EVALUATION OF AN INTEGRATED MANAGEMENT APPROACH FOR THE CONTROL OF PURPLE LOOSESTRIFE, *Lythrum salicaria* L., IN SOUTHERN MANITOBA: BIOLOGICAL CONTROL AND HERBICIDES

A Thesis

Submitted to the Faculty

of

Graduate Studies

by

Donald Charles Henne

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

Department of Entomology

University of Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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Evaluation of an Integrated Management Approach for the Control of Purple Loosestrife, *Lythrum salicaria* L., in Southern Manitoba: Biological Control and Herbicides

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

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ACKNOWLEDGEMENTS

I would like to express my sincerest thanks to the members of my examining committee, Drs. R.E. Roughley, N.J. Holliday, and R. Van Acker. I am grateful to them for the meaningful insights and contributions they made to my research program. I would especially like to thank my supervisor and friend, Dr. R.E. Roughley for his support and patience in mentoring me throughout the years.

Thanks are also extended to my adjunct advisors, Mr. T. Shane Gabor (Institute for Wetland and Waterfowl Research-Ducks Unlimited Canada) and Mr. Cory Lindgren (Manitoba Purple Loosestrife Project). I am indebted to them for allowing me to pursue this research project as a graduate degree. Their confidence in my abilities to carry out this research project to its fruition has allowed me to grow as a scientist.

I would also especially like to my parents, Douglas and Patricia Henne, for their love, support, and encouragement throughout this endeavor. I credit their faith in me for giving me strength and motivation. This thesis is dedicated to them.

I would also like to acknowledge the following individuals for their assistance to me in the field, whether it was recording data or assisting in field cage construction and repairs: Mr. P. Bouchard, Mr. J.K. Diehl, Ms. S. Steige and Mr. Paul Turner. Thanks also to Mr. Irv Gusdal (Environmental Health and Safety Office-University of Manitoba) for providing the use of a luxmeter. Thanks are also extended to Mr. Luke Skinner

(Minnesota Department of Natural Resources) for providing livestock of *Galerucella calmariensis* adults.

This research project was funded by: IWWR (Institute for Wetland and Waterfowl Research-Ducks Unlimited Canada), CMAAS (Canada-Manitoba Agreement on Agricultural Sustainability) and by NSERC grant # A0428 to RER.

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CHAPTER 1. GENERAL INTRODUCTION

Purple loosestrife (*Lythrum salicaria* L.) is a European wetland perennial that was accidentally introduced to North America in the early 1800s (Thompson et al. 1987). In North America, purple loosestrife is an example of an introduced plant species, whose dispersal and spread was allowed not only by the absence of natural enemies but also by disturbance of natural ecosystems by human activities (Malecki et al. 1993). It is said to be an aggressive plant that quickly forms monospecific stands, thereby replacing native plant species that provide food, cover, and breeding areas for a number of wildlife species. Purple loosestrife is also said to degrade natural habitats such as wetlands and riparian areas throughout the temperate regions of the United States and Canada, reducing overall biodiversity (Malecki et al. 1993, Malecki and Rawinski 1979).

Purple loosestrife has existed in North America for nearly two centuries and is now a permanent addition to our flora. The problem with purple loosestrife is that it has become extremely abundant in many areas and appears to be disrupting ecosystems. Many wetland and riparian areas may be at risk because of the problem of growth and spread of purple loosestrife infestations. In essence, purple loosestrife is a weed that needs to be controlled.

Management of purple loosestrife involves four general control approaches: cultural, mechanical, chemical, and biological. Cultural and mechanical control methods have

been largely unsuccessful (McKeon 1959, Malecki and Rawinski 1979, Rawinski 1982). Small and isolated populations of purple loosestrife can be removed by hand-pulling. However, this is not an effective technique for removing older plants or large numbers of plants. Mowing or cutting of adult plants has been shown to decrease plant vigour and retard seed production (Malecki and Rawinski 1979). However, this technique does not destroy the perennial rootstock. Cutting does reduce stem densities, but many repeated cuts are necessary and purple loosestrife has not been eliminated from any site using this technique (Haworth-Brockman et al. 1991).

