	0[1]	-
Harmonia axyridis	11	0	100***	0	60*	0	-	-	-	-
Podisus maculiventris	10	0	-	-	92***	0	-	-	-	-
Pterostichus melanarius	-	-	100***	2	-	-	76	0	-	-

- Not Tested

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

[1] *F. auricularia* did not feed on older adults used in the test, but was observed feeding on teneral adults.

Table 3. Results of Level II predator testing showing the percent of individuals injured/absent compared to loss of individuals in a control (t-test) in each life stage tested.

Species	Percent Injured/Absent			
	2^{nd}-3^{rd} Instar	Control	Pupae	Control
Coccinella septempunctata	52***	2.5	-	-
Coleomegilla maculata	70***	2.0	-	-
Forficula auricularia	70***	5.0	-	-
Harmonia axyridis	40***	4.4	14[1]	6.0
Podisus maculiventris	0	0.0	-	-
Pterostichus melanarius	66[1]**	16.0	54***	10.0

- Not Tested

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

[1] Denotes predation was evident, however, poor recovery in the control resulted in no statistical significance (t-test).

Table 4. Insects observed in the sample quadrats at the two Jackson Count, MI sites during the field predation experiments.

Order: Family	Insect	Abundance[1]
Homoptera	Aphididae	Very Abundant
Hymenoptera	Formicidae	Very Abundant
Hemiptera: Miridae	*Poecilocapsus lineatus*	Present
Coleoptera: Coccinelidae	*Coleomegilla maculata*	Present
Coleoptera: Coccinelidae	*Harmonia axyridis*	Present
Coleoptera: Coccinelidae	*Coccinella septempunctata*	Present
Lepidoptera: Lymantriidae	*Lymantria dispar*	Present

[1] Present = Observed <5 per nine quadrats during a sampling period.

Abundant = Observed 5-10 per nine quadrats during a sampling period.

Very Abundant = Observed >10 per nine quadrats during a sampling period.

Table 5. Total number and percentage of *G. calmariensis* egg masses removed in treatments allowing or excluding predators. Experiments conducted in *L. salicaria*-dominated wetlands in Jackson County, Michigan in 1999.

Egg Mass Density[1]	Egg Masses Removed	Percent Masses Removed*	
		Raw	Corrected
Low	1.9/15	12.7	11.6[a]
Low-Control	0/15	0.0	0.0[a]
Medium	4.6/33	13.9	11.4[a]
Medium-Control	2/33	6.1	1.4[a]
High	3.48/60	5.8	1.3[a]
High-Control	0/60	0.0	0.0[a]

Low = 2-3 masses per plant.

Medium = 5-6 masses per plant.

High = 11 or 13 masses per plant.

Control = Pots with cages to exclude predators.

* Indicates treatments 1-3 were adjusted for mortality observed in control treatments. Letters indicate statistically significant differences (General Models Procedure, d.f.=1, alpha=0.05) between treatments.

Table 6. Total number of adult *G. calimariensis* recovered in emergence pots.

Treatment	Adults Recovered/Released[1]	% Adults Recovered
1	19/90	21.1[a]
2	22/180	12.2[a]
3	61/300	20.3[a]
1C	24/90	26.7[a]
2C	63/180	35.0[a]
3C	176/300	58.7[b]

Numbers with different letters indicate statistically significant differences (Chi-Square, d.f.=1, α=0.05).

[1]Estimated numbers based on average egg mass size of 5-6 eggs/mass

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CHAPTER FOUR

Gypsy Moth (*Lymantria dispar* L.) Feeding on Purple Loosestrife (*Lythrum salicaria* L.) in Michigan

Introduction

The gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae) is a well-known and serious pest of trees and shrubs in the United States. Introduced into eastern Massachusetts in 1869, this generalist herbivore is known to feed on 500+ species in the northeast United States (Liebhold et al. 1995). Most reports focus on feeding of *L. dispar* on woody species (Forbush and Fernald 1896, Mosher 1915, Barbosa and Greenblatt 1979, Lechowicz and Jobin 1983), with fewer noting herbaceous hosts (Forbush and Fernald 1896, Kamalay et al. 1997).

Lythrum salicaria L. was introduced into North America from Eurasia in the early 1800's (Thompson et al. 1987). *Lythrum salicaria* has become an invasive weed in North American wetlands, where mature plants can produce 2.5 million seeds per year and reproduce vegetatively from stems or root crowns (Malecki et al. 1993). Over time *L. salicaria* appears to displace wetland associates, reducing plant diversity with potentially adverse impacts on waterfowl and other wetland wildlife (Thompson et al. 1987). The presence of various insects on *L. salicaria* in North America and Eurasia has been well-documented (Diehl et al. 1997, Barbour and Kiviat 1997, Anderson 1995, Hight 1990). Since no North American insect species controlled *L. salicaria*, A program of

importation biological control was implemented after other methods of control proved ineffective (Malecki et al. 1993). In 1994, the Michigan Department of Natural Resources released *Galerucella calmariensis* L. and *G. pusilla* (Duftschmidt) (Coleoptera: Chrysomelidae), natural enemies imported from Europe, for the control of *L. salicaria.* In 1997, The Purple Loosestrife Project at Michigan State University began conducting large-scale rearing and redistribution of *Galerucella* spp. and has released approximately 300,000 beetles in infested wetlands throughout the state from 1997-1999.

