Biological control of purple loosestrife, *Lythrum salicaria* L. (Lythraceae) with *Galerucella* spp. (Coleoptera: Chrysomelidae): dispersal, population change, overwintering ability and predation of the beetles, and impact on the plant in southern Manitoba wetland release sites.

by Jason K. Diehl

A Thesis Submitted to the Faculty
of Graduate Studies in Partial
Fulfilment of the Requirements
for the Degree of

MASTER OF SCIENCE

Department of Entomology
University of Manitoba
Winnipeg, MB

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BY

Jason K. Diehl

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree

of

MASTER OF SCIENCE

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CHAPTER I

GENERAL INTRODUCTION

Managers of wetlands across eastern North America have struggled for years to break-up monospecific stands of cattail, *Typha* spp. in order to increase the abundance and diversity of wildlife inhabiting such areas (Thompson et al. 1987). Today, instead of cattail, the struggle continues with the introduced species, *Lythrum salicaria* L. (Lythraceae), which outcompetes *Typha* spp. and other wetland plant genera such as *Carex* spp. and *Phalaris* spp., leading to the formation of large monospecific areas of the weed (Smith 1964; Stuckey 1980). Compared to cattail, these large areas of purple loosestrife are believed to provide a less suitable habitat for wetland wildlife (Mckeon 1959; Smith 1964). The importance of prairie wetlands in the population biology of migratory wildfowl is one factor contributing to establishment of a control program against purple loosestrife in the Prairie Provinces.

A classical biological control program using two leaf-feeding chrysomelid beetles, one root-feeding weevil and two flower-feeding weevils has been implemented in North America to limit further spread of this weed. Current research efforts on biological control of purple loosestrife using *Galerucella* spp. are concentrated in southern Ontario (Cooper et al. 1996) and the eastern United States (Blossey et al. 1996), and may not be applicable to the prairie provinces.

In 1993, the two leaf-feeding chrysomelids, *Galerucella pusilla* Dufts. (Coleoptera: Chrysomelidae) and *G. calmariensis* L., were the first biological control

agents released in southern Manitoba to help control the spread of *L. salicaria* (Hight et al 1995). Relative to more southerly latitudes, overwintering mortality of both *Galerucella* spp. may be higher, and the rate of increase and dispersal of these insects lower in prairie Canada. The higher proportion and cold-hardiness of *G. calmariensis* in northern Europe has raised concern about the recruitment potential of *G. pusilla* in areas with low spring temperatures (Blossey 1995a). Beetles were observed in the spring following release in Manitoba, and both species of *Galerucella* are able to survive the winter (Lindgren, personal communication); however, subsequent rates of dispersal and increased abundance in Manitoba appear low (Diehl, unpublished). Low rates of dispersal and increased abundance may not prevent successful biological control, but may dictate modifications in release strategy.

Differences in length of growing season may also influence the impact of *Galerucella* spp. on the stand dynamics of purple loosestrife. The response of plants to herbivore injury is strongly influenced by the plant's growth stage at the time of attack, and the growth stage at the time of attack is dependent upon local climate. It is therefore unwise to extrapolate from other climatic regions to predict the effect of *Galerucella* spp. on loosestrife, rather, it is important to obtain information on the interaction between *Galerucella* spp. and *Lythrum salicaria* under prairie conditions.

The objectives of this thesis are:

i.) To determine the "within site" dispersal rate and rate of increase of *Galerucella* spp. after their release against purple loosestrife in Manitoba.

ii) To investigate the impact of *Galerucella* spp. on purple loosestrife stem density and fruit production under Manitoba conditions.

iii) To estimate overwintering mortality of *G. calmariensis* and *G. pusilla* in Manitoba.

iv) To conduct a survey of insects associated with *L. salicaria* in southern Manitoba.

v) To determine the effect of feeding by the predatory stink bug, *Apoecilus bracteatus* Fitch, on larvae of *Galerucella* spp. under caged conditions.

The first of four sections in this paper style thesis pertains to the dispersal and total egg estimates of *Galerucella* spp., including their impact on *L. salicaria* stem density and fruit production within four release sites in southern Manitoba during the 1995 and 1996 growing seasons. The fourth chapter includes a study determining the ability of caged adult *Galerucella* spp. to overwinter within release sites during the winter of 1995-96. In the summer of 1994, a survey of insects associated with purple loosestrife in southern Manitoba was conducted; the results of which appear in Chapter V. As a result of this survey, a potentially important predator of *Galerucella* spp. larvae was identified within release sites at Libau Marsh, MB. Results of field studies in cages to determine the effect of the predatory stinkbug, *A. bracteatus*, on the larvae of *Galerucella* spp. are presented in Chapter VI of this thesis.

CHAPTER II

REVIEW OF PERTINENT LITERATURE

Purple Loosestrife: Introduction

Purple loosestrife, *Lythrum salicaria* L. (Lythraceae), is a deep rooted, herbaceous perennial weed of Eurasian origin (Thompson et al. 1987) found in a variety of habitats from rock crevices to gravel, clay and organic soils in North America and Europe. Purple loosestrife generally establishes itself in shallow marshes, wet meadows, along stream, pond and riverbanks and lakeshores. Since its arrival early in the 19th century, *L. salicaria* has become a problem weed in wetland and rangeland pastures of north-eastern and north-central United States and Canada (Stuckey 1980). It is estimated that purple loosestrife has invaded more than 121,000 ha of wetland and 41,000 ha of pasture and rangeland in North America (Thompson et al. 1987). The highly competitive nature and aggressiveness of purple loosestrife once established has led to the displacement of native wetland plant species and the formation of large monospecific areas of the weed (Malecki and Rawinski 1979). In some areas, *L. salicaria* has completely displaced native wetland vegetation such as *Typha* spp. (cattails), *Carex* spp. (sedges) and *Juncus* spp. (rushes) (Rawinski and Malecki 1985; Rawinski 1982). In Massachusetts, large areas of purple loosestrife are endangering the local bulrush, *Scirpus longii* Fern. (Coddington and Field 1978), and in New York the dwarf spike rush, *Eleocharis parvula* Rom and J. A. Schultes, is threatened by invasion of this weed (Rawinski 1982). Prolific seed production, high germination rate, resistance to changes in soil moisture and lack of

natural enemies are factors contributing to a competitive advantage of purple loosestrife over wetland plant genera such as *Typha* spp., *Scirpus* spp., *Phalaris* spp. and *Juncus* spp. (Malecki and Rawinski 1979; Gaudet and Keddy 1990). Such reductions in plant diversity are believed to create less suitable nesting habitat for gamebirds, waterfowl and other wetland animal species, leading to a loss in the number and diversity of wildlife inhabiting such areas (Smith 1964; Rawinski and Malecki 1984; Blossey and Schroeder 1992). Economic losses attributed to the impact of purple loosestrife on wildlife and agriculture have been estimated at 45 million dollars per year (Thompson et al. 1987). In some western U. S. states' its establishment in irrigation systems has impeded water flow and generated further cause for concern (Malecki et al. 1993b).

In areas where cattail marshes are not present, the dense growth habit of *L. salicaria* serves as shelter and escape cover for pheasants and cottontail rabbits (Smith 1964). In addition to harbouring an invertebrate food source (Hight 1990; Anderson 1995), newly established stands of *L. salicaria* may provide nest and brood cover for spring breeding waterfowl (Malecki and Rawinski 1979). Unfortunately, the usefulness of cover is often of short duration since the stands become increasingly impenetrable after a couple of years (Malecki and Rawinski 1979). Red-winged black birds also prefer stands of purple loosestrife to those of cattail and they may benefit from the increased abundance of purple loosestrife in North American wetlands (Rawinski and Malecki 1984). Smith (1964) further stated that where cover would otherwise be lacking, the presence of purple loosestrife may be considered to somewhat compensate for its aggressive nature.

A total of 35 species of *Lythrum* occur worldwide, 12 of which are found in North America. Three of the North American species are introductions from Eurasia and include *L. salicaria, L. hyssopifolia* L., and *L. virgatum* L. The remaining nine North American *Lythrum* species are native and none of them are listed as endangered or threatened (Blossey and Schroeder 1991). Of the related plants in North America, none are of any economic importance and no species of *Lythrum* are presently being used as sources of food or fibre (Blossey and Schroeder 1991).

Lythrum alatum (winged loosestrife), *L. lanceolatum* L. and *L. lineare* L. are the three most common native *Lythrum* species in central and northeastern United States and southern Canada (Fernald 1950). Intensive nursery breeding has led to a minimum of 28 varieties of purple loosestrife through hybridization of *L. salicaria* with North American *L. alatum* and European *L. virgatum* (Blossey and Schat 1997; Ottenbreit 1991). Prior to the commercial ban of purple loosestrife in Manitoba, four popular cultivars sold included: *L. virgatum* cv. Morden Pink; *L. salicaria* x *L. virgatum* cv. Dropmore Purple; *L. alatum* x Morden Pink cv. Morden Gleam; and *L. alatum* x Morden Pink cv. Morden Rose (Harp and Collicutt 1983).

In recent comparisons of three North American populations of *L. salicaria* by Blossey and Schat (1997), there are differences in height, biomass production, biomass allocation and root: shoot ratio within and between populations. It is believed this variability between North American populations of *L. salicaria* will have little or no impact on the ability of introduced biological control agents to develop on these plants. Even though related species of *Lythrum* are present in North America, no hybridization is

known to have occurred under field conditions and it is widely accepted that *Lythrum salicaria* is represented by a single, well defined, morphologically uniform, widespread species in North America (Thompson et al. 1987).

Biology of Purple Loosestrife

In North America, mature purple loosestrife plants range in height from 0.5 m-2.0 m and produce a maximum of 30-50 stems, whereas in Europe, plants are generally 1.0 m tall and produce a maximum of 5-10 stems (Blossey and Schroeder 1991). The reduced height and stem number in European populations of purple loosestrife is attributed to the presence of several specialised herbivores that utilise the plant (Blossey and Schroeder 1991).

Lythrum salicaria plants are capable of producing 900-1000 fruit capsules per flowering shoot, each containing 90-120 seeds (Shamsi and Whitehead 1974). Seed production estimates of purple loosestrife have been as high as 90,000 seeds per flowering shoot or 2.7 million seeds per plant with an average stem number of 30 (Thompson et al. 1987). Within marshes of mid-continental North America, the mean density of *L. salicaria* seeds found in the top 5cm of the soil was estimated to be 410,000 seeds /m^2 (Welling and Becker 1990). The seeds of purple loosestrife are approximately 400 x 200 μm with a dry weight equal to 0.5-0.6 mg /seed (Thompson et al. 1987).

There are few estimates of the viability of loosestrife seeds, but it has been shown they will remain viable for a minimum of three years under ideal conditions (Shamsi and Whitehead 1974). However, following a two-year storage period in water, seed viability

decreased from 99% to 80% (Rawinski 1982). Smith (1964) determined that *L. salicaria* seeds remain viable for many years even when submerged in water to depths of 0.5-1.0 meters.

Due to the high temperatures required, fall germination of purple loosestrife seeds seldom occurs. The critical temperature for germination of seeds is 15-20 °C and successful seedling establishment generally occurs on open, moist soils in late spring to early summer when temperatures are high (Shamsi and Whitehead 1974). Shallow marshes are particularly susceptible to invasion by purple loosestrife and the natural lowering of water levels during periods of drought results in large expanses of newly exposed soils available for colonisation (Malecki and Rawinski 1979). Upon germination, the primary root grows deep into the soil and develops branched secondary and tertiary roots. Once seedlings establish themselves' the roots thicken, harden and develop a sclerenchymatous central core that becomes woody as the plants begin to mature (Shamsi and Whitehead 1974).

The common rootstock of a mature purple loosestrife plant is large, but not interconnected with other nearby plants as was once thought (Thompson et al. 1987). Due to the nature of the common rootstock, purple loosestrife does not have an extensive lateral root system (Thompson et al. 1987) and any vegetative growth is restricted to the crown of older established plants (Shamsi and Whitehead 1974). The solid, woody rootstock also serves as a storage organ that provides resources for shoot regrowth after they are cut, burned or killed by herbicide application (Blossey and Schroeder 1991). Research on below ground reserves is limited; however, the root system maintains the life

of the plant during the winter months and in spring, buds present at the top of the rootstock sprout and develop into new stems (Malecki and Rawinski 1979).

The primary reproductive method of purple loosestrife is sexual; however, in some instances, vegetative regrowth can occur from buds present on the stems of the plant. Sexual reproduction is believed to be of primary importance for recent expansions in the distribution of the plant, whereas asexual reproduction by vegetative regrowth is more important on a local scale after the plant has established (Shamsi and Whitehead 1974). In a tristylous plant species such as purple loosestrife, a flower of one style length bears two sets of stamens of different lengths and any pollination is restricted to crosses between the style of one length with stamens of the corresponding length (Darwin 1893). Individual flowers are self-incompatible and must be cross-pollinated by the action of insects or wind that move pollen between the flowers (Thompson et al. 1987; Hight and Drea 1991). Seldom does a purple loosestrife seedling flower during its first year of growth (Malecki and Rawinski 1979).

Origin, Arrival and Spread of Purple Loosestrife in North America

Lythrum salicaria was initially believed to be a native North American plant species; however, it is now widely accepted as a native of Eurasia (Stuckey 1980). The European range of purple loosestrife is much larger than the Asian, extending from Great Britain across Europe to central USSR and from Italy northward to the 65th parallel (Blossey and Schroeder 1991). The main islands of Japan represent the core of the Asian distribution, with additional populations extending across the lowlands of China to

Southeast Asia and Northern India. Accidental and intentional introductions have expanded the distribution of *L. salicaria* to include north and east Africa, Australia, Tasmania, New Zealand, Peru, Chile, United States and Canada (Stuckey 1980; Hight and Drea 1991).

In central Europe, populations of *L. salicaria* are generally small and localized with low abundance (10-100 plants/location). However, near the northern limits of its European distribution in Sweden and Finland, populations of high abundance (>10 000 plants/location) are more common (Blossey 1995b). The original habitats of purple loosestrife along river systems and in marshes have largely been destroyed in central Europe and the plant is now most common in disturbed or man-made locations (Blossey 1995b).

Purple loosestrife was first recorded from the eastern seaboard of North America in 1814 (Stuckey 1980; Malecki and Rawinski 1985) and was introduced as a contaminant of ship's ballast, crop seed, and by the arrival and movement of early settlers (Rawinski 1982; Thompson 1991). European immigrants likely brought the plant with them because of its medicinal properties, including the ability to staunch bleeding, cleanse and heal wounds, ulcers and sores, and treat diarrhoea (Thompson et al. 1987). The plant was initially associated with major waterways in the northeast and it was often characterized as growing in disturbed habitats (Rawinski 1982). Purple loosestrife seeds are buoyant and the movement of water facilitated further expansion of its North American distribution (Thompson et al. 1987). Spring floods are an effective means of dispersing seeds and may account for the abundance of purple loosestrife growing on the

flood plains of major river ways in North America (Malecki and Rawinski 1979). Soon after arriving, *L. salicaria* became well established along the east coast, and during the late 1800's, construction of inland waterways and canals contributed to expansion into the Hudson River Valley, St. Lawrence River Valley and the Great Lakes region (Stuckey 1980). There are many references to purple loosestrife in botanical journals from 1850 to 1900, an indication of the rapid spread of the plant during this period (Rawinski 1982). Purple loosestrife was given little attention until the late 1930's, when its aggressiveness and ability to displace native wetland vegetation was first noticed by wetland wildlife managers (Fernald 1940; Stuckey 1980).

More recent expansion of the North American distribution has coincided with increased land development, road construction, extensive commercial distribution as an ornamental plant and propagation as a forage plant for bees (Stuckey 1980). The bees of commercial honey producers often utilize purple loosestrife as a late season food source (Thompson et al. 1987). The flowers provide a rich pollen and nectar source for bees, and adult flower flies (Syrphidae) (Batra et al. 1986). Additional spread and reproduction was attributable to mud adhering to agricultural machinery, animals and birds, as well as transportation of contaminated seed and foodstuffs (Thompson et al. 1987). The mean rate of spread of *L. salicaria* since 1940 has been estimated to be 645 kilometres per year (Thompson 1991) and the current North American distribution of the plant lies between the 35th to 52nd parallels.

Currently, purple loosestrife is found in all 10 Canadian provinces (DeClerck-Floate 1992) and in southern Manitoba, the plant is found along every major river system,

with large areas of concentration along the Cypress, Assiniboine, and Red Rivers, as well as in the Netley-Libau and Delta marsh systems south of Lake Winnipeg and Lake Manitoba (Diehl et al. 1997).

Control of Purple Loosestrife

Limited control of purple loosestrife can be achieved using physical methods such as cutting, burning, water level manipulation and hand pulling. There is little or no impact of burning *L. salicaria* stems on overall survival, as the rootstocks are likely to survive and generate new growth (Mckeon 1959). Mowing or cutting purple loosestrife stems prior to flowering in June or early July can reduce plant vigour; however, cut shoots remaining on the moist soil will continue growing to produce flowers and viable seed in the same season (Malecki and Rawinski 1979). Mowing purple loosestrife plants in full bloom (August) ensures that sprouts from the cuttings will not grow to produce viable seed; however, by this time cut stems contain viable seed that is available for the next growing season (Malecki and Rawinski 1979). In a separate study, mature plants cut in August were unable to replenish root reserves required for regrowth the following spring, leading to a reduction in stem density (Malecki and Rawinski 1985). However, unless cuttings are removed from the site, late mowing of flowering *L. salicaria* shoots will ensure that viable seed is produced for the following spring. The best time to mow is when cuttings do not contain a large number of viable seeds and do not have enough time to re-grow and produce flowers in the same year (Malecki and Rawinski 1979). In southern Manitoba, this corresponds to the early bloom period of purple loosestrife in the

first or second week of July. In Ontario, Haworth-Brockman et al. (1991) demonstrated no difference in the mean number of *L. salicaria* stems /plot between the control and underwater treatments clipped in early (15 June), mid (15 July) and late summer (15 August). The mature *L. salicaria* plants that were clipped grew new stems and reached control densities by the end of the growing season. Approximately 21 days after germination, the seedlings of purple loosestrife can be mowed as there is not enough time for the plant to develop roots below ground that are capable of regeneration (Gabor and Murkin 1990). However, by 42 days the seedlings have enough below ground root reserves to develop new stems when cut. Gabor and Murkin (1990) recommended cutting seedlings at 21 days to reduce establishment and prevent re-establishment by vegetative sprouting from cut stems. Cutting as a control technique should not be relied upon exclusively, but used in combination with other control techniques such as flooding and burning (Malecki and Rawinski 1985; Haworth-Brockman et al. 1991).

Rawinski and Malecki (1985) demonstrated that under stable water conditions (mean depth of 40 cm), *Typha* spp. can out-compete and suppress purple loosestrife. Other researchers have also demonstrated detrimental effects of flooding on both established and seedling populations of *L. salicaria* (Malecki and Rawinski 1979; Rawinski 1982; Clay 1986; Balogh 1986). Shallow flooding, in which seedlings are initially submerged but able to grow quickly through the water, has little or no impact on seedling densities (Malecki and Rawinski 1979). Raising water levels effectively suppresses newly established seedlings only when they are completely submerged for an extended period (Malecki and Rawinski 1979). Balogh (1986) found that seedlings

10-15 cm in height show significant mortality after five weeks of flooding with 30-100 cm of water and recommended that depths greater than 30 cm are required to control seedlings. In contrast, Haworth-Brockman et al. (1993) demonstrated that four different shallow flooding depths do not affect mean densities of loosestrife seedlings. Young seedlings continued to grow while submerged and eventually emerged from the water and flowered in the same season. They concluded that shallow flooding is not an effective approach to preventing or limiting the establishment of *L. salicaria* seedlings. Negative effects on mature *L. salicaria* plants have been demonstrated when water levels are maintained between 40-60 cm for several consecutive years; however, seasonal flooding has only a limited impact on the plant (Rawinski and Malecki 1984). Purple loosestrife plants are somewhat tolerant of flooding and within days after submergence they develop a porous, spongy bark layer (aerenchyma) on the stem which helps to facilitate gas exchange (Malecki and Rawinski 1979). Complete submergence of mature loosestrife plants should only be considered when dealing with a heavy infestation as this approach can be detrimental to other vegetation (Malecki and Rawinski 1985).

Water level reductions in wetland areas to allow desirable plant species to re-establish may result in an increase in *L. salicaria* densities, as the exposed soil provides a suitable medium for seeds to germinate (Gabor and Murkin 1990). *Lythrum salicaria* stem densities have been shown to increase in comparison with cattail when water levels are low enough to expose mudflats for seedling establishment (Rawinski and Malecki 1985). However, when Japanese millet, *Echinochloa frumentacia* was seeded on exposed mudflats, it successfully competed with purple loosestrife seedlings (Malecki and

Rawinski 1985). The drawdown and reflood approach may be an option for intensively managed wetlands only when used in combination with plant competition.

Hand pulling or digging up purple loosestrife plants is another control technique that works well on newly established seedlings, but not very well on older, mature plants. Control of mature plants by hand pulling or digging is difficult due to the development of an extensive, woody root mass. Any piece of root material remaining in the soil is capable of producing adventitious buds and eventually stems (Rawinski 1982). When plants occur in small concentrated stands, uprooting by hand and ensuring removal of all vegetative material can lead to the elimination of *L. salicaria* from these areas. Unfortunately, the time required to remove even a small area of mature plants is often too great. When stands of purple loosestrife become established and dense, hand removal is usually impractical (Malecki and Rawinski 1979).

Recommended herbicides can provide excellent control of purple loosestrife; however, the plants' preference for wet marshy areas often restricts herbicide use. In dryland situations, the application of glyphosate (Roundup®) at a rate of 1.7 kg/ ha during the period of peak bloom (mid-August) can provide 99% weed control. Unfortunately seed viability from treated plants is cause for concern (Rawinski 1982). Seeds collected from purple loosestrife plants treated with glyphosate in late August had a mean germination rate of 53% compared to untreated plants at a rate of 99% (Rawinski 1982). In the year following control with glyphosate, plots treated in late August contained newly established plants such as *Carex* spp. and *Polygonum* spp., while plots treated in early June contained young purple loosestrife plants (Malecki and Rawinski

1985). The ability of glyphosate to be taken up by the plant, quick inactivation upon exposure to soil, and efficacy against purple loosestrife makes this herbicide attractive in dryland situations.

The herbicides 2,4-D and triclopyr amine may be used to control purple loosestrife but the requirement for repeated applications and high associated costs usually limit their use on a large scale. Smith (1964) reported that 2,4-D provided satisfactory kill of the tops of mature loosestrife plants, however, some resprouting from the base of the plant was observed. Results of treating *L. salicaria* in small plots with various herbicides were variable and in no case did any one year of treatment result in complete destruction of all plants within the test plots (Smith 1964). Overall, the non-selectivity and high cost associated with using these recommended herbicides render them impractical and undesirable in most situations.

The ability of some plants to out-compete the seedlings of purple loosestrife can help prevent establishment in wetlands that are intensively managed. Japanese millet, *E. frumentacia,* grows rapidly, competes well with purple loosestrife and it is able to tolerate the periodic changes in water level that occur in wetlands. Seeding Japanese millet on intensively managed wildlife marshes can provide a barrier that impedes further encroachment by purple loosestrife, and the seed crop it produces may be used by waterfowl (Malecki and Rawinski 1985). In study plots containing both millet and *L. salicaria* seedlings, no loosestrife plants were found at the end of the growing season, even though densities were high at the start of the season (Malecki and Rawinski 1985). The millet seed should be planted soon after receding water exposes the soil, and prior to

the germination and emergence of loosestrife seedlings. Reed canary grass, *Phalaris arundinacia* L., also competes well with purple loosestrife in a mixed seeding, and mature plants can be controlled through replacement by this grass (Novak 1968). It is believed that large areas of Reed canary grass and Japanese millet would be more suitable than purple loosestrife to wetland wildlife, as they utilize the seed as a food source (Malecki and Rawinski 1985; Novak 1968). The competition control strategy focuses on preventing further infestations instead of eliminating existing stands of *L. salicaria*, unfortunately the labour required for such operations makes this technique very costly (Malecki and Rawinski 1985).

Any control leading to a reduction in the number of established purple loosestrife plants may be short lived due to recruitment from the enormous loosestrife seedbank present within most established sites (Welling and Becker 1990). The prevention of recruitment from the seedbank is difficult due to the ability of purple loosestrife seeds to germinate across a broad range of environmental conditions (Shamsi and Whitehead 1974).

Chemical and cultural control techniques can be difficult and expensive to implement when large areas of land are involved. Biological control on the other hand, becomes more economical as the area of infested land increases. In this situation, selection of purple loosestrife as a target weed represents an economical decision in terms of saving time and money. Classical biological control of purple loosestrife represents an economically feasible and less labour intensive approach predicted to have tremendous potential in North America. The biological control program against purple loosestrife is

relatively new and several agents have been released into North America in attempts to control the further reproduction and spread of the plant.

Classical Biological Control of Weeds: An Overview

Biological control of weeds represents one of many alternative approaches to weed control that can help reduce annual pesticide applications. Biological control is based on the theory that introduced natural enemies can reduce the population of a weed, while maintaining a strict host range (Wapshere et al. 1989). H. S. Smith, who advocated the use of pathogens, parasitoids and predators as natural enemies to control insect pests (Smith 1919), coined the term "biological control" in 1919. Debach (1964) later defined biological control as the action of predators, parasites and pathogens in maintaining a target organism's population density at a lower level than would occur in their absence. The National Academy of Sciences (1987) redefined biological control as "the use of natural or modified organisms, genes, or gene products to reduce the effects of undesirable organisms, thus favouring desirable organisms such as crops, trees, animals, beneficial insects and micro-organisms".

In Canada, classical biological control of weeds is regulated by the Plant Protection Act (Harris 1991) and involves the importation of a weed's natural enemies from their area of origin, and subsequent release into a new geographic area occupied by the weed (van Lenteren 1988; Wapshere et al. 1989). Today's classical biological control agents are selected for their ability to attack specific target weeds, while leaving crops and other important plants untouched (Strobel 1991). To ensure this relationship holds true,

rigorous host specificity testing is carried out for agents under consideration for release into a new geographic area (Harris and Zwolfer 1968). The desired end result of a classical control program is a self-perpetuating biological agent population that reduces growth, reproduction and competitive ability of a target weed, making it more susceptible to adverse environmental conditions (Harris 1988; Wapshere et al. 1989). When successful, a classical biological control program does not normally eradicate an invasive weed, but instead establishes a self-sustaining balanced interaction between the target weed and its introduced enemies (Blossey and Schroeder 1991). Residual populations of the target weed are required to serve as reservoirs for the introduced agents as there will likely be oscillations in the populations of both over time. These fluctuations in population are primarily in response to environmental conditions, activities of man and the interaction of the target weed and its associated organisms (Hight and Drea 1991).

During the 1960s, the effort to establish a national control program against purple loosestrife in the United States was at a standstill, largely due to the lack of spread and impact data, the long established presence of the weed in eastern North America and the lack of support from the U. S. Department of Agriculture (Thompson 1991). In the late 1970's, the program was re-evaluated and by 1980, purple loosestrife was approved as a candidate for study as a potential target weed. Purple loosestrife is a suitable candidate weed for classical biological control in North America because it: 1.) exhibits a perennial growth habit, 2) was introduced from Europe, 3) grows in a specific, stable habitat, 4) is taxonomically isolated from other plants, 5) possesses unfilled feeding niches, and 6) has suitable biological control agents found in its native area. During initial European

surveys to identify suitable organisms for introduction into North America, no pathogens attacking *L. salicaria* were found (Blossey and Schroeder 1986).

In surveys of insects associated with purple loosestrife in North America, a total of 68 phytophagous insects attacking purple loosestrife have been identified, some of which caused appreciable damage to individual plants (Hight 1990; Anderson 1995; Voegtlin 1995). However, all observed feeding niches on the plant were believed to be available for exploitation by any future introduced natural enemies (Hight 1990). During the first European survey of insects associated with the plant, a total of 120 species of phytophagous insects were found to be associated with *L. salicaria*, of which 14 were considered host specific (Blossey and Schroeder 1986). From the host specific insects, six were considered as potential biological control agents to be released in North America. These included *Galerucella calmariensis* L. (Coleoptera: Chrysomelidae), *G. pusilla* Duftschmidt, *Hylobius transversovittatus* Goeze (Coleoptera: Curculionidae), two flower-feeding weevils, *Nanophyes marmoratus* Goeze (Coleoptera: Curculionidae), *N. brevis* Boheman, and a gall midge, *Bayeriola salicariae* Kieffer, which reduces seed production by attacking flower buds. The leaf-feeding chrysomelid beetles, *G. calmariensis* and *G. pusilla* and the root-feeding weevil, *Hylobius transversovittatus*, were identified as the most promising candidates for release and establishment in North America (Batra et al. 1986; Blossey and Schroeder 1986). All three agents occur throughout the distributional range of purple loosestrife in Europe and were rigorously tested for host specificity (Blossey and Schroeder 1991). The Animal and Plant Health Inspection Service (APHIS) of the USDA granted approval in 1992 for release of these

three agents into the United States. Approval for release in Canada was granted in the same year by the Plant Protection Division of the Food Production and Inspection Branch, Agriculture and Agrifood Canada. Approximately 25,000 adult *Galerucella* were shipped to the United States in the summer of 1992, passed through quarantine and distributed to rearing centres in Virginia, Maryland, Pennsylvania, New York, Minnesota, Oregon, Washington, Ontario and Manitoba (Malecki et al. 1993a). Despite approval for release in 1992, the Manitoba Purple Loosestrife Project did not introduce the two leaf feeding biological control agents until the summer of 1993 (Lingren, personal communication).

Galerucella spp.: Introduction

In Europe, *Galerucella calmariensis* and *G. pusilla* share the same ecological niche and show strong reproductive isolation (Manguin et al. 1993). In field and laboratory studies conducted by Blossey (1995a), both species had identical competitive abilities and shared the same inclusive fundamental niche on their host plant, *L. salicaria* (Blossey 1995a). The pattern of resource use between *G. pusilla* and *G. calmariensis* in Europe is identical and no differences in adult phenology, winter mortality, colonization pattern and oviposition were observed (Blossey 1995a). In models of stable coexistence of two competitors such as *G. pusilla* and *G. calmariensis*, it is assumed that the inferior species have access to resources that are temporally or spatially unexploited by the superior species (Blossey 1995a). The principle of competitive exclusion predicts the displacement of either *G. pusilla* or *G. calmariensis*; however, according to Blossey

(1995a) this is not the case. On a local scale, both species seem to have identical competitive abilities, and neither species is superior or dominant over the other. However, *G. calmariensis* is more fecund (Ragsdale 1995), possesses a wider host range, and is more abundant in the northern distributional limits of the beetles European range, compared *to G. pusilla* (Blossey and Schroeder 1991). Agren and Fagerstrom (1984) have suggested that competitors may coexist under intense resource competition if they are sufficiently similar in their skills to compete for available resources. According to Blossey (1995a), *G. calmariensis* and *G. pusilla* possess identical competitive abilities, thus allowing them to occupy the same ecological niche on *L. salicaria*.

The taxonomic status of *Galerucella* spp. (*sensu lato*) was revised by Silfverberg (1974) who subdivides the *Galerucella* spp. (*sensu stricto*) into two subgenera; *Galerucella* with *G. nymphaeae* L., *G. grisescens* Joann. *G. placida* Baly, and *Neogalerucella* with *G. limneola* F., *G. tennella* L., *G. calmariensis* L. and *G. pusilla* Dufts. In 1993, Manguin et al. demonstrated through an isozyme comparison that *G. calmariensis* and *G. pusilla* are distinct from the European species *G. grisescens*. Three additional species of *Galerucella* occur in North America: *G. nymphaeae* on water lilies and *L. salicaria*, *G. stefanssoni* Brown, feeding on *Rubus chamaemorus* L. (cloudberry) and *G. quebecensis* Brown, feeding on *Potentilla palustris* (L.) Scop. (marshflower) (Manguin et al. 1993). The introduction of *G. calmariensis* and *G. pusilla* increased the North American number of *Galerucella* to five.

The distribution of *G. pusilla* and *G. calmariensis* is nearly identical, each being found throughout the Palaearctic distribution of *L. salicaria*. Ninety per cent of purple

loosestrife populations in Europe show evidence of attack by *Galerucella* spp. (Malecki et al. 1993a). One difference between the two species is that *G. pusilla* is relatively less abundant than *G. calmariensis* in the northern distributional range of purple loosestrife (Blossey and Schroeder 1991). In central Europe, mixed species beetle communities dominate and the two species are equally abundant (Blossey 1995a). Adult *Galerucella* spp. released in North America were collected from two climatically different sources in Germany; a southern source in Gelnhausen and a northern location in Meggerdorf (Hight et al. 1995). The proportion of *G. calmariensis* collected at the northern collection site was 90% , while in the southern site the proportion of this species was 30% (Hight et al. 1995). Prior to releasing *Galerucella* spp. in North America, adults were exposed to and accepted *L. salicaria* plants collected from Washington, Minnesota and Maryland (Blossey and Schroeder 1991). Despite the source of beetles initially released into North America in 1992, all introductions of *Galerucella* spp. became established the following year and overall survival rate of the beetles was estimated to be 10% (Hight et al. 1995).

Biology of *Galerucella* spp.

In their native habitat, adults and larvae of both species can completely defoliate loosestrife plants and suppress flower production (Blossey 1992). *Galerucella* spp. overwinter as adults and are known to survive extended periods of immersion during spring and summer floods (Hight et al. 1995). In southern Manitoba, adults emerge from hibernation sites in litter from late May to early June. They immediately begin feeding on

the meristematic tissues and young leaf tips of emerging purple loosestrife plants (Blossey et al. 1994). Adults usually drop off the plant when approached; however, feeding damage is easily observed on the shoot tips of newly emerged plants (Hight and Drea 1991). When a large population of adults emerges and feeding starts early in spring, the development of shoots can be retarded or in some instances, completely prevented (Blossey and Schroeder 1991). Any shoots escaping early season defoliation will likely suffer from combined adult and larval feeding later in the growing season. Adults feed for a period of approximately one-week before copulation and oviposition begins (Hight and Drea 1991). Eggs of both species are indistinguishable, opaque, round, covered with a thin line of frass and laid in batches of variable size on the leaves, stems, axils and flowers of the purple loosestrife plant (Malecki et al. 1993). During the first part of the oviposition period, eggs are preferentially laid in small batches at the base of stems, compared to later when eggs are attached to stems, leaves, axils or flowers (Hight and Drea 1991). Blossey (1995a) demonstrated the pattern and duration of oviposition of *G. calmariensis* and *G. pusilla* under field conditions in Europe to be identical. However, under laboratory conditions in Minnesota, *G. pusilla* was shown to distribute its eggs evenly throughout the canopy while *G. calmariensis* laid eggs in the upper third (Ragsdale 1995). The daily oviposition rate of *Galerucella* spp. in Germany was related to temperature, with the highest rates of 10-12 eggs/day occurring at temperatures of 25-30°C and lower rates at temperatures below 20°C (Blossey 1995a). In Minnesota, under greenhouse conditions, the average number of eggs/female/day was 8.2 for *G. pusilla* and 10.5 for *G. calmariensis* (Ragsdale 1995). Ragsdale also observed that greenhouse

colonies of *G. pusilla* produced only half as many adults as colonies of *G. calmariensis*. He attributed the difference to the significantly greater egg hatchability of *G. calmariensis*. Females require 3-4 weeks to reach full egg laying capacity, and oviposition of *G. pusilla* is more negatively affected by lower temperatures than that of *G. calmariensis*. In Germany, decreasing daylength coincides with a drop in the number of eggs laid by both species; however, *G. pusilla* is more affected than *G. calmariensis* (Blossey 1995a). Blossey and Schroeder (1991) determined the average batch size of *G. pusilla* to be 2.9 eggs and that of *G. calmariensis* to be 4.8 eggs under laboratory conditions. Under field conditions in Germany, Blossey (1995a) demonstrated a batch size of 2.9 ± 0.06 eggs for *G. pusilla* and 5.3 ± 0.2 eggs for *G. calmariensis*. Batch size was also significantly larger for *G. calmariensis* (6.6) compared to *G. pusilla* (5.3) under laboratory conditions in Minnesota (Ragsdale 1995). Differences in batch size may be due to the slightly larger size of *G. calmariensis* compared to *G. pusilla*. Oviposition may continue at a reduced rate throughout summer until the end of July when the majority of adults from the overwintered generation have died (Hight and Drea 1991). During an oviposition period from mid-May to mid-July, a female *Galerucella* spp. is capable of producing and laying 400-500 eggs (Hight and Drea 1991; Malecki et al. 1993) however, after four to five weeks of oviposition females become physiologically exhausted and egg laying usually stops.

At a constant temperature of 20°C, first instar larvae hatch approximately 12 days after oviposition (Blossey and Schroeder 1991). Newly hatched first instar larvae move to growing tips where they feed on young meristematic tissues of newly unfolding leaves

(Hight and Drea 1991). Evidence of first instar larval damage is often difficult to observe without spreading apart the young developing leaves (Malecki et al. 1993). Second and third instar larvae feed on all parts of the plant, but generally prefer the undersides of older unfolded leaves or developing flower buds (Hight and Drea 1991). At 20°C, larval development is completed in as few as 14 days (Blossey and Schroeder 1991). Mature third instar larvae move down the stem and drop off the plant to pupate in the leaf litter or top few centimetres of the soil (Blossey 1992). In flooded sites, *Galerucella* spp. larvae may form pupal cells in the thick aerenchyma tissue that develops on plant stems that have been submerged for extended periods (Hight and Drea 1991). The pre-pupal and pupal stages together last approximately 10 days, after which the teneral adults emerge and commence feeding. After feeding for approximately one week, adults may copulate and produce a partial second generation (Blossey and Schroeder 1991). The courtship behaviour of males differs between *G. calmariensis* and *G. pusilla* (Petersen 1994). Warmer summer temperatures over much of the proposed release area in North America compared to northern Germany may speed development from egg to adult resulting in the completion of two generations in the same year for both species of *Galerucella* (Vega et al. 1992).

Natural Enemies of *Galerucella* spp.

Predation of *G. pusilla* and *G. calmariensis* by spiders was observed at all field sites in Europe, and in North America several failures to establish the beetles have been attributed to egg and larval predation by arachnids (Malecki et al. 1993). In Europe,

Galerucella larvae are attacked by *Asecodes mento* Walk. (Eulophidae) and adult beetles by *Centistes rufithorax* Telenga (Braconidae). Predation of eggs, larvae and adults by spiders and ladybird beetles occurs in North America and may offset productivity of the *Galerucella* spp. (Malecki et al. 1993). In studies of the closely related North American species, *G. nymphaeae*, no parasites or pathogens likely to kill the two introduced species were discovered; however, egg predation by the ladybird beetle, *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae) was observed (Malecki et al. 1993). In Manitoba, larvae of both introduced species are attacked by the predatory stink bug, *Apoecilus bracteatus* Fitch (Hemiptera: Pentatomidae) (Diehl et al. 1997).

Host Specificity of *Galerucella* spp.

In host specificity studies carried out by Blossey and Schroeder (1991), plants belonging to one of three groupings were tested; those taxonomically related to *L. salicaria*, associated wetland plants of wildlife importance, and important agricultural plants. A total of 48 plant species were selected and approved by the Technical Advisory Group for the introduction of biological control agents of weeds, U.S.D.A., Plant and Animal Health Inspection Service. Adult *G. calmariensis* and *G. pusilla* will feed on plants outside the genus *Lythrum*; however, oviposition is strictly restricted to this genus (Blossey et al. 1994). *Lythrum salicaria*, *L. alatum* Pursh, *L. californicum* Torr. & Gray and *L. hyssopifolia* L. were all utilized for feeding and oviposition by *G. calmariensis* and *G. pusilla* during laboratory testing of the 48 plant species (Blossey and Schroeder 1991;

Blossey et al. 1994). Development of *G. calmariensis* and *G. pusilla* from larva to adult in larval transfer tests can be completed on *L. alatum* in addition to *L. salicaria* (Blossey and Schroeder 1991). The host range of *G. calmariensis* is wider than that of *G. pusilla*, as it also completes development from larva to adult on *Ammannia auriculata* Willd. and *A. latifolia* L (Blossey et al. 1994). Newly emerged teneral adults of *Galerucella* spp. fed moderately on winged loosestrife, *L. alatum* and swamp loosestrife *Decodon verticillatus* (L.) Ell when potted plants were placed in a naturally occurring *L. salicaria* stand which had been consumed (Blossey et al. 1994). Oviposition under laboratory conditions by *Galerucella* spp. was never observed on plants outside the genus *Lythrum*, and never on potted swamp or winged loosestrife introduced into natural sites (Blossey et al. 1994). The results of Blossey and Schroeder (1991) are an indication that winged loosestrife, *L. alatum* and possibly swamp loosestrife, *D. verticillatus,* may be vulnerable to limited attack by newly emerged adults of *Galerucella* spp.

Impact of *Galerucella* spp. on Purple Loosestrife

In all but one study site in northern Germany during 1988-1990, attack by *Galerucella* spp. caused reduced shoot growth, suppression of flowering, reduced seed output and high plant mortality (Blossey 1992). Plants previously unattacked were unable to compensate for injury caused by three *Galerucella* spp. larvae per centimetre of shoot (Blossey 1992). When densities of *Galerucella* spp. larvae are high, photosynthetic material is removed, fewer carbohydrate reserves are deposited in the roots, and shoot growth may be reduced the following spring (Blossey and Schroeder 1991). Blossey and

Schat (1997) examined the impact of herbivory by *G. calmariensis* on three separate North American populations of *L. salicaria*. They demonstrated significant reductions in plant height, leaf, shoot, root and total plant biomass with increasing levels of attack by *G. calmariensis* for one complete generation. Seedlings of *L. salicaria* compensated for loss of photosynthetic tissues by replacing foliage at the expense of below ground storage reserves (Blossey and Schat 1997). Any herbivore attack resulting in reduced allocation of reserves to root storage is expected to limit the ability of the plant to compete the following spring and increase the risk of winter mortality, especially when young seedlings are attacked (Blossey and Schat 1997). For well-established plants, increased herbivory by *G. calmariensis* causes a significant reduction in allocation of resources to reproduction and only minor diminution in resource allocation to root storage (Blossey and Schat 1997).

Establishment and Dispersal of *Galerucella* spp. in North America

Large numbers of both *Galerucella* species have been reared and released in the U.S. and Canada. Establishment and reproduction of both *G. pusilla* and *G. calmariensis* has been confirmed in North America. Laboratory data demonstrating the effect of *Galerucella* spp. on *L. salicaria* suggest that the beetles can have a measurable impact (Blossey and Schroeder 1991; Blossey 1992; Blossey and Schat 1997). North American data regarding the successful control of purple loosestrife by the two species of *Galerucella* are limited. After examining initial release data from 1992, Hight et al. (1995) concluded that higher survival rates using caged releases were obtained for

introductions of 600 adults compared to 200 adult *Galerucella* spp. They also concluded that the amount of litter, number of standing dead *L. salicaria* stems or density of *L. salicaria* did not affect establishment of the beetles during these initial North American releases. According to Malecki et al. (1993), peak dispersal of overwintered adults is believed to be during the first few weeks of purple loosestrife emergence with a second dispersal period occurring after the new generation of adults has emerged in late summer. There is little information regarding the distances travelled by dispersing adults or the factors influencing migration. In general, there is a need for more detailed studies of the dispersal and growth rates of *Galerucella* spp. within release sites in North America.

Conclusions

The current North American distribution of purple loosestrife is well defined and it is widely accepted that the weed is free from its native natural enemies. This freedom from its natural enemies is thought to contribute to a competitive advantage over other native wetland plant species (Blossey and Schroeder 1986). This does not mean that no natural enemies attack purple loosestrife. In North America, generalist herbivores feed on this weed and some cause significant damage to individual loosestrife plants (Hight 1990; Voegtlin 1995; Diehl et al. 1997). However, negative effects of these native herbivores on entire stands of *L. salicaria* are not usually observed in North America.

Prior to identification of purple loosestrife as a problematic weed, it was one of the best selling ornamental perennial plants, which no doubt contributed to the recent

extensions of its distribution in North America (Thompson et al. 1987). For the greenhouse industry, purple loosestrife represented an economically important plant sold as a garden perennial. Since launching the massive control campaign against purple loosestrife, many greenhouses have stopped selling established 1-year-old seedlings and seed.

Initial concern over the proposed biological control of purple loosestrife was apparent in beekeepers whose bees were utilizing the plant as a source of nectar and pollen (Thompson et al. 1987). However, as beekeepers became aware of the low success rate of classical weed biological control, concern soon faded out. Otherwise, there has been little controversy over the proposed biological control of purple loosestrife in North America.

Documentation of the detrimental effects of purple loosestrife on native vegetation are present in the literature; however, negative impacts on wetland wildlife such as bog turtles have been predicted but not confirmed. Prior to initiation of the control program against *L. salicaria*, more studies should have been conducted to confirm or disprove the negative effects of purple loosestrife on native wetland animal species.