Numerous studies have investigated the effectiveness of herbicides for controlling purple loosestrife (McKeon 1959, Smith 1964, Rawinski 1982, Balogh 1986, Reinartz et al. 1986, Skinner and Hollenhorst 1989, Gabor et al. 1995). Most recently, research has focused on triclopyr amine and glyphosate as potential herbicides for purple loosestrife control. Skinner and Hollenhorst (1989), and Gabor et al. (1995) reported triclopyr amine to be effective for purple loosestrife control. Triclopyr amine is a systemic herbicide selective for broad-leaved weeds. It has a number of other characteristics that make it a potential tool for purple loosestrife control. It is easily absorbed and translocated to the root system and breaks down quickly in soil and water (Dow Chemical 1988). Research indicates that triclopyr amine has no deleterious effects on fish (Mayes et al. 1984, Barron et al. 1991, Janz et al. 1991) or other aquatic organisms (Gersich et al. 1984).

Skinner and Hollenhorst (1989) reported that glyphosate can provide 95% removal of mature purple loosestrife. In addition, Rawinski (1982) found that glyphosate applied during peak bloom provided greater than 90% control and that following application desirable indigenous vegetation was re-established. Glyphosate is a non-selective herbicide that is quickly absorbed by leafy surfaces and translocated to the entire plant (Thompson et al. 1987). Moreover, it has relatively low toxicity to birds (Batt et al. 1980), mammals (Thompson et al. 1987), and aquatic invertebrates and fish (Folmar et al. 1979).

Studies by Skinner and Hollenhorst (1989), and Gabor et al. (1995) indicate that herbicide applications and subsequent removal of the canopy of purple loosestrife results in recruitment of purple loosestrife seedlings from the seed bank. In both studies high densities of purple loosestrife seedlings were reported following herbicide applications. Removal of the purple loosestrife canopy created favourable conditions for purple loosestrife seed germination and growth. Mature purple loosestrife plants can produce an estimated 2.7 million seeds per year (Thompson et al. 1987) and therefore a large seed bank exists in areas where purple loosestrife is well established (Welling and Becker 1990). Thus, an effective method of controlling seedling emergence after herbicide application is required.

Recommended methods for both triclopyr amine and glyphosate herbicides are available depending on site-specific requirements. Triclopyr amine may be effective in areas where

native vegetation still persists while glyphosate may be effective for purple loosestrife control in areas where monospecific stands of adult purple loosestrife dominate. However, neither herbicide will control purple loosestrife re-establishment from the seed bank, unless repeated applications of herbicides are employed.

Current purple loosestrife control efforts include the introduction and establishment of biological control agents as a long-term management option. Biological control of weeds is an attempt to reunite an introduced plant with its natural enemies. Biological control programs for purple loosestrife began across North America in 1992 with release approvals from the Animal and Plant Health Inspection Service, USDA, and by the Plant Protection Division of Food, Production and Inspection Branch of Agriculture Canada (Hight et al. 1995). Species approved for release in Canada are two leaf-eating beetles, *Galerucella calmariensis* L. and *G. pusilla* Duftschmidt, (Coleoptera: Chrysomelidae) which defoliate the plant; *Hylobius transversovittatus* Gauze, a root-mining weevil (Coleoptera: Curculionidae) that attacks the root system and *Nanophyes marmoratus* and *N. brevis* Boheman, flower-feeding weevils that attack the seed pods. Both species of *Galerucella* and *H. transversovittatus* were released in Manitoba in 1992 as part of a biological control program. All three species have successfully overwintered and established in Manitoba.

Herbicides and biological control are the most promising techniques available for controlling purple loosestrife. Biological control is a long-term solution because it may

take many years for the insects to build populations that are effectively large enough to control established stands of adult purple loosestrife. Although herbicides can be effective for killing adult plants, results are short-lived owing to recruitment of purple loosestrife from the seed bank. Repeated sprays may be necessary to prevent subsequent purple loosestrife re-establishment. An integration of the two techniques may be effective and thus provide immediate, as well as long-term, control of this invasive species. An important strategy for purple loosestrife control is encouraging recruitment to exhaust the seed bank but this would require measures to prevent reestablishment of newly recruited seedlings (Welling and Becker 1990). Control of purple loosestrife seedlings could be achieved through the introduction of biological control agents into the herbicide treated areas.