While conducting releases of *Galerucella* spp., *L. dispar* larvae were observed feeding on *L. salicaria* foliage. In the spring of 1998, we conducted studies to estimate *L. dispar* larval density on *L. salicaria* and to determine the percent defoliation attributable to *L. dispar* feeding. We also examined if *L. dispar* was able to complete development on *L. salicaria.*

Materials and Methods

Observations were made at Lake Lansing County Park-North in Meridian Township, Ingham County, MI. The park contains an approximately 16+ hectare wetland infested with *L. salicaria* and surrounded by an oak-dominated forest. Three transects were established 10 meters apart, each beginning at the tree-line and extending 15 m into the wetland. Each transect contained three 1-m^2 quadrats located 5, 10, and 15 m from the tree-line. One-minute timed counts of *L. dispar* larvae/m^2 quadrat were conducted on six different dates from 28 May to 30 June.

On the last three sampling dates, the estimated larval instar and estimated percent defoliation were collected in addition to the number of larvae. Means (± SEM) were reported for each sample date based on a total sample of nine quadrats (n=9). Defoliation was estimated as the percent of total *L. salicaria* leaf area defoliated/m^2 and included feeding by *Galerucella* spp. Weather conditions were recorded on all sample dates. On 12 June, three 2nd or 3rd instars were collected and reared to adult on *L. salicaria* foliage in petri dishes incubated at 24°C and 16L: 8D.

Results and Discussion

The number of *L. dispar* in individual sample quadrats ranged from 0 to 8 although, while collecting data on another experiment, a single *L. salicaria* plant over 50m from the nearest tree-line was found to contain 23 2nd-3rd instar larvae during a one-minute observation. For the period 28 May to 30 June, on average, one *L. dispar* larva was observed in each quadrat and in association with *Galerucella* spp., accounted for 15-17% defoliation of *L. salicaria* (Table 1). We observed *L. dispar* larvae feeding from the margins of the leaf and progressing towards the midvein, leaving irregularly shaped areas of damage. In contrast, *Galerucella* spp. 2nd-3rd instars consume only upper or lower leaf surfaces, creating a "windowpane" effect while *Galerucella* adults chew many small holes through the leaf by feeding briefly at several sites per feeding bout (Blossey and Schroeder, 1991). Of the *L. dispar* (n=3) reared on *L. salicaria* in the lab, all

successfully eclosed as adults, resulting in two males and one female which oviposited about 100 eggs in a single mass.

To our knowledge, this is the first report of *L. dispar* feeding on *L. salicaria*, however, eight species of Lepidoptera have been observed feeding on *L. salicaria* including two species of Lymantriidae (Diehl et al. 1997). Additional sites where *L. dispar* were observed feeding on *L. salicaria* in 1998 occurred in Ingham, Washtenaw, Jackson and Hillsdale Counties, MI. The larvae of *L. dispar* present in *L. salicaria* likely represent survivors of 1st instar ballooning in the spring and thus, may be expected to occur on *L. salicaria* in many areas where *L. dispar* is abundant. At this site, the contribution of *L.* dispar to *L. salicaria* defoliation was small and would not be expected to significantly impact the plant. However, field workers should be trained to differentiate damage of *Galerucella* spp. from damage of generalist herbivores such as *L. dispar* so that estimates of biological control agent impact are not biased.

Table 1. Number of *Lymantria dispar* larvae observed per m^2 on *Lythrum salicaria* at Lake Lansing County Park-North, Ingham County Michigan, 1998.

Sample Date	Weather	Number of *L. dispar*[1]	Mean ± SEM[2] per m^2	Life Stage[3]	% Defoliation
5/28/98	Clear	22	2.44 ± 0.77	-	-
6/2/98	Pt. Cloudy	16	1.77 ± 0.49	-	-
6/8/98	Pt. Cloudy	14	1.55 ± 0.44	-	-
6/12/98	Clear	10	1.11 ± 0.39	2nd-4th	15 ± 5
6/16/98	Clear	11	1.22 ± 0.22	3rd-5th	17.44 ± 4.87
6/30/98	Lt. Rain	2	0.22 ± 0.22	Pupae	17.78 ± 5.12

[1] Total count in all nine quadrats per sample date.

[2] n=9

[3] - indicates data not collected on the sample date.

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APPENDIX 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 2000-2

Title of thesis or dissertation (or other research projects):

PREDATOR EFFECTS ON *GALERUCELLA CALMARIENSIS* L. (COLEOPTERA:CHRYSOMELIDAE), CLASSICAL BIOLOGICAL CONTROL AGENT OF *LYTHRUM SALICARIA* L. (MYRTALES:LYTHRACEAE)

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name (s) (typed)

Donald C. Sebolt

Douglas A. Landis

Date 04/14/00

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America. Bull. Entomol. Soc. Amer. 24:141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.

Copies: Included as Appendix 1 in copies of thesis or dissertation.
Museum(s) files.
Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

APPENDIX 1.1

Voucher Specimen Data

Page 1 of 1 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Galerucella calmariensis L.	MICHIGAN Ingham County East Lansing Michigan State University June 1999 Donald C. Sebolt					30			

(Use additional sheets if necessary)

Investigator's Name(s) (typed)
Donald C. Sebolt
Douglas A. Landis

Date 4/14/00

Voucher No. 2000-2
Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator 14 April 2000
Date