Literature available on the dispersal, biology and distribution of purple loosestrife in North America is abundant. Unfortunately, the same is not true regarding the introduced biological control agents, *G. pusilla* and *G. calmariensis*. Some field research has been conducted in Europe; however, results will likely differ from the climatically distinct areas in North America where beetles have been introduced. Traditionally, classical weed biological control programs have been heavily weighted towards the

selection and release of suitable agents for introduction. By the time control agents are introduced, the majority of available resources are used up and follow-up studies usually involve a determination of whether or not the agent has established. When establishment is not successful, researchers are often at a loss as to explain why. Detailed follow-up studies immediately after release of agents into a new area are necessary so that reasons for success or failure can be supported by scientific data, not personal opinion. This study represents a follow-up immediately after the release of *G. calmariensis* and *G. pusilla* into four wetland sites. The dispersal, overwintering ability, growth rate and predation of *Galerucella* spp. within the four release sites are studied in southern Manitoba.

CHAPTER III

DISPERSAL AND GROWTH RATES OF *GALERUCELLA* SPP. (COLEPTERA: CHRYSOMELIDAE) AND IMPACT ON *LYTHRUM SALICARIA* L. IN SOUTHERN MANITOBA WETLAND RELEASE SITES

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ABSTRACT

During spring of 1995 and 1996, five sites containing purple loosestrife, *Lythrum salicaria* L. (Lythraceae) were sampled for *Galerucella* spp. (Coleoptera: Chrysomelidae) egg masses. In four of these sites, releases of the biological control agents, *Galerucella* spp. were made prior to or during the study. Two release sites were located at Delta Marsh, south of Lake Manitoba and two at Libau Marsh, south of Lake Winnipeg. In 1993, a mixed species introduction of adult *Galerucella calmariensis* (L.) and *G. pusilla* Dufts. was made into one site at each of Libau Marsh and Delta Marsh. The other site at each locality received introductions of *Galerucella* spp. in 1995. A fifth site was selected at Libau Marsh in which there was no *Galerucella* spp. present. Within each study site, a series of transects radiating away from a central origin was established. All sites were sampled in spring for *L. salicaria* stem and *Galerucella* spp. egg densities at predetermined distances along each transect using a $1m^2$ quadrat. In fall, using the same sampling system, purple loosestrife stem densities were determined and seed heads were collected to provide an estimate of the fruit production $/m^2$.

There was a decrease in the density of *Galerucella* spp. eggs with increasing distance from the central release point along all transects sampled during springs of 1995 and 1996. This decrease was most pronounced in both sites that received introductions of adult *Galerucella* spp. in 1995 and less so in 1993 release sites. Purple loosestrife stem density also decreased with distance along the majority of transects sampled during both years. Fruit production by *L. salicaria* was somewhat variable along transects, being either uniform or decreasing with distance from the release point.

The decline of *Galerucella* spp. egg density with increasing distance from the central release point fitted the general non-linear model: density = exp (a + b (distance ^{c})) described by Taylor (1978). Estimates of the a, b, and c parameters were generated for individual transects in all four release sites and used to predict the number of eggs /m² for each transect, up to the last distance sampled. Observed vs. predicted r^2 values were compared to determine how well Taylor's model fit the observed data. Predictions of *Galerucella* spp. egg density along individual transects were used to estimate the total number of eggs within each release site.

In spring of 1995 and 1996, numbers of eggs were highest in sites that received introductions of adult *Galerucella* spp. in the summer of 1993, and lower in sites that received introductions in 1995. The highest number of eggs and largest proportionate increase of egg density from spring of 1995 to 1996 was observed in the Delta Marsh site that received beetles in 1993. In this site, the mean density of purple loosestrife during the 1996 growing season was reduced from 28 stems /m^2 in spring to 4 stems /m^2 in fall; mean fruit production was reduced from 4000 /m^2 in fall of 1995 to less than 25 fruits /m^2 in 1996. Other than at the point of release in the remaining sites, loosestrife stem and fruit production was apparently not reduced by *Galerucella* spp. during the 1995 and 1996 growing seasons.

Broken stick regressions were used to describe the relationship between the density of *Galerucella* spp. eggs and stem and fruit production by *L. salicaria*. From these regressions it was determined that a reduction in loosestrife stem density occurred during the growing season only when the *Galerucella* spp. egg density was $\geq$ 565 eggs /m^2. Below this egg density /m^2 no reduction in the density of purple loosestrife stems

was observed. Fruit production /m^2 was gradually reduced with increasing egg density /m^2 however, when the density of *Galerucella* spp. eggs reached 432 /m^2 the reduction in fruit density /m^2 was more rapid.

INTRODUCTION

Purple loosestrife, *Lythrum salicaria* L. (Lythraceae) was introduced to North America from Eurasia in the early 1800's mainly as a contaminant of ship's ballast (Stuckey 1980; Thompson et al. 1987). Another source of introduction was early settlers who brought the plant with them because of the medicinal properties it was believed to possess (Thompson et al. 1987). Once established along the eastern seaboard, rivers and inland waterways carried the buoyant seeds great distances (Stuckey 1980) and facilitated westward expansions. The ability of *L. salicaria* to outcompete native wetland plant species such as *Typha* spp. has led to the formation of large monospecific areas of the weed that are believed to provide a less suitable habitat for wetland wildlife, compared to displaced vegetation (Malecki and Rawinski 1979; Thompson et al. 1987).

In North America, a classical biological control program using host specific, non-native insects has been implemented to help reduce the population of *L. salicaria* (Hight et al. 1995). Two leaf-feeding beetles, *Galerucella calmariensis* (L.) (Coleoptera: Chrysomelidae) and *G. pusilla* (Dufts.), were introduced to several sites in southern Manitoba during the summer of 1993. In spring of 1994, both species of *Galerucella* survived the winter (Lindgren, personal communication); however, subsequent rates of dispersal and increase in Manitoba appear low (Diehl, unpublished). Successful

biological control may not be prevented by low rates of dispersal and increase; however, modifications of release strategies may be warranted to offset them.

North American data regarding the successful control of purple loosestrife by *G. pusilla and G. calmariensis* are limited. After examining initial release data from 1992, Hight et al. (1995) concluded that higher survival rates using caged releases were obtained for introductions of 600 adults compared to 200 adult *Galerucella* spp. They also concluded that the northern and southern sources of adult *Galerucella* spp. from Germany, amount of litter, number of standing dead *L. salicaria* stems or density of *L. salicaria* did not affect establishment of beetles during initial North American releases. Malecki et al. (1993a) stated that peak dispersal of overwintered adults was believed to be during the first few weeks after the emergence of *L. salicaria.* A second dispersal period usually occurs after the new generation of adults have emerged in late summer. Overall, there is a lack of specific information regarding initial dispersal, distribution and growth rates that occur immediately after introduction of *Galerucella* spp. into North American release sites. Current research projects on biological control of purple loosestrife in North America using *Galerucella* spp. are mainly concentrated in southern Ontario (Cooper et al. 1996) and the eastern United States (Blossey et al. 1996), and may not be applicable to the prairie provinces. The majority of these studies have focused on the impact of *Galerucella* spp. on loosestrife stem and fruit production under caged, field conditions. The response of *L. salicaria* to injury caused by *Galerucella* spp. is dependent on the plant's health and vigor at the time of attack, which is related to local climate. As a result, it may be unwise to extrapolate the impact of *Galerucella* spp. on caged purple loosestrife to conditions outside cages in other climatic areas. Thus, it is

important to obtain baseline information on the interaction between purple loosestrife and the introduced biological control agents in southern Manitoba wetland release sites.

The main objectives of this study are to determine how the population of *Galerucella* spp. changes with time after release and to investigate the impact of the beetles on stem density and fruit production of *L. salicaria* under Manitoba conditions.

MATERIALS AND METHODS

Field Experimental Design

In spring of 1994 five study sites were established in southern Manitoba, three located at Libau Marsh (50° 12 'N, 96° 43 'W) and two at Delta Marsh (50° 11 'N, 98° 18 'W). At each location, there were two release sites in which *Galerucella* spp. were present during the study. The two release sites at Delta Marsh are approximately 0.5 km apart and the two Libau Marsh release sites are 2 km apart. One of the two study sites at each location is an original release site, in which *Galerucella* spp. was introduced for the first time in Manitoba in the summer of 1993 by the coordinator of the Manitoba Purple Loosestrife Project, Corey Lindgren. Sites that received introductions of adult beetles in 1993 are referred to as Delta Marsh-A and Libau Marsh-A. The other release sites at Libau and Delta Marsh received an introduction of adult *Galerucella* spp. in the summer of 1995 and are referred to as Delta Marsh-B and Libau Marsh-B. One site at Libau Marsh (Libau Marsh-C) received no introductions during the course of this study and was used to obtain baseline information on *L. salicaria* stem density and reproductive output in the absence of *Galerucella* spp. The search for a third site to serve as a control at

Delta Marsh was unsuccessful. Libau Marsh-C is approximately 0.5 km from Libau Marsh-B and 2 km from Libau Marsh-A.

Sites differed somewhat in size, shape and drainage, but generally consisted of a central, uniform area of *L. salicaria* surrounded by less dense areas. Delta Marsh-A and Libau Marsh-B release sites were seasonally flooded, and water from spring runoff persisted well into July during the 1995 and 1996 growing seasons. These two release sites appeared similar in terms of loosestrife density: each contained centrally located, dense patches of the weed that became less dense up to the boundary of the site. Site boundaries consisted of roads, ditches, water or wooded areas that limited the spread of the plant. The gradient of loosestrife stems within these two sites was a smooth transition from high to low density with increasing distance from the central point. Libau Marsh-C also contained a centrally located, dense area of purple loosestrife, but only held water from spring runoff until mid to late May. In 1995 and 1996, Delta Marsh-B and Libau Marsh-A were also flooded from spring runoff; however, flooding was not as prolonged as in the other two release sites and surface water disappeared by late June. The densities of *L. salicaria* within Delta Marsh-B and Libau Marsh-A appeared similar; however; there was no single, centrally located dense patch. The distribution of purple loosestrife stems within these two sites was somewhat patchy.

Releases of *G. pusilla* and *G. calmariensis*

Total numbers, sources and release dates of adult *Galerucella* spp. used for all introductions are presented in Table 1. Releases of adult beetles into sites at Libau and

Delta Marsh occurred during the summers of 1993 to 1995. In both sites at Libau Marsh and in Delta Marsh-B there was a single release point near the center of the site (Fig. 1). In Delta Marsh-A there were two centrally located release points, approximately 10 m apart (Fig. 2). The first introduction of adult *G. pusilla* into Delta Marsh-A was made at the east release point and the two late summer introductions of *G. calmariensis* and *G. pusilla* were made at the west point of release (Table 1). In the summer of 1994, additional *G. pusilla* were introduced approximately 15 meters southwest of the west release point. During the course of this study, only the two original release points were considered when sampling *Galerucella* spp. egg masses, as minimal egg laying activity was observed at the third release point in the spring of 1995. Releases into Libau Marsh-A also occurred in 1993 and during the summer of 1995, mixed species releases were made into Libau Marsh-B and Delta Marsh-B. In early September, the second release of *G. pusilla* and *G. calmariensis* in Delta Marsh-B and fourth release into Libau Marsh-B represent additional adults that were required for the overwintering study. All sites received mixed species beetle introductions except Libau Marsh-A, which received only introductions of *G. calmariensis*. The male: female sex ratio of adult *Galerucella* spp. was unknown for all releases and no effort was made to bias it in any way.

The release protocols of Malecki et al. (1993a) were followed for all introductions of adult *Galerucella* spp. Newly emerged adult beetles were placed into a release cage, 1.25 m wide by 1.85 m long by 1.25 m high, within each site. Release cages were provided by the Manitoba Purple Loosestrife Project and consisted of 5 x 10 cm wood framing covered with fine mesh-cloth material. Cages were without floors, weighed approximately 35 kg, and were placed on the ground with no attempt made to anchor

them. A mesh-covered door was present on one side of the release cage so beetles could be introduced and monitored without difficulty. Release points were chosen to correspond to the center of the weed's distribution within each site.

To avoid unnecessary handling of the newly emerged adult *Galerucella* spp., shipping containers were opened and placed directly into the center of release cages at ground level. After a one hour period, loosestrife vegetation inside the shipping containers was removed and placed in the center of the release cage. Beetles were allowed to settle on the purple loosestrife vegetation for approximately 1-week prior to the release cage being removed. Initial dispersal of adults was restricted, but not completely prevented by the cage, as the mesh-covered door was not tightly sealed. Because of the lack of a floor, escape may also have occurred between the bottom of the cage frame and the ground.

Transect Establishment

Within four of the five study sites, a series of eight transects radiating away from a central origin was established and used to sample insects and plants at predetermined distances. In Libau Marsh-A and -B and Delta Marsh-B the origin was located at the point of release (Fig. 1). In the Libau Marsh-C control site, the origin was an arbitrary point close to the center of the weed's distribution in the site. In the fifth site, Delta Marsh-A, the two release points were 10 m apart and formed an east-west line (Fig. 2). In 1995, 14 transects were established and sampled (Fig. 2), while in 1996, the enormous *Galerucella* spp. egg densities forced a reduction in the number of transects sampled to

eight. In 1996, no additional transects were established and centered on the midpoint between RPE and RPW as in spring of 1995 (Fig. 2).

Spring Quadrat Sampling

All four release sites were sampled approximately 2.5 weeks after the start of oviposition by *Galerucella* spp. The sampling dates for each release site in 1995 and 1996 are presented in Table 2. Along established transects within all sites, 1 m^2 wire-framed quadrats were sampled at 1 m intervals from the transect origin to 2 m beyond the furthest distance at which evidence of *Galerucella* spp. was found. Sampling would also stop when the edge of the purple loosestrife stand was reached. All above ground purple loosestrife vegetation within each quadrat was examined for *Galerucella* spp. egg masses. For each 1 m^2 quadrat sampled, the number of purple loosestrife stems, numbers of egg masses and eggs /mass were recorded. Observed adult *Galerucella* spp. were also recorded; however, the majority of adults likely dropped off the vegetation while the quadrat was being placed into position. The long search time involved with this method of sampling allowed release sites to be intensively sampled only once during the peak oviposition period of *Galerucella* spp. Although quadrat sampling involved some disturbance of the plants, efforts were made to keep this to a minimum and avoid any undue trampling of the surroundings.

Fall Quadrat Sampling

Upon completion of flowering and seedset by purple loosestrife in late August to early September of 1995 and 1996, seed heads were collected to provide an estimate of

the fruit production per m^2 (Table 2). A 1 m^2-wire-framed quadrat was placed at a distance of 1, 2, 3, 4 and 5 m from the release point, and a random sample of ten seed heads was collected. Beyond these distances, every second meter was sampled until the point at which no egg masses were found during sampling in the spring of that year. The number of loosestrife stems, numbers of flowering stems and flowering shoots were also recorded for each quadrat sampled. These measures were used to estimate fruit production /m^2 using a slightly modified method of Blossey (1992), in which the average number of fruits from 10 randomly selected flowering shoots was multiplied by the total number of flowering shoots within the 1 m^2 quadrat.

In Libau Marsh-C, a series of transects were sampled using the same techniques, and fruit production /m^2 estimated in the absence of *Galerucella* spp. for the 1995 and 1996 growing seasons. During sampling of egg, stem and fruit densities from all release sites in 1996, samples were taken from the same 1 m^2 area as in 1995.

Estimates of Egg Density

Egg Density-Distance Regressions for Individual Transects

The pattern of reducing egg density with distance from the central release point along transects was fitted to the general model described by Taylor (1978). The model:

$$\text{Density} = \exp(a + b(\text{distance}^{c})) \qquad (1)$$

was fitted to the observed *Galerucella* spp. egg density-distance data and estimates of the a, b, and c parameters generated using non-linear regression techniques. Analysis of all data was conducted using Systat 7.0 for Windows. Gauss-Newton, Quasi-Newton and Simplex estimation techniques were used to generate final parameter estimates by

minimizing the least squares loss function. Estimates of the model parameters were generated for each transect in all four release sites and inserted back into Taylor's model to predict the egg density /m^2 for each distance along transects, up to the last distance sampled. The goodness of fit of Taylor's general model to the observed density-distance data from each transect was assessed based on the observed vs. predicted r^2 value.

Pooled Standardised Egg Density-Distance Regressions

A second set of parameter estimates for equation (1) were obtained using pooled data from all transects in a site during a given year. These parameters, which were fitted as previously described, represent the density-distance relationship for an entire site and were used to make comparisons among sites and years. Visual comparisons of the shape of the density-distance relationship were facilitated by setting the ***a*** parameter of Taylor's general model equal to zero and the ***b*** and ***c*** parameters equal to their fitted values. Standardized density curves were plotted for springs of 1995 and 1996 for all four-release sites.

Total Egg Estimates

The fitted parameters from regressions of *Galerucella* spp. egg density vs. distance along individual transects were used to estimate the total egg population within each release site. First, for each transect, the number of eggs in a 1 m wide annulus was predicted using the formula:

$$\text{Density} = 2\pi(\text{distance})\exp(a + b(\text{distance}^c)) \qquad (2)$$

This procedure was repeated for 1m intervals along individual transects, up to the furthest distance at which *Galerucella* spp. eggs were found. The predicted number of eggs contained in all annuli was summed for each transect. In sites with a single release point, this sum was divided by eight to obtain an estimate of the total egg population within the 1/8 sector of a circle centred on the transect (Fig. 3). The estimates of the number of eggs from all eight sectors were added to obtain an estimate of the total number of eggs within the release site. The number of *Galerucella* spp. eggs at the point of release was included only once during the summation of the total estimate within each site.

The different pattern of transects in Delta Marsh-A required a modification of the total number of eggs estimation procedure. The same estimation and summation technique used in single release point sites as described above provided an estimate of the total number of eggs for the east and west ends of Delta Marsh-A. Egg estimates for the rectangular area between the two release points were made based on a summation of the number of eggs in 1 m-wide rectangles. Estimates of egg density corresponding to the closest North or South transect were used to fill in missing 1 m-wide strips between the two release points. An additional transect running in an east-west line was also sampled at 1 m intervals between the two release points. The observed egg densities along this transect and at the release points were included in the final summation of total egg density only once.

RESULTS

Distances Sampled

Libau Marsh-A & B

The furthest distance sampled in Libau Marsh-A was 75 m along the south transect in 1995 and 56 m along the same transect in the following year. In 1996, time was a limiting factor and sampling could not continue past 56 m, even though *Galerucella* spp. eggs were observed 80 m from the release point. The south transect was bounded by a road running in the east-west direction approximately 130 m from the release point. The shortest distance sampled was 4 m along the northeast transect in 1995 and 7 m along the east transect in 1996. The density of purple loosestrife along these two transects quickly declined to zero. The density of *Galerucella* spp. eggs at the release point was 440 /m^2 in spring of 1995 and 1036 /m^2 in 1996.

The furthest distance sampled from the release point in Libau Marsh-B was 21 m along the northeast transect in 1995 and 15 m along the north and northwest transects in 1996. The shortest distance at which *Galerucella* spp. eggs were sampled was 7 m along the south and west transects in 1995 and 3 m along the northeast and west transects in 1996. This reduction in the shortest distance sampled from spring of 1995 to 1996 was not expected but may be related to the large drop in egg density at the point of release from spring of 1995 to 1996. The density of *Galerucella* spp. eggs at the release point was 15,133 /m^2 in 1995, shortly after cage removal, and 164 /m^2 in spring of 1996.

Delta Marsh-A & B

In Delta Marsh-A, the furthest distance at which *Galerucella* spp. eggs were sampled was 26 m along the west and southwest transects in 1995 and 40 m along the west transect in spring of 1996. Shortest distances sampled were 6 m along the east and south-B transects in 1995 and 16 m along the east transect in 1996. The egg density at the west release point was 275 /m^2 in 1995 and 4220 /m^2 in 1996. At the east release point, the density of *Galerucella* spp. eggs increased from 202 /m^2 in 1995 to 8297 /m^2 in 1996. From spring of 1995 to 1996, a large increase in egg density was observed within a few meters of each release point.

The furthest distance from the release point at which *Galerucella* spp. eggs were found in Delta Marsh-B was 7 m along the south transect in 1995 and 6 m along the northwest transect in 1996, even though purple loosestrife stems were available. Along seven of eight transects sampled during 1995, no *Galerucella* spp. eggs were found beyond a distance of 1 m from the release point; the south transect was the exception. In 1995, the cage was removed from the release point approximately 1 week prior to sampling and many eggs were laid while females were confined. The density of eggs at the release point decreased from 693 /m^2 in 1995 to 338 /m^2 in spring of 1996.

Galerucella spp. Egg Density-Distance Data

Estimates of the ***a***, ***b***, and ***c*** parameters and associated r^2 values obtained by fitting equation (1) to the density-distance data along individual transects in the four release sites are depicted in Tables 3-6. To illustrate the relationship of *Galerucella* spp. egg density to distance and the fit of the regression line, five representative plots from the

potential 66 plots are shown in Figures 4-8. Pooled estimates of the ***a***, ***b*** and ***c*** parameters for each site in 1995 and 1996 appear in Table 7. Pooled standardized estimates of numbers of *Galerucella* spp. eggs /m² are plotted against distance from the central release point (Fig. 9-12) to allow visual comparison of egg density distribution curves from 1995 to 1996. The 1995 and 1996 observed *Galerucella* spp. egg density-distance data from each release site are presented in Figures 13-16. The non-linear regression curve appearing in each plot is of the form of equation (1), and represents data from all transects radiating from the release point. Final distances in Figures 13-16 do not necessarily represent the furthest distance at which *Galerucella* spp. eggs were sampled. In order to fit regression curves and make comparisons from 1995 to 1996, distances were fixed within each release site and some data points beyond the last distance were not utilized. Estimates of the total number of *Galerucella* spp. eggs from four of the release sites in spring of 1995 and 1996 are also compared to obtain information on rates of increase or decrease from one year to the next (Table 8).

Libau Marsh-A: 1995 and 1996

Egg Density-Distance Regressions for Individual Transects

Eight transects were established and sampled in Libau Marsh-A during spring of 1995 and the same eight transects were sampled again in 1996. The smooth transition from high to low egg density with distance from the release point along the east transect in Libau Marsh-A: 1995 (Fig. 4) was characteristic of 6 of 8 individual transects within this site during 1995 and 1996. Some variability in the density of eggs was observed along the northwest transect in 1996 (Fig. 5). Along this transect, the density of

Galerucella spp. eggs at a distance of 12 m was higher than at the release point; however, beyond this distance, the egg density quickly decreased to zero. As a result, equation (1) is not a good representation of the observed data and the regression line depicted in Figure 5 is the poorest fit of all transects from this site.

Taylor's a, b, and c Parameters

The single-shared release point ensures the similarity of *a* parameter estimates within each year and in 1996, estimates of *a* were higher than in 1995, reflecting an increase in the egg density at the release point (Table 3). As expected, values of the *b* parameter were negative for all eight transects during spring of both years and 12 of 16 observed vs. predicted r^2 values indicate a very good fit of Taylor's model to the observed density-distance data. In 1996, the r^2 values of the north and northwest transects were lowest, suggesting a poor fit of the model to the observed density-distance data. The low r^2 values associated with these two transects were a result of the density of eggs being larger at some distances along the transect than at the point of release. Excluding the west transect in 1996, all estimates of the *c* parameter were less than 2 in both years.

Pooled Standardised Egg Density-Distance Regressions

As expected, the pooled value of *a* parameter for Libau Marsh-A in 1995 was lower than in 1996 (Table 7), reflecting the increase in egg density at the release point. The pooled value of the *b* parameter was also negative and the *c* parameter estimate less than 0.5 in spring of both years (Table 7). In spring of 1995 and 1996, the pooled standardized estimate of egg density in Libau Marsh-A dropped gradually with increasing distance from the central release point (Fig. 9). The observed egg density-distance data and associated regression curves support this reduction and also demonstrate that in 1995,

a lower proportion of eggs were within 10 m of the release point than was the case in 1996 (Fig. 13). Densities at most distances from the release point also ranged higher in 1996 than 1995, being most evident within 20 m of the release point (Fig. 13).

Libau Marsh-B: 1995 and 1996

Egg Density-Distance Regressions for Individual Transects

The rapid decline in the number of *Galerucella* spp. eggs /m² from the central release point to the next sampled distance of 1 m along the south transect (Fig. 6) is characteristic of the other transects in Libau Marsh-B during spring of 1995 and 1996. Even though slight variability in the number of eggs /m^2 was observed along some transects in this site, the rapid reduction of *Galerucella* spp. egg density with distance was consistent (Fig. 15). Excluding the point of release, the number of *Galerucella* spp. eggs /m^2 along individual transects in Libau Marsh-B were low, especially in 1995. This pattern was likely due to the introduction of adults in spring of 1995 and their initial confinement to the release point during the early part of their oviposition period.

Taylor's a, b, and c Parameters

As in Libau Marsh-A, the same eight transects established in 1995 were sampled during the spring of 1996 and the single release point ensured similar ***a*** parameter estimates within years (Table 4). Estimates of the ***a*** parameter were lower in 1996 compared to 1995, reflecting the drop in egg density at the point of release from one spring to the next. The high ***a*** parameter value in 1995 was somewhat artificial and can be explained by the newly introduced adults being caged during the first part of their egg laying period. All ***b*** parameter estimates were negative for individual transects,

supporting the overall reduction in egg density with distance from the central release point. Observed vs. predicted r^2 values indicate an excellent fit of Taylor's model to the observed density-data along each transect and all estimates of the *c* parameter in Libau Marsh-B were less than 0.5 in 1995 and 1996 (Table 4).

Pooled Standardised Egg Density-Distance Regressions

The pooled value of the *a* parameter decreased from 1995 to 1996 (Table 7), reflecting the drop in egg density at the point of release. This trend is opposite to Libau Marsh-A, where egg density at the point of release increased from one year to the next. All pooled estimates of the *b* parameter were negative and estimates of the *c* parameter less than 0.5 (Table 7). The observed vs. predicted r^2 values from the pooled data indicated an excellent fit of Taylor's model to the observed data. The pooled standardized density estimates (Fig. 10) and observed egg density-distance data (Fig. 14) from Libau Marsh-B demonstrate the rapid decline in the number of *Galerucella* spp. eggs /m^2 with increasing distance from the central release point during spring of both years. The tails of each standardized density curve demonstrate that a greater proportion of eggs were sampled in 1996 than in 1995 beyond a distance of 1 meter from the release point (Fig. 10).

Delta Marsh-A: 1995 & 1996

Egg Density-Distance Regressions for Individual Transects

Due to high *Galerucella* spp. egg densities and associated sampling times in 1996, Delta Marsh-A could not be as intensively sampled as other sites and only 6 of 12 original transects established in 1995 were sampled in 1996. The smooth. gradual

decrease in egg density with distance from the release point along the southeast transect (Fig. 7) was also observed along 15 of 18 transects in Delta Marsh-A during 1995 and 1996. The half bell-shaped egg distribution curve along the southeast transect (Fig. 7) occurred along 7 of 12 transects in spring of 1995. In spring of 1996, this half bell-shaped distribution occurred in only 2 of 6 transects. Along the North-A transect in 1996, the density of eggs was greater at 2 m than at the point of release (Fig. 8). This pattern of egg density was similar to the northwest transect in Libau Marsh-A: 1996 (Fig. 5); however, the reduction in goodness of fit of Taylor's model to the observed data along this transect was not as pronounced. Despite some variability in egg density with distance (Fig. 8), a smooth transition from high to low egg density was observed along the majority of transects in Delta Marsh-A.

Taylor's a, b, and c Parameters

The two release points (RPW & RPE) each possess different numbers of eggs /m^2 and estimates of the *a* parameter (Table 5). Overall, values of the *a* parameter were higher in 1996 than in 1995 and all *b* parameter values were negative, indicating a reduction in egg density with distance away from the release point. For 1995, eleven of 12 r^2 values indicated a good fit of Taylor's model to the observed density-distance data. The fit of the observed data along the southwest transect, as indicated by the low r^2 value (Table 5), is poor. The poor fit is a result of the density of eggs being larger at distances of 2 and 18 m than at the point of release. In 1996, all six values of r^2 indicate a good fit of Taylor's general model to the observed density-distance data. Estimates of the *c* parameter in 1995 were most variable of all release sites during both years, ranging from 0.64 to 5.57. In 1995, five of 12 transects had values of the *c* parameter greater than 2.

The remaining 7 transects in 1995 and all 6 transects in 1996 had values of the ***c*** parameter less than two.

Pooled Standardised Egg Density-Distance Regressions

The pooled estimate of the ***a*** parameter increased from 1995 to 1996 (Table 7), demonstrating the increase in the number of *Galerucella* spp. eggs at the release point from one year to the next. The pooled estimate of the ***b*** parameter was negative and the ***c*** parameter estimate was less than 2 in 1995 & 1996. The r^2 (observed vs. predicted) values associated with the pooled data from Delta Marsh-A: 1995 and 1996 were similar to Libau Marsh-A. Graphical presentation of the pooled egg density-distance data (Fig 14) from Delta Marsh-A visually demonstrates the overall increase in the density of eggs from 1995 to 1996. The majority of eggs sampled during spring of 1995 and 1996 were within 15 meters of the release point. In 1996, the egg density distribution curve extends further from the release point than in 1995, demonstrating the greater spread of *Galerucella* spp. eggs from the release point (Fig. 11). The egg density distribution curves from Delta Marsh-A also demonstrate the subtle half bell-shaped curves observed along some of the individual transects in this site. Standardized egg density distribution curves from Delta Marsh-A also illustrate the gradual reduction in egg density with distance from the release point (Fig. 11). Compared to the other three release sites, the observed decline in egg density with increasing distance occurs more slowly.

Delta Marsh-B: 1995 and 1996

Egg Density-Distance Regressions for Individual Transects

No transects from Delta Marsh-B: 1995 & 1996 are included in Figures 4-8; however, all plots are similar to those in Libau Marsh-B: 1995 & 1996, in which a rapid decline in *Galerucella* spp. egg density with distance was observed.

Taylor's a, b, and c Parameters

A single, shared release point is common to all eight transects and as a result, estimates of the ***a*** parameter within each year are identical (Table 6). In 1995, values of the ***a*** parameter were higher than in 1996, reflecting the decrease in egg density at the release point from one year to the next. During both years, all values of the ***b*** parameter were negative, indicating an overall reduction in *Galerucella* spp. egg density with distance away from central release point. All r^2 values indicate an excellent fit of the model to the observed density-distance data. Values of the ***c*** parameter were more variable in 1996 than in 1995, ranging from 0.01 to 4.07. Along 6 of 8 transects in 1995, values of the ***c*** parameter were less than 2 (Table 6). In 1996, higher estimates of the ***c*** parameter were generated for 5 of 8 transects, as few *Galerucella* spp. eggs were found beyond a distance of 2 meters from the release point.

Pooled Standardised Egg Density-Distance Regressions

The pooled estimate of the ***a*** parameter in Delta Marsh-B decreased from 1995 to 1996 (Table 7), corresponding to the drop in *Galerucella* spp. egg density at the point of release. Pooled estimates of the ***b*** parameter were negative and ***c*** parameter values were < 2 in spring of both years. Pooled standardized density estimates (Fig. 12) and observed density-distance data with associated regression curves (Fig. 16) from Delta Marsh-B are

similar to those in Libau Marsh-B (Figs. 10, 15) and rapidly decrease with distance from the central release point. Similarly, the tail of the standardized egg density estimate in 1996 is greater than in 1995, indicating that a larger proportion of eggs were sampled beyond 1 m in 1996 compared to 1995 (Fig. 12). Observed vs. predicted r^2 values from both years indicate an excellent fit of the Taylor's model to the pooled data (Table 7).

The non-linear regression curves generated from the Delta Marsh-B and Libau Marsh-B: 1995 & 1996 density-distance data are nearly identical and rapidly decline within the first few meters of the central release point. The reduction in egg density is more pronounced for both sites in the initial year of adult introduction (1995). In the spring following the introduction (1996), the rapid drop in egg density is still apparent, but less pronounced compared to 1995. All r^2 values from the two 1995 release sites confirmed an excellent fit of Taylor's model to the observed density-distance data.

Both Libau Marsh and Delta Marsh-A received initial introductions of adult *Galerucella* in 1993 and the pooled density-distance data looks very different from the two sites that received introductions in spring of 1995. In 1993 release sites, density-distance data was more variable and Taylor's model was not always a good fit to the observed data. However, an increase in the ***a*** parameter from 1995 to 1996, similar ***b*** parameter estimates and r^2 values were observed in the Libau Marsh-A and Delta Marsh-A release sites. The half bell-shaped distribution of eggs along the southeast transect (Fig. 7) in Delta Marsh-A: 1995 was only observed in sites that received introductions of *Galerucella* spp. in 1993.

Total Egg Estimates from Four Release Sites

In sites where releases were made in spring of 1993, the total number of *Galerucella* spp. eggs increased from spring of 1995 to 1996. The largest increase in spring egg density occurred in Delta Marsh-A, followed by Libau Marsh-A. A small increase in total egg density from one year to the next occurred in Delta Marsh-B and in Libau Marsh-B a decrease occurred. Both Libau and Delta Marsh-B release sites received introductions of adult *Galerucella* in the spring of 1995.

Density of *L. salicaria*: Stem and Fruit Data

In spring and fall of 1995 and 1996, *L. salicaria* stem densities and fall fruit densities were recorded from the same locations as egg densities along all transects radiating from the central release point. This allowed for comparison of stem densities from spring and fall of the same year and provided some measure of the change in loosestrife stand density. The number of *Galerucella* spp. eggs /m^2 and the spring stem density were used to calculate the number of eggs /stem within each quadrat sampled. Figures 17-31 represent *L. salicaria* stem and fruit densities and number of *Galerucella* spp. egg /stem in 1 m^2 quadrats at various distances from release points along individual transects. Due to the large number of plots generated from each release site in 1995 and 1996, a representative sample was selected.

Libau Marsh-A: 1995 & 1996

Excluding the west transect in 1996, the density of *L. salicaria* stems in Libau Marsh-A gradually decreased with distance from the release point. A similar stem

density as the release point at a distance of 3 m suggests that the reduction in stem density with distance was not as pronounced along the west transect (Fig. 19A). The density of *L. salicaria* stems at the release point in Libau Marsh-A: 1995 increased slightly from spring to fall of 1995, and no obvious reduction in loosestrife stem density was observed along the other transects during the 1995 growing season (Fig. 17A). At most distances along the west transect, except for 2 m from the release point, *L. salicaria* stem density was similar from spring to fall of the 1996 growing season (Fig. 19A). A slight increase in stem density at a distance of 2 m was observed, even though the number of *Galerucella* spp. eggs /stem was highest at this distance. As in 1995, no obvious reduction of *L. salicaria* stems was observed along transects during the 1996 growing season, despite an increase in the number of eggs /stem along the majority of transects from spring of 1995 (Fig. 17B) to 1996 (Fig. 19B).

In general, fruit production in Libau Marsh-A was variable along transects, either being the same (Fig. 18) or gradually decreasing (Fig. 20) with distance from the release point. The density of fruits at the point of release declined from fall of 1995 to 1996 (Fig. 18, 20). At most distances along the southeast transect, fruit production was variable from one year to the next, although at 16 and 18 meters, a decrease in fruit production /m^2 was observed from fall of one year to the next (Fig. 18). At these distances, the numbers of eggs /stem were highest and only slight reductions in stem density were observed during the 1995 growing season (Fig. 17A & B). However, by fall of 1996, fruit production at these distances was negligible (Fig.18) and even though stems were present at these distances, minimal fruit production occurred. No stem or egg data were collected from beyond 6 meters along the southeast transect due to the presence of a large

willow (*Salix* spp.). At all distances along the west transect, except the point of release, the density of fruits /m^2 increased from fall of 1995 to 1996 (Fig. 20).

Libau Marsh-B: 1995 & 1996

Characteristic of transects in Libau Marsh-B, the number of loosestrife stems /m^2 along the northeast (Fig. 29A) transect was uniform up to the last distance sampled during both years. In 1995, the highest number of *Galerucella* spp. eggs /stem was sampled at the release point (Fig. 29B) and a reduction in the number of *L. salicaria* stems was observed from spring to fall (Fig. 29A). However, this reduction in stem density at the release point was expected to be larger due to the enormous density of eggs present there in 1995. Beyond the release point, numbers of eggs /stem along the northeast transect decreased until no eggs were sampled at a distance of 19 meters. In spring of 1996, the numbers of eggs /stem were lower than 1995, especially at the point of release.

Unfortunately, no matching fruit density data are available for the northeast transect in Libau Marsh-B: 1995 as only the north, south, east and west transects were sampled in fall. Also, the distances at which stem density data were collected along these four transects in fall of 1995 and 1996 did not match distances sampled in spring of the same years. However, similar to stem density, fruit production /m^2 along transects in Libau Marsh-B was relatively uniform up to the last distance sampled during fall of 1995 and 1996.

Delta Marsh-A: 1995 & 1996

Lythrum salicaria stem densities along the south-A transect (1995) were uniform up to the last distance sampled during both years (Fig. 21A). However, as with the majority of transects in this site, the density of stems along the east transect (1996) gradually decreased with distance from the central release point (Fig. 23A). Along the south-A transect in Delta Marsh-A: 1995 (Figs. 21A & B) the numbers of eggs /stem are relatively low and the stem densities from spring to fall of the same growing season are somewhat variable at each distance. Despite the highest number of eggs /stem at the release point, the stem density increased slightly from spring to fall of 1995 (Fig. 21B). As in Libau Marsh-A, an overall increase in the number of *Galerucella* spp. eggs /stem was observed from spring of 1995 (Fig. 21B) to 1996 (Fig. 23B). During the 1996 growing season, loosestrife stem densities along the east transect were reduced to zero at the release point and a distance of 2 meters (Fig. 23A). The same reduction in stem density near the point of release was observed along all other transects in Delta Marsh-A.

In general, fruit production was variable along transects, being either relatively constant (Fig. 24) or decreasing (Fig. 22) with distance from the release point. The fruit production /m^2 along the south-A and east transects in Delta Marsh-A was reduced from high numbers in fall of 1995 to zero in 1996 (Fig. 22, 24). A similar reduction was consistent along remaining transects in Delta Marsh-A during the fall of 1996 (Fig. 24).

Delta Marsh-B: 1995 & 1996

Along 7 of 8 individual transects in Delta Marsh-B, the number of loosestrife stems /m^2 decreased with distance from the central release point during 1995 and 1996

(Fig. 25A, 27A). The stem density along the south transect remained relatively uniform up to the last distance sampled during the 1995 growing season. During both growing seasons, a reduction in stem density was observed at the point of release (Fig. 25A, 27A). Few *Galerucella* spp. eggs were sampled in this site during spring of 1996, and eggs were only found at the point of release along the south transect (Fig. 27B).

In 1996, the density of fruits along the south transect (Fig. 28) was somewhat variable and did not decrease with distance from the release point as along the east transect in 1995 (Fig. 26). The numbers of eggs /stem decreased from spring of 1995 (Fig. 25B) to 1996 (Fig. 27B) and fruit production /m^2 at the release point increased five fold during this period (Fig. 26), despite a reduction in stem density at the release point during both years. Overall, the density of *Galerucella* spp. eggs in this site was low and sampling began approximately 2 weeks after initial adult introduction.

Libau Marsh-C

Along the east transect in Libau Marsh-C, the density of *L. salicaria* stems gradually decreased with distance from the center of the site (Fig. 30). Similarly, fruit production along the west transect also decreased with distance from the center of the site in fall of 1995 and 1996 (Fig. 31). Sampling in Libau Marsh-C began at an arbitrary point near the center of the weed's distribution and no *Galerucella* spp. eggs, larvae or adults were found in this site during the course of this study. In the absence of *Galerucella* spp. (Libau Marsh-C) the density of purple loosestrife stems clearly increased from spring to fall during the 1995 & 1996 growing seasons. A slight increase

in fruit production /m^2 by purple loosestrife was also observed along the majority of transects from fall of 1995 to 1996.

In all four release sites, the overall variability in the number of *Galerucella* spp. eggs /stem and fruit production /m^2 and the reduction of stem density with distance from the release point was consistent along the majority of transects sampled during 1995 and 1996. The reduction in loosestrife stem density with increasing distance from the release point is somewhat artificial and a result of the release points being chosen to correspond to the weed's center of distribution within each site. Also, the density of *Galerucella* spp. eggs appears to mirror the reduction in stem density with distance from the release point.

Pooled Stem and Fruit Data

Mean spring and fall stem density, fall fruit density and *Galerucella* spp. spring egg density from five study sites during the 1995 and 1996 growing seasons are presented in Table 9. Data from the four release sites represent the pooled egg, stem and fruit densities along transects radiating away from the center of each site. Mean spring stem density from Libau Marsh-C was obtained by sampling along a single transect running in an east-west direction.

In Libau Marsh-A, the density of *Galerucella* spp. eggs increased approximately three fold from spring of 1995 to 1996; however, no difference between mean spring and fall stem densities was observed during either growing season. Mean fall fruit production increased slightly from fall of 1995 to 1996, despite an overall reduction in mean stem density from one year to the next. In Delta Marsh-A, the spring density of eggs increased fifteen fold from 1995 to 1996. In 1995, no difference between spring and fall stem

densities was observed; however, in 1996 a sizable reduction in loosestrife stem density and fruit production /m^2 occurred in Delta Marsh-A. In Delta Marsh-B and to a greater extent in Libau Marsh-B, a reduction in the mean number of *Galerucella* spp. eggs /m^2 was observed from spring of 1995 to 1996. The reduction in mean loosestrife stem density during both growing seasons was larger in Delta Marsh-B compared to Libau Marsh-B, in which only a slight reduction was observed. A large increase in mean fruit production /m^2 was observed in Libau Marsh-B from fall of 1995 to 1996, while in Delta Marsh-B a decrease in mean fruit density occurred.

In 1995 and 1996 the log transformed spring stem density decreased with distance from the release point in all study sites (Fig. 32) ($r = 0.13$, d.f. = 379, $p<0.05$). Fall stem density also decreased with increasing distance from the release point (Fig. 33) ($r = 0.11$, d.f. = 278, $p<0.05$) and the relationship between spring and fall stem densities (Fig. 34) is positive. ($r = 0.57$, d.f. = 231, $p<0.05$). The relationship between fruit density and distance was also negative ($r = 0.06$, d.f. = 262, $p<0.05$) (Fig. 35).

A series of density dependent plots were generated using the pooled data from individual release sites in 1995 and 1996. There was no significant relationship between fruits /stem and $\log_{10}$(egg density /stem), $\log_{10}$ (1996 egg density data) and $\log_{10}$(1995 egg density data), $\log_{10}$(spring stem density) - $\log_{10}$(fall stem density) and $\log_{10}$(egg density /stem), or fall stem density/spring stem density and $\log_{10}$(egg density /stem). Due to the relationship between stem density and distance from the release point, as well as that between egg density and distance, the effect of *Galerucella* spp. on purple loosestrife is not easily observed. To elucidate these relationships, it is necessary to

examine the differences between *L. salicaria* spring stem density and subsequent fall stem or fruit density during the same growing season.

Effects of *Galerucella* spp. on *L. salicaria* stands

Broken Stick Regression

The difference between the number of fall and spring *L. salicaria* stems /m^2 provides a measure of the change in stand density during a particular growing season. When the difference is positive the density of loosestrife stems has increased during the growing season and when negative, stem density has decreased from spring to fall. When this difference is plotted against the number of *Galerucella* spp. eggs /m^2 we can determine the egg density at which a reduction in stem density was observed. After initial examination of plots it appeared that there was a break point at which a further increase in egg density resulted in a reduction of loosestrife stem density. To quantify this break-point, spring and fall loosestrife stem densities and *Galerucella* spp. egg data from each quadrat were pooled from all release sites in both years. In Figure 36, the log transformed *L. salicaria* fall stem density - spring stem density was plotted against the log transformed *Galerucella* spp. egg density. The egg density at which an overall reduction in stem density occurred during the growing season was 2.753 on the log scale which is equivalent to 566 *Galerucella* spp. eggs /m^2. The observed vs. predicted r^2 was 0.533 with 234 degrees of freedom. Below this egg density the slope of the regression line is not significantly different from zero (95% Wald Confidence Interval) and the difference between fall and spring stem densities is minimal.

A similar break-point is expected when the difference between the number of fruits /m^2 and spring stems /m^2 is plotted against the number of *Galerucella* spp. eggs /m^2. In most cases this difference is positive, meaning that more fruits are produced per meter squared than stems in spring. Since each stem has the potential to produce 10-200 fruits, we expect this difference to be positive most of the time. The intercept of the regression equation represents an estimate of the number of fruits /spring stem and is 2.2 on the log scale which translates into 158 fruits /spring stem. When the difference is negative, fruit production /m^2 has been reduced to less than the spring stem density /m^2. The log transformed *L. salicaria* fruit density - spring stem density was plotted against the log transformed *Galerucella* spp. egg density (Fig. 37). The breakpoint is 2.636 which is equivalent to 432 *Galerucella* spp. eggs /m^2. The observed vs. predicted r^2 was 0.468 with 232 degrees of freedom. The negative slope of the regression line prior to the break-point is significantly different from zero (95% Wald Confidence Interval). Below the breakpoint, a reduction in the difference between fruit and spring stem densities occurred with increasing egg density. In other words, with increasing egg density there was a small rate of reduction in fruit production until the breakpoint was reached; above the breakpoint, there was a rapid reduction in fruit production with increasing egg density.