The rationale behind combining the two techniques is that the process of biological control could be accelerated with the aid of herbicide application. Chemical control is often avoided with biocontrol agent releases. This decision is usually made with little or no knowledge of the effects of a herbicide on a biocontrol agent (Rees and Fay 1989). Studies examining the compatibility of biocontrol agents with herbicides report that herbicides either do not affect biocontrol agent survival (Haag 1986, Kok 1980, Tipping 1991), herbicides reduce biological control agent survival (Haag 1986, Tipping 1991, Rees and Fay 1989), or that proper timing of herbicide application is crucial to ensure biocontrol agent survival (Story et al. 1988, Trumble and Kok 1979,1980a). There is the

direct risk of the herbicide to the biocontrol agent and the indirect risk of starvation through removal of its host plant at a critical time in its development.

Integrating herbicides with biological control agents is a relatively novel approach to controlling weeds. As the term 'integrated' implies, Integrated Vegetation Management (hereafter called IVM) combines different weed management practices so that the reliance on any one weed control technique is reduced (Kelner et al. 1996), and which are effective, economical, environmentally and socially acceptable (Mulder and Doll 1993, Swanton and Murphy 1996). IVM is a multidisciplinary, ecological approach to managing weed populations. IVM requires advanced managerial skills and often more time investments than traditional approaches such as herbicide applications or mechanical disturbance such as discing (Vangessel et al. 1996). Certainly in the case of purple loosestrife, wildlife managers will be faced with the decision as to what method of control will be best under their particular situation. The integration of different weed control strategies is a reality in many aquatic situations, but most employ herbicides. The increasing purple loosestrife problem along with restrictions imposed on the use of herbicides will make the development of an effective IVM system in wetlands even more critical. Therefore, information on effective purple loosestrife control techniques is extremely important to wetland and wildlife managers faced with purple loosestrife infestations. Using biological control agents in partnership with herbicides may accelerate purple loosestrife management efforts. Malecki et al. (1993) predicted that

purple loosestrife could be reduced to approximately 10% of its current level over 90% of its range using biological control.

Experimental studies under caged, field conditions in southern Manitoba were conducted to provide information needed to develop an effective and efficient IVM approach and an effective national purple loosestrife control strategy. The objective of this study was to investigate an IVM approach for purple loosestrife control using biological control agents and herbicides. Field experiments (1996-98) were conducted in an established stand of purple loosestrife at Netley-Libau Marsh to determine effectiveness of single techniques and combinations of techniques. The biological control insect used was *G. calmariensis*. This species was chosen because it is easily reared, has high reproductive potential and has established and over-wintered successfully in Manitoba. The closely-related *G. pusilla* has also established and over-wintered successfully in Manitoba and could have been used in this study as well. The remaining biological control insects (*H. transversovittatus*, *N. marmoratus*, *N. brevis*) would not be suitable for an IVM approach. Herbicides kill the adult loosestrife plants and therefore eliminate the food source for the root boring and flower-feeding weevils. *Galerucella calmariensis* can move from adult purple loosestrife plants to untreated areas ("refugia") and then to the target plants, the emerging purple loosestrife seedlings.

There are a number of important considerations regarding the biology of the insects and the timing of the herbicide application. Two general combinations of biological control

and herbicides may be considered. First, mature purple loosestrife plants could be removed using a broad-leaf plant selective herbicide such as triclopyr amine. Following mature purple loosestrife removal, biological control agents would be released to control purple loosestrife re-establishment from the seed bank. A second approach would involve releasing insects, allowing them to establish, and then using triclopyr amine or glyphosate to eliminate the mature plants.

Triclopyr amine is generally applied in July, at a time when adult *G. calmariensis* are active. Following herbicide application, potted purple loosestrife plants would be introduced. These areas would provide feeding sites ("refugia") for the adult insects until purple loosestrife seedlings emerged. Glyphosate would be applied in late August following adult insect dormancy. In the spring, adult insects would emerge and be sustained by purple loosestrife seedlings. It is expected that the integration of biological control agents with herbicides will effect long-term control of purple loosestrife much faster than either method used alone.

CHAPTER 2. REVIEW OF THE LITERATURE

2.1 INTRODUCTION

Plants that we refer to as weeds present special problems. They compete with other plants for limited resources such as moisture, light and nutrients. As a result, some plants lose the struggle to survive against such competitive odds, and are displaced. Often, these displaced flora are economically important and/or rare plants. More than 1,800 species of weeds are responsible for serious economic losses each year, of which about 200 can be found with any particular cultivated crop (Shaw 1982). Weed problems do not exist solely in agroecosystems, however. Natural systems, such as wetlands and grasslands are also susceptible to weed infestations. Invasions by non-native plant species are said to degrade biological communities, threatening survival of many native species (Randall 1996). Purple loosestrife, *Lythrum salicaria* L. is often cited in the literature (e.g. Thompson et al. 1987, Malecki et al. 1993) as being responsible for the degradation of wetland habitats and reductions in wetland biodiversity.

The primary aim of this literature review is to discuss purple loosestrife history, biology and control practices and to discuss the development of an Integrated Weed Management (IWM), also called Integrated Vegetation Management (IVM), program for control of this invasive weed. Examples of control practices against other weeds are included in this review in order to evaluate methods that may or may not apply to a purple loosestrife

management program, and why most purple loosestrife control strategies have largely failed.

2.2 DEFINITION OF A WEED

The literature contains many definitions of the term weed. Some definitions are vague and subjective while others are broader and include specific negative impacts of certain plants on their surrounding flora, such as inhibiting growth by competing for light, water and nutrients. Flint and Van den Bosch (1981) define weeds as plants that grow in places where they are not wanted. Similarly, DeOng et al. (1972) define weeds as any vegetation which grows in an undesired location. By these definitions any plant can, under certain circumstances, become a weed. For example, plants such as volunteer Canola (*Brassica napus* L., *B. rapa* L., (Cruciferae)) and volunteer wheat (*Triticum aestivum* L., (Graminaceae: Hordeae)) which are of value as important crops may themselves become weeds where, for aesthetic or economic reasons, they are not wanted (DeOng et al. 1972). Even trees, may be regarded as weeds. To better understand why certain plants become weeds, it is necessary to examine certain traits that weedy plants possess.

2.2.1 CHARACTERISTICS OF WEEDS

Weeds are classified as being either monocotyledonous (grasses and sedges-Monocotyledonae) or dicotyledonous (broadleaf plants-Dicotyledonae). Dicot weeds, or broadleaf weeds, including loosestrife appear to comprise the vast majority of weed

problems. Weeds are also commonly classified according to the length of their life cycles: annuals (one-year life cycle), biennials (two-year life cycle) or perennials (living for more than two years), (Cranshaw 1992). Perennial weeds such as loosestrife can live for many years and are regarded as being among the most difficult weeds to control. Many perennials produce extensive root systems that allow the plants to resist destruction by practices that kill only the aboveground portions of the plants (Cranshaw 1992). These practices include burning, mowing and flooding. The effects of each of these practices on purple loosestrife are discussed within this review.

Over 700 different species of plants, now considered to be weeds, were introduced into North America from Europe and Eurasia (Cranshaw 1992). There is a tendency for plants to become a nuisance in areas where they have been introduced. McWhorter and Shaw (1982) find that little useful information has been developed to explain why new, exotic weed species, or even some previously inconspicuous native species, emerge into agroecosystems (and natural systems) as a major threat, or what factors determine and regulate the rate of spread. One cause may be that the physical and environmental conditions in some parts of the world are at least as suitable and/or more extensive than in the weed's original habitat (Wilson 1964). Another cause is that these plants are under effective, naturally occurring, biological control in their native habitats (Flint and Van den Bosch 1981). According to Hight and Drea (1991) exotic plants that are introduced to new regions usually lack the guild of phytophagous insects, mites, and pathogens that normally suppress populations of plants in their native area. The greater persistence of

purple loosestrife in North America than in Europe is thought to be due to the relative absence here of its co-evolved European parasites, predators and competing plant species (Mal et al. 1992). Blossey and Notzold (1995) hypothesized that introduced invasive plants possess shifted biomass allocation patterns. In the absence of herbivores, selection will favor genotypes with better competitive abilities and reduced resource allocation for defense against herbivores (which are now absent). These competitive abilities can be enhanced by increasing vegetative growth or reproductive efforts. If exotic weeds have these shifted allocation patterns then they may be more vulnerable to herbivory once they are reunited with their co-evolved herbivores.