DISCUSSION

Many researchers have used some indication of insect presence as a measure of dispersal or density (Wadley and Wolfenbarger 1944; Wolfenbarger 1940; Annand et al. 1932). Such is the case using the egg masses of *Galerucella* spp. as a measure of adult

dispersal, and ultimately total population. Although time consuming and somewhat difficult to count, egg masses of the introduced biological control agents provided direct evidence of the prior presence of ovipositing female *Galerucella* spp. Other researchers have used adult or larval feeding damage (Wolfenbarger 1940; Hawley 1934; Gaines 1932) as indicators of insect dispersal. However, during the survey of insects associated with purple loosestrife in southern Manitoba several species of insects were found that damaged the leaves of the plant (Diehl et al. 1997). Egg masses were chosen as an indicator of dispersal as they are quantifiable and easily distinguished from all other eggs by the thin line of frass deposited on each egg after it has been laid.

Dispersal has been defined as any movement within or away from a population, resulting from either long distance migration or short meandering movements limited to one particular habitat (Southwood 1966). Freeman (1977) defined dispersal as the movement of flying insects from a central release point in such a way as to lower their population density with increasing distance. However, movement within release sites may not always be away from the central point and there is usually some random component involved (Freeman 1977). For purposes of this study, elements of both definitions are considered appropriate. Although not observed, long distance movement of adult *Galerucella* spp. from southern Manitoba wetland release sites is likely occurring (Lindgren, personal communication). Adult *Galerucella* spp. have been observed sitting on the windshields of research vehicles while driving distances of 1 or 2 km from release sites (Diehl, personal observation). However, the short meandering movements of adults within release sites were the focus of this particular dispersal study.

According to Wadley and Wolfenbarger (1944), several elements contribute to the change in insect density with distance from a center release point. They stated that the density of dispersing insects would grow less as the circumference expands and this observed trend would be modified primarily by the tendency of insects to stop after they have found a satisfactory place. According to Kennedy (1961), migrants temporarily inhibit their responsiveness to vegetative stimuli; when the inhibition ceases, the vegetative stimuli induce cessation of movement. In contrast, non-migrants or insects with short meandering movements are readily interrupted by behavioral responses to such stimuli. In addition to a considerable random element, dispersal of insects also occurs so that all individuals do not compete for the same resources or so they can find and utilize new resources (Freeman 1977). When independent dispersal and reproduction are combined with the previously mentioned conditions, movement of insects can be partly described as a two-dimensional diffusion process (Freeman 1977; Hengevold 1994). The c parameter of Taylor's (1978) general model can be used to describe random diffusion and non-random processes. Only when $c = 2$ is movement considered to be random, in which there is neither repulsion nor attraction among diffusing individuals. Taylor (1978) stated that any deviation from $c = 2$ is considered non-random and believed to result from some combination of differences in the populations dispersal power (Skellam 1951) and a breakdown in the assumption of independent behavior (Bateman 1950). According to Taylor and Woiwood (1978), individuals in a population are not likely to behave independently of one another and as a result, truly random dispersal is rare in nature.

1993 Release Sites: Libau and Delta Marsh-A

Observed density-distance data and standardized density estimate plots from both Libau & Delta Marsh-A clearly depict the reduction in egg density with increasing distance from the release point. Compared to 1995 release sites, this reduction is more gradual and expected, since the beetles are well established and have passed through a number of generations within these two sites. Initial dispersal of adults in Libau & Delta Marsh-A was limited during the first few weeks by the presence of a release cage. However, by the second and third years after initial introduction, *Galerucella* spp. had several opportunities to disperse and egg masses were found further from the release point compared to 1995 release sites. The half bell-shaped distribution of eggs observed along some transects only in 1993 release sites is an indication that within a few meters of the release point the density of eggs was relatively uniform.

The reduction in the density of insects with increasing distance from a central release point has also been observed in many other dispersal studies (Dobzhansky & Wright 1943; Wadley & Wolfenbarger 1944; Finch & Skinner 1975; Withers et al. 1997). Density-distance plots from these studies also show a steep reduction or falling off from the point source with the slope becoming less steep as distance increases. The leveling off of the downward slope of *Galerucella* spp. egg masses with increasing distance was also observed by Wadley and Wolfenbarger (1944) during their dispersal study of the European Elm bark beetle, *Scolytus multistriatus* (Marsham) (Coleoptera: Scolytidae) and by Macleod and Donnelly (1963) during their dispersal study of blow flies (Diptera: Calliphoridae).

The density of *Galerucella* spp. expressed as the number of eggs /stem vs. distance appears highly variable compared to the number of eggs /m^2 vs. distance. The same reduction in the density of eggs with distance is not observed when plotting the number of eggs /stem vs. distance. However, presenting the egg density-distance data as the number of eggs /stem vs. distance does in some instances help explain some of the changes in loosestrife stem density-distance data during the growing season.

The value of the ***b*** parameter represents the slope of Taylor's general non-linear model equation. Since the *Galerucella* spp. egg density decreases with increasing distance away from the central release point along all transects, the value of the ***b*** parameter is always negative. The negative value of the ***b*** parameter estimate from individual transect and pooled data in 1995 and 1996 is also symptomatic of the reduction in egg density with distance from the release point in both sites. Taylor's (1978) ***a*** parameter estimates from the pooled data and those from individual transects demonstrate similar patterns in both 1993 release sites. The value of the ***a*** parameter increased from spring of 1995 to 1996, reflecting the increase in *Galerucella* spp. egg density at each release point. Despite the presence of two release points in Delta Marsh-A, similar individual estimates of the ***a*** parameter corresponding to RPW or RPE were obtained. According to Taylor (1978), the *c* parameter value is a measure of non-randomness of dispersal. When $c = 2$ insect dispersal is considered random, when $c < 2$ insects tend to aggregate close to the point of release and when $c > 2$ insects are demonstrating repulsion from one another, leading to a more uniform distribution (Taylor 1978). Along all transects in Libau Marsh-A, excluding the west of 1996, *c* parameter values were less than 2. Along the west transect, numbers of *Galerucella* spp. eggs /m^2 were higher at a

distance of 3 m than at the release point, contributing to the higher *c* parameter value (>2). In 1995, estimates of the *c* parameter from transects in Delta Marsh-A were somewhat variable with 5 estimates > 2 and 7 estimates < 2. However, in 1996 all individual *c* parameter estimates were less than 2, as was the case in Libau Marsh-A. The pooled estimate of the *c* parameter from Taylor's model was obtained using the combined density-distance data from individual transects, and for this reason is the most important indicator of repulsion or attraction between adult beetles within release sites. In both 1993 release sites, the pooled value of the *c* parameter was less than 2, indicating a tendency for females to oviposit close to the point of release, despite the completion of a number of generations. During the 1996 growing season, the number of eggs /m^2 dramatically increased and a severe reduction in stem and fruit density was observed in Delta Marsh-A. Despite this large population increase of *Galerucella* spp. in spring of 1996, the distribution of eggs within the site did not appear to differ from the previous year. The majority of egg masses sampled in spring of 1996 were found close to the point of release.

Observed vs. predicted r^2 values from individual transects appeared to be equally variable in 1993 release sites; however, the r^2 values associated with the pooled data from these two sites were similar in 1995 and 1996. In most instances, a poor fit of Taylor's model, as indicated by a low r^2 value, was explained by the density of eggs being higher at some distance along the transect than at the point of release.

Taylor's (1978) general model was successfully applied to the observed *Galerucella* spp. egg density-distance data and estimates of the total number of eggs within release sites were obtained. For the summation technique used to obtain the final

estimate of the total egg number within release sites, it was assumed that loosestrife stems and *Galerucella* spp. eggs were present for all estimated spaces between individual quadrats and for the 1/8 annulus corresponding to each transect. Beyond a distance of 5 m from the release point, the distribution of loosestrife stems in 1993 release sites became somewhat patchy (visually) and estimates of egg density perhaps less accurate than close to the point of release, where the distribution was more uniform.

The total egg estimate from Libau Marsh-A increased from 116, 474 in spring of 1995 to 306, 689 in 1996 (Table 8), representing a 163 % increase in the number of *Galerucella* spp. eggs within this site from one spring to the next. A total of 680 adult *G. calmariensis* were released into Libau Marsh-A, 605 in the summer of 1993 and 75 in 1994. Upon the start of sampling in spring of 1995, two generations were likely completed after the 605 adults released in 1993 and one after the 75 adults released in 1994. The total egg estimate from Delta Marsh-A also increased from 87, 054 in spring of 1995 to more than 2 million in 1996, representing a 2537 % increase in the number of *Galerucella* spp. eggs in the site (Table 8). A total of 716 adult *G. calmariensis* and *G. pusilla* were released into Delta Marsh-A, 616 in the summer of 1993 and 100 in 1994. At the start of sampling in spring of 1995, two generations were likely completed after the 616 adults released in 1993 and one after the 100 adults released in 1994. In Libau & Delta Marsh-A release sites, the increase in mean spring egg density and total egg estimate from spring of 1995 to 1996 are a suggestion that male and female *Galerucella* spp. are effectively finding each other for mating purposes. The Allee effect as described by Allee (1931) in which individuals in a small population have a disproportionally low

recruitment rate is not applicable to 1993 release sites due to the higher densities of eggs during the 1996 growing season.

With few exceptions, the density of purple loosestrife stems along individual transects in Libau & Delta Marsh-A declined with distance from the release point. Release point data from 1993 sites clearly demonstrates that fruit production decreased from fall of 1995 to 1996, even though stem densities at the release point in Delta Marsh-A: 1995 and Libau Marsh-A: 1995 & 1996 were similar. In Libau Marsh-A, no obvious reduction in stem density was observed at the point of release during the 1995 and 1996 growing seasons (Figs. 17, 19). In fact, there was a slight increase in the number of loosestrife stems during the 1995-growing season. Despite this, fruit production at the point of release was reduced from >3000 fruits /m^2 in 1995 to <1000 fruits /m^2 in fall of 1996 (Fig. 18, 20). This reduction of fruit density at the release point in Libau Marsh-A suggests that even though the stem density was relatively constant, an impact of *Galerucella* spp. on fruit production may have been observed.

A slight increase in loosestrife stem density at the release point was also observed in Delta Marsh-A during the 1995 growing season (Fig. 21), suggesting no impact of the beetles on stem densities at the release point. However, during the 1996 growing season, loosestrife stem and fruit densities were reduced to 0 at and within a few meters of the release point (Fig.23) along all sampled transects.

From spring of 1995 to 1996, mean number of *Galerucella* spp. eggs /m^2 (Table 9) and corresponding number of eggs /stem increased in both 1993 release sites. In Libau & Delta Marsh-A an increase in mean egg density was mirrored by an increase in the total egg estimate from one year to the next. The response of *L. salicaria* mean stem

density was similar in both sites during the 1995-growing season; however, during 1996 this was not repeated. In Libau Marsh-A, mean *L. salicaria* stem densities were relatively constant from spring to fall of the same year, however, from 1995 to 1996, an overall reduction of 5 stems /m^2 was observed (Table 9). Despite this slight reduction in mean stem density, fruit production /m^2 in Libau Marsh-A appeared to increase from fall of 1995 to 1996. However, upon consideration of the standard errors associated with each mean fruit density and stem density /m^2, observed differences from spring or fall of 1995 to 1996 are minimal. During the 1995-growing season in Delta Marsh-A, mean *L. salicaria* stem densities were the same in spring and fall. However, in 1996 the mean density of purple loosestrife was reduced from 28 to 4 stems /m^2 (Table 9) and fruit production was reduced to nearly 0 fruits /m^2 along the majority of transects sampled during the growing season (Fig. 22, 24).

In Libau Marsh-A, a mean egg density of 84 to 227 eggs /m^2 was not sufficient to cause a reduction in the mean number of stems /m^2 during the 1995 and 1996 growing seasons. It is unclear whether the slight reduction in mean stem density from 1995 to 1996 was a result of the feeding action of the introduced agents or simply natural variation in stand density caused by unfavorable environmental factors. Fruit production at the release point was reduced from fall of 1995 to 1996, suggesting that *Galerucella* spp. had a measurable impact on fruit production by purple loosestrife. Even though *G. calmariensis* are well established in Libau Marsh-A, a measurable impact on *L. salicaria* stem density was difficult to observe during the 1995 and 1996 growing seasons.

Although there is no direct evidence that stem density affects *Galerucella* spp. egg density, the reduction in egg density with distance closely mirrors the decline in

loosestrife stem density along the majority of transects sampled in all release sites. As a result, oviposition at the periphery of the loosestrife stand may never reach a level close to that at the point of release due to the limited stem density and dispersal in the site may have already reached a peak. I believe this occurred in the Delta Marsh-A release site, in which the dramatic population increase of *Galerucella* spp. during the 1996 growing season caused a measurable reduction of mean loosestrife stem and fruit density. A contributing factor to the population increase in Delta Marsh-A was the completion of two generations during each of the 1995 and 1996 growing seasons (Diehl, personal observation). The ability of *Galerucella* spp. to complete more than one generation per year in North America was predicted by Vega et al. (1992). This was not observed in any of the remaining three release sites during the course of this study. The reductions in mean stem and fruit density during the 1996 growing season in Delta Marsh-A are consistent with a direct result of the feeding action of the introduced biological control agents, *G. pusilla* and *G. calmariensis* (Blossey and Schat 1997). In Delta Marsh-A, a mean egg number of 1771 eggs /m^2 was enough to contribute to a reduction in the number of *L. salicaria* stems and fruits /m^2. The observed reduction in stem density and fruit production during 1996 was almost certainly due to introduced agents and not a result of variation in stand density caused by unfavorable environmental factors. Both species introduced into Delta Marsh-A in 1993 are well established and a measurable impact on *L. salicaria* stem density and fruit production was observed during the 1996 growing season.

1995 Release Sites: Libau & Delta Marsh-B

Introductions into Libau & Delta Marsh-B were mixed species releases of both *G. pusilla* and *G. calmariensis*. Observed density-distance data (Fig. 15, 16) and standardized density estimate plots (Fig. 10, 12) from both sites clearly demonstrate the rapid reduction in *Galerucella* spp. egg density with distance during spring of 1995 and 1996. This reduction in egg density with distance was more rapid compared to1993 release sites and based on the observed data, dispersal of newly introduced adults was minimal during the initial and subsequent year of introduction into Libau Marsh-B and Delta Marsh-B. Dispersal of adults during the first year was limited by the presence of a cage positioned over the release point for a one-week period after introduction. In both 1995 release sites, the standardized density curve generated from the 1996 observed data is slightly greater than the curve in 1995. Thus, the proportion of *Galerucella* spp. eggs was lower close to the point of release in 1996 than in 1995.

Examination of Taylor's parameter estimates from individual transects (Tables 4, 6) and the pooled data (Table 7) from both 1995 release sites clearly show a reduction in the ***a*** parameter value from one year to the next, reflecting the drop in *Galerucella* spp. egg density at the release point. As expected, egg density decreased with distance from the release point in both sites and the ***b*** parameter estimates from the pooled and individual transect data were negative in 1995 and 1996. Compared to 1993 release sites, values of the ***b*** parameter from 1995 sites were more negative, further demonstrating the more rapid reduction in egg density with distance from the release point. Pooled estimates of the ***c*** parameter were considerably less than 2 in both sites (Table 7) and the overall tendency was for beetles to lay a majority of their eggs close to the point of

release, after the completion of one and two generations. As expected, pooled *c* parameter estimates were lower in 1995 release sites compared to sites having received introductions in 1993. Although "within site" dispersal during the first few years after release is limited, there is still a tendency for beetles to slowly extend their egg laying away from the point of release. Observed vs. predicted r^2 values from individual transects and pooled data indicated an excellent fit of Taylor's model to the observed density-distance data from both 1995-release sites.

From spring of 1995 to 1996 in Libau Marsh-B, the estimate of the total number of eggs decreased by 87% (Table 8). In 1995, a total of 892 adult *G. calmariensis* and *G. pusilla* were released into Libau Marsh-B; however, at the start of sampling, only one oviposition period had been completed by 835 adults. The remaining 57 adult *G. calmariensis* were introduced into this site during fall of 1995 as part of the overwintering study and did not lay eggs in 1995. Mate finding during the period of initial confinement to the release cage in Libau Marsh-B was likely not difficult and no Allee effect (Allee 1931) would be likely to occur.

Despite a slight reduction in mean spring egg density from spring of 1995 to 1996, the estimate of the total number of eggs in Delta Marsh-B increased by 35% (Table 8). This result was not expected, especially since the mean egg density in Delta Marsh-B decreased slightly from spring of 1995 to 1996. Of the 877 eggs estimated to be in Delta Marsh-B in 1995, 80% were sampled at the point of release and 20% were estimated from the remainder of the site. In 1996, only 25% of the total number of eggs were sampled at the release point and 75% were estimated from the remainder of the site. The distances along individual transects at which *Galerucella* spp. eggs were estimated were

greater in 1996 and as a result, more eggs were estimated away from the point of release compared to 1995. This contributed to a higher total egg estimate from Delta Marsh-B during the 1996-growing season. As in Libau Marsh-B, the 800 adult *G. pusilla* and *G. calmariensis* released in spring of 1995 completed only one oviposition period prior to sampling in 1995. The remaining 118 adult *G. pusilla* and *G. calmariensis* were introduced during fall of 1995 as part of the overwintering study, and no oviposition occurred in that year.

Growth rates, as measured by the total egg estimate from 1996/1995, indicate that there are differences between some release sites. Excluding Libau Marsh-B, an increase in the total egg estimate was observed from spring of 1995 to 1996 in all release sites. Delta Marsh-B and Libau Marsh-A possessed similar site characteristics and rates of growth, despite having received introductions a couple of years apart. However, the initial density of eggs at the release point in Delta Marsh-B was smaller than expected due to presence of a fungal pathogen, believed to be *Beauvaria* spp., attacking adults inside the release cage. The growth rate in Delta Marsh-B was expected to be similar to that in Libau Marsh-B, but instead a small increase in the total egg estimate was observed. Growth rates from these two sites represent very low rates of population increase. Despite the large reduction in mean egg density observed in Libau Marsh-B from 1995 to 1996, the total egg estimate in 1996 was still 3.5 times higher than in Delta Marsh-B.

In both 1995 release sites, the density of purple loosestrife stems quickly decreased with increasing distance from the release point in 1995 and 1996. A reduction in the density of loosestrife stems was also observed at the point of release in both sites

during the 1995 and 1996 growing seasons (Figs. 25, 27, 29). However, in Libau Marsh-B, loosestrife stem density was reduced only 35% from spring to fall of 1995, despite the enormous density of *Galerucella* spp. eggs at the release point in spring. Releases of adult *Galerucella* spp. into this site were made at the start of June in 1995. By this time, plants had already begun spring regrowth, and possibly were able to tolerate feeding by first instar *Galerucella* spp. larvae. The number of eggs /stem was highest at the release point due to the enormous egg density sampled in spring of 1995. At a distance of 13 m along the northeast transect, the lowest number of eggs /stem appears to have caused a reduction in loosestrife stem density (Fig. 29). This apparent reduction may have been due to the 1 m^2 wire-framed quadrat not being placed in exactly the same position from spring to fall of the same year. During sampling with the quadrat, care was taken to place it in the same location as the previous; however, there may have been occasions when this did not occur. Along with environmental factors, misplacement of the quadrat may account for some of the variation observed in stem density from spring to fall or from one year to the next.

Fruit production data were collected from Libau Marsh-B in fall of 1995 and 1996. Unfortunately only four of eight transects sampled in 1995 could be repeated in 1996 and the distances from which data were collected did not match. As in Libau Marsh-B, fruit production by purple loosestrife in Delta Marsh-B was variable along transects, either not differing or gradually decreasing with distance from the release point (Fig. 26, 28).

A large reduction in the mean *Galerucella* spp. egg density was observed in Libau Marsh-B from spring of 1995 to 1996, whereas in Delta Marsh-B only a slight reduction

was observed (Table 9). The large reduction observed in Libau Marsh-B was somewhat of an artifact and related in part to the enormous density of eggs present at the point of release in spring of 1995.

The low mean spring egg density recorded from Delta Marsh-B: 1995 compared to Libau Marsh-B: 1995 was surprising and expected to be similar, since the number of adults introduced and the release techniques utilized were similar in each site. In spring of 1995, heavy rainfall was recorded for 3 days after adults were introduced into the release cage. Humidity inside the release cage was high and a fungal pathogen, assumed to be *Beauvaria* spp., killed some of the introduced adults prior to oviposition. Consistent with infection by *Beauvaria* spp. (Hight et al. 1995), many adult cadavers covered in white fungal spores were observed on the leaves of purple loosestrife plants. *Beauvaria* spp. is a soil-born fungus; however, it is unknown whether it was present inside cages prior to adult release or if the beetles were infected with spores during laboratory rearing at Cornell University.

There was an increase in mean fall stem and fruit density from 1995 to 1996 in Libau Marsh-B, and in the year of initial adult introduction, stem density and to a greater extent fruit production /m^2 may have been negatively affected by the beetles. In Libau Marsh-B, a mean egg number of 285 eggs /m^2 may have been sufficient to contribute to a slight reduction in the number of stems and larger reduction in the number of fruits /m^2 during the 1995 growing season. In 1996, a low mean spring egg density had no measurable impact on purple loosestrife stem or fruit production. This result was opposite of Delta Marsh-B, in which mean stem and fruit production decreased from one

year to the next. This also demonstrates that mature, well-established purple loosestrife plants do not respond similarly in all sites.

During the summer of 1997 a large increase in the *Galerucella* spp. population was observed in Libau Marsh-B by the coordinator of the Manitoba Purple Loosestrife Project, (Lindgren, personal communication) and minimal flower production was observed. During the 1998 growing season, loosestrife stem density in addition to flower production were both visibly reduced by the feeding action of the introduced biological control agents. Libau Marsh-B was the only site in which the total egg estimate decreased from spring of 1995 to 1996. Similarities between Delta Marsh-A and Libau Marsh-B include the dense, uniform centrally located areas of purple loosestrife, the ability of these sites to hold water from spring runoff well into July and the release of both *G. pusilla* and *G. calmariensis*.

Opposite to Libau Marsh-B, and as one would expect, the mean fruit density was further reduced from fall of 1995 to 1996 in Delta Marsh-B, suggesting an impact of the beetles on fruit production during the 1996-growing season. Indeed, the larger reductions in mean stem density from spring to fall of 1995 and 1996 in Delta Marsh-B seem to support this. However, mean egg densities in spring of 1995 and 1996 were consistently low and so these observed reductions may not have been the direct result of feeding by *G. pusilla* and *G. calmariensis*. Instead, they may be due to unfavorable environmental factors and/or plant competition. Although there is no quantitative data for support, the composition of plants in Delta Marsh-B changed markedly during the 1995 and 1996 growing seasons. On the basis of visual assessments, the density of *Typha* spp., *Phragmites* spp. and *Bromus* spp. increased during 1995. This trend continued in 1996,

and may have ultimately contributed to the slight reduction in loosestrife stem density during both growing seasons. Evidence of marsh plants such as reed canary grass, *Phalaris arundinacia* L., competing well with and even replacing mature purple loosestrife plants has been documented (Novak 1968).

Broken Stick Regression

Although some variability in stem and fruit density was apparent from one year to the next along individual transects, mean stem and fruit densities from release sites did provide a way of measuring the effect of the introduced biological control agents. In Delta Marsh-A, feeding by *G. calmariensis* and *G. pusilla* during the 1996-growing season reduced both the mean stem density and fruit production from spring to fall. Due to the effect of *Galerucella* spp. on loosestrife stem density and fruit production in all release sites, the broken stick regression technique successfully identified the egg density at which subsequent effects of the introduced agents were observed. Compared to Delta Marsh-A:1996, the overall impact of *Galerucella* spp. on loosestrife stand density in most of the remaining three release sites was minimal. However, at the point of release within these sites, a measurable impact on either stem and fruit density was often observed and as a result, the importance of these data points to the broken stick regression cannot be understated. Some of the data points appearing after the breakpoint in the downslope of the broken stick regression are from the other three release sites, suggesting that the regressions are not just an artifact of the observed data from Delta Marsh-A during the 1996 growing season. Instead, these regressions appear to represent a generally

applicable relationship between the density of *Galerucella* spp. eggs /m^2 and *L. salicaria* stem and fruit production within release sites in southern Manitoba wetlands.

CONCLUSIONS

1.) The introduced biological control agents, *G. pusilla* and *G. calmariensis*, were successfully established in the two 1995 wetland release sites; one located at Delta Marsh and one at Libau Marsh.

2.) *Galerucella* spp. egg density and *L. salicaria* stem density decreased with increasing distance from the central release point in all four-release sites.

3.) Fruit density of *L. salicaria* decreased slightly with increasing distance from the release point.

4.) Taylor's general non-linear model fit the observed *Galerucella* spp. egg density-distance data and can be used to predict the total egg population within release sites.

5.) The numbers of eggs were larger in 1993 release sites compared to sites receiving beetles in 1995. Based on egg mass sampling, "within site" dispersal of *Galerucella* spp. during the first few years after introduction is slow. Taylor's pooled *c* parameter estimate was less than two in all four release sites, indicating a tendency for female *Galerucella* spp. to lay their eggs within a few meters of the release point.

6.) *Galerucella* spp. did have a measurable effect on the density of *L. salicaria* stems and fruits in Delta Marsh-A during the 1996-growing season. In the other three release sites, measurable effects on plants were confined to the point of release.

7.) Densities $\geq$ 566 *Galerucella* spp. eggs /m^2 were associated with reductions in fall stem density relative to spring stem density.

8.) Densities $\geq$ 432 *Galerucella* spp. eggs /m^2 were associated with rapid reductions in fruit production relative to spring stem density. At egg densities $<$ 432 /m^2, a slight reduction in the number of fruits is still observed.

Table 1. Release date, number of adults, species and source of beetles introduced into study sites in southern Manitoba.

Site	Release Date	No. Released	Species Released	Source of Beetles
Libau A	30.vii.93	355	*G. calmariensis*	Cornell University
Libau A	10.viii.93	250	*G. calmariensis*	Cornell University
Libau A	20.vii.94	75	*G. calmariensis*	University of Guelph
	*Total Number Released 680 Adults			
Libau B	8.vi.95	520	*G. calmariensis*	University of Guelph
Libau B	21.vi.95	125	*G. calmariensis*	AAFC, Lethbridge
Libau B	4.vii.95	190	*G. calmariensis* & *G. pusilla*	Cornell University
Libau B	29.ix.95	57	*G. calmariensis*	Cornell University
	*Total Number Released 892 Adults			
Delta A	21.vii.93	262	*G. pusilla*	University of Guelph
Delta A	10.viii.93	250	*G. calmariensis*	University of Guelph
Delta A	20.viii.93	104	*G. pusilla*	University of Guelph
Delta A	20.vii.94	100	*G. pusilla*	University of Guelph
	*Total Number Released = 716 Adults			
Delta B	29.vi.95	800	*G. calmariensis* & *G. pusilla*	Cornell University
Delta B	2.ix.95	118	*G. calmariensis* & *G. pusilla*	Delta Marsh-A
	*Total Number Released = 918 Adults			

Table 2. Sampling dates of spring egg density and fall fruit density within study sites in southern Manitoba.

Location	Site	Year	Egg Density Sampling Date	Fruit Density Sampling Date
Libau Marsh	A	1995	09,16,21.vi	28.viii
		1996	26-27.vi, 02-04 .vii	07-08.ix
Delta Marsh	A	1995	06-07, 13-14.vi	31.viii, 01,05,07.ix
		1996	17-21.vi, 24-25.vi	03,05.ix
Libau Marsh	B	1995	23,27.vi, 06,09.vii	25,29.viii
		1996	09-11.vii, 15-18.vii	10,12.ix
Delta Marsh	B	1995	11,18.vii	31.viii
		1996	20.vi, 08.vii	05.ix
Libau Marsh	C	1995	09.vii	29.viii
		1996	NA	12.ix

vi = June; vii = July; viii = August; ix = September
NA = Date not available, but sampling did occur.

Table 3. Estimated values of a, b, c parameters from the nonlinear model density = $\exp(a + b*\text{distance}^c)$ and associated r^2 values for each transect in Libau Marsh-A.

Year	Transect	a	b	c	r^2
1995	East	6.087	-0.772	1.266	0.998
	North	6.069	-0.561	0.434	0.616
	Northeast	6.086	-0.477	1.426	0.999
	Northwest	6.095	-0.312	0.529	0.619
	South	6.030	-0.542	0.449	0.745
	Southeast	6.086	-1.352	0.105	0.745
	Southwest	6.060	-0.387	0.564	0.698
	West	6.083	-1.230	0.230	0.730
1996	East	7.039	-1.000	0.808	0.912
	North	7.331	-0.165	0.950	0.506
	Northeast	6.941	-1.486	0.450	0.994
	Northwest	6.933	-0.535	0.295	0.416
	South	6.945	-0.415	0.550	0.890
	Southeast	6.981	-0.721	0.615	0.879
	Southwest	6.980	-0.491	0.565	0.932
	West	6.732	-0.015	2.450	0.787

Table 4. Estimated values of ***a***, ***b***, ***c*** parameters from the nonlinear model density = $\exp(a + b*\text{distance}^c)$ and associated r^2 values for each transect in Libau Marsh-B.

Year	Transect	a	b	c	r^2
1995	East	9.624	-3.881	0.180	0.999
	North	9.624	-3.531	0.289	0.999
	Northeast	9.624	-2.604	0.350	0.999
	Northwest	9.624	-4.049	0.200	0.999
	South	9.624	-4.742	0.326	0.999
	Southeast	9.624	-3.543	0.235	0.999
	Southwest	9.624	-3.741	0.289	0.999
	West	9.624	-5.503	0.124	0.999
1996	East	4.833	-1.489	0.147	0.937
	North	5.097	-1.200	0.338	0.932
	Northeast	5.099	-3.315	0.192	0.998
	Northwest	5.097	-2.370	0.008	0.972
	South	5.099	-2.492	0.129	0.967
	Southeast	5.099	-2.835	0.060	0.986
	Southwest	5.099	-3.730	0.028	0.998
	West	5.099	-2.667	0.324	0.999

Table 5. Estimated values of a, b, c parameters from the nonlinear model density = $\exp(a + b*\text{distance}^c)$ and associated r^2 values for each transect in Delta Marsh-A.

Year	Transect	a	b	c	r^2
1995	East	5.832	-0.051	2.465	0.814
	Northeast	5.689	-0.0002	4.530	0.988
	Northwest	5.467	-0.0001	5.194	0.766
	North-A	5.298	-0.373	0.640	0.848
	North-B	5.738	-0.029	1.968	0.536
	North-C	6.150	-0.082	1.358	0.927
	Southeast	5.551	-0.004	3.202	0.985
	Southwest	5.454	-0.077	0.731	0.132
	South-A	5.403	-0.134	1.202	0.665
	South-B	5.702	-0.001	5.575	0.983
	South-C	6.093	-0.234	0.819	0.754
	West	5.445	-0.063	1.551	0.836
1996	East	9.098	-0.144	1.716	0.999
	North-A	8.687	-0.119	1.250	0.785
	North-B	9.196	-0.333	0.750	0.908
	South-A	8.361	-0.313	0.759	0.904
	South-B	9.158	-0.414	0.750	0.873
	West	8.341	-0.549	0.411	0.771

Table 6. Estimated values of ***a***, ***b***, ***c*** parameters from the nonlinear model density = $\exp(a + b*\text{distance}^c)$ and associated r^2 values for each transect in Delta Marsh-B.

Year	Transect	a	b	c	r^2
1995	East	6.541	-3.976	1.667	1.0
	North	6.541	-3.019	0.373	0.999
	Northeast	6.541	-3.045	2.281	1.0
	Northwest	6.541	-5.848	1.630	0.999
	South	6.541	-4.901	0.042	0.999
	Southeast	6.541	-3.902	2.027	1.0
	Southwest	6.541	-4.749	1.833	1.0
	West	6.541	-5.958	1.561	0.999
1996	East	5.823	-2.601	3.570	1.0
	North	5.823	-3.327	3.482	1.0
	Northeast	5.823	-4.437	4.067	1.0
	Northwest	5.823	-2.523	0.013	0.999
	South	5.823	-2.601	3.570	1.0
	Southeast	5.823	-2.386	0.204	0.978
	Southwest	5.823	-2.849	0.067	0.995
	West	5.823	-0.354	2.754	0.999

Table 7. Comparison of pooled estimates of the *a*, *b* and *c* parameters fromTaylor's nonlinear model density = exp(a + b (distance^c)) and associated r^2 values.

Location	Site	Sampling Year	*a* parameter	*b* parameter	*c* parameter	r^2 value
Libau Marsh	A	1995	6.080	-0.772	0.336	0.68
		1996	6.983	-0.378	0.635	0.57
Delta Marsh	A	1995	5.744	-0.079	1.409	0.56
		1996	8.740	-0.074	1.338	0.64
Libau Marsh	B	1995	9.625	-3.626	0.278	0.99
		1996	5.098	-2.525	0.069	0.96
Delta Marsh	B	1995	6.541	-3.994	0.335	0.99
		1996	5.823	-2.903	0.148	0.99

Table 8. Estimates of the total number of eggs within study sites during spring of 1995 and 1996.

Location	Site	Release Year	Total Egg Estimate: 1995	Total Egg Estimate: 1996	N96 / N95
Delta Marsh	A	1993	87 054	2 209 324	25.37
Delta Marsh	B	1995	877	1 355	1.54
Libau Marsh	A	1993	116 474	306 689	2.63
Libau Marsh	B	1995	36 518	4 759	0.13

Table 9. Mean spring egg density of *Galerucella* spp. (eggs /m^2), spring and fall stem density and mean fall fruit density (# /m^2) of purple loosestrife within five study sites in southern Manitoba.

Location	Sampling Year	Mean Spring Egg Density	Mean Spring Stem Density	Mean Fall Stem Density	Mean Fall Fruit Density
Libau Marsh-A	1995	84.70 ± 8.79	24.06 ± 1.65	24.11 ± 2.69	684.71 ± 94.91
	1996	226.35 ± 31.55	18.32 ± 1.54	19.93 ± 1.96	852.58 ± 88.78
Delta Marsh-A	1995	118.95 ± 13.82	31.86 ± 2.45	29.09 ± 2.10	4014.77 ± 315.98
	1996	1771.43 ± 288.80	28.39 ± 3.48	4.37 ± 0.65	22.70 ± 9.65
Libau Marsh-B	1995	285.70 ± 213.06	92.32 ± 5.38	80.08 ± 3.87	7610.71 ± 409.54
	1996	12.66 ± 3.32	98.66 ± 4.18	93.43 ± 6.12	21033.31 ± 2324.56
Delta Marsh-B	1995	27.23 ± 23.02	67.03 ± 9.76	50.00 ± 7.51	4780.20 ± 1025.86
	1996	23.67 ± 12.37	48.56 ± 8.21	33.30 ± 6.07	2913.18 ± 789.43
Libau Marsh-C	1995	0.0	39.85 ± 9.59	60.53 ± 5.59	12290.36 ± 1808.34
	1996	0.0	44.63 ± 7.68	62.82 ± 7.21	14091.68 ± 2200.27

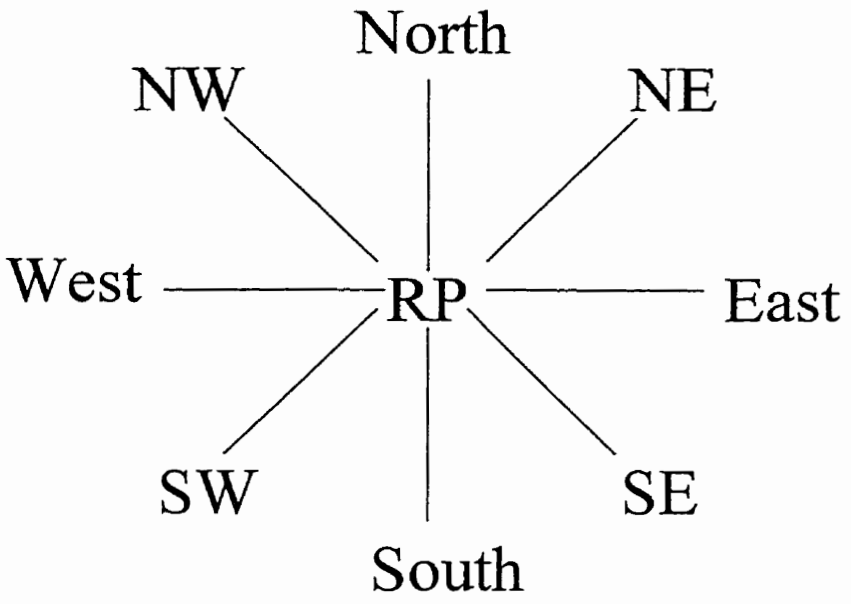


Figure 1. Pattern of transects used to sample *Galerucella* spp. egg and loosestrife stem density at various distances from the central release point. Libau Marsh-A & -B, and Delta Marsh-B contain a single release point (RP) and the pattern of transect establishment was the same in 1995 and 1996.

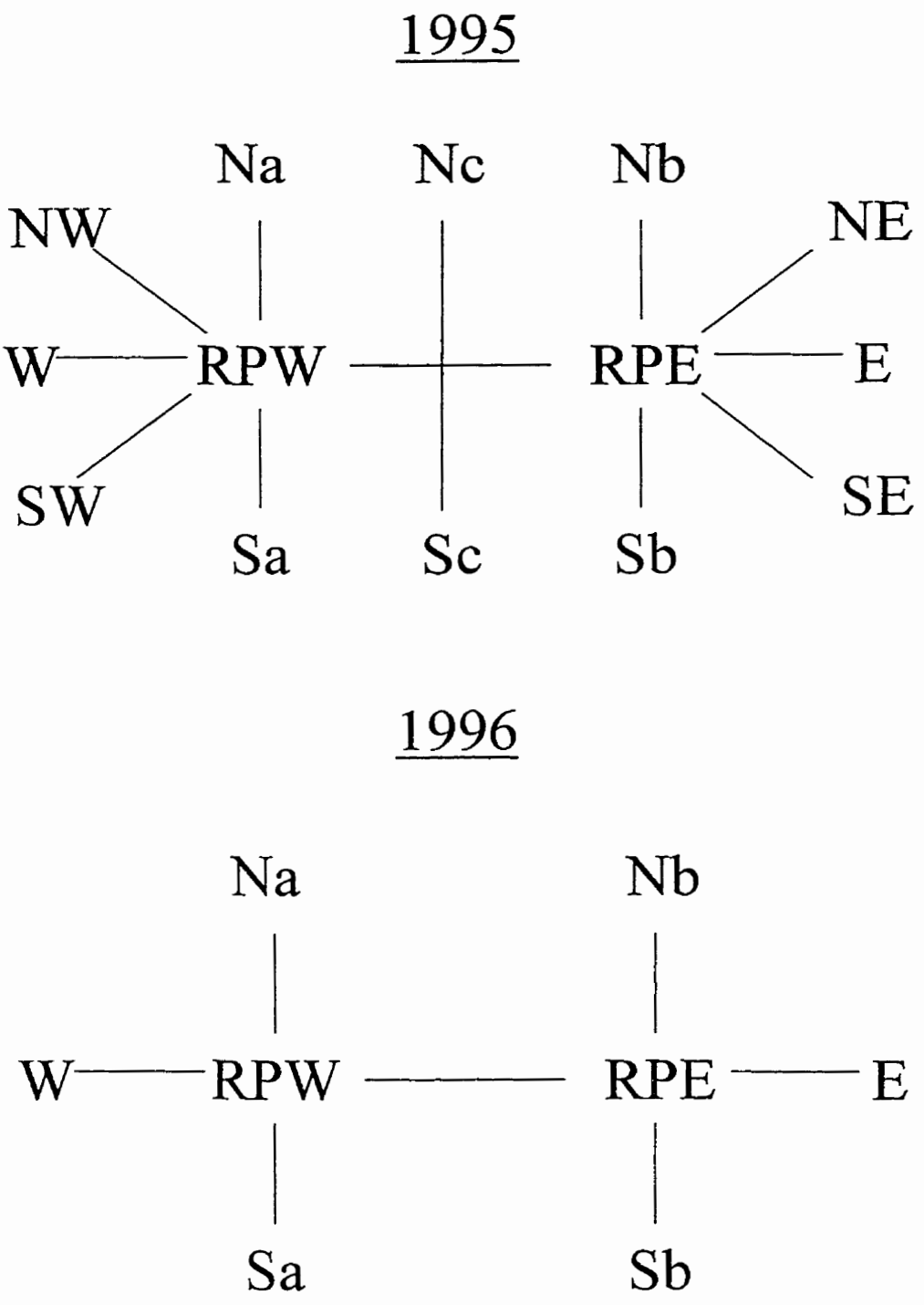


Figure 2. Pattern of transects used to sample *Galerucella* spp. egg and loosestrife stem densities at various distances from the central release point. In Delta Marsh-A release point west (RPW) and release point east (RPE) are approximately 10 meters apart.

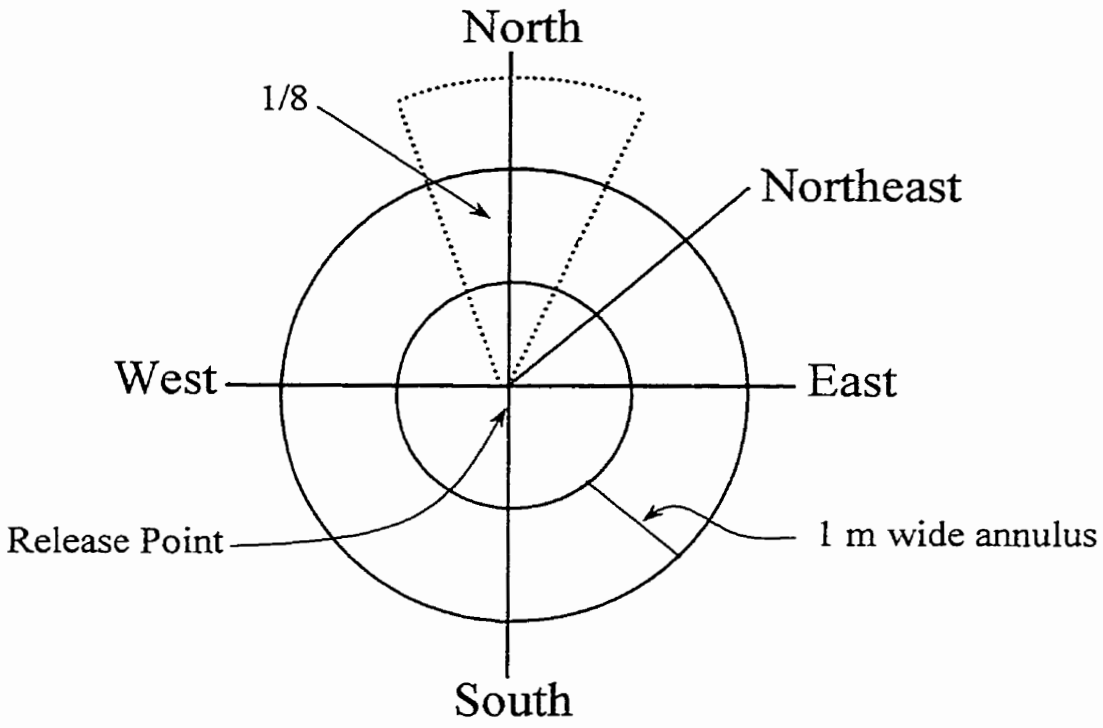


Figure 3. Diagram of release point surrounded by two-1 metre wide annuli. Dashed lines represent a 1/8 sector of a circle.

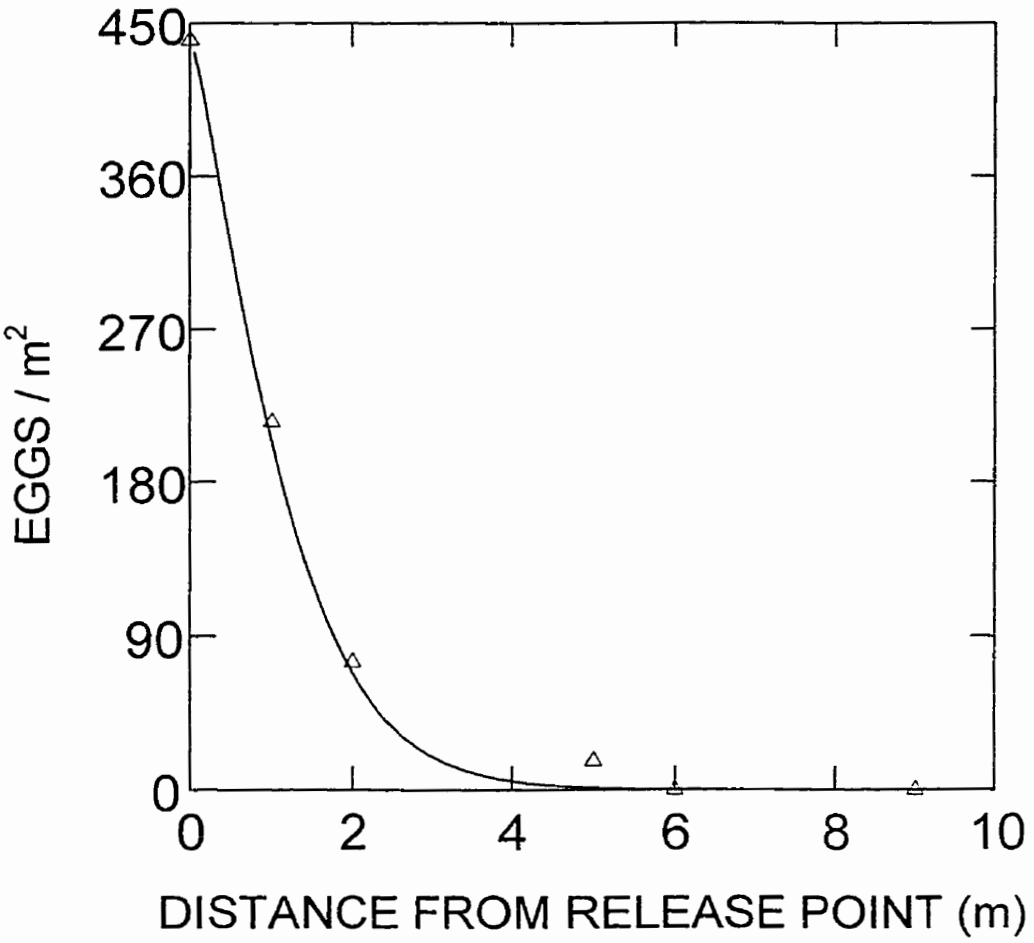


Figure 4. *Galerucella* spp. egg density at various distances from central release point along east transect in Libau Marsh-A: 1995. Observed values Δ and fitted line (see Table 3 for parameter values).