Shaw (1982) states that weeds comprise an early stage of plant succession that terminates with the climax vegetation that is characteristic of the area. For example, there is a persistent ecological pressure to change agroecosystems back to weeds, forage, shrubs and trees. In cropland and other disturbed areas, numerous ecological niches are initially unfilled, creating opportunities for the establishment of aggressive species of plants such as weeds (Klingman 1991).

One view of competition between plants emphasizes competition for resources, niche differentiation and resource partitioning as the primary mechanisms whereby competition and coexistence in plant communities can be described and explained (Johansson and Keddy 1991). Another view emphasizes competition for space, dominance control and competitive hierarchies (Johansson and Keddy 1991). The problem with weeds is that

they aggressively compete with other plants for resources such as light, water, space and nutrients (Cranshaw 1992). In wildlands, weeds alter ecosystems and communities, using resources that would have been otherwise available to and utilized by native plant species (Randall 1996).

Most weeds also tend to be highly aggregated in distribution. Johnson et al. (1995) studied the spatial distribution of broadleaf weeds in several maize and soybean fields in eastern Nebraska. They found that individual species as well as species assemblages are highly aggregated. Indeed, purple loosestrife is often cited as forming large monospecific stands where it has become well established.

Invasive plants can displace native species, suppress native species recruitment, support non-native animals by providing food and cover, harbor fungi or microbes and hybridize with native species, altering gene pools (Randall 1996) thereby degrading or eliminating habitat for native animals. Purple loosestrife is a strong competitor and tends to displace neighboring vegetation (Hight 1990). For example, shading of native vegetation by weeds such as purple loosestrife can slow early growth of these plants considerably. Obstruction of light by taller weeds can prevent native plants from realizing their optimum photosynthetic rates and ability to procure available moisture and nutrients (Flint and Van den Bosch 1981).

Purple loosestrife is highly successful in inter-specific competition in sites with high or low nutrient supply levels (Shamsi and Whitehead 1977). For instance, purple loosestrife is tolerant of a wide range of ecological conditions and is able to make direct developmental responses. For example, purple loosestrife can produce aerenchyma in submerged stems and can adjust leaf size in response to reduced illumination (Thompson et al. 1987).

2.3 THE PURPLE LOOSESTRIFE PROBLEM

Purple loosestrife belongs to the loosestrife family Lythraceae, which consists of ≥30 species, mostly of north temperate regions (White et al. 1993). It is an aquatic plant found in wetlands, coastal areas, ditches, and along stream banks (Hight 1990). Purple loosestrife is readily identified during the period from late June to early September, when the terminal, spike-like panicles are covered with reddish-purple blooms (Hight 1990). The period from germination to flowering is short (about 8-10 months) and flowering continues until September to October in some areas of Europe (Shamsi and Whitehead 1974). Purple loosestrife is an erect, perennial, herbaceous, dicotyledonous plant 0.5 to over 3.5 m tall with up to 30-50 stems arising from a persistent perennial tap root and spreading rootstock of up to 0.5 m in diameter (Thompson et al. (1987), Mal et al. (1992), Mullin (1998)). Purple loosestrife shoots do not persist above ground through the winter but resume growth the following spring (Shamsi and Whitehead 1977). This large taproot is the main organ of vegetative reproduction (Shamsi and Whitehead 1974). Vegetative shoots may be capable of reaching maturity and producing seed within the

same growing season (Shamsi and Whitehead 1974). Purple loosestrife is said to be an invasive weed and certain variables can be used in identifying invasive species. Begon et el. (1990) describe traits that these species possess. These include: 1) colonizes habitats that are unpredictable in nature (i.e. disturbed), 2) reproduces rapidly, 3) matures early, 4) high reproductive outputs, 5) short juvenile periods and 6) small seed sizes. These variables point to an underlying r-K selection continuum (i.e. early-late successional roles), (Rejmanek and Richardson 1996). So-called 'r strategists' are the most successful invaders, with most invasions occurring in disturbed habitats. The biological process of invasion has two distinct phases: 1) initial establishment of a species at a single spatial location, and (2) the spread of a species in space. Purple loosestrife is an early successional species on all types of open, wet soils, where it forms dense, monospecific stands (Blossey 1993). Unlike other successional species, purple loosestrife can persist for years, re-growing out of the rootstock, even after certain mechanical control attempts (e.g. mowing, cutting), (Blossey 1993, Smith 1964). In North America, infestations of purple loosestrife follow a pattern of establishment, maintenance at low numbers and then a dramatic population increase under optimal conditions (Mullin 1998). In Europe, populations of purple loosestrife are rapidly invaded by other plant species, later forming a regular but infrequent component of mixed wetland vegetation (Shamsi and Whitehead 1974, Blossey 1992). Purple loosestrife is a prolific seed producer. There are estimates that one plant may produce as many as 30-50 stems (Thompson et al. 1987). A single stem may produce as many as 900-1000 seed capsules (Shamsi and Whitehead 1974, Thompson et al. 1987). A single rootstock, then, is capable of producing as many as 2.5-