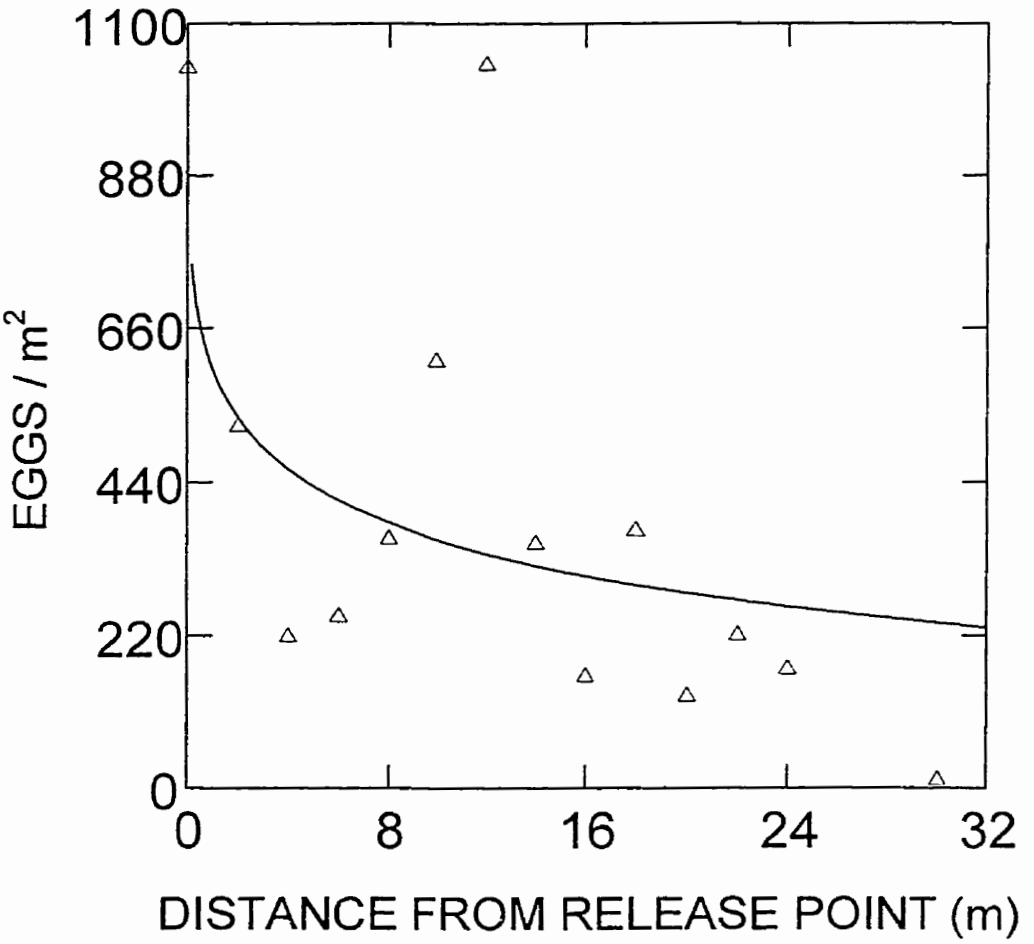


Figure 5. *Galerucella* spp. egg density at various distances from central release point along northwest transect in Libau Marsh-A: 1996. Observed values Δ and fitted line (see Table 3 for parameter values).

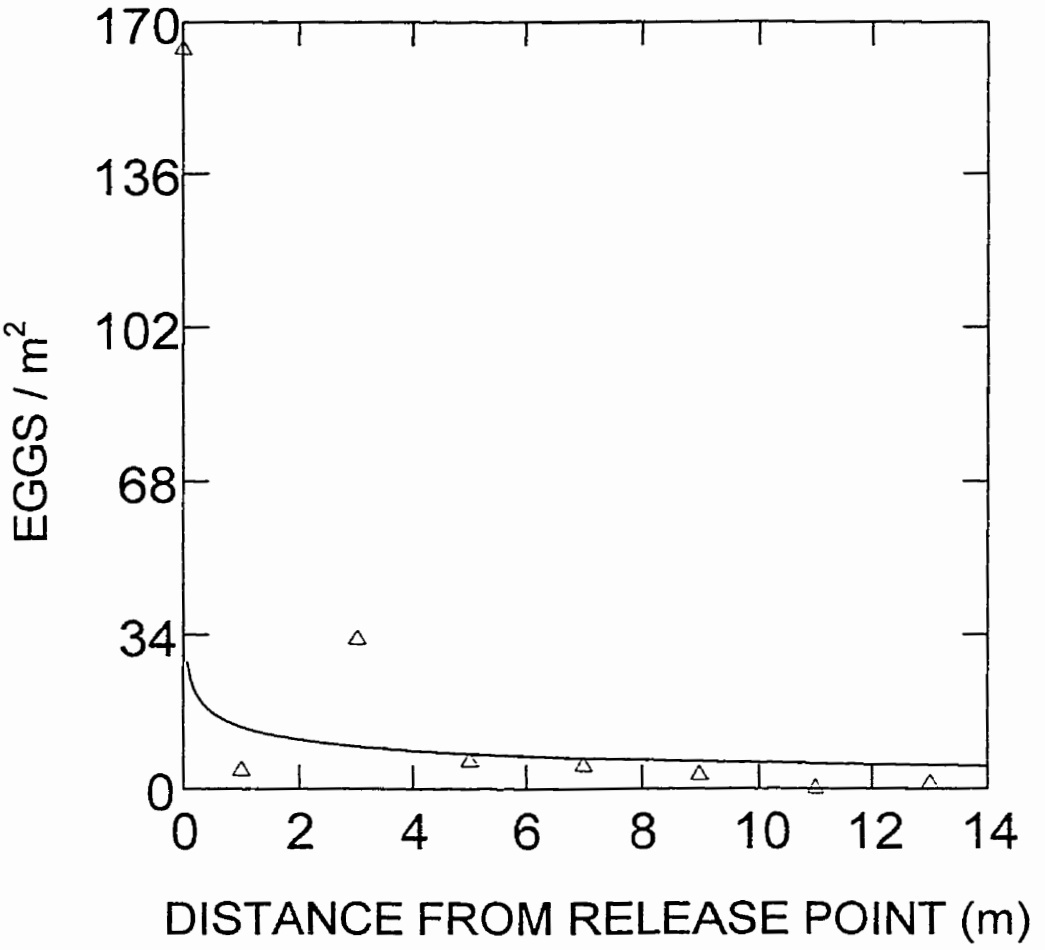


Figure 6. *Galerucella* spp. egg density at various distances from central release point along south transect in Libau Marsh-B: 1996. Observed values Δ and fitted line (see Table 4 for parameter values).

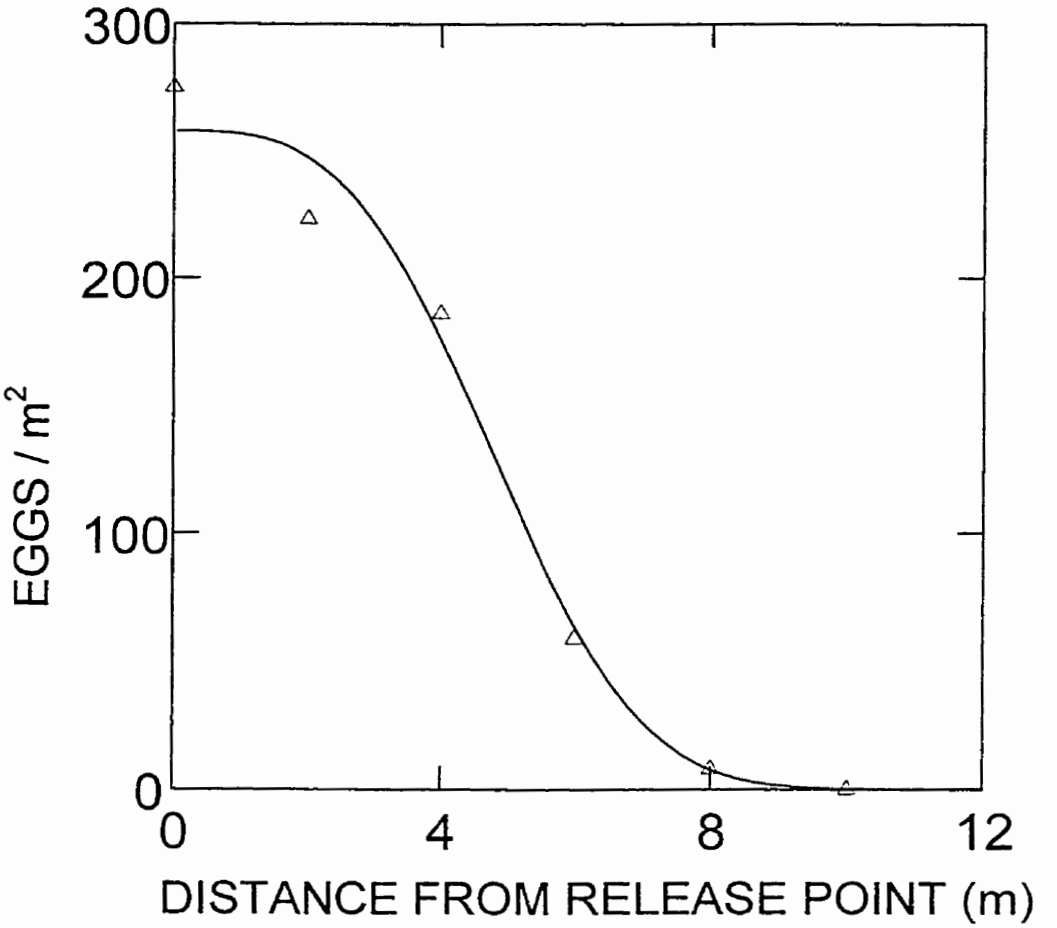


Figure 7. *Galerucella* spp. egg density at various distances from central release point along southeast transect in Delta Marsh-A: 1995. Observed values Δ and fitted line (see Table 5 for parameter values).

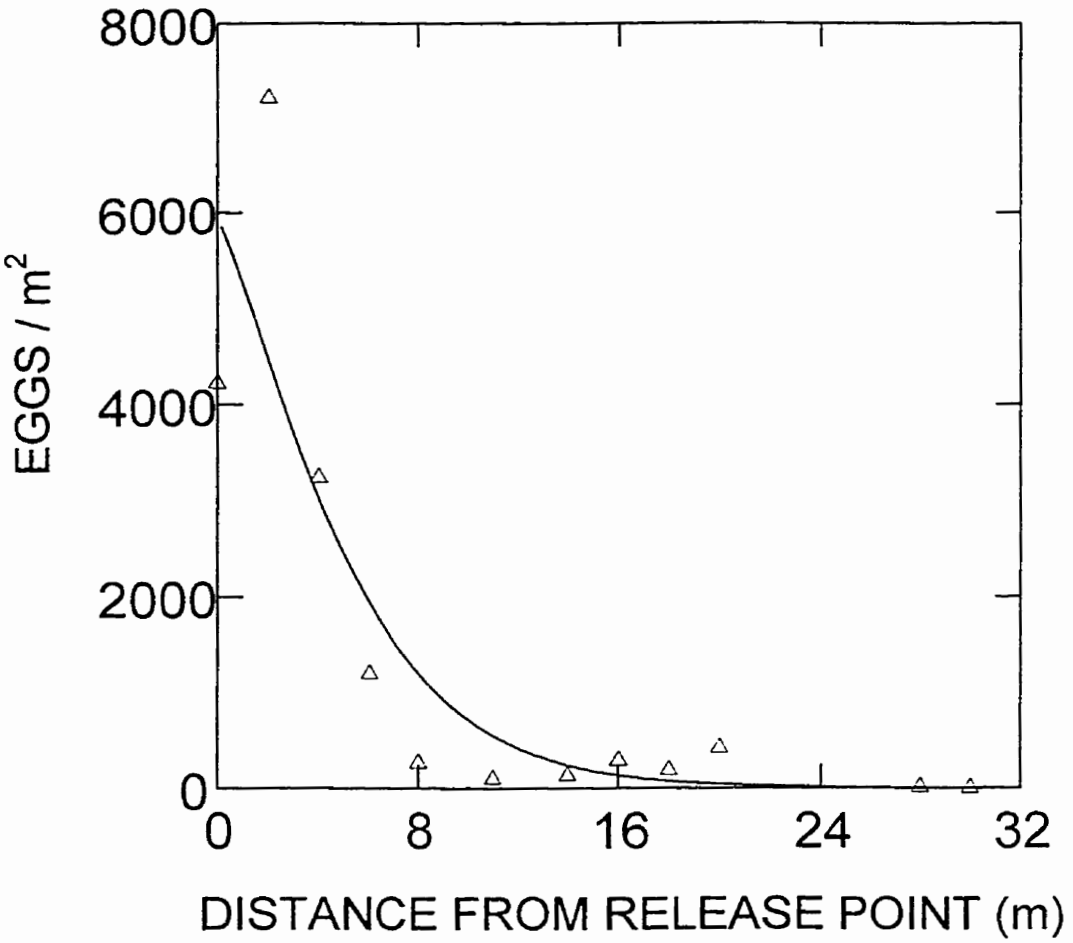


Figure 8. *Galerucella* spp. egg density at various distances from central release point along north-A transect in Delta Marsh-A: 1996. Observed values Δ and fitted line (see Table 5 for parameter values).

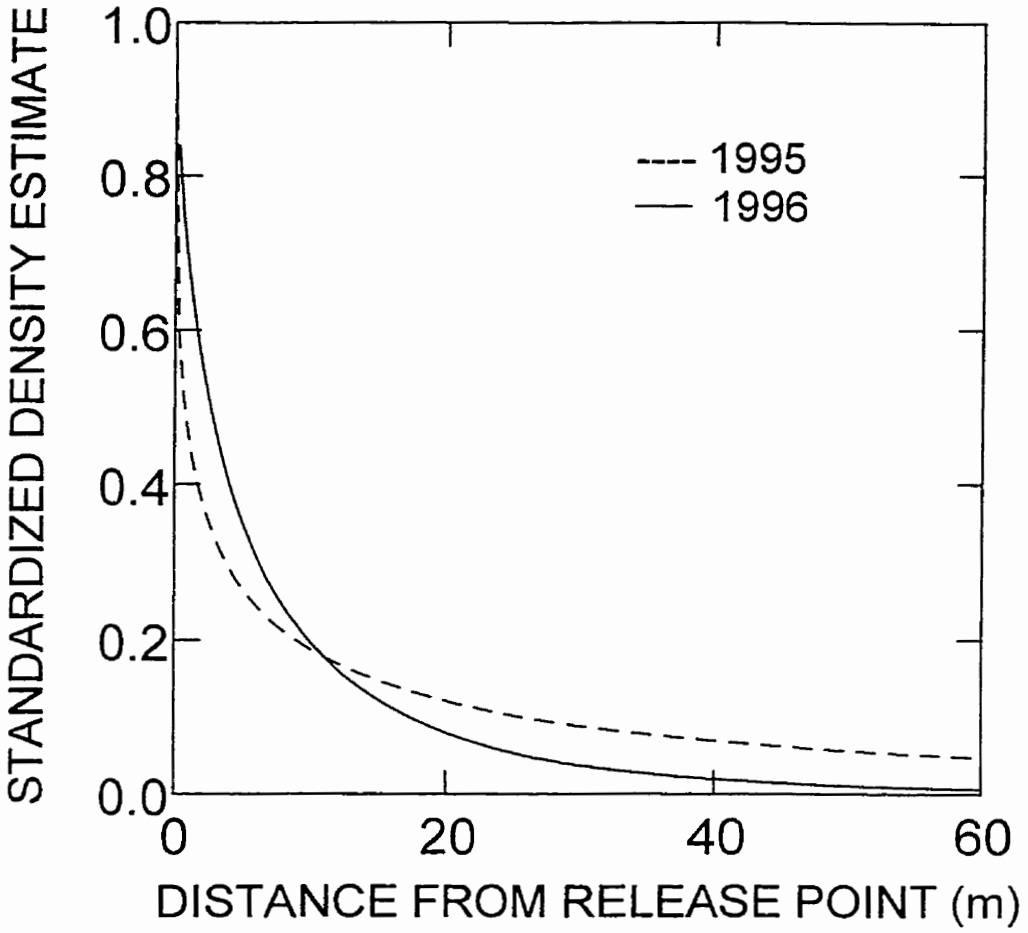


Figure 9. Standardized estimate of *Galerucella* spp. egg density in relation to distance from central release point in Libau Marsh-A.

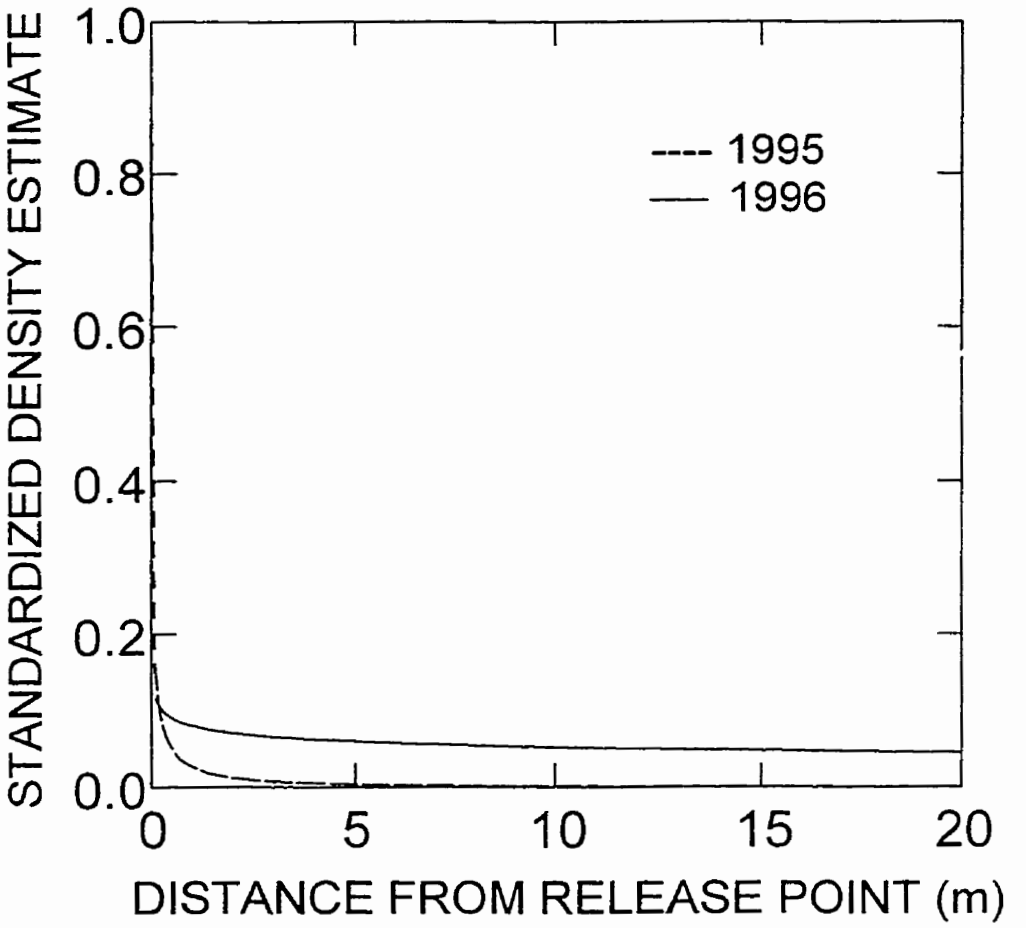


Figure 10. Standardized estimate of *Galerucella* spp. egg density in relation to distance from central release point in Libau Marsh-B.

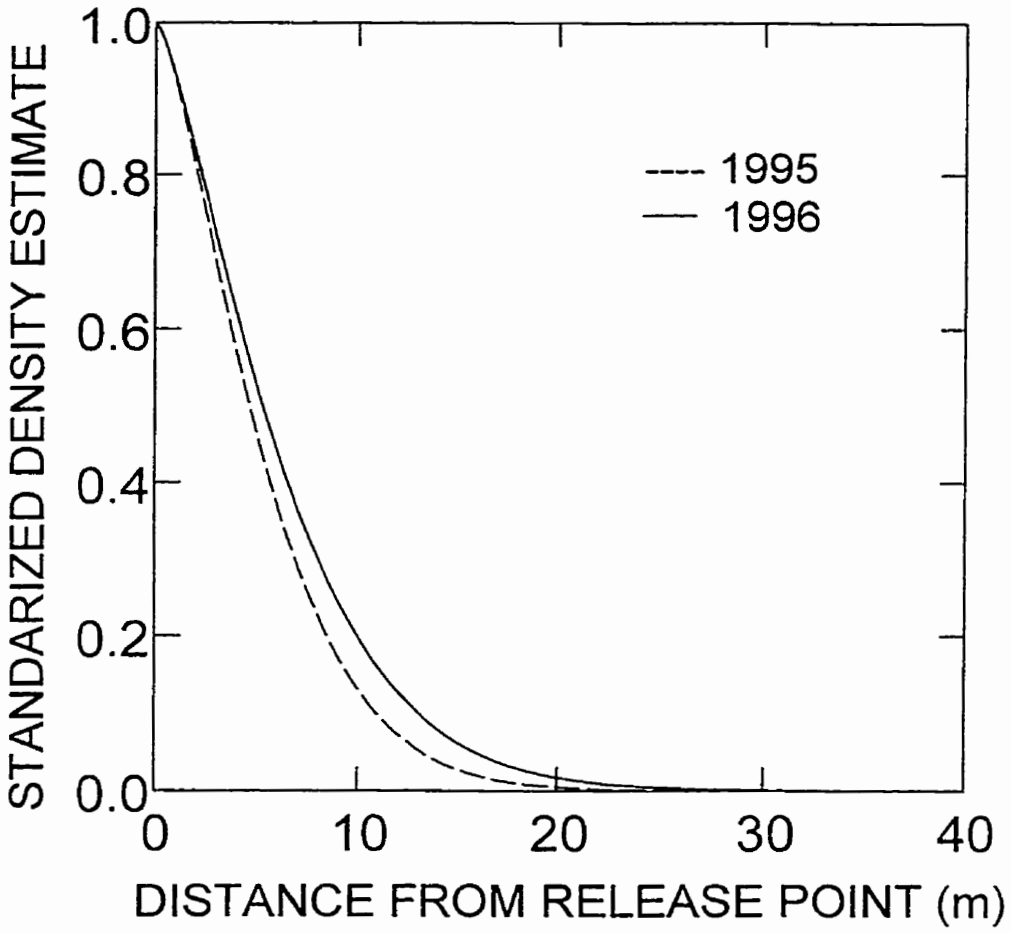


Figure 11. Standardized estimate of *Galerucella* spp. egg density in relation to distance from central release point in Delta Marsh-A.

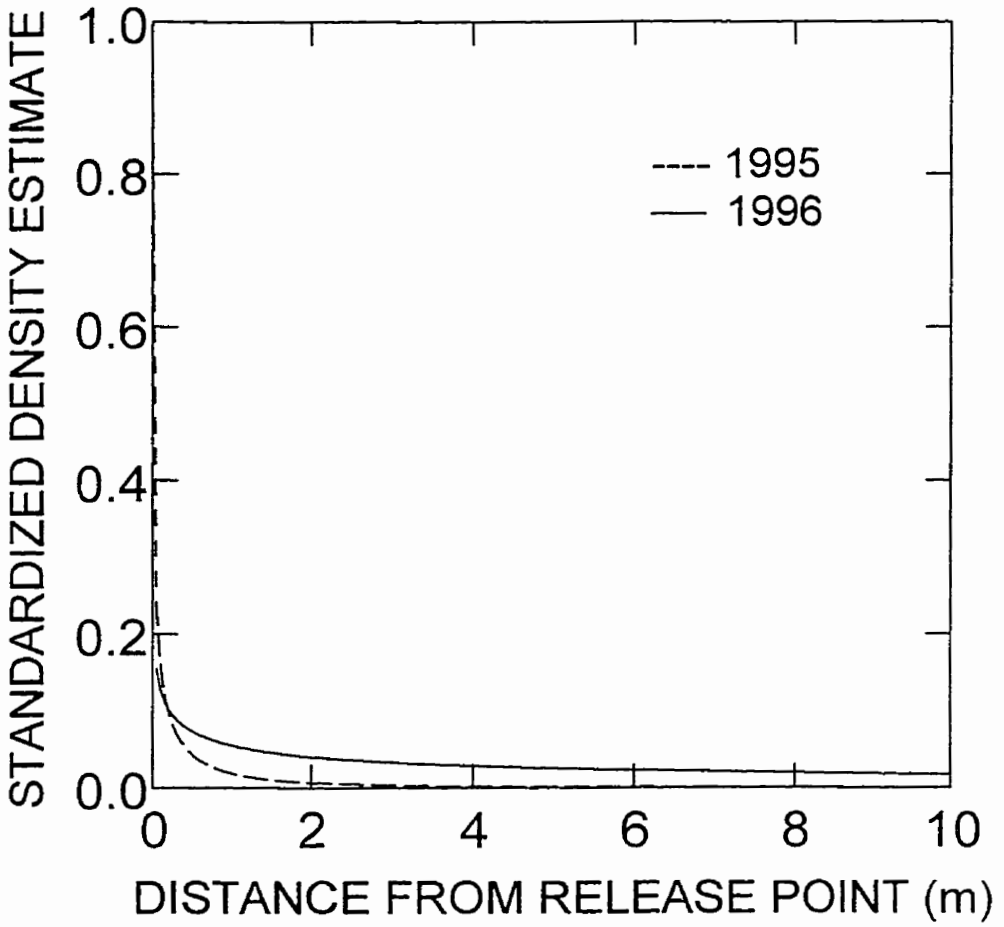


Figure 12. Standardized estimate of *Galerucella* spp. egg density in relation to distance from central release point in Delta Marsh-B.

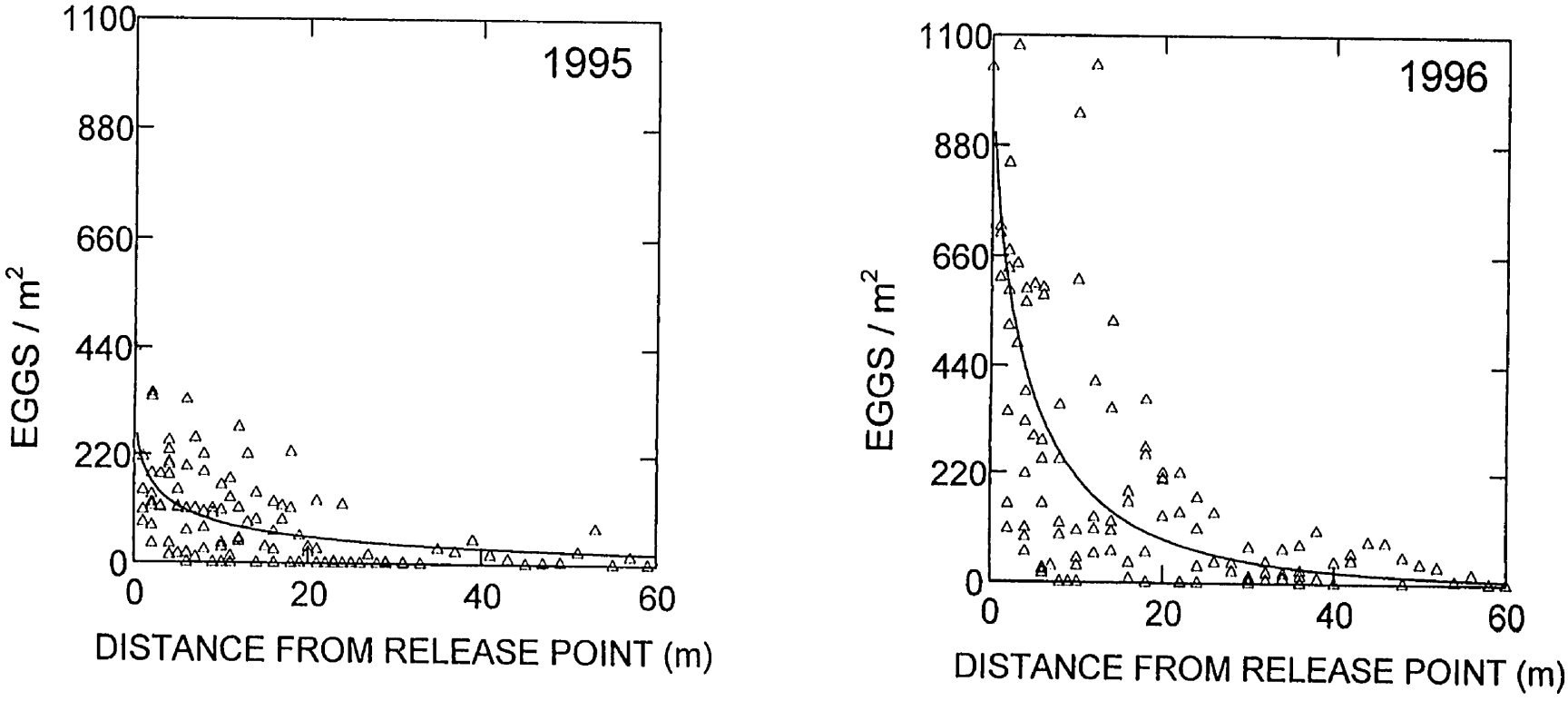


Figure 13. Relationship of *Galerucella* spp. egg density to distance from release point in Libau Marsh-A. Data represent eight transects.

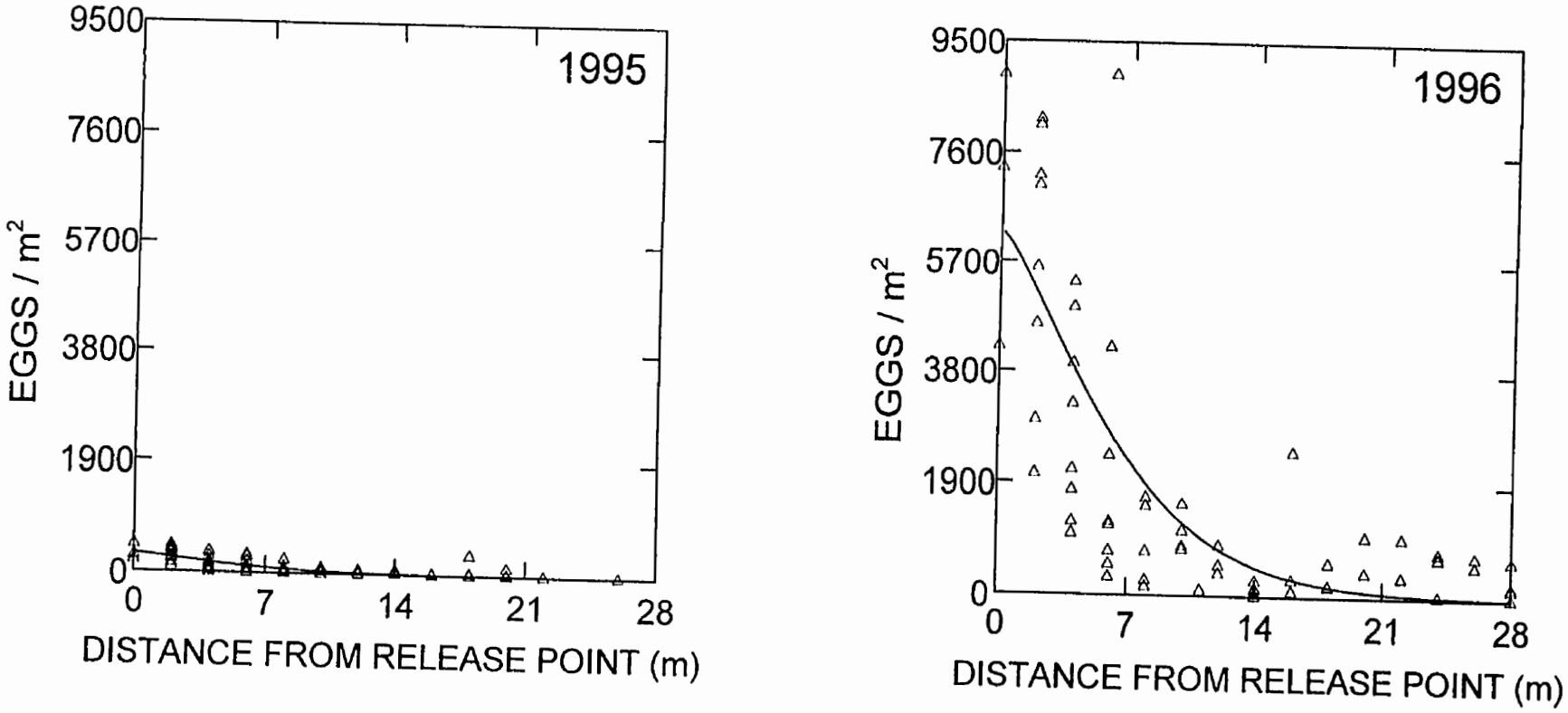


Figure 14. Relationship of *Galerucella* spp. egg density to distance from release point in Delta Marsh-A. Data represent eight transects.

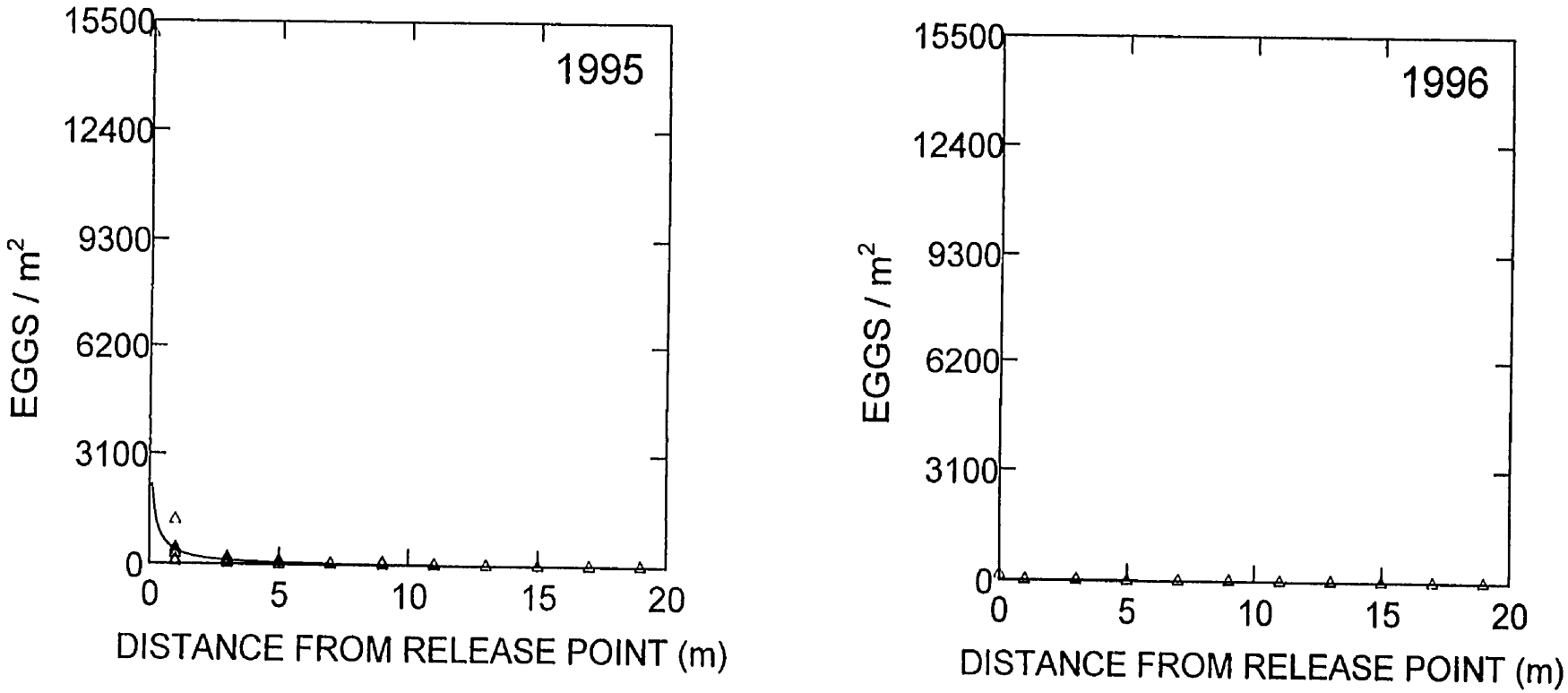


Figure 15. Relationship of *Galerucella* spp. egg density to distance from release point in Libau Marsh-B. Data represent eight transects.

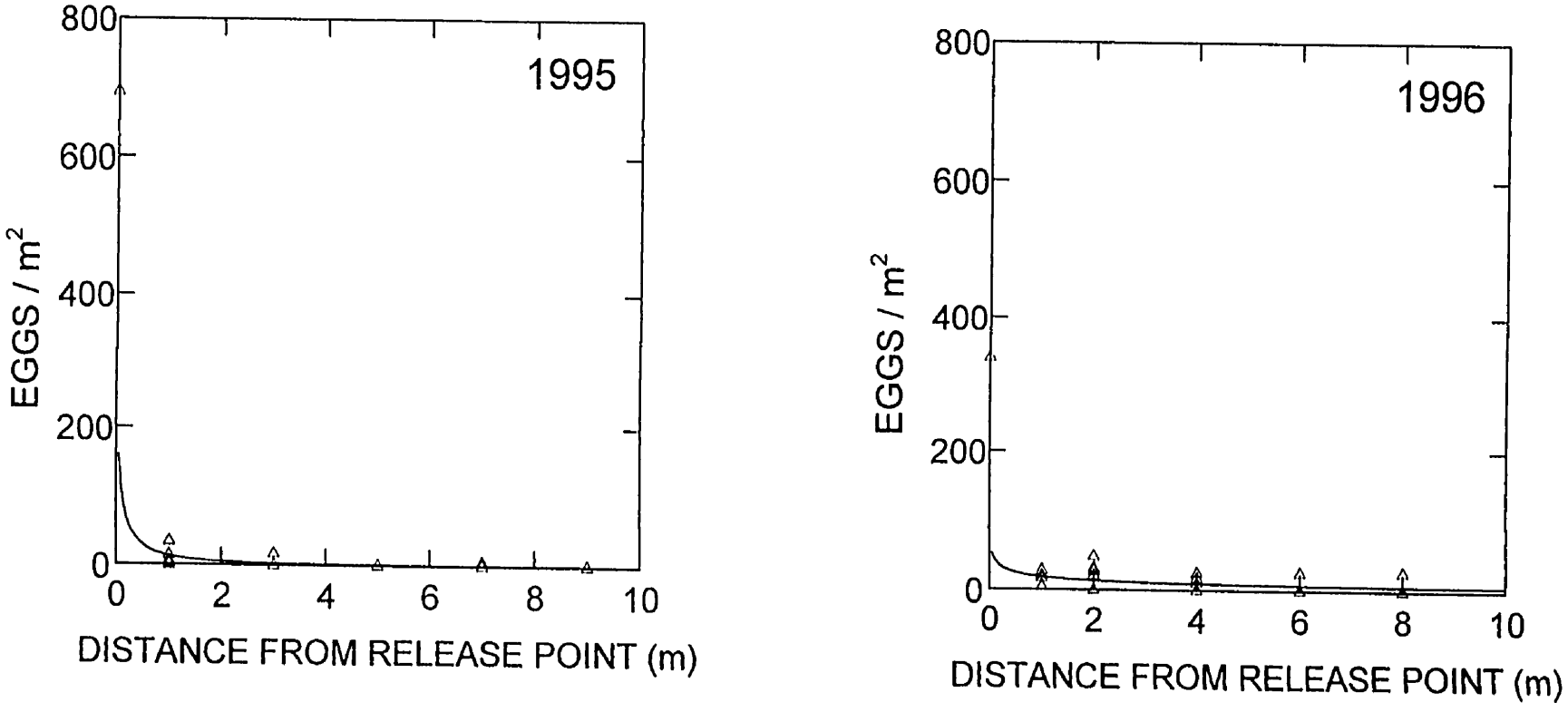


Figure 16. Relationship of *Galerucella* spp. egg density to distance from release point in Delta Marsh-B. Data represent eight transects.

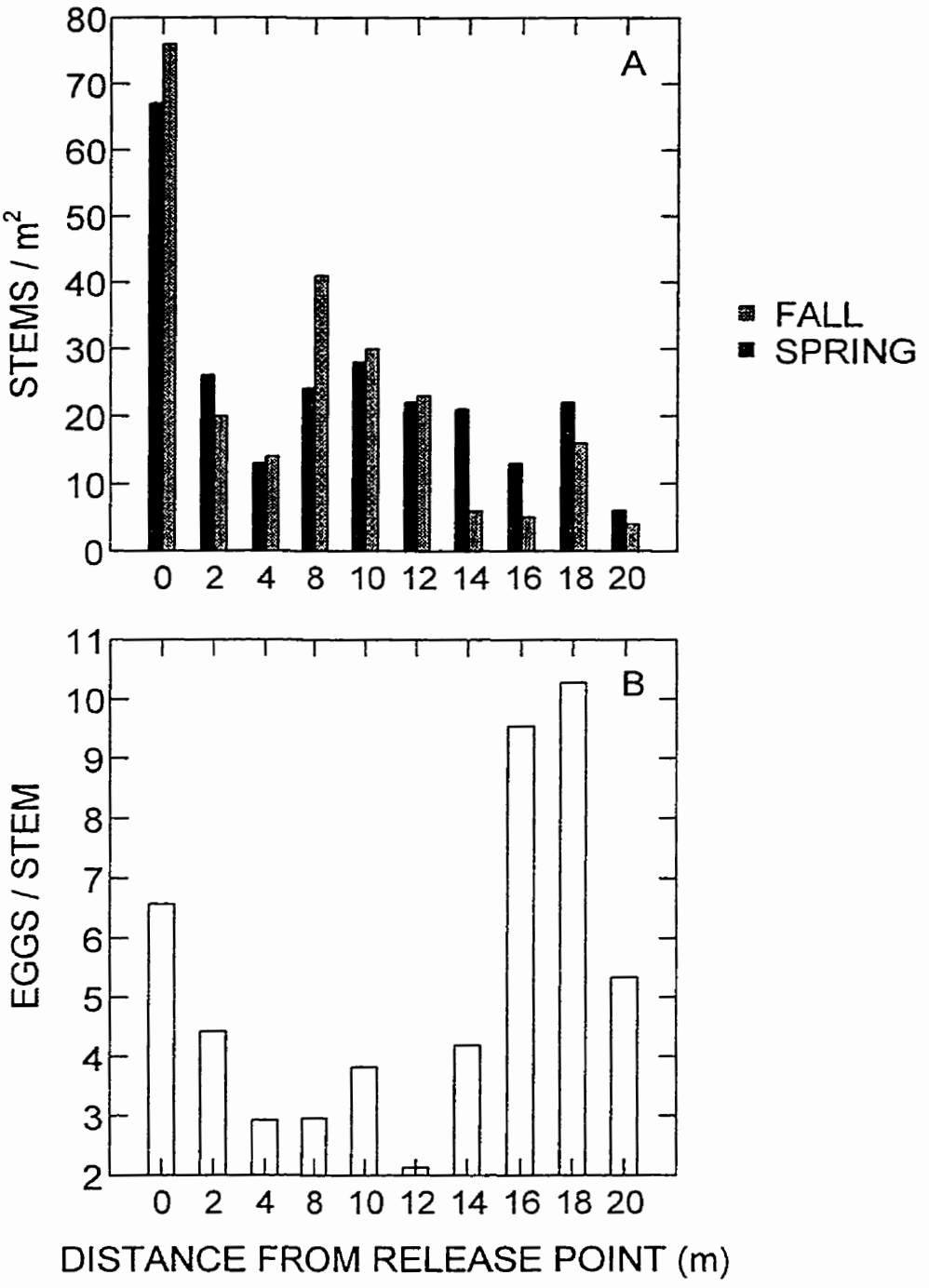


Figure 17. A. *Lythrum salicaria* stem densities in spring and fall of 1995 along the southeast transect in Libau Marsh-A. B. *Galerucella* spp. eggs /stem in spring at various distances from central release point.