2.7 million seeds annually (Thompson et al. 1987, Malecki et al. 1993). Perennial plants such as purple loosestrife may survive for decades (Blossey and Schat 1997) and can thus produce many millions of seeds throughout their lifetime. These seeds are extremely small, weighing approximately 0.5 mg (Blossey and Notzold 1995), and are produced in seed capsules (Hight 1990). The great abundance of purple loosestrife wherever it has become established provides an enormous supply of seed for further invasion of other wetland areas (Smith 1964). Seeds germinate best on moist, open ground and plants will survive in 50% full sunlight (Keddy 1988). Rawinski (1982) found that seed viability decreased from 99% to 80% after two years of storage in a natural body of water. Fresh seed and seed that was stored under cool, dry conditions for three years showed no differences in percent germination (Shamsi and Whitehead 1974). Maximum germination occurs when mean maximum temperature is 19.5° C (Shamsi and Whitehead 1977). No germination of purple loosestrife occurs below 14° C and the critical temperatures for germination are between 15 and 20° C (Shamsi and Whitehead 1974). On germination, the primary root grows deep into the soil and soon develops branched secondary and tertiary roots (Shamsi and Whitehead 1974). Later, the roots thicken, harden and develop a sclerenchymatous central core. At early developmental stages, seedlings are vulnerable to loss of shoots and leaves but can compensate for loss of photosynthetic tissue at the expense of underground storage to ensure survival until winter (Blossey and Schat 1997). Invasiveness of woody species having dry fruits and a mean seed mass of <2.0 mg are very often limited to wet habitats and exposed mineral soils (Rejmanek and Richardson 1996). Welling and Becker (1990) found mean purple

loosestrife seed densities of over 400,000 seeds m^{-2} in the top 5 cm of soil. Under certain natural conditions, seedling densities of 10,000 to 20,000 plants m^{-2} have been documented (Rawinski 1982). When the right disturbance conditions occur, a large population increase can occur within one or two years (Thompson et al. 1987). In North America, it is estimated that purple loosestrife is rapidly spreading at a mean rate of 645 km^2 per year (Thompson 1991). It is these aforementioned attributes that are thought to be the primary reasons that have allowed purple loosestrife to outcompete and eliminate other species in areas where it exists (Malecki et al. 1993).

Approximately 14% of Canada, or 1.27 million km^2 is covered by wetlands, primarily concentrated in Manitoba, Ontario, and the Northwest Territories (Mal et al. 1992). Purple loosestrife aggressively invades wetlands (Mal et al. 1992; Hight et al. 1995), forming huge monospecific stands that are thought to reduce the biodiversity of ecosystems that they infest (Stuckey 1980, Mal et al. 1992, Blossey 1993, Blossey et al. 1994). Purple loosestrife may have displaced more than 50% of the native vegetation biomass in some wetlands (Thompson et al. 1987). As a result, many wildlife species are thought to be on the decline. This decline is interpreted as being due to changes in physical and trophic structure with a concomitant reduction in habitat diversity (Mal et al. 1992). Specific detrimental effects of purple loosestrife on gamebirds and waterfowl include 1) the creation of unsuitable nesting habitat and breeding cover for many wetland waterfowl inhabitants, and 2) the low food potential of purple loosestrife and the displacement of other food plants, such as *Typha* spp. (Batra et al. 1986, Hight 1990,