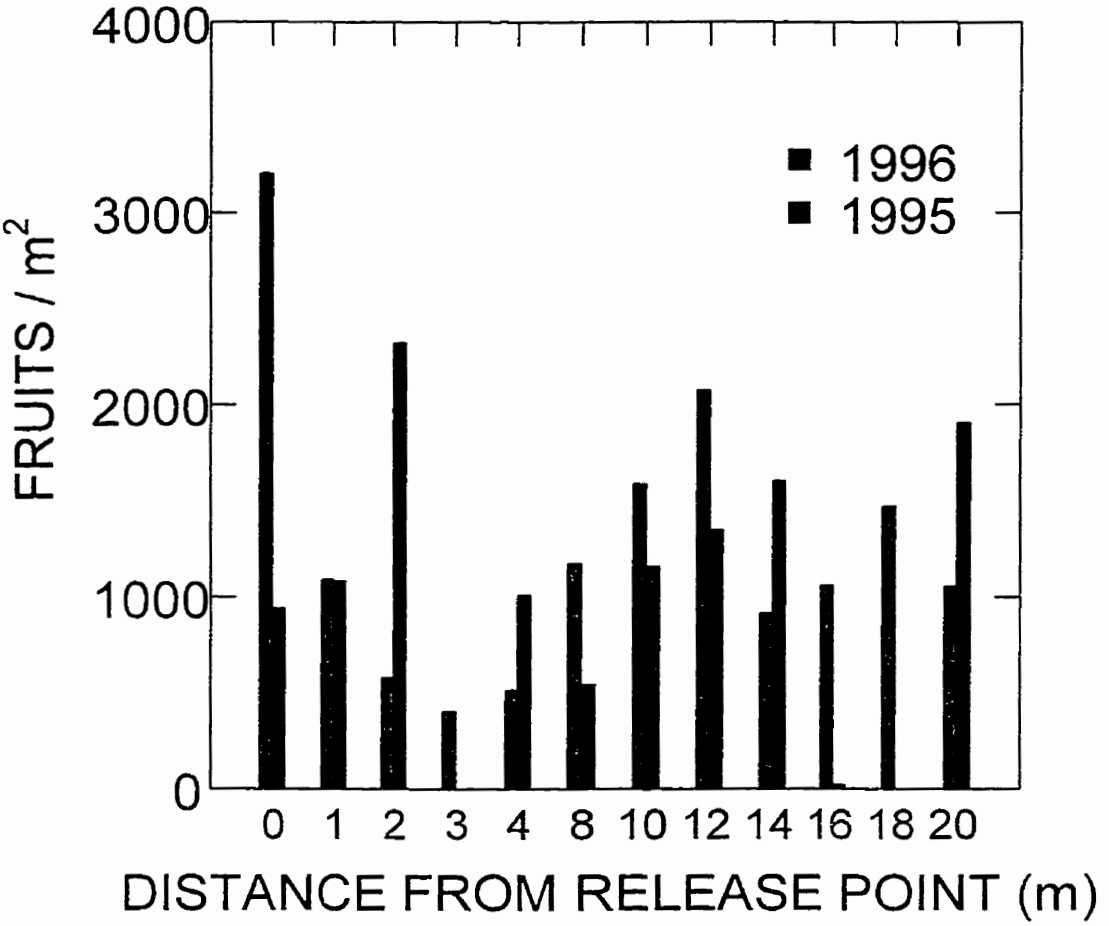


Figure 18. Fruit density at various distances from central release point in 1995 and 1996 along the southeast transect in Libau Marsh-A.

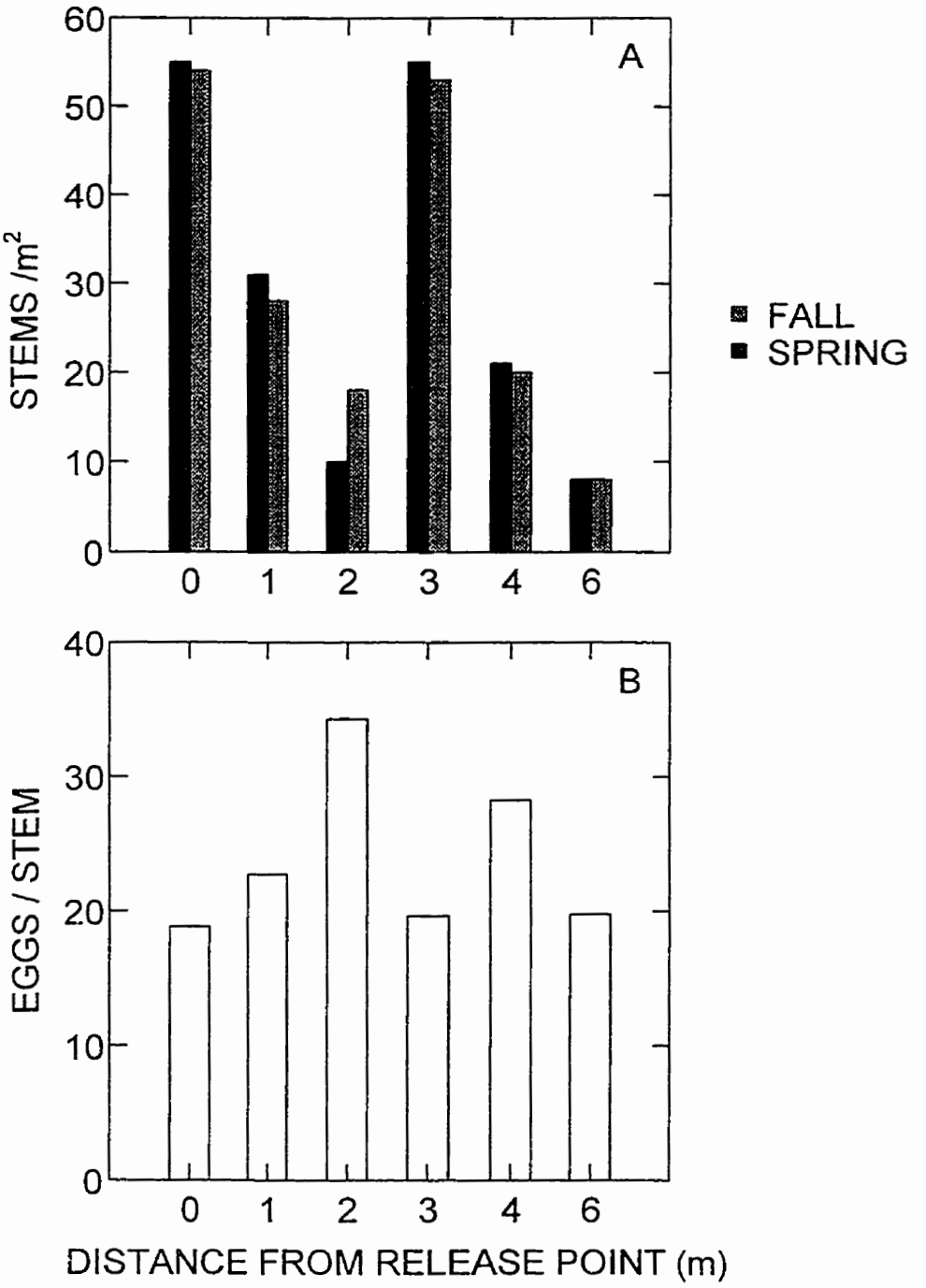


Figure 19. A. *Lythrum salicaria* stem densities in spring and fall of 1996 along the west transect in Libau Marsh-A. B. *Galerucella* spp. eggs /stem in spring at various distances from central release point.

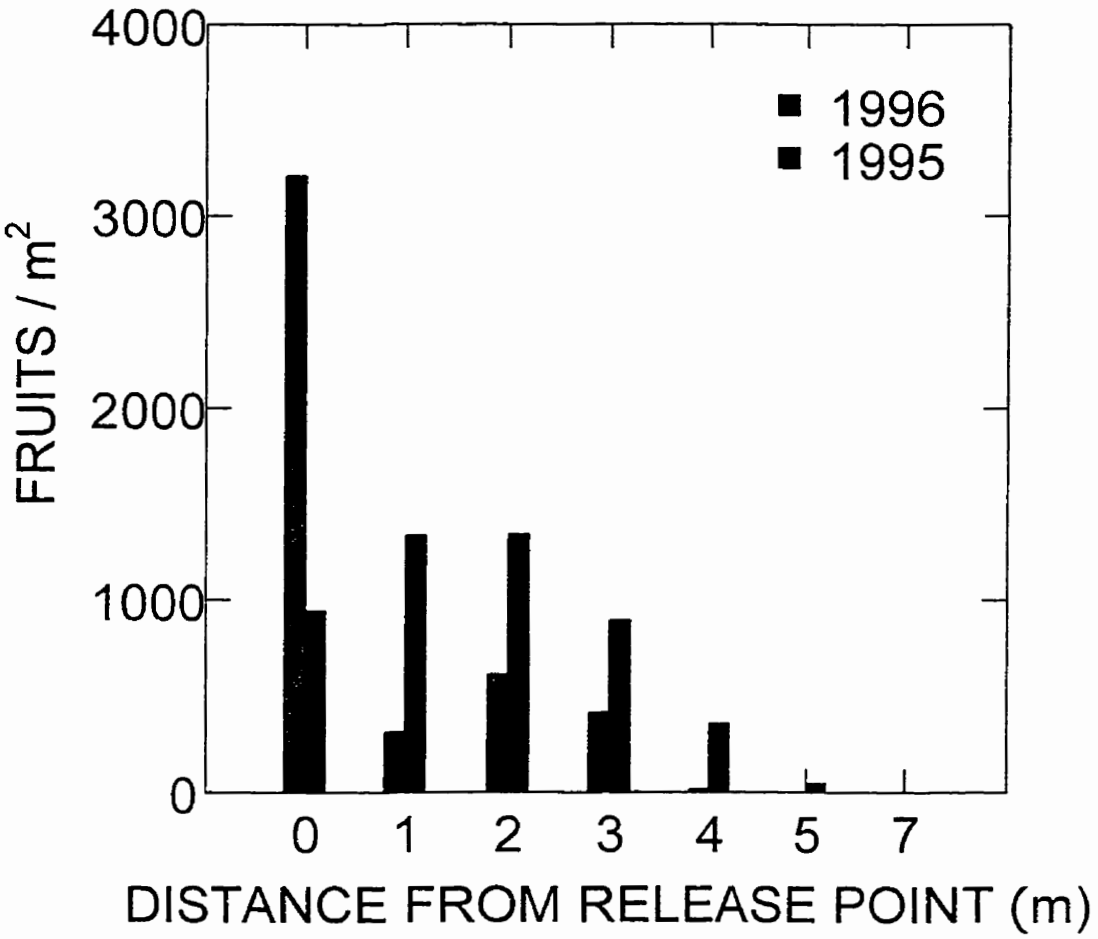


Figure 20. Fruit density at various distances from central release point in 1995 and 1996 along the west transect in Libau Marsh-A.

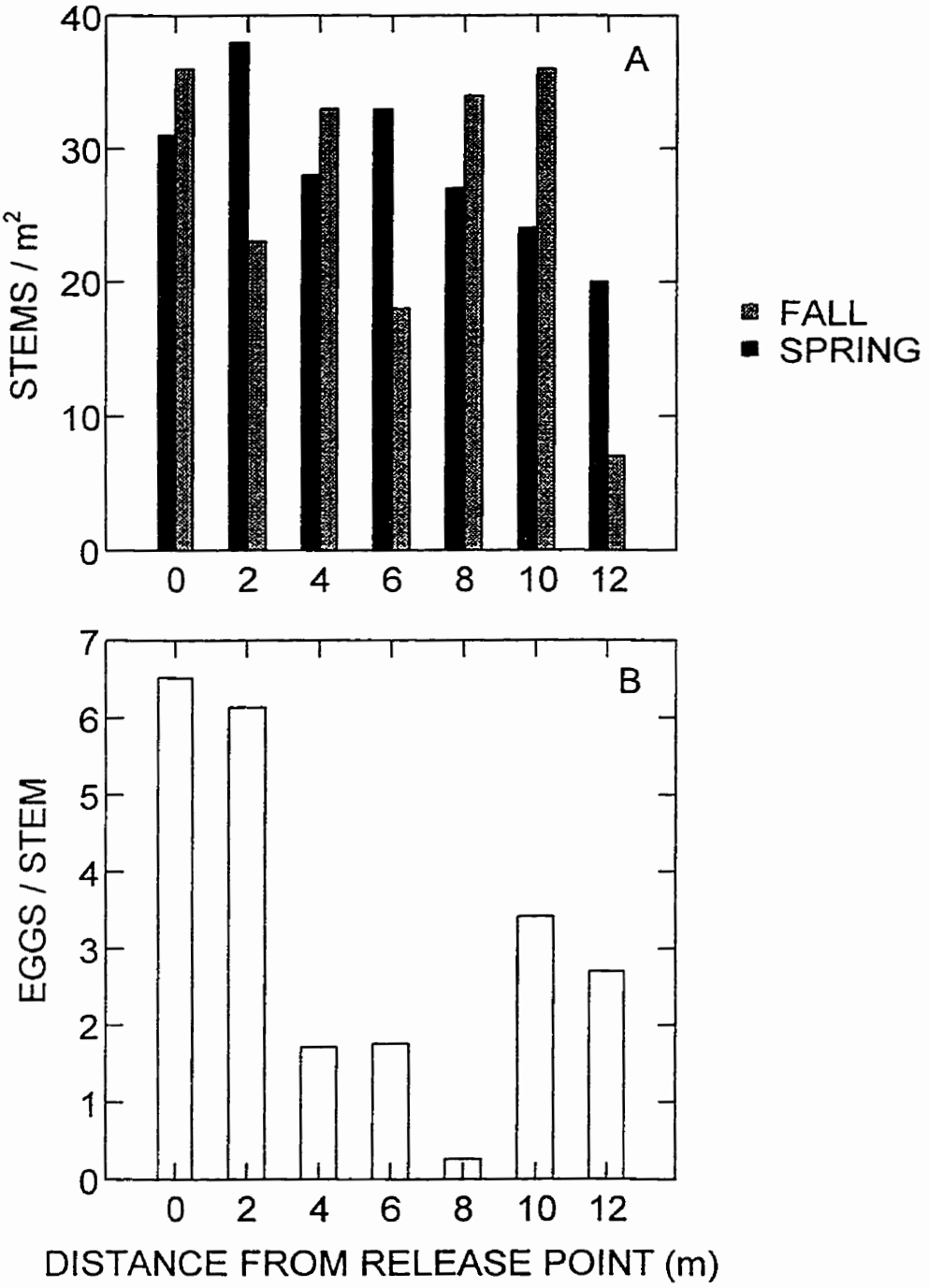


Figure 21. A. *Lythrum salicaria* stem densities in spring and fall of 1995 along the south-A transect in Delta Marsh-A. B. *Galerucella* spp. eggs /stem in spring at various distances from central release point.

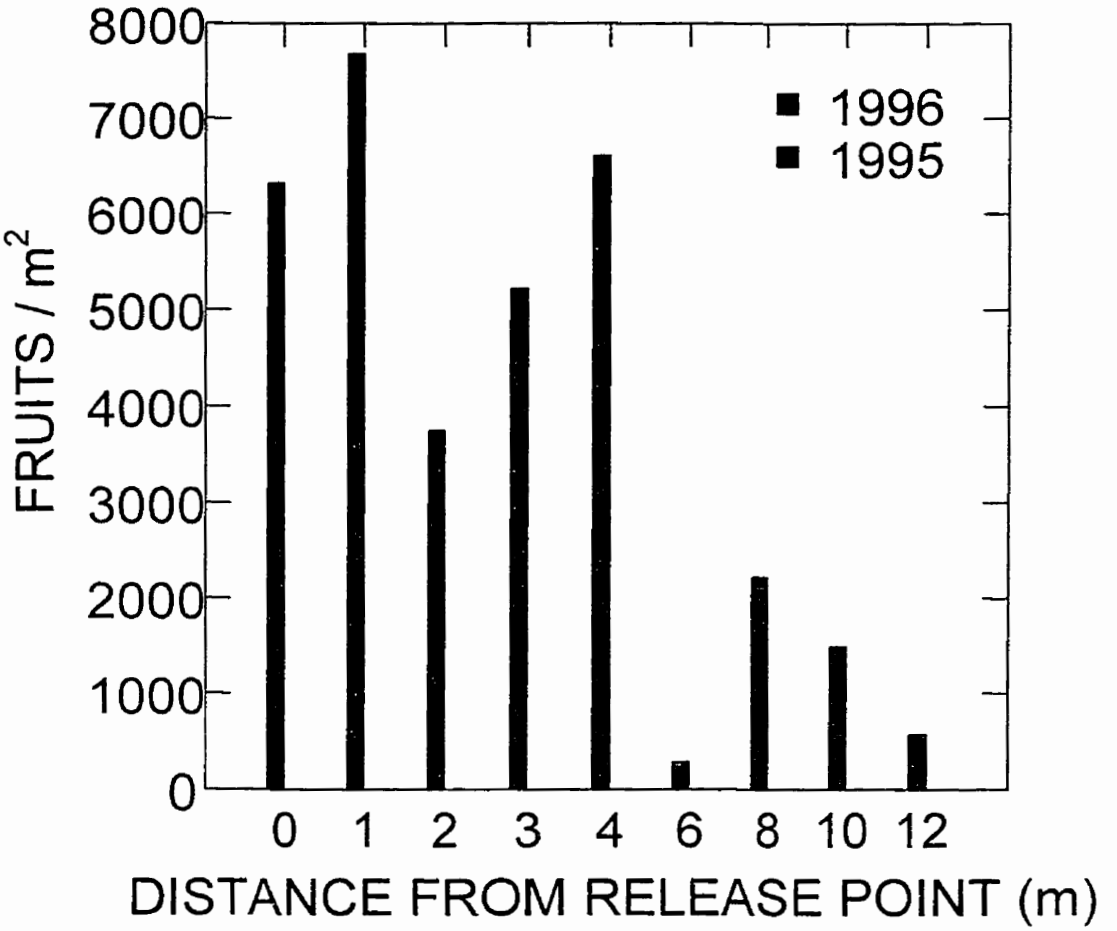


Figure 22. Fruit density at various distances from central release point in 1995 and 1996 along the south-A transect in Delta Marsh-A

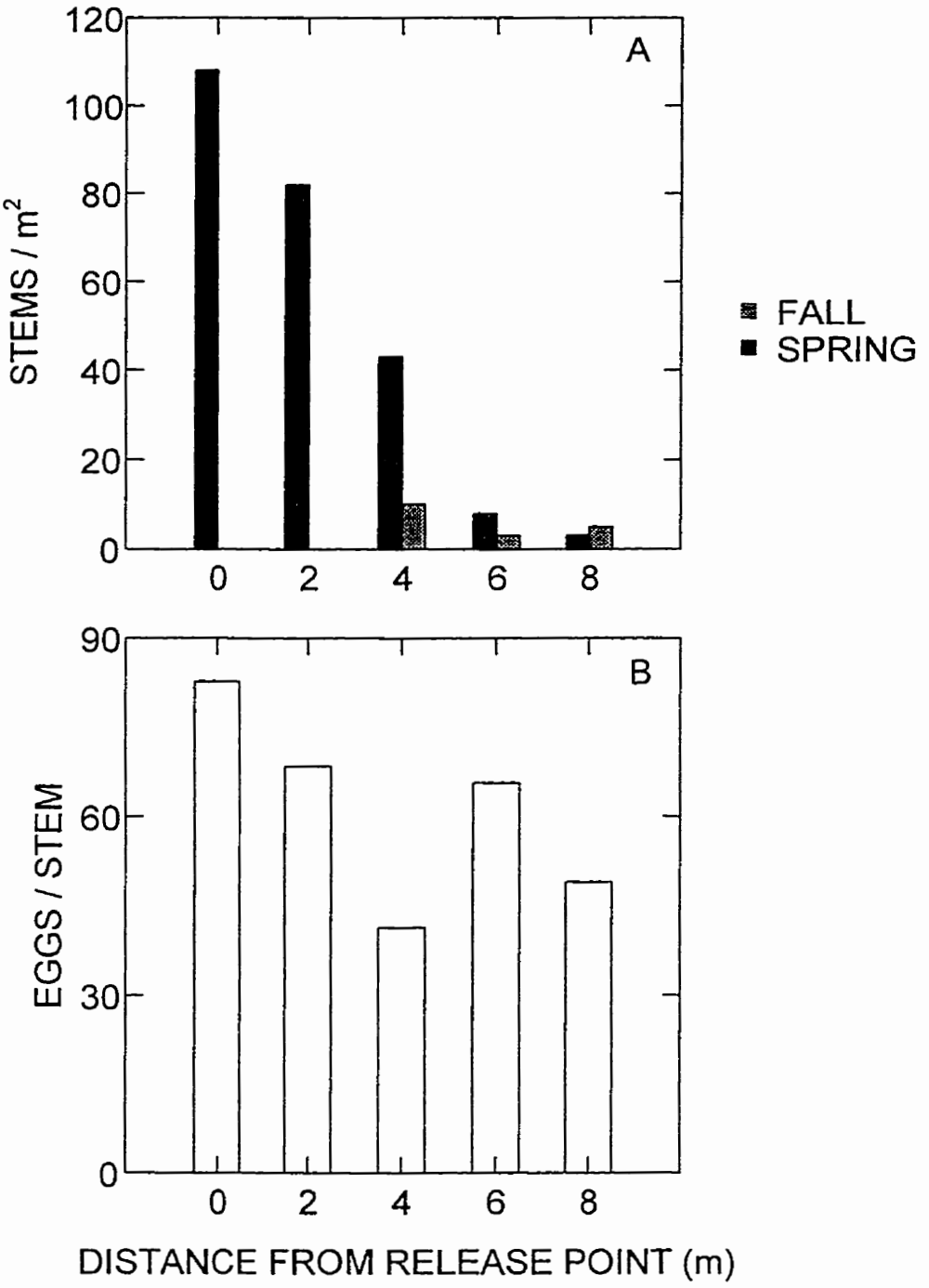


Figure 23. A. *Lythrum salicaria* stem densities in spring and fall of 1996 along the east transect in Delta Marsh-A. B. *Galerucella* spp. eggs /stem in spring at various distances from central release point.

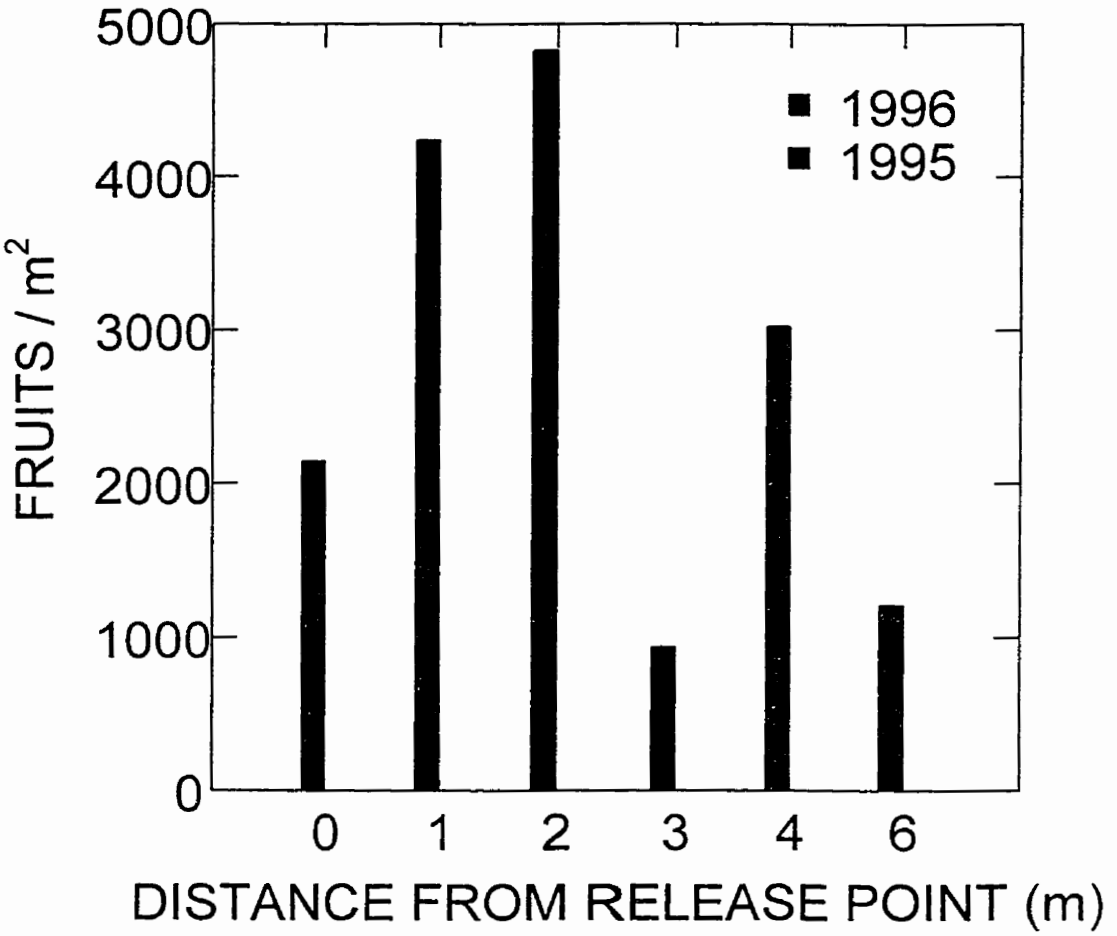


Figure 24. Fruit density at various distances from central release point in 1995 and 1996 along the east transect in Delta Marsh-A.

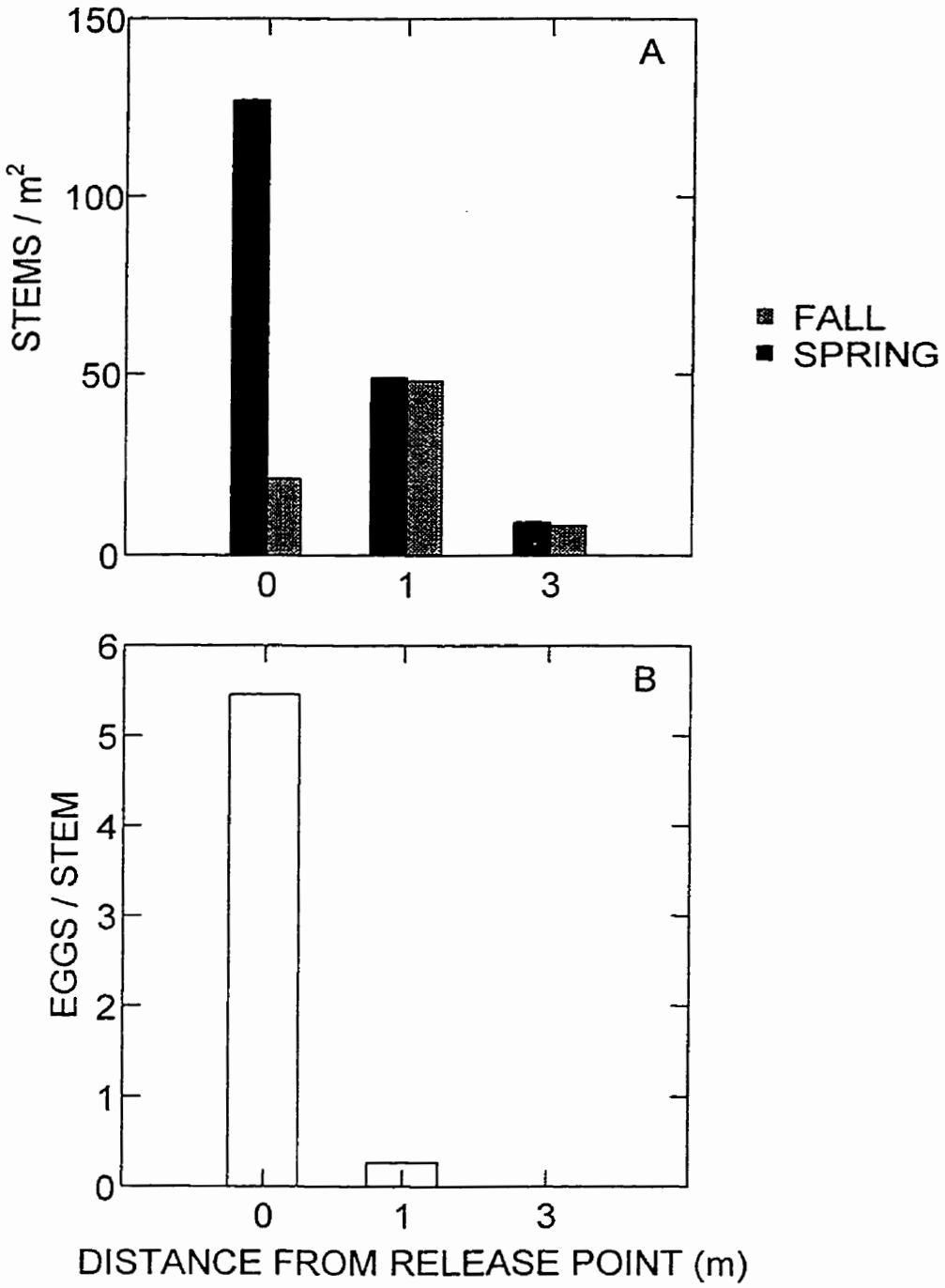


Figure 25. A. *Lythrum salicaria* stem densities in spring and fall of 1995 along the east transect in Delta Marsh-B. B. *Galerucella* spp. eggs /stem in spring at various distances from central release point.

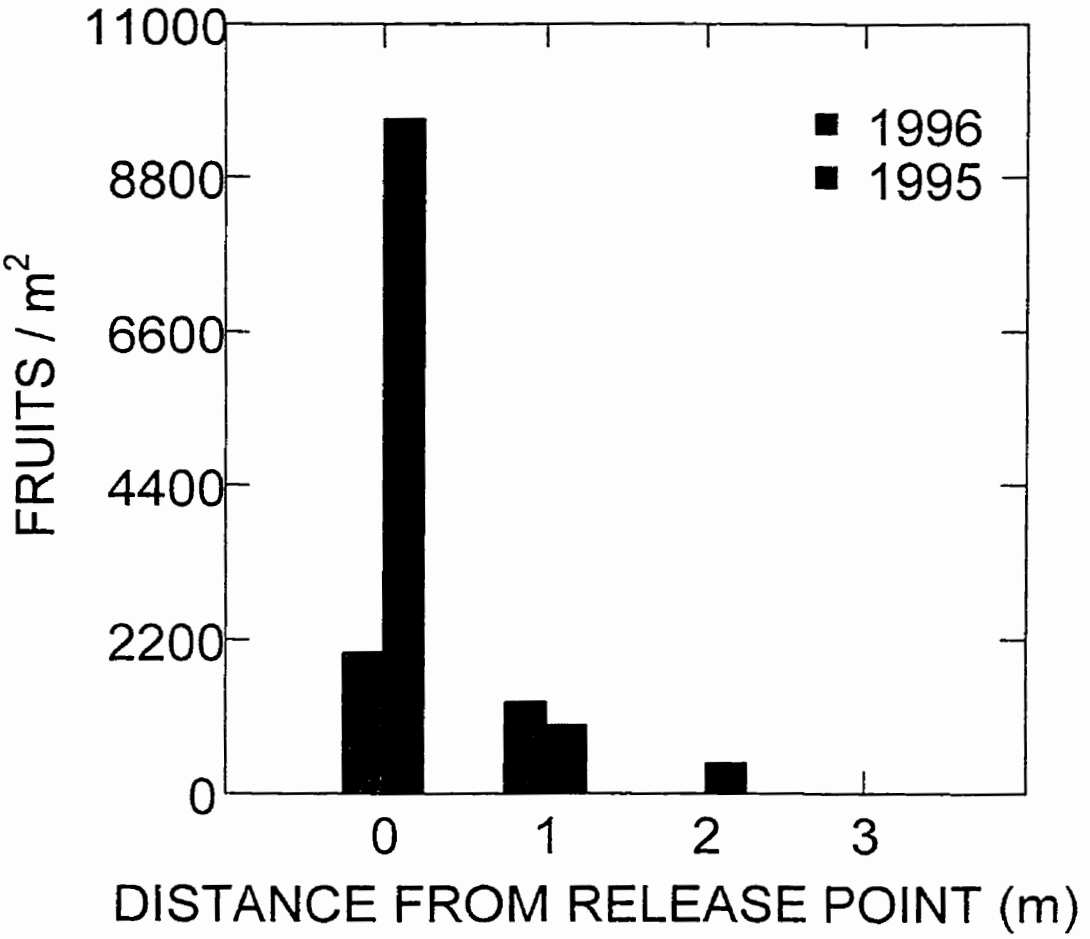


Figure 26. Fruit density at various distances from central release point in 1995 and 1996 along the east transect in Delta Marsh-B.

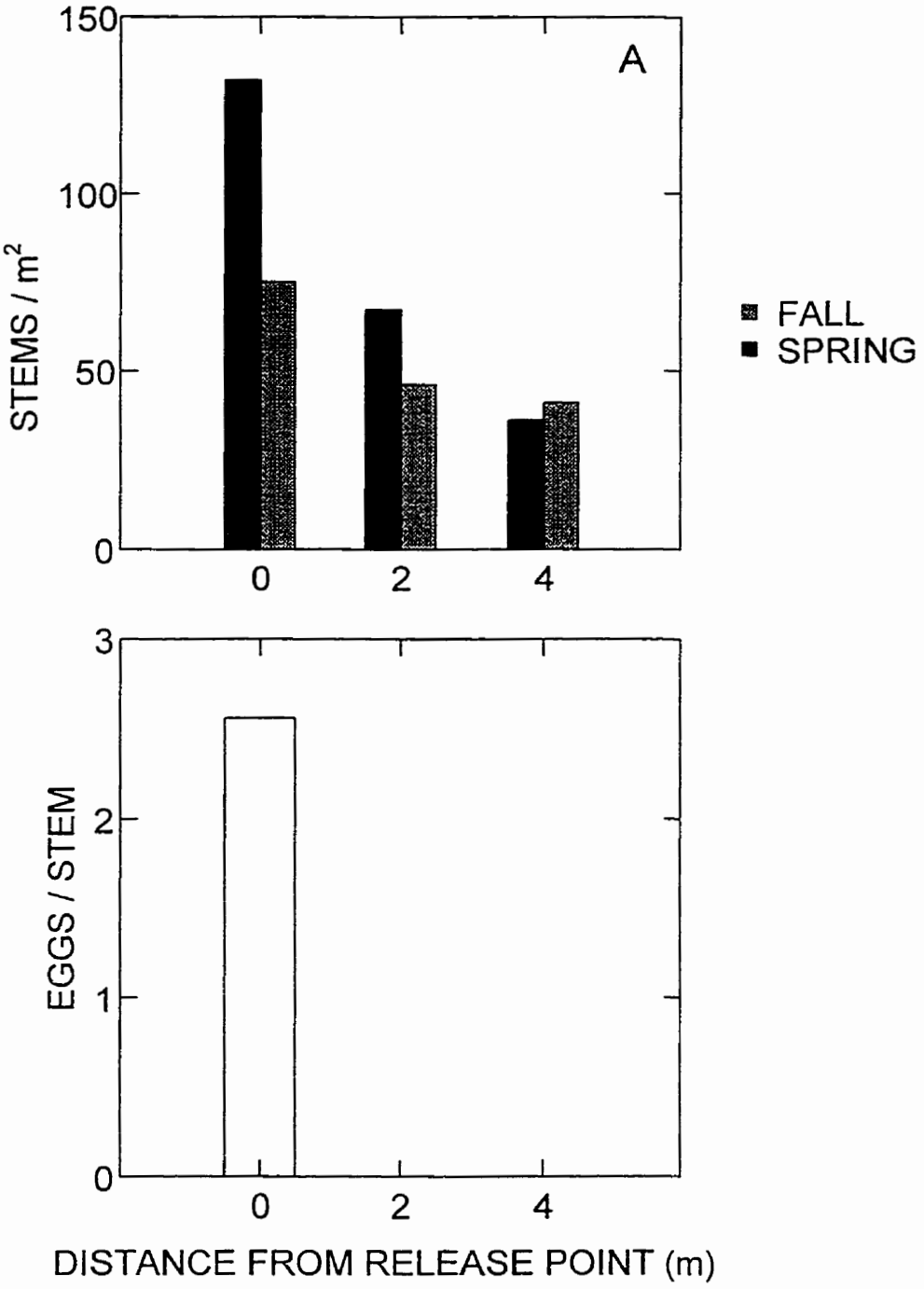


Figure 27. A. *Lythrum salicaria* stem densities in spring and fall of 1996 along the south transect in Delta Marsh-B. B. *Galerucella* spp. eggs /stem in spring at various distances from central release point.

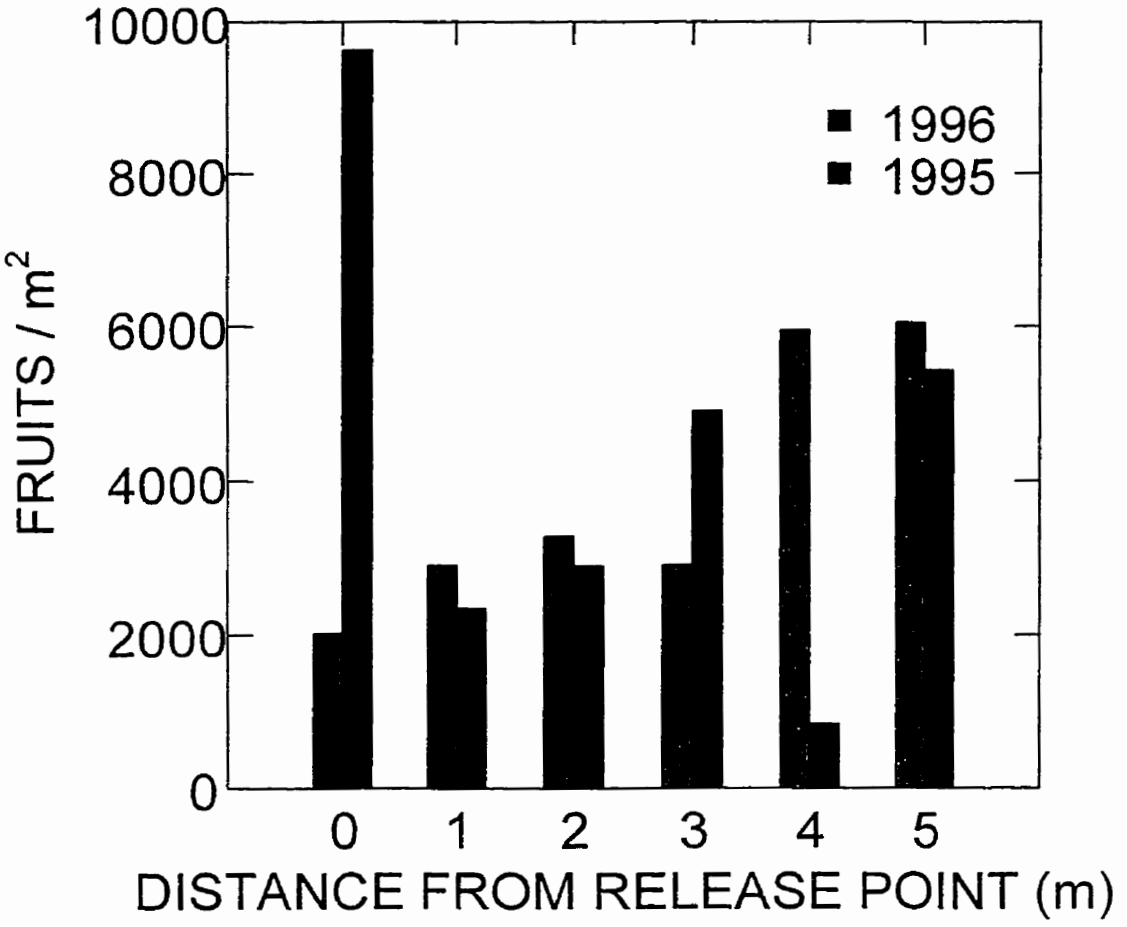


Figure 28. Fruit density at various distances from central release point in 1995 and 1996 along the south transect in Delta Marsh-B.

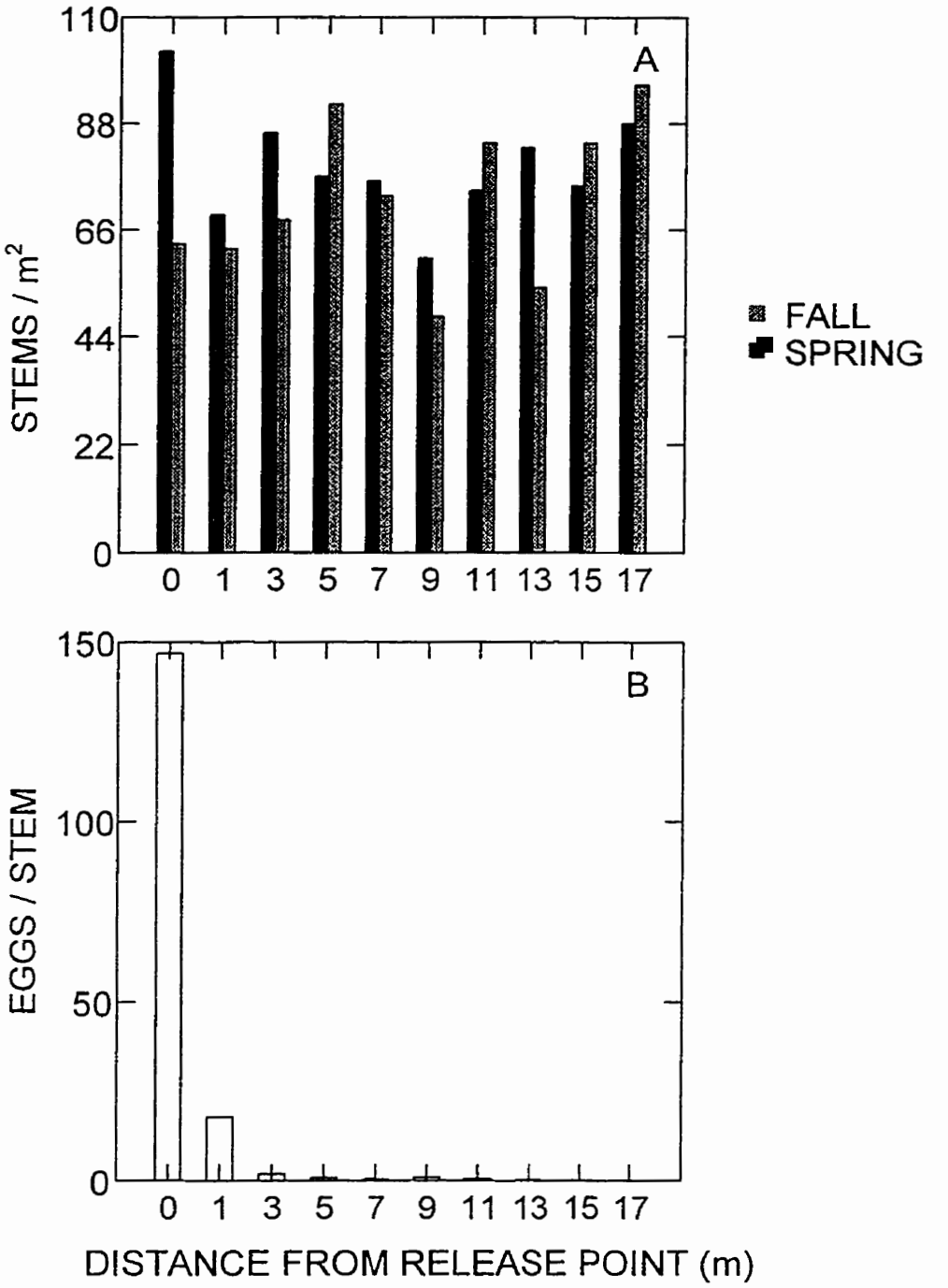


Figure 29. A. *Lythrum salicaria* stem densities in spring and fall of 1995 along the northeast transect in Libau Marsh-B. B. *Galerucella* spp. eggs /stem in spring at various distances from central release point.

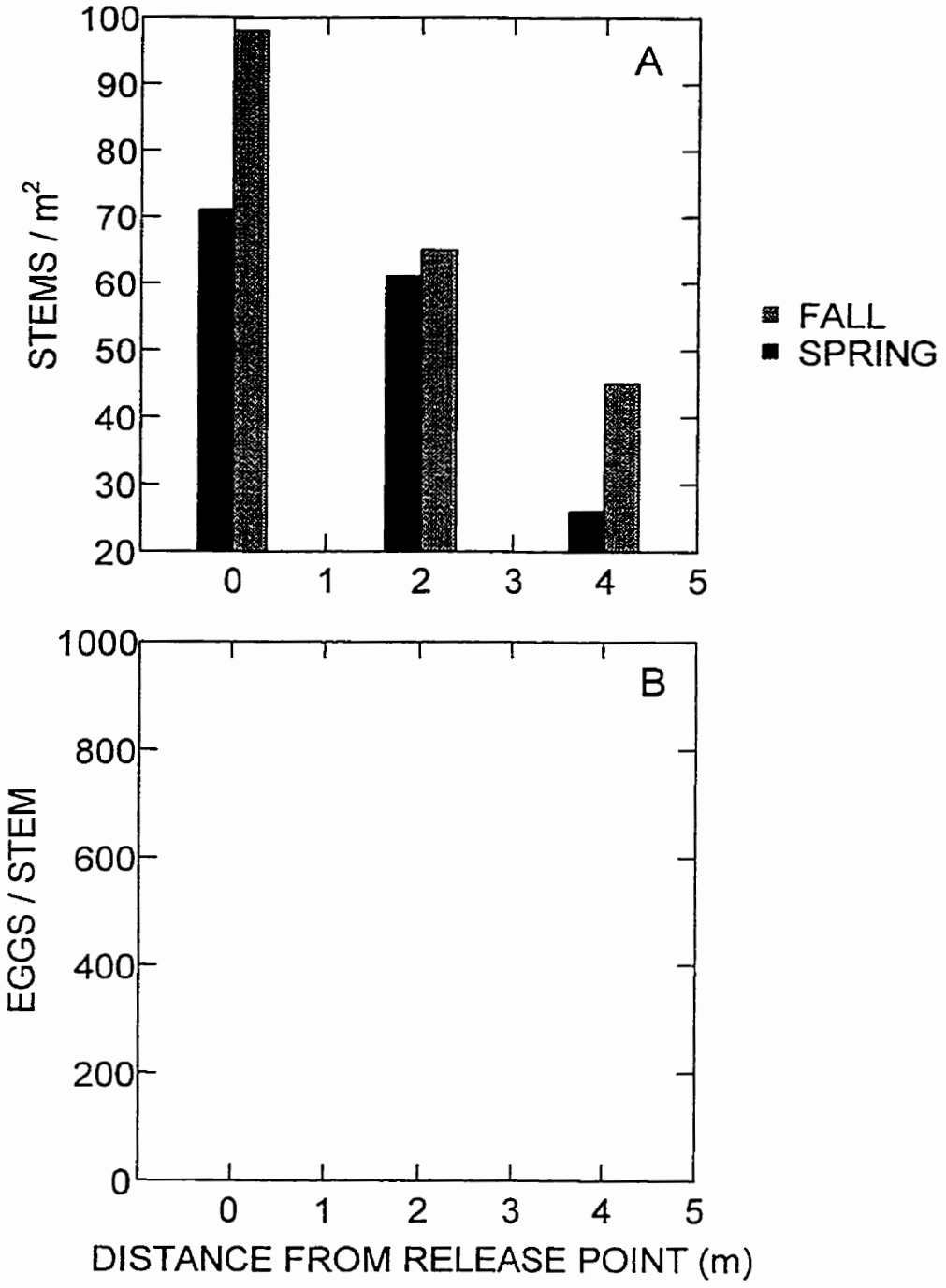


Figure 30. A. *Lythrum salicaria* stem densities in spring and fall of 1995 along the east transect in Libau Marsh-C. B. *Galerucella* spp. eggs /stem in spring at various distances from central release point.

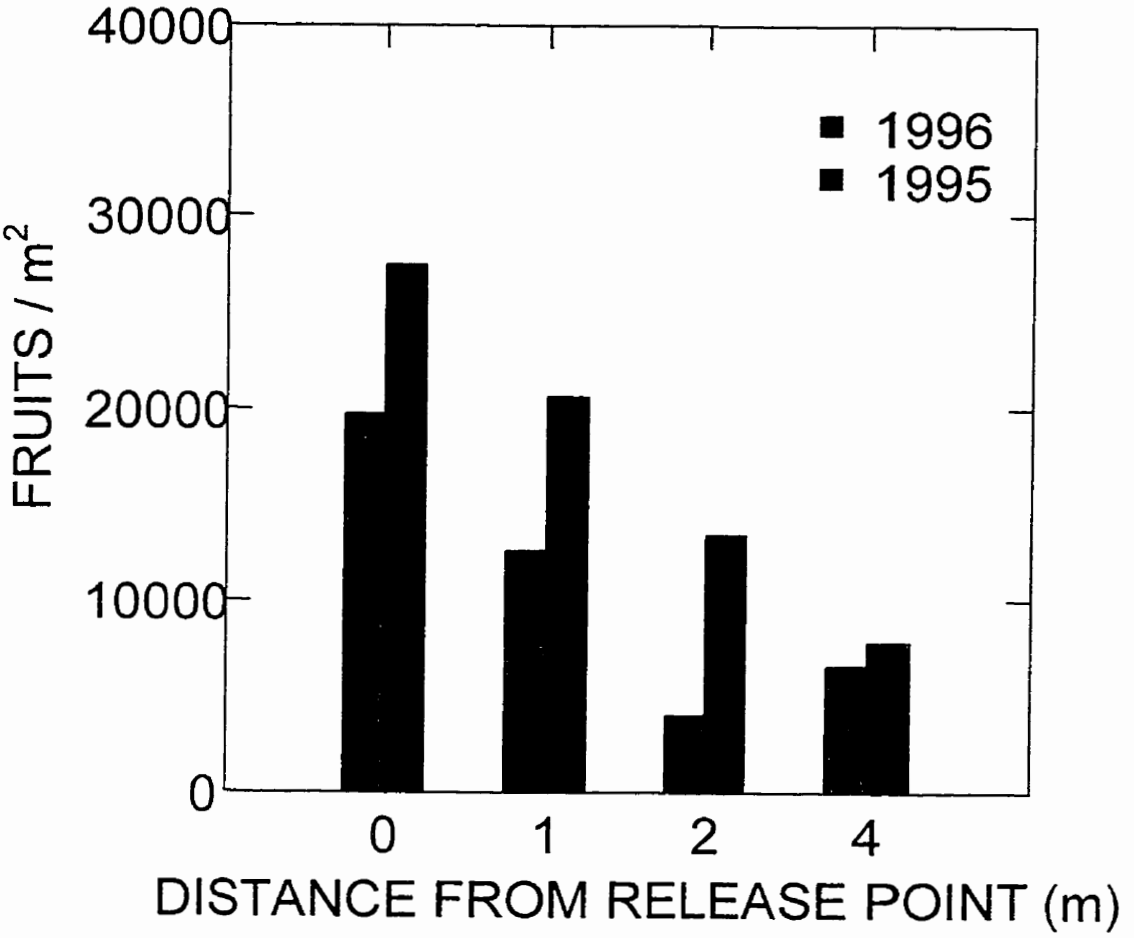


Figure 31. Fruit density at various distances from central release point in 1995 and 1996 along the west transect in Libau Marsh-C.

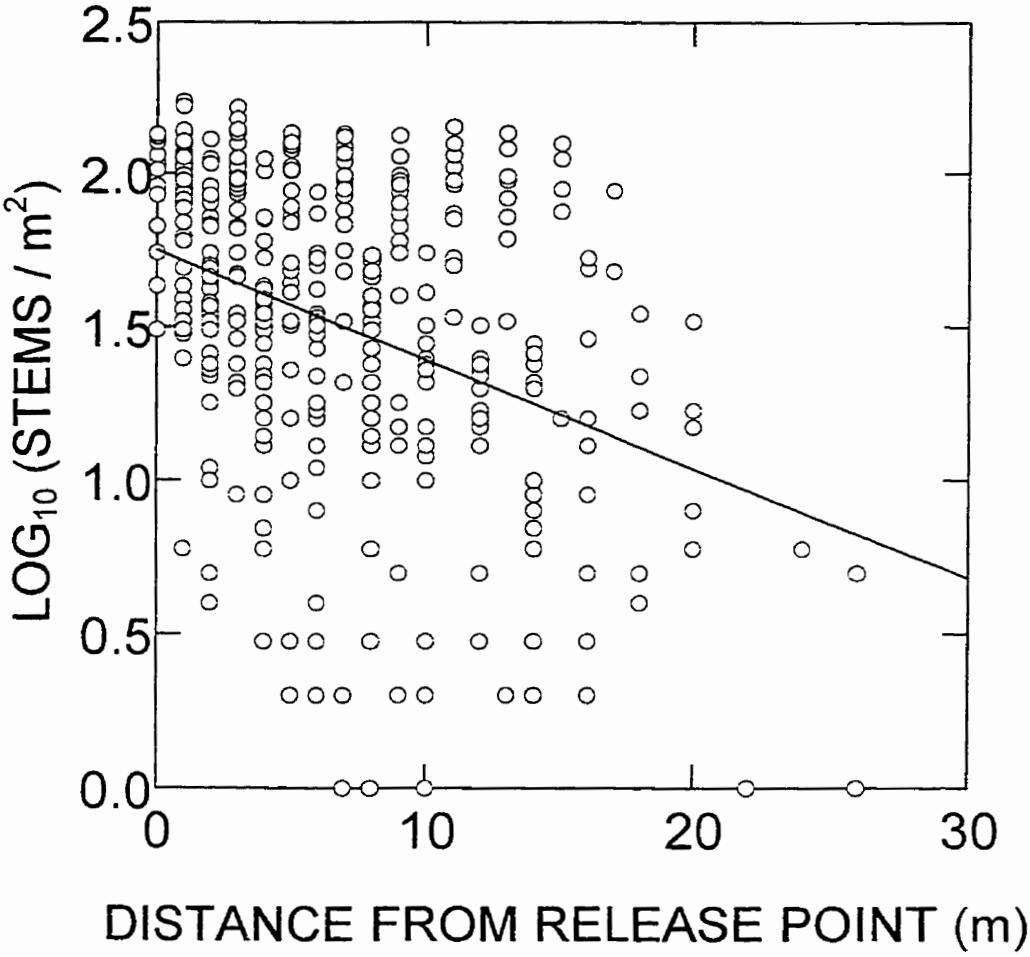


Figure 32. Density (log_{10} transformed) of *L. salicaria* stems in spring in relation to distances from central release point. Figure includes data from all four release sites in 1995 and 1996.

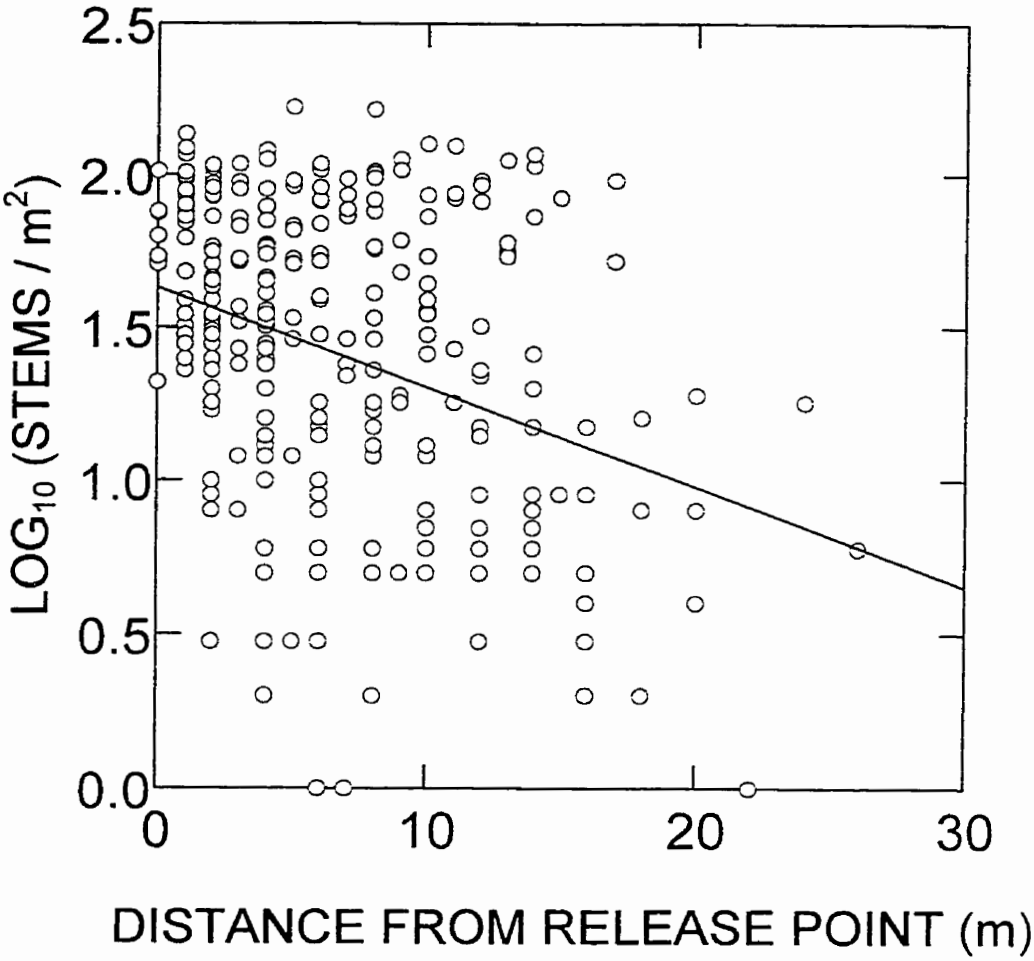


Figure 33. Density (log_{10} transformed) of *L. salicaria* stems in fall in relation to distances from central release point. Figure includes data from all four release sites in 1995 and 1996.

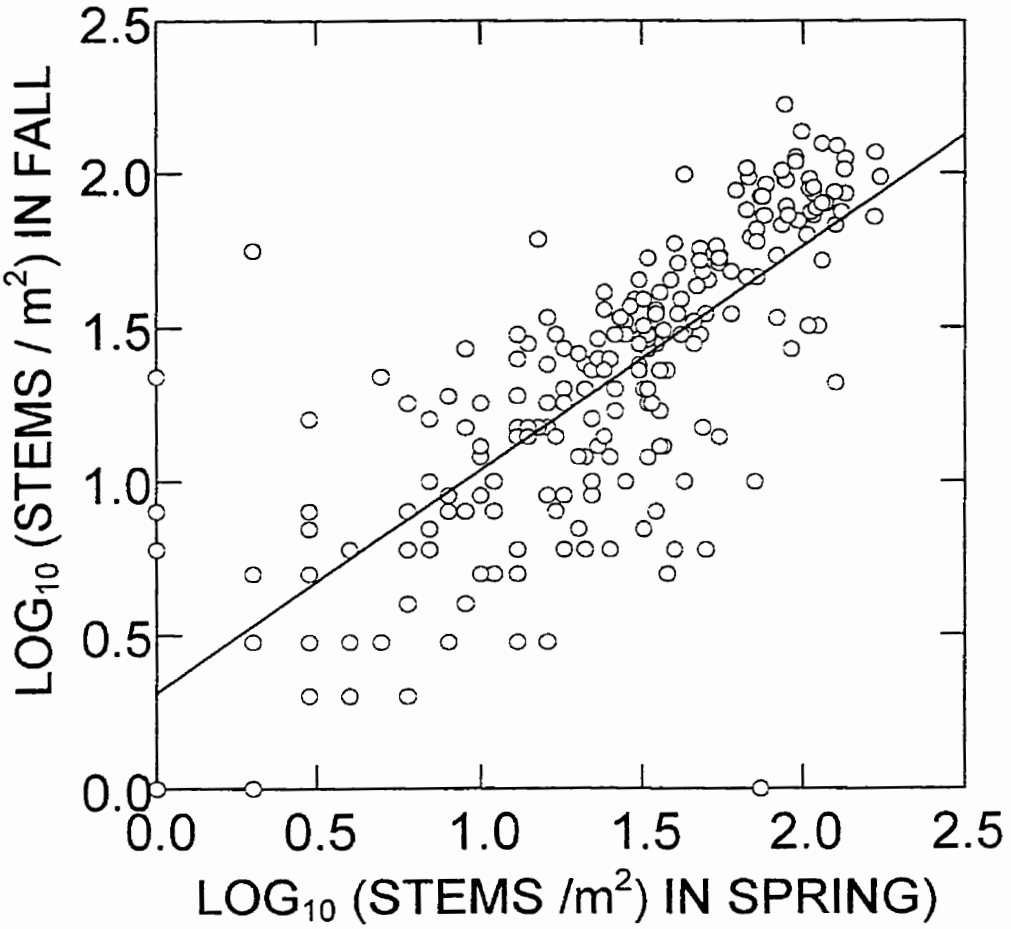


Figure 34. Density (log_{10} transformed) of *L. salicaria* stems in fall in relation to stem density (log_{10} transformed) in the proceeding spring. Figure includes data from all four release sites in 1995 and 1996.

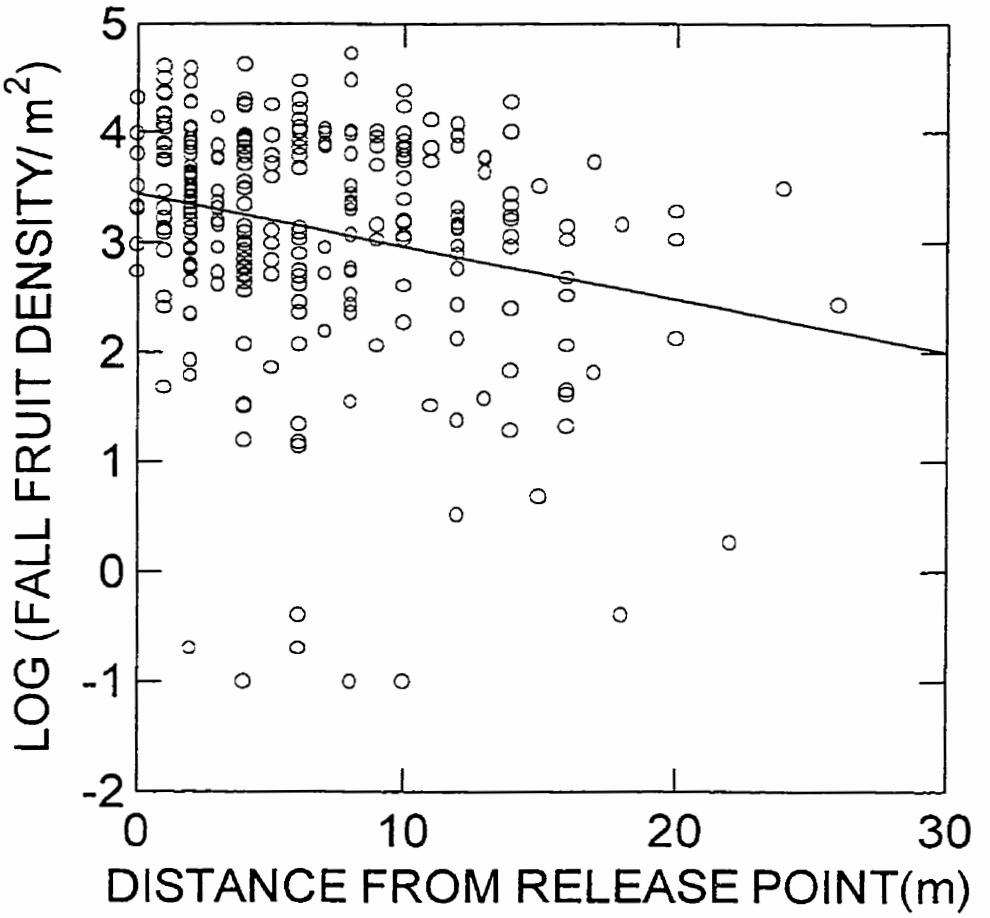


Figure 35. Density (log_{10} transformed) of *L. salicaria* fruits in fall in relation to distances from central release point. Figure includes data from all four release sites in 1995 and 1996.

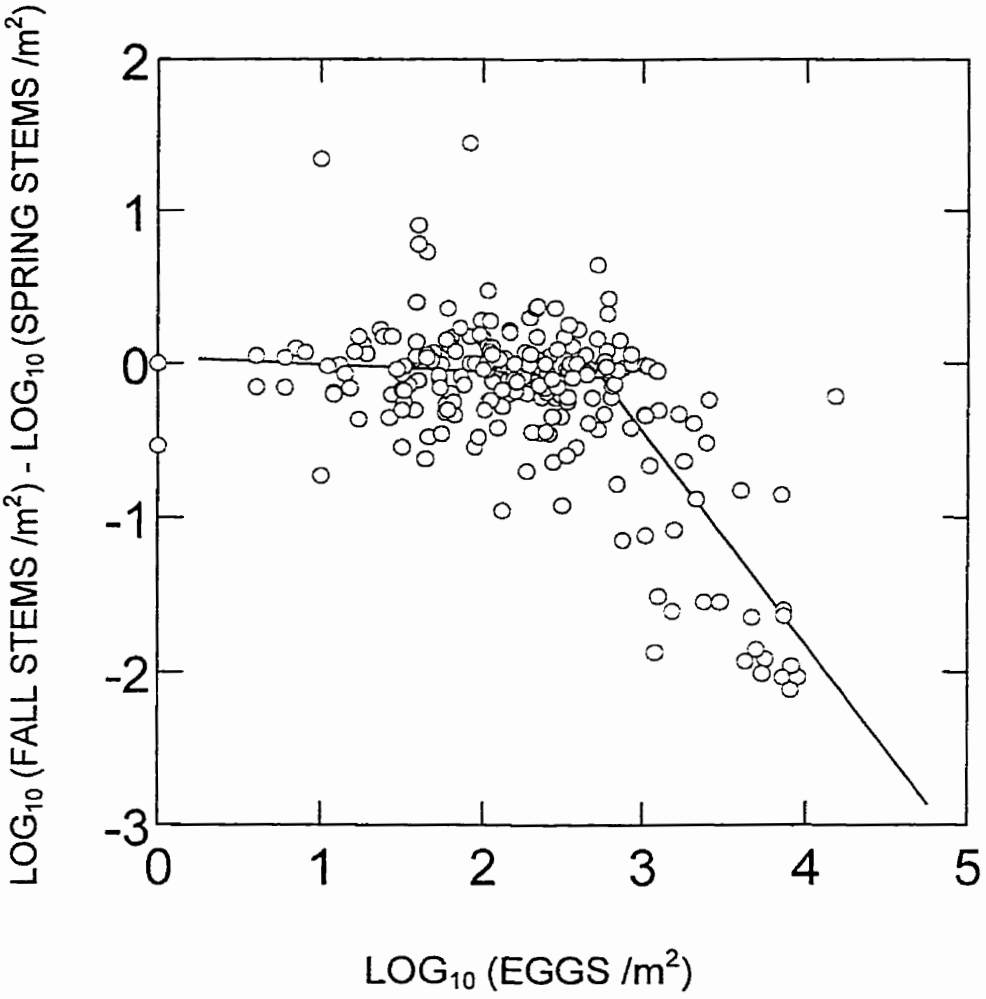


Figure 36. Fall stem density - preceding spring stem density (log transformed) plotted against *Galerucella* spp. egg density (log transformed). Figure includes data from all four release sites in 1995 and 1996.

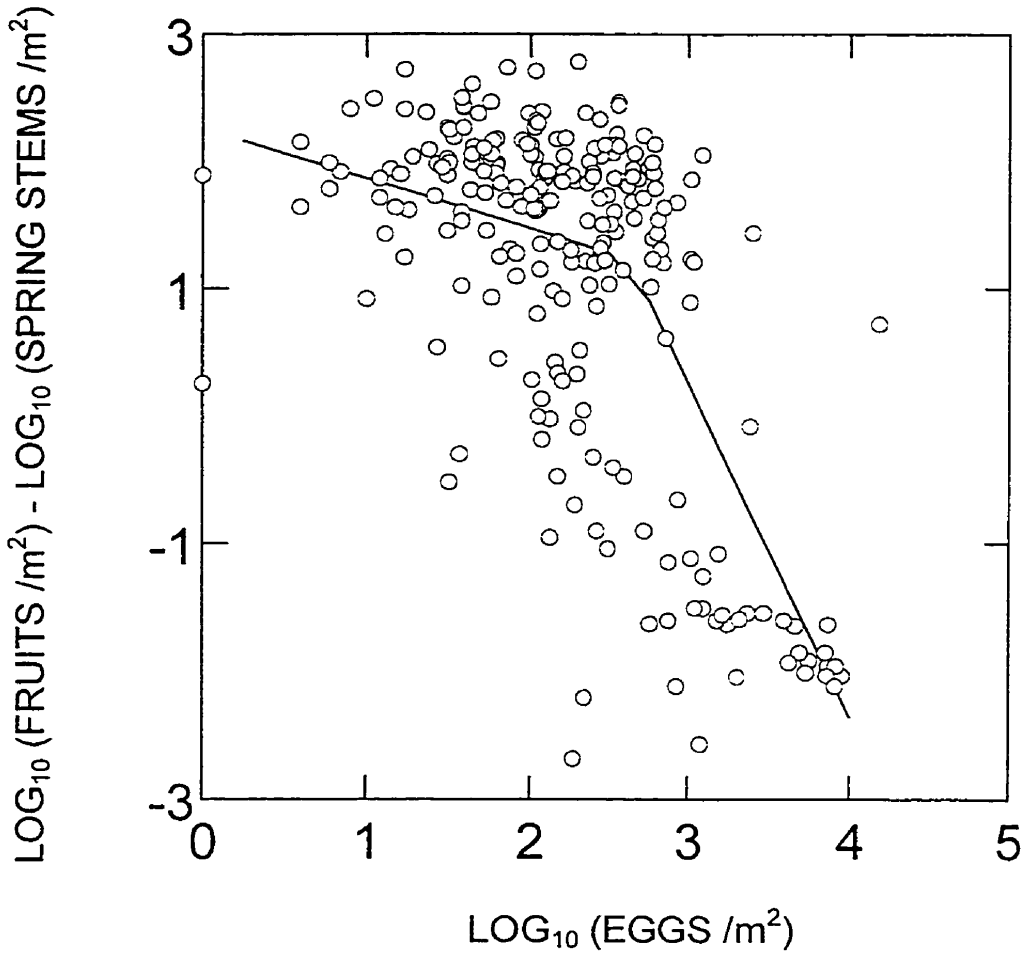


Figure 37. Fruit density – spring stem density (log transformed) plotted against *Galerucella* spp. egg density (log transformed). Figure includes data from all four release sites in 1995 and 1996.

CHAPTER IV

OVERWINTERING ABILITY OF ADULT *GALERUCELLA CALMARIENSIS* (COLEOPTERA: CHRYSOMELIDAE) AND *G. PUSILLA* IN SOUTHERN MANITOBA RELEASE SITES.

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ABSTRACT

During the winter of 1995-96, plots were set up within four release sites in southern Manitoba to examine the overwintering ability of the introduced biological control agents, *Galerucella pusilla* (Dufts.) and *G. calmariensis* (L.) (Coleoptera: Chrysomelidae). In August of 1995, 1-m^2 areas of purple loosestrife vegetation close to the point of release were repeatedly vacuumed with a D-Vac sampler to obtain adult *Galerucella* spp. Collected adults were taken to the laboratory to determine sex, identify to species, and mark. Cages were setup over the vacuumed 1-m^2 areas, and marked beetles were introduced into them. Cages were removed in late September when loosestrife foliage had senesced and all adult *Galerucella* spp. entered the soil. In spring of 1996, cages were replaced and adult *Galerucella* spp. were collected from all five cages during a two week period of emergence ending 12 June, 1996. Overwintered adult *G. calmariensis* were recovered from five of five cages and *G. pusilla* from two of three cages. While outside air temperature dropped below -38.7°C in release sites, temperature at the soil surface or 5 cm below remained relatively stable at -8°C to -2°C, respectively. Despite these constant temperatures, per cent recovery was highly variable within and between overwintering sites in southern Manitoba. There was no difference between the survival of males or females (p-value = 0.56) and no consistent difference between the survival of *G. pusilla* and *G. calmariensis*. A highly significant three-way interaction between species, survival and cage suggests the pattern of species survival was different between cages. It was also determined that adult *Galerucella* spp. can tolerate prolonged periods of spring flooding as overwintered adults with marks were recovered from cages completely flooded by spring runoff.

INTRODUCTION

In summer of 1993, the two European leaf-feeding chrysomelid beetles, *Galerucella calmariensis* (L) (Coleoptera: Chrysomelidae) and *G. pusilla* (Dufts.) were introduced into southern Manitoba to help control purple loosestrife, *Lythrum salicaria* L. (Lythraceae). Both *Galerucella* spp. have similar life cycles and the adults overwinter in leaf litter or soil at the base of *L. salicaria* plants. Hight et al. (1995) stated that adults will also overwinter in hollow loosestrife stems and old inflorescences. Emergence occurs in late May to early June, and adults immediately begin feeding on the meristematic tissues and young leaf tips of emerging purple loosestrife plants (Blossey et al. 1994). Adults feed for a period of one-week before copulation and oviposition begins (Hight and Drea 1991). Eggs of both species are opaque, round, covered with a thin line of frass and laid in batches of variable size on the leaves, stems, axils and flowers of the purple loosestrife plant (Malecki et al. 1993). Newly hatched first instar larvae move to growing tips where they feed on young tissues of newly unfolding leaves (Hight and Drea 1991). Second and third instar larvae feed on all parts of the plant, but generally prefer the undersides of older unfolded leaves or developing flower buds (Hight and Drea 1991). Mature third instar larvae move down the stem and drop off the plant to pupate in the leaf litter or top few centimetres of the soil (Blossey 1992). The pre-pupal and pupal stages together last approximately 10 days, after which the teneral adults emerge and commence feeding. After feeding for approximately one week adults may copulate and produce a partial second generation before overwintering (Blossey and Schroeder 1991).

The distribution of *G. pusilla* and *G. calmariensis* in Europe and Asia is nearly identical, each being found throughout the Palaearctic distribution of *L. salicaria* (Blossey and Schroeder 1991; Blossey, 1995a). In Europe, ninety per cent of purple loosestrife populations show evidence of attack by *Galerucella* spp. (Malecki et al. 1993a). One difference between the two species is that *G. pusilla* is relatively less abundant than *G. calmariensis* in the northern parts of the distribution such as Finland and Sweden. (Blossey and Schroeder 1991; Blossey 1995a). Adult *Galerucella* spp. released in Manitoba were the descendants of insects collected from two different sources in Germany; a southern source in Gelnhausen and a northern location in Meggerdorf (Hight et al.1995). The proportion of *G. calmariensis* collected at the northern collection site was 90%, while in the southern site the proportion of this species was 30% (Hight et al. 1995). In central Europe, mixed species beetle communities dominate and the two species are equally abundant (Blossey 1995a).

Studies in which adult *Galerucella* spp. were forced to overwinter outdoors in PVC tubes lined with fresh moss or oak leaves demonstrated highly variable mean survival rates ranging from 0-40 per cent, and no differences between *G. calmariensis* and *G. pusilla* (Blossey 1995a). During this overwintering study, outside air temperatures averaged between 0 and -5°C and adult beetles were active during warm days in February (Blossey 1995a). The ability of adult *Galerucella* spp. to survive the harsh prairie winters is no longer in doubt and in North America, Hight et al. (1995) estimated the mean survival of *Galerucella* spp. at all 1992 release sites to be 10 per cent. None of the sites used in this overwintering study were included in the initial

estimate of mean survival, as releases of *Galerucella* spp. in southern Manitoba were not made until the summer of 1993.

Climatic conditions of the northern and southern sources of *Galerucella* spp. in Germany are mild and humid with temperatures in the northern collection site ranging from 0 to 5°C during the winter and those in the southern site averaging around 5°C (Hight et al. 1995). Even though *G. calmariensis* was more abundant in the northern collection site, the two sources of adult beetles released in North America were climatically similar and no differences in overwintering ability were observed during initial caged studies conducted in New York and Pennsylvania (Hight et al. 1995). Temperatures during the winter months in these two locations are milder than in southern Manitoba; however, it is known that both species are able to survive extreme winter temperatures on the prairies. Since there is no specific information on survival rates in southern Manitoba, the purpose of this study was to estimate the overwintering mortality of *G. calmariensis* and *G. pusilla* under caged field conditions at several release sites.

MATERIALS AND METHODS

Five overwintering plots were setup in study sites; two cages in Delta Marsh-A, and one each in Delta Marsh-B and Libau Marsh-A and B. Each plot was a 1-m^2 area of purple loosestrife close to or at the point of release. In August of 1995, after adults emerged from pupation, but prior to them entering the soil to overwinter, each plot was repeatedly vacuumed using a D-Vac sampler with a nozzle diameter of approximately 25 cm. When no further *Galerucella* spp. were collected, each 1-m^2 plot was covered

with a 1m-x1m-x1.3m cage. Cages consisted of fine, white colored, polyethylene mesh material held in place using 2.54cm diameter steel tubing at the corners. The gauge of the monofilament mesh screen was number 8 with 25 squares per 2.54 cm horizontally and 23 squares per 2.54 cm vertically. The bottom perimeter of the cage was dug into the ground to prevent adults from escaping.

Collected *Galerucella* spp. were taken to the laboratory to determine sex, identify to species and mark. Two techniques were used to mark adult beetles; the first consisted of a small amount of waterproof nail polish placed on a single elytron and the second was the branding of an elytron with a red-hot pin. After marking, adult beetles were allowed to recover for 24 hours in the laboratory where they were supplied with an abundance of fresh purple loosestrife vegetation. In most cases, the adult *Galerucella* spp. were reintroduced to plots from which they were initially collected. There were no *Galerucella* spp in Delta Marsh-B, so adults from Delta Marsh-A were marked and released into the overwintering cage in this site. In the case of Libau Marsh-B, adults were obtained from the Brookside Greenhouse rearing facility in Winnipeg, MB. (Table 10). Numbers of adults in these two sites were small and it was difficult to obtain enough individuals for experimental purposes.

Overwintering cages were removed in late September when loosestrife foliage had senesced and adult *Galerucella* spp. were not visible above ground. The steel tubing which anchored the four corners of the cage was left in the ground and the mesh portion of the cage was removed. Air temperature and temperature at the soil surface and at a depth of 5 cm in overwintering cages were measured using Hobo XT™ temperature data loggers. Data loggers placed into Libau Marsh-B to monitor air and

soil temperature failed and no data was collected. In Libau Marsh-A, only daily mean air temperature and temperature 5 centimeters below the soil surface were recorded during the winter of 1995-96. In Delta Marsh sites, Hobo™ data loggers functioned correctly and all temperature data were obtained. In mid-March of 1996, just as the snow began to melt, cages were replaced over the steel tubing in the exact position as before. Initially, cages were not dug into the ground due either to the presence of a thick layer of ice or the ground still being frozen. Beginning at the end of May, sites were visited weekly, and any adult *Galerucella* spp. in cages were removed and taken to the laboratory to examine what proportion of each species and sex survived the winter. Sampling ceased and cages were removed when no additional beetles emerged. Data were analyzed using a loglinear modeling technique of contingency tables within the Loglin module of Systat for Windows, standard version 7.0 with delta = 0.5. This technique was used to analyze the relationship of survival with species, sex and cage.

RESULTS

Adult *Galerucella* spp. Survival

Fall release and spring recovery data of marked adult *Galerucella* spp. from overwintering cages in four release sites are presented in Table 10. In spring of 1996, marked adult *Galerucella* spp. were recovered from each cage during a two-week emergence period ending 12 June. Adult *G. calmariensis* were recovered from all five overwintering cages and *G. pusilla* from two of three cages. The majority of marked beetles recovered from cages were identified by the presence of waterproof nail polish

on the elytron and only a few beetles with branded elytra were recovered. The technique of elytral burning caused higher mortality during the 24 hour recovery period compared to marking the elytron with nail polish (Diehl, personal observation). Many adult *Galerucella* spp. without marks were also recovered from overwintering cages and it was decided not to include these data in the analysis.

Per cent recovery of *G. calmariensis* ranged from 2 % in Libau Marsh-B to 82 % in Delta Marsh-B, while that of *G. pusilla* ranged from 0 % in Delta Marsh-B to 47 % in Delta Marsh-A. There was no significant difference between the survival of males or females (Log ratio $\chi^2 = 0.28$, d.f. = 3, $p > 0.05$). However, a significant effect of species on survival ($\chi^2 = 13.56$, d.f = 3, $p < 0.05$) was identified. However, the significance of this interaction was heavily influenced by the results obtained from the cage in Delta Marsh-B. In Delta Marsh-A & -B, the overwintering cage did not significantly affect survival ($\chi^2 = 0.76$, d.f. = 2, $p > 0.05$), however, the interaction of cage and species significantly affected survival ($\chi^2 = 58.75$, d.f. = 2, $p < 0.05$); thus the pattern of species survival was different in different cages.

Conditions in Plots

At the time of adult re-introduction in fall of 1995, all overwintering sites were dry and purple loosestrife plants had finished flowering. However, in spring of 1996, plots in Delta Marsh-A and Libau Marsh-B release sites were completely flooded with 40-50 cm of water from spring runoff. Delta Marsh-B and Libau Marsh-A overwintering plots remained dry, even though water from spring runoff was present in these sites.

Daily mean air temperature in Libau Marsh-A (Fig. 38) was variable, reaching - 38.7°C or lower at the start of February and gradually warming to above 0°C in April. Hobo™ data loggers do not record temperatures below -38.7°C and at Gimli Industrial Park, MB (50° 37' 38" N 97° 02' 04" W), approximately 45 km North of Libau, outside air temperature dropped to – 42.6°C at the start of February. Daily mean temperature 5 cm below the soil surface was constant at - 2°C from the end of December to the beginning of May, after which time it warmed up and reached a peak of 22°C near the start of June (Fig. 39). Even though outside air temperatures were below - 30°C near the end of January, the temperature 5 cm below the soil surface was constant at - 2°C during the entire winter of 1995-96.

Daily mean temperature at the soil surface in Delta Marsh-A (West Release Point) was relatively constant and ranged from -8.5°C at the beginning of December to - 1°C near the end of February (Fig. 40). At the east release point soil surface temperatures were less variable, ranging from -4.5°C in early February to -1.5°C near the end of March (Fig. 41). Data loggers monitoring outside air temperature during the winter months in Delta Marsh-A failed and no data were recorded. In spring of 1996, daily mean outside air temperature (Fig. 42) and temperature 5 cm below the soil surface (Fig. 43) were recorded from the west release point in Delta Marsh-A. Soil temperatures were constant at -1°C in April and gradually increased to 18°C near the end of May. While temperatures 5 cm below the soil surface were constant at - 1°C for the month of April, outside air temperatures were above 0°C.

During the winter of 1995-96, air temperatures at Delta Marsh-B ranged from a high of 0°C to a low of -38.7°C during the middle of January (Fig. 44). Temperatures at

the soil surface remained were constant at about - 2°C from 1 December to the beginning of April (Fig. 45).

DISCUSSION

Some adult *Galerucella* spp. were recovered from overwintering cages without marks and it is possible that some beetles lost their marks during the winter. Another alternative is that some beetles were not vacuumed and remained in the 1 m^2 area when the cage was erected. As neither scenario can be proven, it was decided not to include unmarked individuals in the data set.

Adult *Galerucella* spp. released in North America in 1992 were the descendants of insects collected from either a southern source in Gelnhausen, Germany or a northern location in Meggerdorf (Hight et al. 1995). Despite the source of *Galerucella* spp., the majority of initial introductions established the following year and overall survival of the beetles was estimated to be 10% (Hight et al. 1995). In southern Manitoba introduced *Galerucella* spp. are able to survive the harsh prairie winter; however, the overwintering ability of beetles was highly variable, ranging from 0-82 %. Some of this variability can be explained by a lack of replication within sites combined with differences within and among sites such as local topography, fall moisture, amount of snow cover & spring runoff and the source of beetles. Due to differences between sites, it was decided not to pool the data to determine mean recovery rates of either species.

Results of the overwintering study in Germany were also variable with survival ranging from 0-40 % in PVC tubes containing soil lined with oak leaves or *Sphagnum* moss kept outdoors or in a concrete bunker (Blossey 1995a). In agreement with

Blossey (1995a), we observed no difference in survival between males and females of either species. According to Hight et al. (1995), the source of beetles, amount of litter, number of erect dead stems, and density of host plants did not influence overwintering success and subsequent establishment of adult *Galerucella* spp. under caged field conditions in New York and Pennsylvania.

Protection from low winter temperatures depends primarily on the insulating power of the substrate and of any overlying snow (Danks, 1991). Temperature data measured in release sites demonstrate that while outside air temperature dropped below -38.7°C, the temperature at and 5 cm below the soil surface was relatively constant at -8°C and -2°C, respectively. Adequate snow cover on top of leaf litter and overwintering adults helped to maintain a constant temperature just below freezing at or below the soil surface.

Along with cold temperature, predators and parasites are likely sources of adult mortality during the late fall and early spring, prior to overwintering and emergence. Unfortunately, in North America, there is no information regarding predators and parasitoids that attack overwintering adult *Galerucella* spp. in leaf litter. In southern Manitoba, the predatory stink bug, *Apoecilus bracteatus* Fitch (Hemiptera: Pentatomidae), only attacks the soft-bodied larval stage of *Galerucella* spp. (Diehl et al. 1997) and is not a factor resulting in winter mortality. While studying the subnivean invertebrate activity in southern Manitoba, Aitchison (1977) found that generalist predators such as staphylinids were extremely abundant in October and November, carabids were active down to – 4°C and 49 species of arachnids were active during the entire winter. As temperatures at the soil surface ranged from –1°C to –8.5°C, it may

be possible for some winter active invertebrates to utilise overwintering adult *Galerucella* spp. as a potential food source, thus contributing to mortality during the winter. Also, handling of individual adults was extensive during the procedure of sex and species identification and it is possible that some adult beetles may have died after the 24 hour recovery period.

Two overwintering plots were present in Delta Marsh-A and per cent recovery of both *G. pusilla* and *G. calmariensis* varied between them. Both plots had a similar amount of snow cover during the winter; however, temperatures at the soil surface were slightly different. At the east release point the lowest recorded temperature at the soil surface was -4.5°C, compared to -8.5°C at the west release point; both species of *Galerucella* survived at the colder plot. Some of the variability in per cent recovery may be explained by differences in microclimate between the two cages.

Adult *Galerucella* spp. recovered in Delta Marsh-B were originally from Delta Marsh-A and a similar per cent recovery of adults was expected from both sites; however, this did not occur. The highest per cent recovery of *G. calmariensis* and lowest per cent recovery of *G. pusilla* were obtained from Delta Marsh-B. Despite low daily mean air temperatures during late January, temperatures at the soil surface were constant at -1 °C to -3°C. Temperatures at the soil surface were slightly warmer compared to Delta Marsh-A and recovery of *G. calmariensis* was highest in this site. No *G. pusilla* were recovered from the overwintering cage in Delta Marsh-B, despite the release of more than 100 individuals. The highly variable per cent recovery of both species from the overwintering cage in Delta Marsh-B was responsible for the nearly significant

interaction between species and survival. For this reason we conclude that no consistent difference in survival exists between *G. pusilla* and *G. calmariensis*.

In Libau Marsh-A, per cent recovery of adult *G. calmariensis* was close to 50%. Snow cover was good during the winter of 1995-96 and temperatures 5 cm below the soil surface were constant at -2°C, while outside air temperatures dropped below -38.7°C at the start of February. Good per cent recovery of adult *G. calmariensis* from Libau Marsh-A was expected as beetles have completed three generations and survived two winters since initial introduction in 1993. The source of adult *G. calmariensis* used in the Libau Marsh-B overwintering study was the Brookside greenhouse rearing facility in Winnipeg, MB. *Galerucella calmariensis* introduced into the overwintering cage in Libau Marsh-B were newly emerged adults never exposed to low temperatures. The poor recovery rate of adults the following spring may have been indicative of the lack of adaptation of cultured insects to field conditions.

Amount and duration of spring runoff in overwintering plots did not appear to influence recovery of adult *Galerucella* spp. Plots in Delta Marsh-A and Libau Marsh-B were completely submerged in 40-50 cm of water for extended periods in spring of 1996. Marked adult *Galerucella* spp. were found on spring regrowth of purple loosestrife which had broken the water's surface, thus demonstrating their ability to survive complete submergence with water prior to their emergence from overwintering areas. Adults are known to overwinter in dead stems and inflorescences; however, heavy snow cover during the winter lodged stems from the previous growing season. In spring these stems were completely covered with water from spring runoff and the mechanism by which they survived under these conditions is unknown.

CONCLUSIONS

The introduced biological control agents, *G. calmariensis* and *G. pusilla* are capable of overwintering in southern Manitoba under field conditions, and the rate of survival did not consistently differ between species or sexes. Adult *Galerucella* spp. in the field experienced fairly constant temperatures of -8°C to -2°C, while air temperatures dropped below -38.7°C. Recovery of marked adults was highly variable, ranging from 0 to 81 %. Reasons for this may include loss of marks, microclimatic variation, predation and the number of winters of field selection since release in Manitoba. Both species of *Galerucella* were able to tolerate prolonged spring flooding of the overwintering site.

Table 10. Fall release and spring recovery data of marked *G. calmariensis* and *G. pusilla* adults from overwintering cages within four release sites. Experiment was conducted during winter of 1995-96. Note Delta Marsh-A contained two separate overwintering cages.