Anderson 1995). Purple loosestrife also provides poor habitat for muskrats (*Ondatra zibethicus spatulatus* (Osgood) (Mammalia: Muridae)) and marshwrens (*Cistothorus palustrus* (Wilson) (Passeriformes: Troglodytidae)) but provides more suitable habitat for red-winged blackbirds (*Agelaius phoeniceus* (L.) (Icteridae)) (Rawinski 1982). Purple loosestrife is also reported to be of little value as food or shelter for many wetland species (e.g. Thompson et al. 1987). Native plant species normally used for food and shelter may become scarce as a result of displacement by purple loosestrife. Large, spreading infestations of purple loosestrife may threaten many plants and animals such as bulrushes (*Scirpus longii* Fern.) in Massachusetts, dwarf spikerush [*Eleocharis parvula* (Roemer and Schultes) Link] in New York and the bog turtle (*Celmmys muhlenbergii* Schoepff (Reptilia: Emydidae)) in the northeastern United States (Rawinski et al. 1982). The impact of this scarcity of resources on wildlife has not been subjected to proper scientific investigation. Several authors (e.g. Malecki and Rawinski 1979) cite convincing observations that would support the hypothesis that purple loosestrife infestations in wetlands result in a reduced ability for these ecosystems to support a wide range of animals. In some western states, purple loosestrife establishment in irrigation systems impedes water flow (Malecki et al. 1993). Purple loosestrife forms dense, brush-like stands that are difficult to penetrate by boat and on foot (Balogh and Bookhout 1989), and forms wide crowns that dominate the herbaceous canopy (Thompson et al. 1987). The impact of loosestrife on agriculture is the reduction in quality of wetland pasture and wetland wild hay meadows because it is less palatable to livestock than the grasses and sedges that it displaces (Thompson et al. 1987, Hight 1990).

2.3.1 INTRODUCTION TO NORTH AMERICA

The introduction of purple loosestrife to North America has been thoroughly investigated by several authors (e.g. Thompson et al. (1987), Hight (1990), Mal et al. (1992), Blossey et al. (1994); Hight et al. (1995). Purple loosestrife was thought to have been introduced to North America from Eurasia in the early 19th century as a contaminant of the ballast of sailing ships trading between various European countries and the eastern seaboard of North America. These ships would often use moist sand from tidal flats as ballast, and unload it on North American shores upon arrival. This sand probably contained purple loosestrife seeds (McAvoy et al. 1997). By the 1830s, purple loosestrife was already well established along the New England seaboard (Malecki et al. 1993). According to Mal et al. (1992), primary colonization of loosestrife in Canada began in the Maritime Provinces, migrating through rivers, canals and other waterways. In addition, European settlers intentionally introduced purple loosestrife as a medicinal herb and as an ornamental (Blossey et al. 1994). Today, purple loosestrife is often cultivated in gardens from which it can escape into nearby wetlands and become established along edges of ponds, in roadside and railroad ditches, riparian pastures and grazing lands, agricultural areas, and in low, wet meadows and marshes that are submerged in the spring and become dry in the summer (Stuckey 1980, Hight 1990, Manguin et al. 1993). Purple loosestrife cultivars (forms of *L. virgatum*, a native *Lythrum* species) developed in the mid 1900s in Manitoba were originally thought to be sterile but Ottenbreit (1991) found that wild purple loosestrife artificially crossed with certain cultivars produced hybrid plants that were fully fertile. Similarly, Lindgren and Clay (1993) conducted field tests and found that one such

cultivar, 'Morden Pink', will cross-pollinate with wild purple loosestrife plants and produce viable seeds. Some taxonomists consider *L. salicaria* and *L. virgatum* to be the same species, since they differ by only a few minor diagnostic characteristics and freely interbreed (Vick 1992).