Site	Species	Beetle Source	Release Cage	Number Released	Number Recovered	Percent Recovery
Delta Marsh-A	*G. calmariensis*	DM-A	East-A	37	7	18.9
	G. pusilla	DM-A	East-A	14	2	14.3
Delta Marsh-A	*G. calmariensis*	DM-A	West-A	31	2	6.5
	G. pusilla	DM-A	West-A	19	9	47.4
Delta Marsh-B	*G. calmariensis*	DM-A	Central-A	11	9	81.8
	G. pusilla	DM-A	Central-A	107	0	0.0
Libau Marsh-A	*G. calmariensis*	LM-A	Central-A	56	26	46.4
Libau Marsh-B	*G. calmariensis*	Greenhouse	Central-A	57	1	1.70

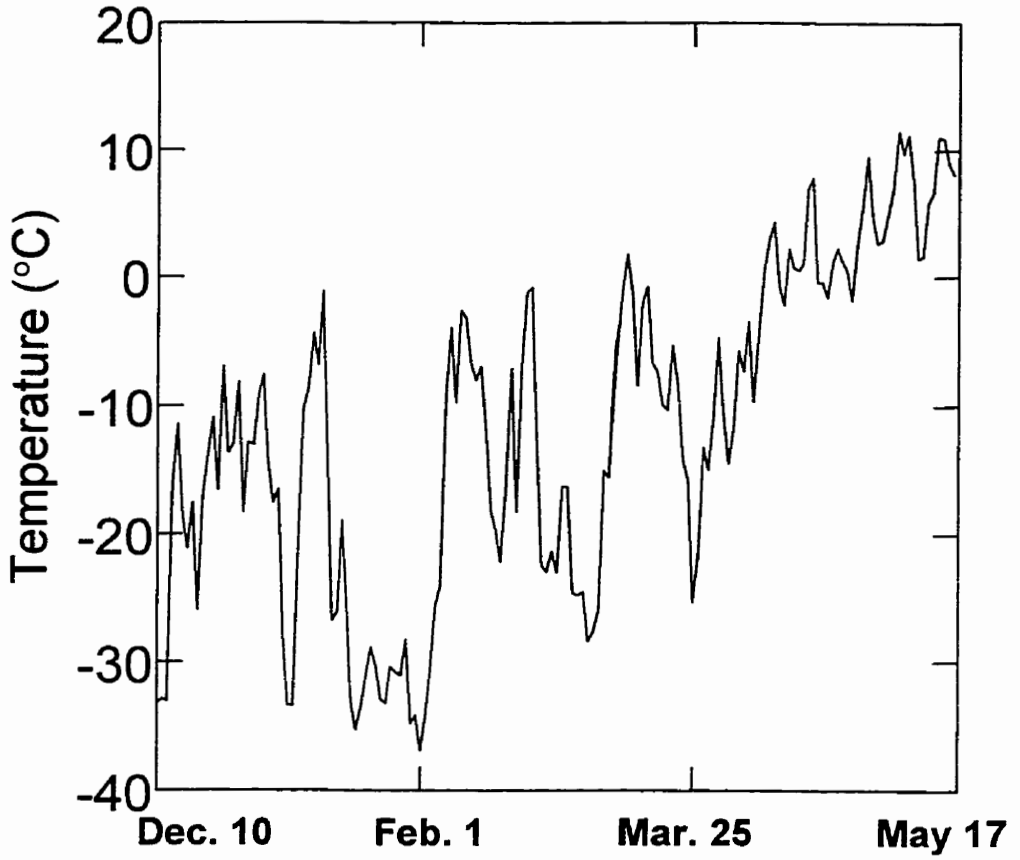


Figure 38. Daily mean air temperature at Libau Marsh-A during winter of 1995-96.

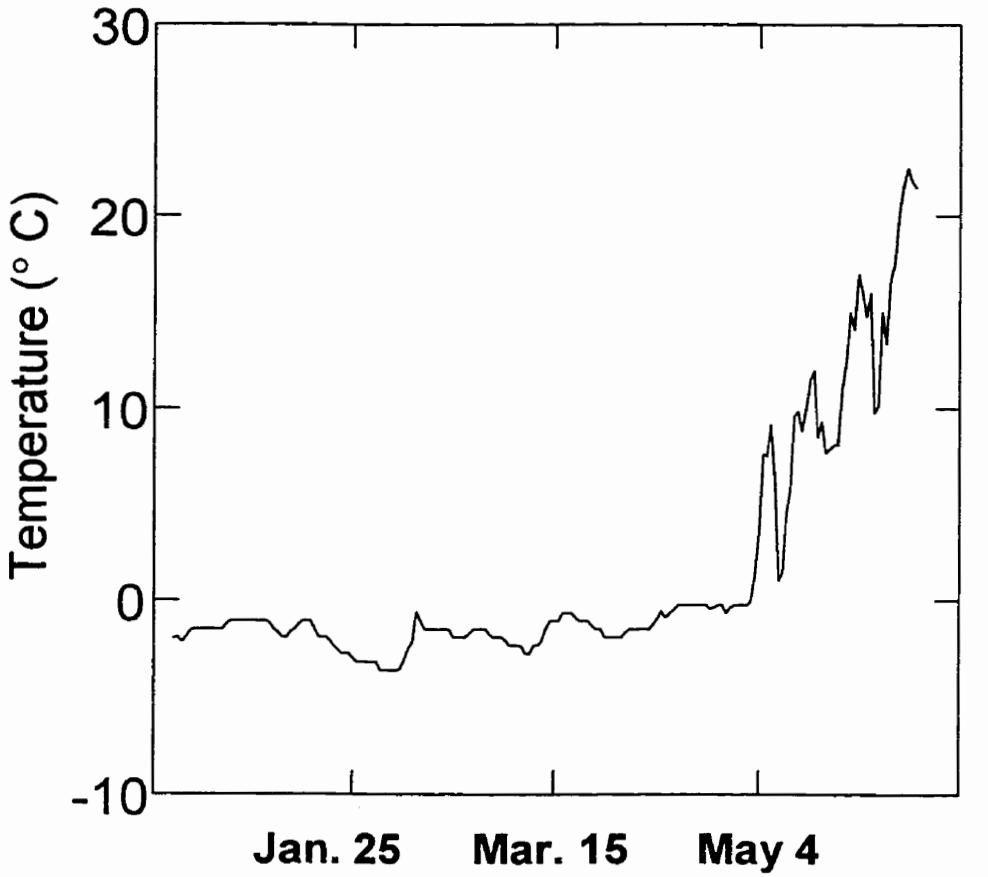


Figure 39. Daily mean temperature 5 cm below soil surface at Libau Marsh-A during winter of 1995-96.

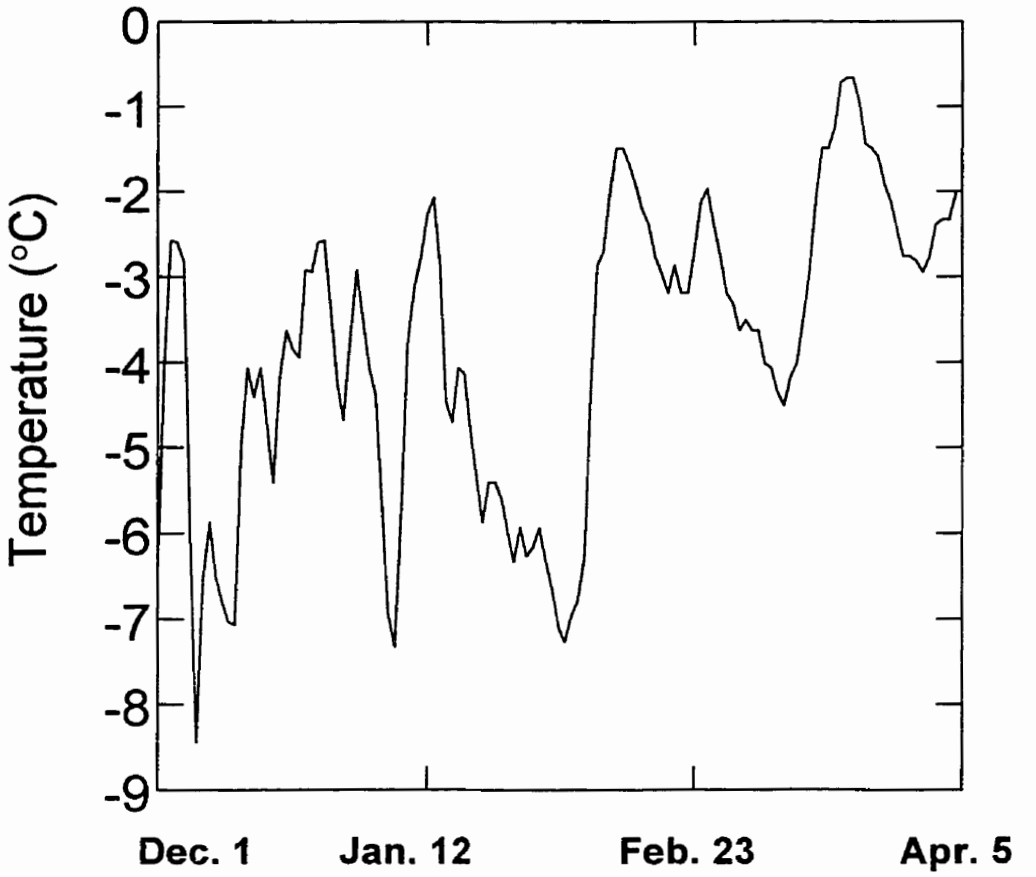


Figure 40. Daily mean temperature at soil surface in Delta Marsh-A (West Release Point) during winter of 1995-96.

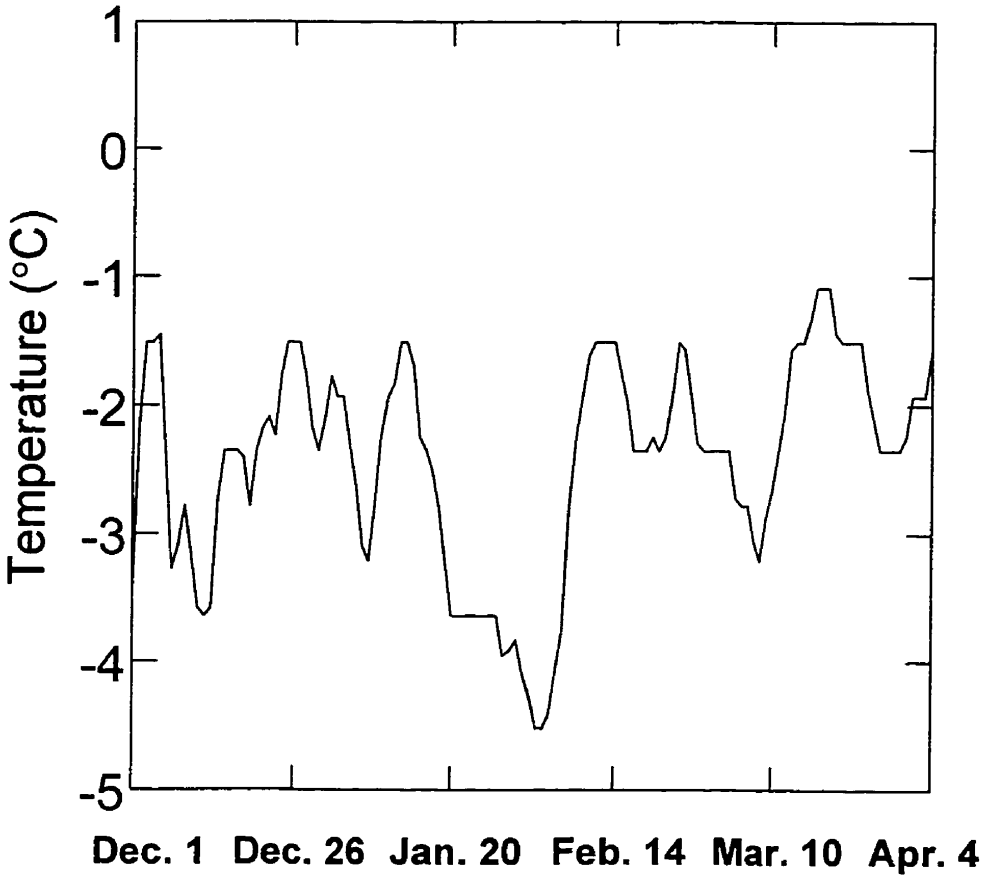


Figure 41. Daily mean temperature at the soil surface in Delta Marsh-A (East Release Point) during winter of 1995-96.

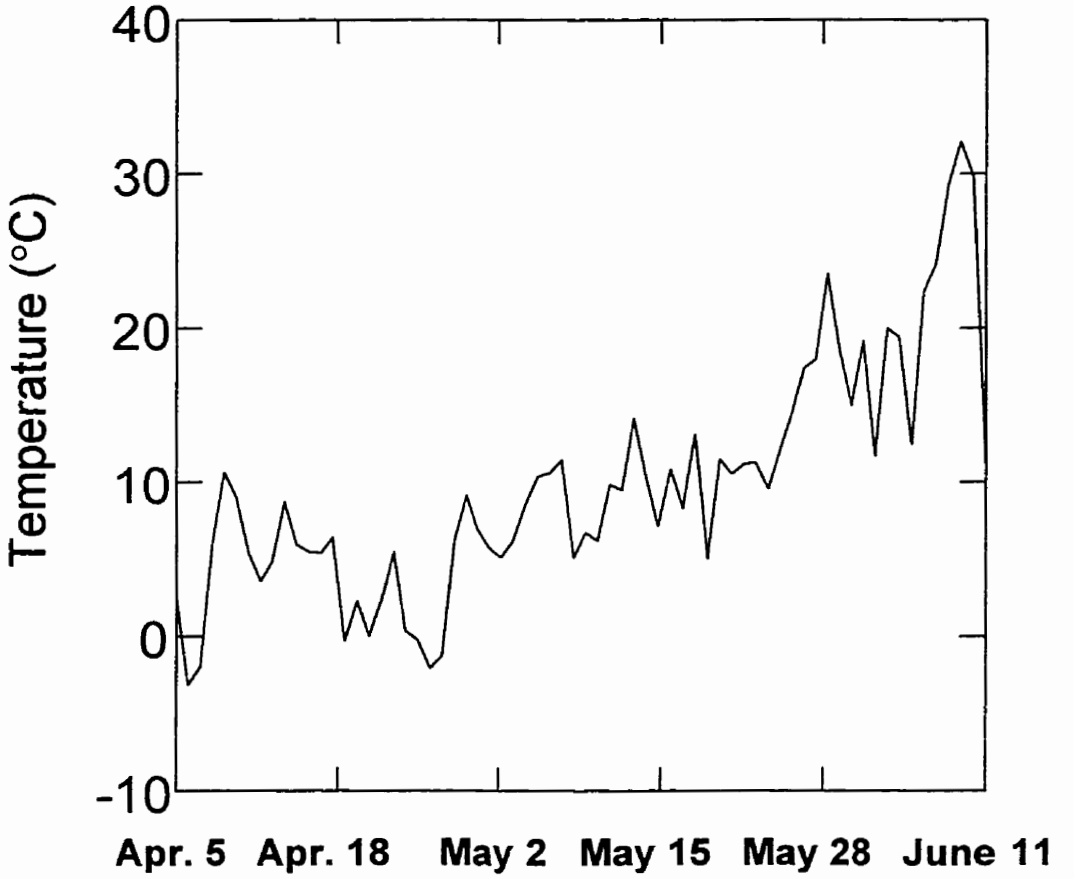


Figure 42. Daily mean air temperature at Delta Marsh-A during spring of 1995-96.

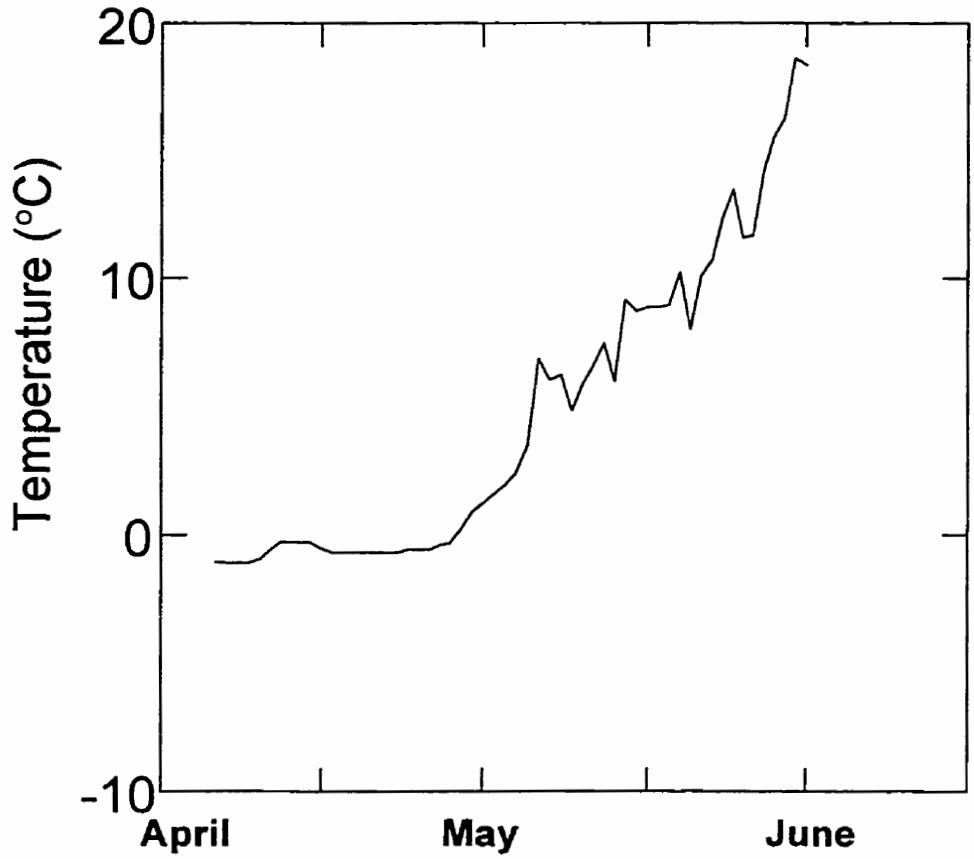


Figure 43. Daily mean temperature 5 cm below soil surface at Delta Marsh-A (West Release Point) during spring of 1995-96.

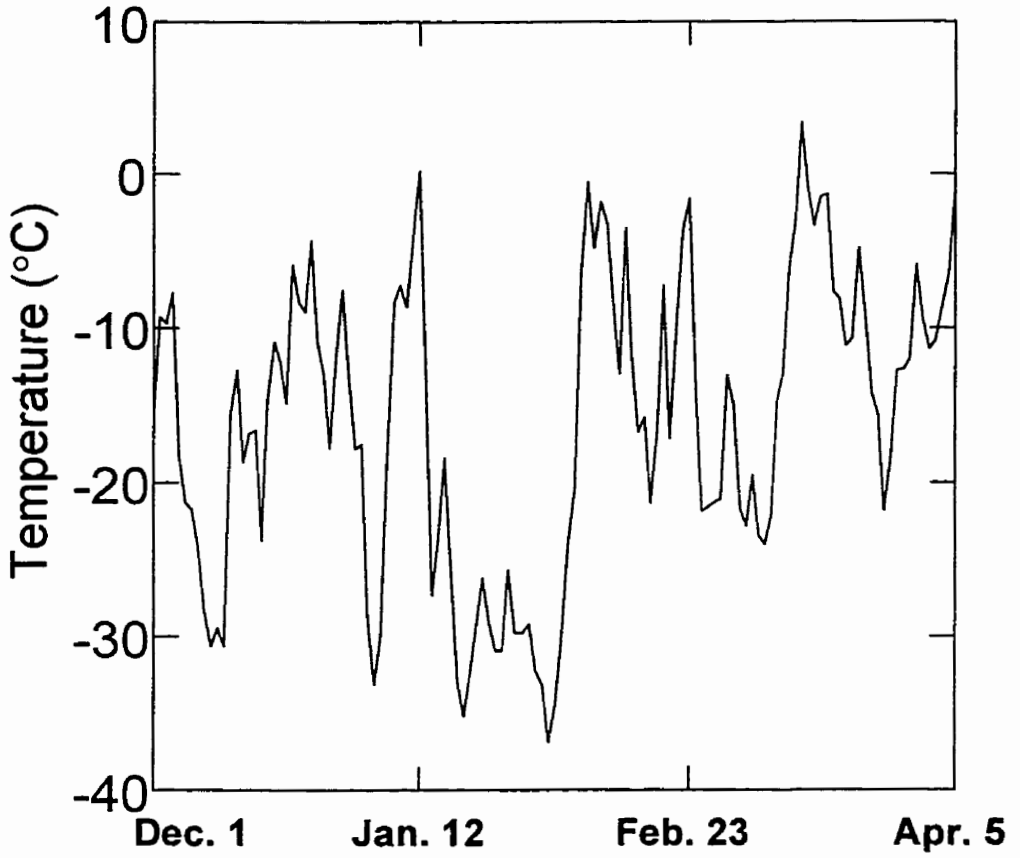


Figure 44. Daily mean air temperature at Delta Marsh-B during winter of 1995-96.

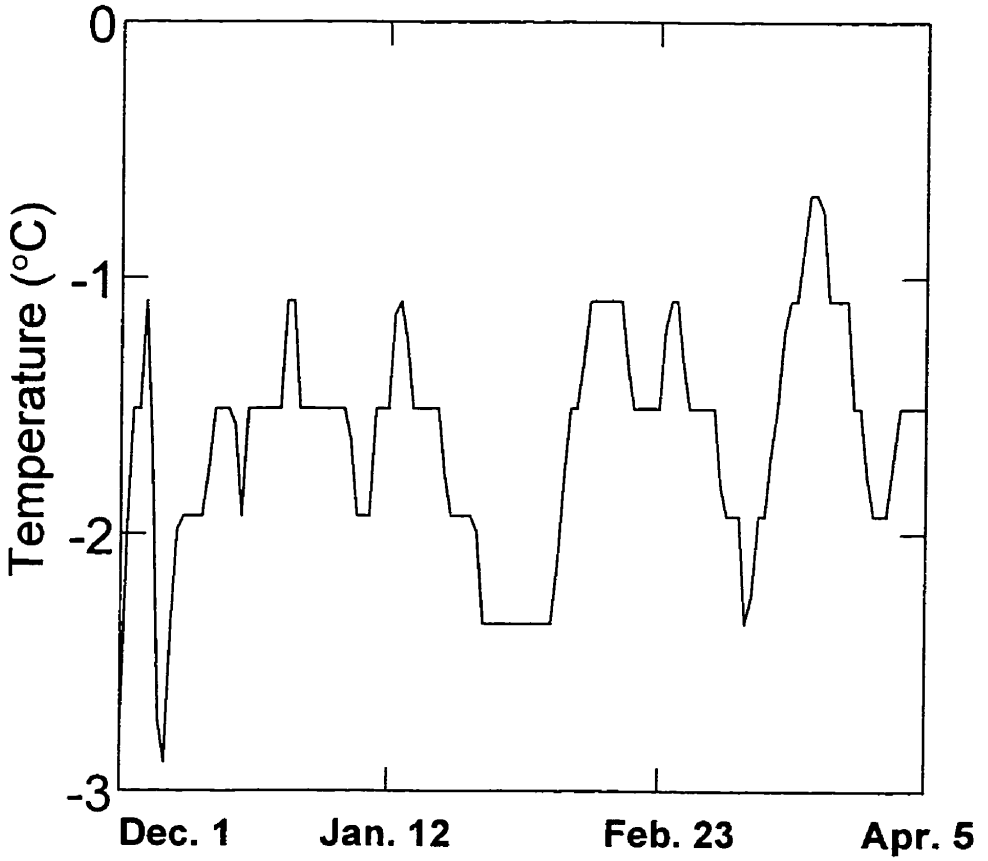


Figure 45. Daily mean temperature at soil surface in Delta Marsh-B during winter of 1995-96.

CHAPTER V

INSECTS ASSOCIATED WITH PURPLE LOOSESTRIFE, *LYTHRUM SALICARIA* L., IN SOUTHERN MANITOBA.

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ABSTRACT

In 1994 and 1995, insects were collected from several sites in southern Manitoba, where purple loosestrife, *Lythrum salicaria* L., is dominant. Collection techniques included the use of trap plants, sweep netting, vacuum sampling, and hand collecting. Insects from 38 families were collected, and grouped into four general feeding guilds: herbivores, predators, omnivores and fungivores. Herbivores that directly fed on *L. salicaria* included: *Galerucella nymphaeae* Linné (Coleoptera: Chrysomelidae), *Myzus lythri* (Schrank) (Homoptera: Aphididae), *Poecilocapsus lineatus* (Fabricius) (Hemiptera: Miridae), *Kleidocerys resedae* (Panzer) (Hemiptera: Lygaeidae), *Ametastegia glabrata* (Fallén) (Hymenoptera: Tenthredinidae), *Orgyia leucostigma* (Smith) (Lepidoptera: Lymantriidae), *Lophocampa maculata* (Harris) (Lepidoptera: Lymantriidae), *Spilosoma virginica* (Fabricius) (Lepidoptera: Arctiidae), *Simyra henrici* (Grote) (Lepidoptera: Noctuidae), *Dichomeris ligulella* (Hübner) (Lepidoptera: Gelechiidae), *Melanchra picta* (Harris) (Lepidoptera: Noctuidae), *Hyles galli* (Rottenburg) (Lepidoptera: Sphingidae), and *Biston betularia cognataria* (Gn) (Lepidoptera: Geometridae). These herbivores do not severely limit the density of purple loosestrife in southern Manitoba and any interactions with introduced biological control agents should be minimal. The predatory stink bug, *Apoecilus bracteatus* Fitch (Hemiptera: Pentatomidae) was identified as a potentially important predator of the introduced biological control agents, *Galerucella pusilla* Duftschmidt and *G. calmariensis* (L.) (Coleoptera: Chrysomelidae).

INTRODUCTION

Purple loosestrife, *Lythrum salicaria* L., is a herbaceous, perennial wetland plant that was introduced from Europe to North America in the early 1800's (Hight 1990; Stuckey 1980). After its introduction, purple loosestrife quickly established itself along the eastern seaboard of the United States and gradually spread westward across New York State into the St. Lawrence River valley (Stuckey 1980). By the mid 1800s' large areas of the plant were well established along the St. Lawrence River in southern Ontario and Quebec (Thompson et al. 1987). Currently in Canada, purple loosestrife can be found in all ten Canadian provinces (DeClerck-Floate 1992). In southern Manitoba, *L. salicaria* is found along every major river system, with large areas of concentration along the Cypress, Assiniboine, and Red Rivers, as well as in the Netley-Libau and Delta marsh systems, south of Lake Winnipeg and Lake Manitoba.

The highly competitive nature of purple loosestrife in North America has led to the displacement of native wetland plant species and the formation of large monospecific areas of *L. salicaria* (Thompson et al. 1987; Malecki and Rawinski 1979). A classical biological control program using host-specific, non-native insects has been implemented in Canada to help control populations of purple loosestrife (Hight et al. 1995). In Manitoba, the host-specific, leaf-feeding beetles, *Galerucella pusilla* (Dufts.) (Coleoptera: Chrysomelidae), *Galerucella calmariensis* (L.) and the root-feeding weevil, *Hylobius transversovittatus* (Goeze) (Coleoptera: Curculionidae) have been released.

In addition to introduced organisms, the naturally occurring insect fauna may play an important role in reducing plant fitness, or otherwise interact with the introduced

biological control agents. Inventories of naturally occurring insects on *L. salicaria* have been compiled for Europe (Batra et al. 1986) and eastern North America (Hight 1990; Anderson 1995), but no information is available for the northern great plains region. The main objective of this study was to characterize the insect fauna associated with the above-ground parts of *L. salicaria* in southern Manitoba and identify any species that may interact with the introduced biological control agents, *G. calmariensis* and *G. pusilla*.

MATERIALS AND METHODS

During the summer of 1994, insect collections were made at six sites in southern Manitoba dominated by purple loosestrife. Two sites were located at Delta Marsh (50° 11′ N 98° 18′ W), three at Libau Marsh (50° 12′ N 96° 43′ W), and one site in Winnipeg (49° 53′ N 97° 09′ W), Manitoba. A single site at Delta Marsh and one site at Libau had previously received introductions of the biological control agents, *G. pusilla* and *G. calmariensis* in the summer of 1993; there were no introductions in the remaining four study sites. In 1994, all sites were visited weekly from 15 May, soon after emergence of purple loosestrife, until 15 September, when the aboveground parts of the plants had died. In 1995, insect herbivores of purple loosestrife were also collected; however, sites were not as intensively sampled as in 1994. The summers of 1995 and 1996 were dedicated to the completion of *Galerucella* spp. releases and subsequent egg mass sampling within the four release sites.

Four collection methods were used to obtain insects from purple loosestrife. Trap plants, consisting of five potted seedlings, were placed along transects radiating from a central point, soon after spring emergence of the plant. The purpose of trap plants was to attract herbivores to seedlings, which could then be removed from sites with minimal disturbance to the surrounding area. Plants were watered as necessary and each week one pot was collected from a predetermined distance along each transect. The pots were brought back to the laboratory and the aboveground portion of the plant sampled for insects. Initially, the trap and wild plants were similar in size and appearance; however, as the season progressed trap plants were overshadowed by the naturally occurring purple loosestrife.

The majority (75 - 80%) of collections from *L. salicaria* were made using a sweep net; however, collections were also made using a vacuum sampler which sucked insects from the vegetation and flowers of purple loosestrife. Aperture size of the D-Vac was approximately 25 cm in diameter. All weekly sweep net and vacuum sampling occurred during the day and involved intensive sampling of individual plants from randomly chosen areas within the site. The two collection techniques were targeted on the vegetation and flowers of *L. salicaria*; occasionally plants of other species may have been sampled. Plants in the field were examined *in situ* and any insects were hand picked and taken to the laboratory to test for herbivory on *L. salicaria*.

Once in the laboratory, insects were placed into petri dishes containing fresh purple loosestrife vegetation and observed for feeding activity. If feeding was observed,

insects were reared to adulthood and identified. Voucher specimens of all taxa are deposited in the J. B. Wallis Museum of Entomology at the University of Manitoba.

In this study, an insect was classified as a herbivore of purple loosestrife based on one of three criteria: observation of feeding on *L. salicaria* in the field, observation of feeding in the laboratory, or collection of adults and immatures from *L. salicaria* and confirmation from the literature of herbivory on herbaceous plants (if none was directly observed on purple loosestrife). Observations and information from the literature were used to characterize remaining insect taxa as predators, omnivores or fungivores.

RESULTS AND DISCUSSION

A complete list of insects collected is provided in Table 1. In total, 63 insect genera, representing 38 families and seven orders were collected from *L. salicaria* during the study. The introduced biological control agents, *G. pusilla* and *G. calmariensis* are included in the 63 genera collected. Our discussion concentrates on common taxa (>50 specimens collected), herbivores of purple loosestrife and insects that may interact with the introduced biological control agents.

Coleoptera. *Galerucella calmariensis* and *G. pusilla* were collected from sites at Libau and Delta Beach in which there were previous introductions. Eggs, larvae, and adults of both European species were collected from the leaves, flowers, and stems of purple loosestrife. Neither species were present in sites where there had not been introductions. The biology and feeding habits of these beetles has been described in detail by Hight and

Drea (1991) and Blossey and Schroeder (1991). Larvae and adults of the water lily beetle, *G. nymphaeae* (Linné), were also observed feeding on *L. salicaria* at Libau and Great Falls, MB. Larval feeding damage to the leaves of purple loosestrife was comparable in type to that of the introduced *Galerucella* spp.; however, numbers of *G. nymphaeae* appeared too low to have a significant impact on purple loosestrife in southern Manitoba. The European immigrant, *G. nymphaeae* occurs throughout the United States and Canada and feeds on plants belonging to the genera *Nuphar* Sm. (Nymphaeaceae), *Polygonum* L. (Polygonaceae) and occasionally on native and introduced *Lythrum* species (Lythraceae) (Manguin et al. 1993; Hight 1990).

Members of the coccinellid genera, *Hippodamia* Dejean, *Coccinella* Linné and *Anisosticta* Dejean, are predatory and feed on aphids belonging to many different genera, including *Myzus* (Gordon 1985). Adults of *H. tredecimpunctata tibialis* (Say) and *C. septempunctata* (L.) were frequently collected from *L. salicaria*. Coccinellid larvae were also observed on the leaves of purple loosestrife, but were not collected. Larval stages of the Coccinellidae are known to feed on the eggs of *Galerucella sagittariae* (Hippa et al. 1984) and *G. nymphaeae* (Hippa et al. 1982) in Europe. Therefore, in southern Manitoba, it is possible that coccinellids are consuming eggs and early larval instars of *Galerucella* spp., as well as aphids such as *Myzus lythri*.

The family Scirtidae was represented by members of the genus *Cyphon* Paykull., of which marshes are believed to be the typical habitat (Young and Stribling 1990). In both sites at Libau, adults were commonly collected from the vegetation and flowers of purple loosestrife; no adults were collected from Delta Beach. Larval Scirtidae are

aquatic in lentic habitats (Stribling and Young 1990), and feed on algae, fungi and other organic matter (Woodward et al. 1988). Adults of this genus are commonly observed on foliage and have been collected from purple loosestrife in Europe (Batra et al. 1986). Adults of the genus *Cortinicara* Johnson (Coleoptera: Lathridiidae) were very abundant on the foliage of purple loosestrife at both Delta Beach and Libau. Adult *Cortinicara* are commonly found on foliage, flowers, or in leaf litter, and the larvae feed on moulds and decomposing leaf material (Johnson 1975). Although adults of *Cortinicara* and *Cyphon* are abundant within sites, these beetles are likely having minimal impact on the purple loosestrife.

Hemiptera. *Poecilocapsus lineatus* (Fabricius) (Hemiptera: Miridae) typically occurs on weeds in moist, shaded areas, and its host plants include more than 250 species in 57 families (Wheeler and Miller 1981). This mirid is widely distributed in Canada (Kelton 1982b), and its eggs (Wheeler and Miller 1981), nymphs and adults (Hight 1990) have been previously recorded from *L. salicaria* in North America. In our sites, much of the feeding damage caused by *P. lineatus* nymphs occurred on purple loosestrife; however, from observations in the field, Canada thistle, *Cirsium arvense* (L.) Scop. (Compositae) and field mint, *Mentha arvensis* L. (Labiatae) were also fed upon. First and second instar nymphs were frequently collected in early-June and caused noticeable damage to the young leaves of newly emerged purple loosestrife shoots. However, plants showed no persistent damage and few adult *P. lineatus* were collected late in the growing season. Despite conspicuous black necrotic spots on the leaves resulting from nymphal feeding

(Wheeler and Miller 1981), damaged purple loosestrife plants usually recover by terminal bud or lateral shoot growth (Hight 1990).

Adult *Lygus lineolaris* (Pallisot de Beauvois) and *L. rubrosignatus* (Knight) (Hemiptera: Miridae) were commonly collected during the initial bloom period of *L. salicaria*, which began in mid-July. In addition, relatively small numbers of *L. lineolaris* nymphs were collected before bloom. *Lygus lineolaris* is polyphagous, and host plants include at least 328 species or subspecies including *Lythrum lanceolatum* Ell. (Young 1986). *Lygus lineolaris* feeds on leaf buds and reproductive structures such as flower buds and developing fruits (Pack and Tugwell 1976) and it is likely that *L. salicaria*, with its long bloom period, is serving primarily as a mid-late season host for adults and nymphs of *L. lineolaris*. No adults of *Lygus* spp. were collected from newly emerged *L. salicaria* in spring, so it is likely that adults migrate to purple loosestrife from earlier emerging host plants that are utilized at the beginning of the growing season.

Adults of *Plagiognathus medicagus* Arrand and *P. obscurus* Uhler (Hemiptera: Miridae) were obtained in late season collections from the leaves and flowers of *L. salicaria*. *Plagiognathus obscurus* has been collected from a wide variety of herbaceous plants (Kelton 1982a) and adults of this species have been previously recorded from purple loosestrife in North America (Hight 1990). *Plagiognathus obscurus* is both phytophagous and predacious on mites, codling moth larvae and aphids (MacPhee and Sanford 1954).

Kleidocerys resedae (Panzer) (Hemiptera: Lygaeidae) is a Holarctic species that feeds on the seeds of many plants, including *L. salicaria* (Hight 1990). In our study,

nymphs were abundant prior to, and during the early bloom stages of purple loosestrife, whereas adults were collected from the reproductive structures of the plant and from fall collections of seed heads. Seed production of *L. salicaria* may be depressed if numbers of *K. resedae* exceed 30 nymphs and adults per flowering shoot (Hight 1990); however, in our sites a maximum of 5-10 *K. resedae* per flowering shoot was observed only on a few isolated plants.

Orius tristicolor (White) (Hemiptera: Anthocoridae) is widely distributed across Canada and is abundant on the flowering heads of herbaceous plants (Kelton 1978). Adults and nymphs of *O. tristicolor* are generalist predators on small arthropods and supplement their diet by feeding on pollen (Salas-Aguilar and Ehler 1976).

The predaceous stink bug, *Apoecilus bracteatus* Fitch (Hemiptera: Pentatomidae), attacks a wide range of soft-bodied insects that feed on leaf surfaces (Evans and Root 1980). Within release sites, nymphs and adults of *A. bracteatus* were observed consuming the larvae of the two *Galerucella* spp. biological control agents. In sites that did not receive introductions of the *Galerucella* spp., predaceous stinkbugs were not found. It is not known whether *A. bracteatus* was present in release sites prior to the introduction of the biological control agents, or if they invaded these sites from the surrounding area in response to the presence of *Galerucella* spp.

Homoptera. Adults of *Macrosteles quadrilineatus* Forbes (Homoptera: Cicadellidae) were most abundant during the summer and early fall; however, nymphs were not collected from *L. salicaria*. Nymphs of *M. quadrilineatus* feed on grasses and cereals in the spring, then migrate to herbaceous plants in the early summer (Beirne 1952). The

presence of adults on *L. salicaria* during the summer is consistent with the literature. However, the lack of nymphs for *M. quadrilineatus*, as well as the Delphacidae and Psyllidae suggest that these insects are not using *L. salicaria* as a reproductive host.

The winter hosts of the European aphid, *Myzus lythri* (Schrank) (Homoptera: Aphididae) are *Prunus* spp. L. (Rosaceae) and the summer hosts are *Lythrum* and *Epilobium* spp. L. (Onagraceae) (Voegtlin 1995). It has been suggested that *M. lythri* could be manipulated to contribute to the biological control of purple loosestrife (Voegtlin 1995); however, the numbers of *M. lythri* collected in our study sites were small and there was no noticeable effect on the loosestrife plants. This may have been due to a shortage of *Prunus* spp. near sites, or because of environmental factors inhibiting migration (Voegtlin 1995).

Lepidoptera. *Orgyia leucostigma* (Smith) (Lepidoptera: Lymantriidae) is a native defoliator that feeds on both deciduous and coniferous trees (Wilson 1991). Eggs and larvae of this insect have been previously recorded from purple loosestrife in North America (Hight 1990). In the field, larvae typically fed in the shade, on the underside of leaves and in the laboratory we observed feeding on all parts of the purple loosestrife leaf, including the mid-rib.

Spilosoma virginica (Fabricius) (Lepidoptera: Arctiidae) is common throughout most of North America (Peterson et al. 1993), and its host plants include more than 100 plant species, particularly low growing forbs, grasses, sedges, and herbaceous plants (Dethier 1988). Larvae of *S. virginica* were abundant and frequently observed feeding on *L. salicaria* within sites at Delta Beach.

Simyra henrici (Grote) (Lepidoptera: Noctuidae) is common in moist habitats across North America and its host plants include *Typha* spp. L. (Typhaceae), *Polygonum* sp., *Salix* sp. L. (Salicaceae) and a number of grasses and sedges (Decker & Maddox 1971). Larvae of this native species have not been previously recorded from purple loosestrife in North America. The feeding habit of the first and second instar larvae is described as leaf skeletonization, while later instars scallop the leaf margins (Decker & Maddox 1971). This description of feeding is consistent with the observed feeding habit of this caterpillar on *L. salicaria* within collection sites.

Larvae of *Melanchra picta* (Harris) (Lepidoptera: Noctuidae) were hand collected from the foliage of *L. salicaria* at Libau and Winnipeg. The larvae feed on the leaves of many crop plants (Rockburne and Lafontaine 1976) and have also been recorded from *Salix*, *Vaccinium* L. (Ericaceae), and *Trifolium* L. (Fabaceae) (Covell 1984). In the field, larvae of this species were usually collected in groups of >10 individuals from isolated purple loosestrife plants. In five days, under laboratory conditions, 10-12 larvae completely defoliated a plant consisting of 10 stems. However, the occurrence of larvae in the field was infrequent and noticeable damage to stands of purple loosestrife within collection localities was not observed.

The larvae of *Dichomeris ligulella* (Hübner) (Lepidoptera: Gelechiidae) feed by skeletonizing leaf material and host plants of economic importance include *Quercus* spp. L. (Fagaceae), and *Malus* spp. L. (Blumenthal and Simons 1980). Larvae of this native species have been previously recorded from purple loosestrife in the United States (Hight 1990).

Hyles galli (Rottenburg) (Lepidoptera: Sphingidae) is a Holarctic, native sphinx moth whose hosts include *Epilobium*, *Galium* L. (Rubiaceae), *Asperula* L. (Rubiaceae) and *Goderia* (Hodges 1971). A single larva of this species was collected from *L. salicaria* and reared in the laboratory where it consumed 4-5 leaves of purple loosestrife per day. Larval densities of this hawkmoth were too low to cause significant damage to the purple loosestrife within study sites.

Larvae of *Biston betularia cognataria* (Gn) (Lepidoptera: Geometridae) were collected from the foliage of purple loosestrife in Winnipeg, MB. Some host plants of this Holarctic species include *Salix, Cornus* L (Cornaceae)., *Acer* L. (Aceraceae), *Populus* L. (Salicaceae) and *Prunus* (McGuffin 1977). Larvae of this species have not been previously recorded from purple loosestrife in southern Manitoba.

The adult stage of *Nymphalis antiopa* Linnaeus (Lepidoptera: Nymphalidae) was collected from the flowers of *L. salicaria* but no larvae of this species were observed feeding on the plant.

Diptera. Adult Chloropidae were the most frequently collected flies in this study. *Elachiptera* spp. occur in marshy areas and the adults are secondary invaders of damaged plant tissues, feeding on associated bacteria (Sabrosky 1948). The adults of many species of Sciomyzidae and Otitidae generally feed on liquids and are commonly collected while resting on vegetation or flowers near the larval habitat. Beyond invading and feeding on already damaged loosestrife plant material, members of the Chloropidae and Otitidae likely have minimal impact on purple loosestrife.

Members of the genus *Helophilus* Meigen (Diptera: Syrphidae) are commonly found in wet areas such as marshes and polluted ponds, where the larvae are filter feeders in marshy soil or water (Hartley 1961). Larvae of *Sphaerophoria contigua* Macquart (Diptera: Syrphidae) are generalist predators and feed on a variety of aphids including members of the genus *Myzus* (Knutson 1973). Adult syrphids were frequently collected while resting on vegetation and flowers of purple loosestrife near the larval habitat.

Hymenoptera. The larvae of *Ametastegia glabrata* (Fallén) (Hymenoptera: Tenthredinidae) are early-mid season feeders on the purple loosestrife foliage and were commonly collected from the underside of leaves, on the lower half of the plant. Adults were common late in the growing season and easily collected using a sweep net. *Ametastegia* spp. feed on plants from the genera *Rumex* sp. (Polygonaceae), *Polygonum* sp. and *Viola* sp. (Violaceae) (Smith and Barrows 1987). Both adults and larvae of *A. glabrata* have been previously recorded from *L. salicaria* in Europe (Batra et al. 1986), but not from North America.

Hylaeus spp. (Hymenoptera: Colletidae) are solitary bees that were frequently collected from the flowers of *L. salicaria*. Little is known about the biology of the *Hylaeus* spp. because adults carry pollen internally and are believed to nest in abandoned galls or pithy stems, including those of *Rhus* L. (Anacardiaceae) and *Sambucus* L. (Caprifoliaceae) (Scott 1994). Adults of the Apidae and Megachiliidae were also collected from the flowers of purple loosestrife during the bloom period of the plant. Other than collecting pollen and fertilizing the plant, these Hymenoptera should have minimal interactions with introduced biological control agents.

CONCLUSIONS

Of the 41 genera of herbivorous insects collected, members of 14 were observed feeding on purple loosestrife in the field or laboratory. Eight genera of Lepidoptera were collected in this study (Table 1), of which three have been previously recorded from *L. salicaria* (Batra et al. 1986). The other six herbivorous genera observed feeding on *L. salicaria* were: *Myzus lythri* (Schrank), *Poecilocapsus lineatus* (Fabricius), *Kleidocerys resedae* (Panzer), *Ametastegia glabrata* (Fallén) and *Galerucella nymphaeae* (L.), all of which have been previously recorded from purple loosestrife either in Europe or the United States. Because of the presence of immatures on the plant, it was also inferred that *Lygus lineolaris* (Pallisot de Beauvois) was herbivorous on *L. salicaria.* Several species of *Lygus* have been recorded from purple loosestrife in Europe (Batra et al. 1986) and *L. lineolaris* was collected from the plant in the northeastern United States (Hight 1990). The remaining genera, although collected from purple loosestrife, were represented by few specimens and apparently had little or no effect on the plant. The majority of these herbivores were likely incidentals collected by vacuum sampling, a technique which collected insects not only resting on purple loosestrife, but also on other vegetation in close proximity.

The predatory guild of insects represented 17 of the 63 genera collected during the survey. Of these, only one appears likely to limit the success of the introduced biological control agents. The predatory stink bug, *Apoecilus bracteatus* Fitch (Hemiptera: Pentatomidae), was consistently found in sites which had received

introductions of the biological control agents, *G. calmariensis* and *G. pusilla*. Nymphs and adults of this species were most abundant within 2 m of the release point of *Galerucella* spp. and predation by *A. bracteatus* on the larvae of the biological control agents was observed under laboratory and field conditions. Adults and late instar nymphs were observed with *Galerucella* spp. larvae impaled on their mouthparts. Because of their abundance near the point of release, this predatory species may threaten the continued reproduction and spread of the *Galerucella* spp. biological control agents after establishment.

A number of naturally occurring herbivores of *L. salicaria* in southern Manitoba were identified as a result of this survey. By using *L. salicaria* as a host plant, all of these herbivores have the potential to interact with the introduced biological control agents. However, during the survey, it was observed that populations of naturally occurring herbivores did not reach high enough levels to cause noticeable damage to the large, central areas of purple loosestrife present within survey sites. For this reason, we conclude that the existing herbivores of *L. salicaria* in southern Manitoba will not limit the amount of loosestrife available for the introduced biological control agents. Instead, these herbivores may supplement the increased herbivore load on purple loosestrife that is being provided by the introduction of the European biological control agents.

Table 11. Insects associated with purple loosestrife, *Lythrum salicaria* L. (Lythraceae), in southern Manitoba.

TAXON	STAGE COLL[1]	COLLECTION MONTH	FEEDING GUILD	PLANT PART[2]	COLLECTION TECHNIQUE	COLL FREQ[3]
COLEOPTERA						
Chrysomelidae						
Galerucella calmariensis (L.)	E, A, l	vi,vii,viii	Herbivore	L,F,S	sweep,hand	<10
G. pusilla (Duftschmidt)	E, A, l	vi,vii,viii	Herbivore	L,F,S	sweep,hand	<10
G. nymphaeae (L.)	A, l	vi,vii	Herbivore	L,S	hand	<50
Disonycha procera Casey	A	vii,vii	Herbivore	L	trap plant	<10
Curculionidae						
Otiorhynchus ovatus (L.)	A	vii	Herbivore	L	sweep, hand	2
Tychius picirostris (Fabricius)	A	viii	Herbivore	L	D-vac	<10
Cerambycidae						
Clytus ruricola (Olivier)	A	vi	Herbivore	L	sweep	1
Coccinellidae						
Hippodamia convergans Guerin	A	viii,ix	Predator	L,F	D-vac,sweep	<75
H. tredecimpunctata tibialis (Say)	A	ix	Predator	L,F	"	<10
Coccinella septempunctata (L.)	A	vii,viii,ix	Predator	L,F	"	<50
Anisosticta bitriangularis (Say)	A	iii,ix	Predator	L,F	"	<10
Cantharidae						
Species 1	A	vi,vii	Predator	L	sweep	<10
Species 2	A	vi,vii	Predator	L	sweep	<10
Species 3	A	vi,vii	Predator	L	sweep	<10
Staphyllinidae						
Stenus sp.	A	vii,viii	Predator	L	D-vac,sweep	<10
Scirtidae						
Cyphon sp.	A	vii,viii,ix	Fungivore	L,F	D-vac,sweep	<75

TAXON	STAGE COLL[1]	COLLECTION MONTH	FEEDING GUILD	PLANT PART[2]	COLLECTION TECHNIQUE	COLL FREQ[3]
Cryptophagidae						
Atomaria sp.	A	viii	Fungivore	L	D-vac	1
Lathridiidae						
Cortinicara sp.	A	vii,viii,ix	Fungivore	L	D-vac	<75
Mycetophagidae						
Typhaea sp.	A	vii	Fungivore	L	sweep	<10
HEMIPTERA						
Miridae						
Poecilocapsus lineatus (Fabricius)	A,N	vi,vii,vii	Herbivore	L,F	sweep,hand	<75
Lygus lineolaris (Pallisot de Beauvois)	A,N	vii,viii	Omnivore	L,F	D-vac,sweep	<75
L. rubrosignatus Knight	A,N	vii,viii	Herbivore	L,F	"	<75
Plagiognathus obscurus Uhler	A	vii,viii	Omnivore	L,F	"	<50
P. medicagus Arrand	A	vii,viii	Herbivore	L,F	"	<50
Lygaeidae						
Kleidocerys resedae (Panzer)	A,N	vii,viii,ix	Herbivore	L,F	D-vac,sweep	<50
Xyonysius californicus (Stal)	A	viii	Herbivore	L,F	D-vac	<10
Nabidae						
Nabis sp.	A,N	vii	Predator	L	D-vac,sweep	<10
Nabicula sp.	A	vii	Predator	L	"	<10
Saldidae						
Saldula sp.	A	vii	Predator	L	D-vac,sweep	<10
Salda sp.	A	vii	Predator	L	"	<10
Anthocoridae						
Orius tristicolor (White)	A	vi,vii,viii,ix	Predator	L,F	D-vac,sweep	<50
Anthocoris musculus (Say)	A	vii,viii,ix	Predator	L,F	D-vac,sweep	<10

TAXON	STAGE COLL[1]	COLLECTION MONTH	FEEDING GUILD	PLANT PART[2]	COLLECTION TECHNIQUE	COLL FREQ[3]
Pentatomidae						
Apoecilus bracteatus Fitch	A,N	vi,vii,viii	Predator	L,S	sweep,hand	<50
HOMOPTERA						
Cicadellidae						
Macrosteles quadrilineatus Forbes	A	vii,viii,ix	Herbivore	L	D-vac,sweep	<75
Cicadula melanogaster Provancher	A	vii	"	L	D-vac	<50
C. vittipennis Sand. & DeL.	A	viii	"	L	"	<10
C. subcupraea (Provancher)	A	viii	"	L	"	<10
Cosmotettix bilineatus (Gill. & Bak.)	A	vii	"	L	"	1
Forcipata loca Delong & Caldwell	A	viii,ix	"	L	"	<10
Limotettix sphagneticus Emeljanov	A	vii	"	L	"	1
Cuerna septentrionalis (Walker)	A	viii	"	L	"	<10
Euscelis sp.	A	vii,viii	"	L	D-vac,sweep	<10
Empoasca sp.	A	viii,ix	"	L	D-vac,sweep	<50
Psyllidae						
Aphalara sp.	A	viii	Herbivore	L	D-vac	<10
Delphacidae						
Bakerella muscotana Beamer	A	vii	Herbivore	L	D-vac	<10
Stenocranus pallidus Beamer	A	viii	"	L	D-vac	<50
Megamelus spp.	A	viii	"	L	D-vac,sweep	<10
Aphididae						
Myzus lythri (Schrank)	A,N	vii,viii	Herbivore	L,S	D-vac,sweep	<50
THYSANOPTERA						
Thripidae						
Frankliniella tritici (Fitch)	A	viii	Herbivore	F	D-vac,sweep	<50
F. tenuicornis (Uzel)	A	viii	"	F	D-vac,sweep	<10
Chirothrips patruelis Hood	A	viii	"	L,F	D-vac,sweep	<10

TAXON	STAGE COLL[1]	COLLECTION MONTH	FEEDING GUILD	PLANT PART[2]	COLLECTION TECHNIQUE	COLL FREQ[3]
Phlaeothripidae						
Haplothrips kurdjumovi Karney	A	ix	Predator	L,F	D-vac,sweep	<10
LEPIDOPTERA						
Noctuidae						
Simyra henrici (Grote)	l	vii	Herbivore	L	hand	<10
Melanchra picta (Harris)	l	vii	Herbiovre	L	hand	<10
Lymantriidae						
Orgyia leucostigma (Smith)	l	vi	Herbivore	L	hand	<10
Lophocampa maculata (Harris)	l	vii	"	L	hand	<10
Arctiidae						
Spilosoma virginica (Fabricius)	l	vii	Herbivore	L	hand	<50
Gelechiidae						
Dichomeris ligulella (Hubner)	l	vi	Herbivore	L	hand	<10
Sphingidae						
Hyles galli (Rottenburg)	l	vii	Herbivore	L	hand	<10
Geometridae						
Biston betularia cognataria (Gn)	l	vii	Herbivore	L	hand	<10
Nymphalidae						
Nymphalis antiopa (L.)	A	ix	Hebivore	F	sweep	1
DIPTERA						
Chloropidae						
Elachiptera vittata Sabrosky	A	viii,ix	Herbivore	L,F	D-vac,sweep	<50
E. angustistylum Sabrosky	A	vii,viii,ix	"	L,F	"	<10
E. pechumani Sabrosky	A	viii,ix	"	L,F	"	<10
E. erythropleura Sabrosky	A	viii	"	L,F	"	<10
E. costata (Loew)	A	viii	"	L,F	"	<10

TAXON	STAGE COLL[1]	COLLECTION MONTH	FEEDING GUILD	PLANT PART[2]	COLLECTION TECHNIQUE	COLL FREQ[3]
Syrphidae						
Helophilus latifrons Loew	A	viii	Herbivore	L,F	sweep	<10
H. faciatus	A	viii	"	L,F	sweep	<10
Sphaerophoria contigua Macquart	A,l	vii	Predator	L	hand	<10
Dolichopodiae						
Dolichopus penicillatus Van Duzee	A	vii	Predator	L	D-vac,sweep	<10
D. barbicauda Van Duzee	A	vii	"	L	"	1
D. plumipes Scopeli	A	viii	"	L	"	1
D. bifractus Loew.	A	vii	"	L	"	<10
Otitidae						
Chaetopsis sp.	A	viii	Fungivore	L	D-vac,sweep	<10
HYMENOPTERA						
Tenthredinidae						
Ametastegia glabrata (Fallén)	A,l	vi,vii,viii	Herbivore	L	hand,sweep	<50
Apidae						
Apis melifera (L.)	A	vii,viii	Herbivore	F	sweep	<50
Bombus sp.	A	viii	"	F	sweep	<50
Megachilidae						
Megachilie inermis Provancher	A	viii	Herbivore	F	sweep	<10
Colletidae						
Hylaeus sp.	A	vii	Herbivore	F	sweep	<50

[1]Stage collected; E = egg; l = larva; N = nymph; A = adult.

[2]Plant part collected from; L = leaf; S = stem; F = flower.

[3]Collection frequency = number of specimens collected in study

CHAPTER VI

AN ASSESSMENT OF PREDATION BY THE STINK BUG, *APOECILUS BRACTEATUS* FITCH (HEMIPTERA: PENTATOMIDAE), ON *GALERUCELLA* SPP. LARVAE UNDER FIELD CONDITIONS IN SOUTHERN MANITOBA

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ABSTRACT

During the summer of 1994, nymphs of the predatory stink bug, *Apoecilus bracteatus* Fitch (Hemiptera: Pentatomidae), were observed in release sites at Libau Marsh (50° 12′ N 96° 43′ W) consuming larvae of the introduced biological control agents, *Galerucella pusilla* Dufts. (Coleoptera: Chrysomelidae) and *G. calmariensis* L.

A study using different densities of *A. bracteatus* nymphs attacking second and third instar *Galerucella* spp. larvae was conducted to determine the predatory abilities of this stink bug. Five densities of stink bug nymphs were introduced into field cages each containing 21 second and third instar *Galerucella* spp. larvae previously established on potted *Lythrum salicaria* L. (Lythraceae) plants. The number of *Galerucella* spp. larvae observed on the above ground portion of the plants was recorded each day until the end of the experiment. After the first and second days, there was a significant difference (p-value < 0.05) in the number of *Galerucella* spp. remaining between cages with stink bugs and those without. These differences were attributed to predation by the stink bug, *A. bracteatus*. In Libau Marsh-A, where *A. bracteatus* nymphs were most abundant, introduced agents have been present since 1993. Compared to another site having received beetles in 1993, a smaller increase in the total egg estimate was observed from 1995 to 1996. The predatory stink bug may be a contributing factor to the slow population increase observed in Libau Marsh-A.

INTRODUCTION

In southern Manitoba, the two leaf-feeding chrysomelid beetles, *Galerucella calmariensis* (L.) (Coleoptera: Chrysomelidae) and *G. pusilla* (Dufts.) were introduced in 1993 to help limit the population of purple loosestrife, *Lythrum salicaria* L. (Lythraceae). Prior to initial introduction of adult *Galerucella* spp. in North America, beetles collected in Germany were quarantined for several generations after arriving to the United States to ensure freedom from native parasites and diseases.

Predation of *G. pusilla* and *G. calmariensis* by spiders has been observed in many European field sites, and in North America, several failures to establish beetles have been attributed to egg and larval predation by arachnids (Malecki et al. 1993b). In studies of the closely related North American species *G. nymphaeae*, no parasites or pathogens of that species likely to attack *G. calmariensis* or *G. pusilla* have been discovered. However, egg predation by the ladybird beetle, *Coleomegilla maculata* (Coleoptera: Coccinellidae), was observed (Malecki et al. 1993b). Predation of eggs, larvae and adults by spiders and ladybird beetles does occur in North America and may affect populations of the introduced *Galerucella* spp. (Malecki et al. 1993b). Some failures to establish in western Canada have also been attributed to the soil-borne fungal pathogen, *Beauvaria bassiana* (Balsamo), which attacks the larval and adult stages of *Galerucella* spp. (Hight et al. 1995).

In southern Manitoba, larvae of *G. calmariensis* and *G. pusilla* are attacked by the predatory stink bug, *A. bracteatus* (Hemiptera: Pentatomidae) (Diehl et al. 1997). Nymphs and adults of this stink bug are solitary hunters, attacking a wide range of soft-bodied insects that feed on leaf surfaces (Evans & Root 1980). Within a few meters of

release points at Libau Marsh (50° 12′ N 96° 43′ W), individual *A. bracteatus* nymphs were observed consuming first, second and third instar *Galerucella* spp. larvae (Diehl, unpublished data). No stink bug nymphs were observed in release sites at Delta Marsh, MB (Diehl, personal observation).

There is no information on the predatory abilities of *A. bracteatus* on the larval population of the introduced biological control agents; however, their abundance in close proximity to the point of release may threaten population increases of *Galerucella* spp. after establishment. The purpose of this study was to examine the predatory abilities of the stink bug, *A. bracteatus* on the larvae of *G. calmariensis* and *G. pusilla* in field cages and predict what affect, if any, they may have on their continued reproduction and spread in southern Manitoba.

MATERIALS AND METHODS

In 1996, 15 field cages were set up on a grassy area at the Point Field Laboratory on campus of the University of Manitoba in Winnipeg. Cages were 1m x 1m x 1m, and consisted of fine, white coloured, polyethylene mesh material held in place using 2.54cm steel tubing at the corners. The gauge of the monofilament mesh screen was number 8 with 25 squares per 2.54 cm horizontally and 23 squares per 2.54 cm vertically. The bottom perimeter of the cage was dug into the ground to prevent insects from entering or escaping. Cage bottoms were lined with heavy gauge, black polyethylene sheeting to reduce grass growth and vacuumed using a D-Vac sampler (aperture diameter = 25 cm) to remove any insects that were present. Three pots, each containing two healthy, single-stemmed purple loosestrife plants previously established in a growth room (22°C and

16:8hr; dark:light) were grouped in the center of each cage. Plants used in this experiment were established from naturally occurring purple loosestrife collected from the bank of the Assiniboine River in Winnipeg, MB.

The five treatments were replicated three times and included the following combinations of *Galerucella* spp. larvae and *A. bracteatus* nymphs: 0 larvae, 0 nymphs; 21 larvae, 0 nymphs; 21 larvae, 1 nymphs; 21 larvae, 2 nymphs and 21 larvae, 3 nymphs. Experimental cages were placed in three rows of five treatments arranged in a randomized complete block design. Second and third instar *Galerucella* spp. larvae were collected from purple loosestrife in the Libau Marsh area. Larvae of *Galerucella* spp. are indistinguishable (Manguin et al. 1993) and no effort was made to identify them to species. Stink bugs were collected from release sites at Libau Marsh and held in a growth chamber at 22°C, 16:8hr; dark:light, until initiation of the experiment two weeks later. While in the growth chamber, *A. bracteatus* nymphs were provided with an abundance of *Galerucella* spp. larvae as a food source. Prior to stink bug introduction on 15 July, purple loosestrife in each pot was inoculated with seven 2^{nd} or 3^{rd} instar *Galerucella* spp. larvae, for a total of 21 larvae per cage. A period of 12 hours was allowed for the larvae to establish themselves on the potted loosestrife plants. Immediately prior to the introduction of stink bugs, the number of *Galerucella* spp. larvae on stems was recorded. Fourth instar nymphs of *A. bracteatus* were introduced by placing the 5cm x 5cm clear-plastic holding container in the center of the group of three potted plants. Counts of *Galerucella* spp. larvae were made each day until the end of the experiment. The number of larvae observed on the above ground portions of the loosestrife plants was recorded during each observation period.

Mortality of *Galerucella* spp. larvae due to predation (%) was estimated after applying Abbott's (1925) formula to correct for losses due to pupation or mortality not associated with predation. The per cent larvae living through the previous day or "apparent survival" was determined by dividing the mean number of larvae living after a specific day by the mean number of the previous day. At the start of the experiment (Day = 0), 100% of the *Galerucella* spp. larvae were living and available for predation. For days 0, 1 and 2, Abbott's formula was used to determine the corrected predation (%) in each treatment. The per cent larvae living through the previous day in the control was subtracted from that for the corresponding day in each treatment. This value was divided by the per cent larvae living through the previous day in the control and multiplied by 100 to give the per cent corrected predation. Contingency tables were used to determine whether *Galerucella* numbers were affected by the presence of stink bugs. Tables were analysed by loglinear modeling with delta = 0.5 (Bishop et al. 1975).

RESULTS

The number of stink bugs, mean number of *Galerucella* spp. larvae per cage ± SEM, per cent larvae living through the previous day after 1 and 2 days and corrected predation values are presented in Table 12. In control cages with no stink bugs, there was a gradual reduction in the mean number of larvae observed after day 1 and 2. The larvae of *Galerucella* spp. pass through three instars and this reduction is attributed to movement of 3rd instar larvae into the soil for pupation. Also apparent is the quick reduction of larvae in cages containing 1, 2 and 3 stink bugs compared to the control cage. The number of stink bugs per cage affected the number of *Galerucella* spp. larvae

present both on Day 1 (LR $\chi^2 = 14.72$; d.f.= 3; $p<0.001$) and on day 2 (LR $\chi^2 = 55.63$; d.f.= 3; $p<0.001$). After Day 1, there was no difference in the number of *Galerucella* spp. larvae between cages containing 1, 2, and 3 stinkbugs (LR $\chi^2 = 0.79$; d.f.= 2; $p = 0.67$); however, after Day 2, differences were observed (LR $\chi^2 = 28.55$; d.f. = 2; $p<0.001$). Analysis of data from Day 2 included only changes in the number of *Galerucella* spp. larvae observed from Day 1 to Day 2. As a result, it was concluded that differences in the mean number of larvae per cage between the control and other treatments are due to the predatory ability of the stink bug, *Apoecilus bracteatus*.

Per cent-corrected predation after 24 hours was similar between cages containing 1, 2, or 3 stink bugs; however, after two days, differences between treatments were observed. In cages containing a single stink bug nymph, corrected predation decreased from 35.4% after one day to 2.4% after day two. Sometime during the initial 24 hour period, the stink bug nymph inside each cage consumed enough *Galerucella* spp. larvae to temporarily satisfy its appetite and stop feeding. Feeding continued after the first day in cages with two stink bug nymphs and 80.1% of the remaining *Galerucella* spp. larvae were consumed. In cages containing three stink bug nymphs, all available *Galerucella* spp. larvae were consumed after 2 days. All *A. bracteatus* nymphs introduced into cages were alive and recovered after completion of the experiment.

DISCUSSION

In all three treatments, approximately 30 % of the available *Galerucella* spp. larvae were consumed during the first 24-hour period. In cages containing a single stink bug, the mean number of larvae at the start of the experiment was 18 and after 24 hours,

corrected predation was equal to 35.4 %. This translates into a consumption rate of 6.40 *Galerucella* spp. larvae /nymph during the first 24 hours inside cages containing a single stink bug. During the second day, the consumption rate decreased to 0.24 larvae /nymph. In cages with two stink bug nymphs, the consumption rate during the first 24 hours was 2.5 *Galerucella* spp. larvae per nymph and 4.10 during the second 24-hour period. In cages with 3 stink bugs, consumption rates per nymph were 2.2 *Galerucella* spp. larvae during the first 24 hours and 2.9 larvae during the second 24 hour period. Larval consumption rates were very similar during the first 24 hour period in cages containing 2 and 3 stink bug nymphs. However, during the second 24 hour period the consumption rate of *Galerucella* spp. larvae in cages with 3 nymphs was lower than in cages containing two stink bug nymphs. This result is somewhat artificial and can be explained by the limited number of *Galerucella* spp. larvae available for predation by the stink bug. In cages containing three *A. bracteatus* nymphs, all available larvae (mean = 8.7) were consumed during the second 24 hour period. If more larvae were available in cages with three stink bugs, the rate of consumption during the second day might have been higher. Group feeding by *A. bracteatus*, in which a single *Galerucella* spp. larvae is attacked and fed upon by two or more stink bug nymphs at the same time, was observed in release sites at Libau Marsh, MB (Diehl, personal observation). Although not observed in cages during periods of observation, this type of feeding behaviour might tend to reduce the number of *Galerucella* spp. larvae killed in cages with many stink bugs.

Stink bugs used in this study were recently molted fourth instar nymphs, which likely possess larger appetites than first, second or third instars. Consumption rates

during the second 24-hour period in cages with 3 stink bugs are presumably an underestimate due to the limited number of *Galerucella* spp. larvae. As a result, these data are not used in the calculation of the mean consumption rate of fourth instar stink bug nymphs. Based on corrected predation rates, the mean consumption rate of fourth instar *A. bracteatus* nymphs was estimated to be 3.1 ± 1.2 *Galerucella* spp. larvae during a 24 hour period. Despite being supplied with an abundance of *Galerucella* spp. for two weeks prior to initiation of the experiment, *A. bracteatus* nymphs continued to consume larvae. It is assumed at some point, the stink bug's appetite will be satisfied or some other cue will cause it to stop consuming larvae. Such a cue to stop feeding may occur prior to molting when stink bugs detect pheromones, causing them to aggregate together on a single host plant (Evans & Root 1980). Feeding by *A. bracteatus* nymphs was sometimes observed under field conditions while they were in molting aggregations (Diehl, personal observation). However, after completion of molting, individual stink bugs would leave the group to begin a solitary hunt for prey. Nymphs remained in close proximity to the release point of *Galerucella* spp. and were often observed with second and third instar larvae impaled on their mouthparts. The development of stink bugs from nymph to adult appeared to coincide well with *Galerucella* spp. larval development in release sites at Libau Marsh (Diehl, personal observation). First and second instar *A. bracteatus* nymphs were observed consuming first instar *Galerucella* spp. larvae early in the season, while older stink bug nymphs fed on second and third instar larvae later in the season.

In Europe, the larvae of *Galerucella* spp. are attacked by *Asecodes mento* Walk. (Hymenoptera: Eulophidae) and adult beetles by *Centistes rufithorax* Telenga

(Hymenoptera: Braconidae), both parasitic wasps. In northern Germany, the second flight period of *C. rufithorax is* synchronized with emergence of the new generation of adult *Galerucella* spp. during summer and mortality in excess of 80 per cent has been observed from field-collected adults (Blossey and Schroeder 1991). First instar *C. rufithorax* larvae overwinter inside adult *Galerucella* spp., continuing development in spring and quickly killing their host before egg laying begins. In North America, two species of *Centistes* are recorded (Muesebeck et al. 1951) and both are believed to be capable of utilizing adult *Galerucella* spp. in some release areas (Blossey and Schroeder 1991).

Early in the season, second and third instar *Galerucella* larvae are attacked by another European parasitic wasp, *Asecodes mento.* Rates of parasitism as high as 35 per cent have been reported from *Galerucella* spp. larvae collected in northern Germany (Blossey and Schroeder 1991). In North America, the genus *Asecodes* has not been recorded and will pose no threat to the introduced biological control agents.

In addition to attacking the introduced biological control agents, the predatory stink bug also attacked other soft-bodied insects within release sites including the sawfly, *Ametastegia glabrata* (Fallén) (Hymenoptera: Tenthredinidae) and the marsh caterpillar, *Simyra henrici* (Grote) (Lepidoptera: Noctuidae). Feeding on the larvae of *Galerucella* spp. by *A. bracteatus* is likely a result of their concentration near the point of release in Libau Marsh-A & B. No density estimates for stink bug nymphs were obtained from Libau Marsh-A; however, the largest number of nymphs observed at one time was a molting group of 15 second instars aggregated on a single loosestrife plant close to the point of release. It is unknown whether *A. bracteatus* was present in release sites prior to

the introduction of adult beetles, or if they invaded these sites from the surrounding area in response to the presence of *Galerucella* spp. In either case, introduced biological control agents were able to establish themselves in both release sites at Libau Marsh and the predatory stink bug should not be a factor preventing successful establishment.

Fourth and fifth instar nymphs and adults are assumed to possess higher rates of consumption compared to earlier instars. Each fourth instar nymph is estimated to consume 21.6 *Galerucella* spp. larvae per week. Therefore, this estimation of consumption is likely an underestimate for fifth instars and adults, which likely consume more larvae than fourth instars. Assuming a fixed consumption rate of 21.6 *Galerucella* spp. larvae per week for all growth stages and a development time of one week for each nymphal instar, a single stink bug may be capable of removing up to 130 *Galerucella* spp. larvae from the release site over as six week period. In Libau Marsh-A, 8-15 individual stink bugs may consume 1036-1944 *Galerucella* spp. larvae during their developmental period. If no other food source is available, removal of this many larvae from close proximity to the release point in Libau Marsh-A may be one of the factors contributing to the slow build up of *Galerucella* spp. in this site.

In Libau Marsh-A, where *A. bracteatus* nymphs are most abundant, *Galerucella* spp. have been present since 1993. Although total egg estimates have increased in this site from 1995 to 1996, population growth is much lower than that observed in other release sites after 2-3 years. The predatory stink bug may be one of the factors contributing to the slow population increase observed in Libau Marsh-A.

CONCLUSIONS

The predatory stink bug, *A. bracteatus* readily consumes *Galerucella* spp. larvae within release sites at Libau Marsh and under experimental conditions. A consumption rate of 3.4 *Galerucella* spp. larvae per day for fourth instar *A. bracteatus* nymphs has been estimated. Predation by this stink bug is likely a contributing factor to the slow growth rate observed in the Libau Marsh-A release site.

Table 12. Results of 2 day predation study using 3 densities of the predatory stink bug, *Apoecilus bracteatus* Fitch (Hemiptera: Pentatomidae), attacking 2nd and 3rd instar *Galerucella* spp. larvae caged under field conditions.

Stink Bugs/ Cage	Day	Mean Larvae/ Cage ± SEM	% Larvae Living Through Previous Day	Corrected[1] Predation (%)
0	0	14.3 ± 0.7	-	0
	1	12.3 ± 0.9	86	0
	2	8.0 ± 1.2	65	0
1	0	18.0 ± 1.2	-	0
	1	10.0 ± 1.0	56	35
	2	6.3 ± 2.3	63	2
2	0	17.0 ± 0.6	-	0
	1	10.3 ± 1.5	61	29
	2	1.3 ± 0.9	13	80
3	0	16.7 ± 0.3	-	0
	1	8.7 ± 1.2	52	40
	2	0.0	0.0	100

[1] Corrected for loss due to causes other than predation using Abbott's formula (Abbott 1925).

CHAPTER VII

GENERAL DISCUSSION

Insects Associated with *L. salicaria* in southern Manitoba

Surveys conducted in Europe (Batra et al. 1986) and eastern North America (Hight 1990; Anderson 1995) have identified many insects associated with *L. salicaria*, however no information is available for the northern Great Plains region. The main objective of the initial European survey conducted by Batra et al. (1986) was to identify potentially important candidates for the North American biological control program. In the survey conducted by Hight (1990), unexploited feeding niches in the roots, leaves and flowers of the plant were identified in the eastern United States. During my survey of insects associated with *L. salicaria*, 38 families of insects were collected in southern Manitoba. Fourteen species of herbivorous insects were found directly feeding on purple loosestrife, eight of which were lepidopteran larvae. Three of these lepidopteran larvae and the remaining six herbivorous insects were previously recorded from *L. salicaria* either in Europe or the United States (Batra et al. 1986; Hight 1990; Anderson 1995). Important non-lepidopteran herbivores identified in more than one survey include: the aphid, *Myzus lythri* (Schrank) (Homoptera: Aphididae), which Voegtlin (1995) suggested could be manipulated to contribute to the biological control of purple loosestrife; the four-lined plant bug, *Poecilocapsus lineatus* (Fabricius) (Hemiptera: Miridae); the sawfly, *Ametastegia glabrata* (Fallén) (Hymenoptera: Tenthredinidae) and the tarnished plant bug, *Lygus lineolaris* (Pallisot de Beauvois) (Hemiptera: Miridae). Other than small, localized effects on individual plants, these herbivores did not appear to limit the amount of *L. salicaria* available to *G. calmariensis* and *G. pusilla* in southern Manitoba.

Therefore, any interactions between herbivores and the introduced biological control agents in southern Manitoba should be minimal. Insects utilizing the plant will likely supplement the increased herbivore load being provided by the introduction of these two biological control agents. During this survey, the predatory stink bug, *Apoecilus bracteatus* Fiche (Hemiptera: Pentatomidae), was also identified as a potentially important predator of the introduced agents. Caged field studies using *Galerucella* spp. larvae and different densities of *A. bracteatus* nymphs were conducted to determine the predatory ability of this stink bug.

Natural Enemies of *Galerucella* spp.

In Europe, *Galerucella* spp. larvae are attacked by *Asecodes mento* (Hymenoptera: Eulophidae) and the adults by *Centistes rufithorax* (Hymenoptera: Braconidae) (Blossey and Schroeder 1991). Both insects are parasitic wasps; however, only *Centistes* is believed to be capable of utilizing adult *Galerucella* spp. as a potential host in North America (Blossey and Schroeder 1991). The genus *Asecodes* has not been recorded from North America and is not likely to be a factor in the survival of the introduced biological control agents. There are no records of North American parasitoids of the introduced *Galerucella* spp. and techniques utilized during my survey of insects associated with purple loosestrife did not detect any attacking the egg, larval and adult stages of these introduced agents. However, predation of all stages of *Galerucella* spp. by arachnids, and of eggs by Coccinellidae has been observed either in European field sites or in the United States (Malecki et al. 1993). The soil-borne fungal pathogen, *Beauvaria bassiana* (Balsamo) Vuillemin also attacks larvae and adults of *Galerucella*

spp. and has been associated with several failures to establish these agents in western Canada (Hight et al. 1995).

During the survey of insects associated with *L. salicaria* in southern Manitoba, the predatory stink bug was observed consuming the larvae of the introduced biological control agents in release sites at Libau Marsh. *Apoecilus bracteatus* is a generalist predator, attacking many types of soft-bodied insects that feed on leaf surfaces (Evans and Root 1980). In addition to consuming *Galerucella* spp. larvae, stink bug nymphs have also been observed feeding on lepidopteran larvae and *Ametastegia glabrata* (Hymenoptera: Tenthredinidae) larvae within release sites at Libau Marsh (Diehl, personal observation). In cage studies, predation by the stink bug may play a role in delaying or preventing the buildup of *Galerucella* spp. within the Libau Marsh-A release site. A group of 8-15 stink bug nymphs may be capable of consuming up to 1950 *Galerucella* spp. larvae during their developmental period from nymph to adult. Consumption of this many *Galerucella* spp. larvae in close proximity to the point of release may be a factor contributing to the slow population buildup in this site.

Overwintering Ability of *Galerucella* spp.

The recovery of adult *Galerucella* spp. in the spring following their initial introduction in 1993 demonstrated the predicted ability of adults to successfully overwinter and establish in North America (Blossey and Schroeder 1991). In Germany, Blossey (1995a) demonstrated variable overwintering survival ranging from 2 – 40% for adults of both species. Until now, no comparable studies on the overwintering ability of adult *Galerucella* spp. have been reported since their release into North American sites.

In southern Manitoba, per cent recovery of adult *Galerucella* spp. from wetland release sites was more variable, ranging from 0 – 80%. Variability may be explained by considering the source of beetles used in each overwintering experiment, in addition to the techniques of marking and handling individual adult *Galerucella* spp.

Survival was expected to be higher for individuals that were previously exposed to winter temperatures for one or two generations. Poor survival of adults obtained from the greenhouse rearing facility in Winnipeg did support this; however, no *G. pusilla* were recovered from the Delta Marsh-B cage in spring of 1996, despite these insects being descendents of those that overwintered in Delta Marsh-A during the previous winter. In agreement with Blossey (1995a), no consistent differences were observed between the overwintering ability of *G. calmariensis* and *G. pusilla*; however, better replicated studies are required to determine what differences if any exist between adults that have been overwintered in a previous generation and those that have not.

Survival Under Conditions of Spring Flooding

The introduced biological control agents, *Galerucella calmariensis* and *G. pusilla* successfully overwintered and established in several southern Manitoba wetland release sites. Apparent failure of these agents to establish in a third release site at Libau Marsh was thought to be associated with unusually high water levels in the year following introduction (Lindgren, personal communication). However, our recovery of marked individuals from cages flooded with water from spring runoff is evidence that overwintered adults are capable of surviving some degree of flooding. During the fourth year after the apparent failure to establish, large numbers of adult *Galerucella* spp., and

visibly reduced loosestrife stem densities were observed in this third Libau Marsh release site (Henne, personal observation). Survival of adult *Galerucella* spp. under flooded conditions has been previously reported from Pennsylvania and adults are known to overwinter inside the erect dry purple loosestrife stems (Hight et al. 1995). Some dead stems were standing inside overwintering cages during the winter; however, by spring, all were flattened and completely covered with water. The mechanism by which adult *Galerucella* spp. survived periods of prolonged flooding is unknown and warrants further investigation.

Dispersal and Population Growth of *Galerucella* spp.

According to Freeman (1977), most mathematical models of dispersal are used for the purposes of description, explanation or prediction of biological processes. The general nonlinear model as described by Taylor (1978) assumes the rate of change of insect density with distance is proportional to the number of eggs found at a given distance. Taylor also stated that any semilogarithmic curves generated using density-distance data do not specifically represent functional forms for dispersal, instead they are simple empirical descriptions of the phenomenon (this also applies to Taylor's general nonlinear model). In this study, Taylor's empirical model was used not only as a descriptive tool, but also to predict the total population of *Galerucella* spp. eggs in release sites.

The application and extension of Taylor's non-linear model to obtain estimates of total egg density within release sites have never been attempted using data from the introduced biological control agents, *G. pusilla* and *G. calmariensis*. In doing so, I have

demonstrated that within site dispersal of *Galerucella* is relatively slow and the majority of females tend to lay their eggs within 5 m of the initial point of release. Compared to sites receiving beetles in 1995, limited expansion of egg mass distribution close to the point of release was observed from spring of one year to the next in both 1993 release sites.

In 1995 release sites, rates of increase as measured by 1996 total egg estimate divided by that from 1995 are lower compared to sites that received beetles in 1993 (Table 8). The rate of increase of *Galerucella* spp. appears to be slow for the first couple of years after initial introduction. A slow rate of increase was also observed during the control program against tansy ragwort, *Senecio jacobaeae* L. (Compositae), in which the population of introduced cinnabar moth, *Tyria jacobaeae* L. (Lepidoptera: Arctiidae) did not begin to increase until the fourth year after initial release (Harris et al. 1971). Similarly, the chrysomelid beetles, *Chrysolina* spp. (Coleoptera: Chrysomelidae), released against St. John's Wort, *Hypericium perforatum* L. (Hypericaceae), in British Columbia took 5-13 years for their population to build up (Harris and Peschken 1971). For some species, a sharp reduction in numbers during the first year is observed and may be related to selection during a period of climatic adaptation (Harris 1984). A large reduction in the total egg estimate and number of adults from the 1995 to 1996 growing season in Libau Marsh-B supports this (Table 13). The number of adults also decreased from one year to the next in Delta Marsh-B, which received beetles in the spring of 1995. According to Harris (1971), if an agent introduced for weed control survives the sharp reduction in numbers during the first year, it may persist at low densities for several generations before the population starts to increase. This appears to hold true for

Galerucella spp. introduced for control of purple loosestrife in some southern Manitoba release sites. During the first couple of years after initial introduction, measurable impacts of the beetles on stem density and large increases in their population are generally not observed. However, by the third year after release into Delta Marsh-A, the population of *Galerucella* spp. increased to a level at which measurable impacts were observed on loosestrife stem density and fruit production. In Libau Marsh-B, a visible reduction in stem density and fruit production was observed during the 1998 growing season, four seasons after initial introduction in spring of 1995 (Lindgren, personal communication). Libau Marsh-B was the only release site in which a reduction in the total egg estimate was observed from spring of 1995 to 1996. Despite this initial drop, the population was able to cause a visible impact on the plants by the fourth year. Unfortunately, there are no data on population changes during the first two years after initial releases were made into Libau Marsh-A and Delta Marsh-A.

A slow initial buildup of the population may also be evidence of the Allee effect (Allee 1931), in which individuals seeking mates have difficulty finding them when their numbers are low. It is unknown whether chemical attraction plays any role in mate finding by *Galerucella* spp.; however, differences in male courtship behaviour between *G. pusilla* and *G. calmariensis* have been observed (Petersen 1994). If mate finding is solely based on visual cues, and adults spend most of their time wandering until they come into close proximity, then an Allee effect is more likely to be observed. Another factor contributing to the slow rate of increase is the presence of predators, pathogens and possibly parasites that utilize the newly introduced beetles. Although no parasites have been recorded from *Galerucella* spp. in North America, the stink bug, *A. bracteatus*, has

been identified as a potentially important predator of the introduced biological agents (Diehl et al. 1997). Stink bug predation is one factor likely contributing to the slow population increase in the Libau Marsh-A release site. Several failures to establish adult *Galerucella* spp. in western Canada have also been attributed to the fungal pathogen, *Beauvaria bassiana* (Hight et al. 1995). This fungal pathogen appeared to play a role in the poor oviposition observed in Delta Marsh-B after initial introduction in spring of 1995 (Diehl, personal observation).

Observed egg density-distance data collected during this study represent a snapshot in time, approximately midway or 2.5 weeks through the oviposition period of overwintered adult *Galerucella* spp. The average period of oviposition by *Galerucella* spp. is 4-5 weeks; however, eggs can continue to be laid at a reduced rate until the end of July in northeastern United States (Hight and Drea 1991). Based on the mean egg production /day by newly emerged female *G. calmariensis* and *G. pusilla* during an entire period of oviposition (Ragsdale 1995), the number of eggs laid 2.5 weeks after the start of an oviposition period, can be determined. The average number of eggs /female /day was 10.5 for *G. calmariensis* and 8.2 for *G. pusilla* (Ragsdale 1995). Using an average of 9.35 for both species, the number of eggs produced /female during a 2.5 week oviposition period is estimated to be 117.6. There is no available literature describing the shape of the oviposition curve for *Galerucella* spp. and it is unknown whether the majority of eggs are laid at the start, middle, or end of their oviposition period. This estimate of egg production does not take into account the effects of temperature on oviposition by adult *Galerucella* spp. Blossey (1995a) determined that the highest rates of 10-12 eggs /female

/day occur when temperatures are 25-30°C and lower rates occur at temperatures below 20°C.

In 1993 release sites, the number of adult female *Galerucella* spp. required to lay the total egg estimate for each year and the total number of adults are estimated using Ragsdale's data (Table 13). The number of *Galerucella* released into Libau and Delta Marsh-B is known and does not have to be estimated in 1995. However, in spring of 1996, the total egg estimate is used to predict the number of adult *Galerucella* spp. within these two sites (Table 13). As the initial number of females is known for 1995 release sites (assumed 50:50 sex ratio), the total egg estimate from 1995 is used to predict the number of eggs laid /female during the 2.5 week oviposition period. In Libau Marsh-B, each female laid approximately 87.40 eggs during the 2.5 week period, whereas in Delta Marsh-B the number dropped to 2.20 eggs /female. The number of eggs /female from Libau Marsh-B (87.40) compares well with the estimates obtained using Ragsdale's data (117.6). The difference between the two rates of oviposition may be partly attributed to egg predation by the numerous arachnids and other invertebrates present in Libau Marsh-B (Diehl, personal observation). Predation of *Galerucella* spp. eggs by spiders has been previously observed in European field sites (Malecki et al. 1993). The low number of eggs /female observed in Delta Marsh-B during spring of 1995 was a likely due to infection by the *Beauvaria* spp. fungus which killed a large number of adults prior to egg deposition (Diehl, personal observation).

Excluding Delta Marsh-A, which had a second generation of beetles in 1995 and 1996, estimates of egg to adult mortality were determined for the remaining three release sites (Table 13). Since beetles have completed a number of generations in Libau & Delta

Marsh-A, per cent egg to adult mortality was expected to be lower than in 1995 release sites. However, this was not observed and mortality was similar in all three sites, ranging from 95.52% in Libau Marsh-A to 99.78% in Libau Marsh-B. In Libau Marsh-A, where the predatory stink bugs are most abundant, per cent egg to adult mortality represents the low end of the range. Therefore, feeding by *A. bracteatus* may not be such an important factor limiting the population increase of *Galerucella* spp. in Libau Marsh-A as suggested by the cage studies and further investigation is warranted.

Two generations of *Galerucella* spp. were completed in Delta Marsh-A during 1995, and egg density estimates were made only for the first generation. Therefore, the average per cent overwintering mortality from two cages in this site (78.25%) was used to estimate the number of adults (F2 generation) in this site during fall of 1995 (Table 13). The number of eggs laid by the newly emerged adults (F1 generation) in spring of 1995 is estimated by dividing the number of adults in fall of 1995 (F2 generation) by a survival rate of 80.69% (0.8069). The per cent survival from egg to adult, prior to overwintering, was obtained by subtracting the average per cent overwintering mortality in Delta Marsh-A (78.25%) from the average per cent egg to adult mortality in the remaining three sites (97.56%). The result was then subtracted from 100% to obtain the survival rate of 80.69%. A large proportion of the mortality from egg to adult occurs during the process of overwintering. This is supported by data from the overwintering study of *Galerucella* spp., which yields an average mortality of 72.88% from the five cages. Prior to adult *Galerucella* spp. overwintering, approximately 20% mortality is observed during the period of egg deposition and adult emergence from the pupal stage in Delta Marsh-A during 1995.

Despite a similar distribution of eggs within all four release sites, a significant impact on stem and fruit production was only observed in the Delta Marsh-A release site during the 1996 growing season. This site differed from the other three sites by the presence of two release points approximately 10 meters apart. Although difficult to prove, this was likely an important factor in the establishment of *Galerucella* spp. in Delta Marsh-A. The completion of two generations by *Galerucella* spp. during the 1995 and 1996 growing seasons was an important factor likely contributing to the population increase observed in Delta Marsh-A, especially during the 1996 growing season. During the 1998 growing season, the mass emergence of F2 adults during a two week period starting the end of August was observed in a third site at Libau Marsh (Henne, personal communication). The completion of a second generation by *Galerucella* spp. in these two sites has proven the suggestion by Vega et al. (1992) that beetles might complete more than one generation in North America.

FUTURE RESEARCH

1.) Overwintered adult *Galerucella* spp. were recovered from flooded cages. The mechanism by which they survive under flooded conditions could be determined and further investigated.

2.) There is a lack of published information on predators, parasites and pathogens attacking all stages of *Galerucella* spp. in North America. Observed instances of predation, parasitism and activity of pathogens against these introduced agents could be summarized and published.

3.) The importance of the predatory stink bug, *A. bracteatus*, as a factor contributing to the slow population increase observed in the Libau Marsh-A release site remains unclear. Consumption rates have been determined and accurate density estimates of stink bugs would help answer the question of their importance as a predator of *Galerucella* spp. in this site.

4.) Developmental rates and diapause cues of *Galerucella* spp. could be further investigated to help determine in what geographical areas the completion of two generations may be possible.

Table 13. 1995 and 1996 total egg estimates, numbers of adult *Galerucella* spp. and per cent egg to adult mortality from spring 1995 to spring 1996.

Site	Release Year	1995 No. Adults [1]	1995 Total Egg Estimate I	1995 Total Egg Estimate II	1995 No. Adults Fall (F2)	1996 No. Adults [1]	1996 Total Egg Estimate	% Egg-Adult Mortality 95-96 [2]
Libau-A	1993	1,981	116,474	-	-	5,216	306,689	95.52
Delta-A[3]	1993	1,480	87,054	214,096 [6]	172,754 [5]	37,574	2,209,324	97.56 (78.25 [4])

Site	Release Year	1995 No. Released	1995 Total Egg Estimate I	1995 Total Egg Estimate II	1995 No. Adults Fall (F2)	1996 No. Adult [1]	1996 Total Egg Estimate	% Egg-Adult Mortality 95-96 [2]
Libau-B	1995	836	36,518	-	-	81	4,759	99.78
Delta-B	1995	800	877	-	-	23	1,355	97.37

[1] (1995 or 1996 Total Egg Estimate / 117.6) X 2.
[2] [1 - (1996 No. Adults) / 1995 Total Egg Estimate] X 100.
[3] 2 complete generations observed in 1995 and 1996.
[4] Average 1995-96 overwintering mortality from 2 cages in Delta Marsh-A.
[5] 1996 No. Adults / 0.2175
[6] 1995 No. Adults Fall / 0.8069.
F2 refers to second generation adults

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