In its native range, purple loosestrife is a pioneer plant of fen communities, ranging from Great Britain to central Russia and it is locally abundant in areas of Sweden and Finland, with total plant populations exceeding 10,000 plants (Shamsi 1976, Blossey 1995). The northern limits of purple loosestrife are near the 65th parallel and the southern limits are along the Mediterranean basin in North Africa (Blossey 1995). In southern European countries along the warm coastal areas, purple loosestrife is not hindered by delay in germination and growth, and not only undergoes vigorous growth but also successfully competes with other plants, establishing itself as a dominant plant (Shamsi and Whitehead 1977). Purple loosestrife is now well established and can be extremely abundant throughout the northeastern and mid-western United States. It also occurs in scattered locations in the northwestern states, the Pacific Northwest, and California (Manguin et al. 1993). There is also an area of high abundance of purple loosestrife in the southernmost part of Texas (Mal et al. 1992). Purple loosestrife occurs in all 10 Canadian provinces with the largest and more serious infestations located in southern Ontario, Quebec, and Manitoba (DeClerck-Floate 1992). In southern Manitoba, purple loosestrife is found along major rivers, with large concentrated areas along the Cypress,

Red, and Assiniboine rivers, as well as at Netley-Libau and Delta marshes south of Lakes Winnipeg and Manitoba respectively (Diehl et al. 1997).

Purple loosestrife has been declared a noxious weed under the weed control acts of various provincial or state governments of Canada (Manitoba) and the United States (California, Idaho, Minnesota, Ohio, and Wisconsin), (Mal et al. 1992). Manitoba and Prince Edward Island have placed a total ban on the sale of *Lythrum* varieties and many nurseries in other provinces are voluntarily withdrawing *Lythrum* from sale (DeClerk-Floate 1992). There are problems associated with such an act, such as enforcement and recognition of the plant as a pest (Vick 1992).

2.4 INTEGRATED WEED MANAGEMENT-(IWM)

As the term 'integrated' implies, integrated weed management (hereafter as IWM) combines different weed management practices so that the reliance on any one weed control technique is reduced (Kelner et al. 1996). IWM approaches are said to be effective, economical, environmentally and socially acceptable (Mulder and Doll 1993, Swanton and Murphy 1996). IWM is a multidisciplinary, ecological approach to managing weed populations. The aim of IWM is to unite different components in a coordinated effort to limit the impact of weeds (Schweizer 1988). These components are numerous and require the integration of weed biology with management strategies (Vangessel et al. 1996). The use of biological control agents (insects and pathogens) should include monitoring at regular intervals for annual and perennial weeds, removing

weeds by hand-pulling, selecting appropriate herbicides, and spot treating with herbicides.

A single method cannot control all weeds. The notion behind IWM is that if more than one weed control strategy is utilized, weeds can be prevented or delayed from adapting to a single method of control. This is accomplished by creating conditions that are unfavorable to weeds, often by exerting such pressure that growth of native vegetation is favored over that of weeds, while maintaining suitable conditions for the beneficial vegetation (Walker and Buchanan 1982, Slife 1991). To be considered successful, a weed control method must reduce weed densities to the economic threshold level or less (Messersmith and Adkins 1995). However, no such economic threshold has been established for purple loosestrife. In the case of purple loosestrife, eradication is not the aim of control efforts. The primary goal is to stop the spread of this invasive plant and to reduce the density of existing infestations.

Some view IWM as a means of protecting the environment from excessive pesticide use (Shaw 1982) but it requires advanced managerial skills and often more time investments than traditional approaches (Vangessel et al. 1996), at least in the short-term. Certainly in the case of purple loosestrife, wildlife managers will be faced with the decision as to what method of control will be best under their particular situation. McWhorter and Shaw (1982) caution that problems may be encountered in developing weed-control strategies in aquatic systems. One problem is that aquatic areas are often multiple-use areas.

Conflicts of interest may result if there is a lack of consensus about control strategies. There is the perception by some that purple loosestrife is beneficial because of its aesthetic attractiveness, and because of its role as a source of nectar for honey bees. Another problem is the use of herbicides in aquatic systems. The integration of different weed control strategies is a reality in many aquatic situations, but most employ herbicides. The increasing purple loosestrife problem along with restrictions imposed on the use of herbicides will make the development of an effective IWM system in wetlands more difficult in the future.

2.5 REVIEW OF PURPLE LOOSESTRIFE CONTROL

According to Gabor et al. (1996), the development of an effective control strategy for purple loosestrife has important implications for wetland management throughout the continent, which is primarily the recovery of wetlands that are degraded by purple loosestrife infestations. Traditional control of purple loosestrife has utilized water level manipulation (flooding and drawdowns), mowing and cutting, burning, and the application of herbicides (2,4-D, glyphosate, triclopyr), (Blossey et al. 1994). These techniques can successfully eliminate small, young stands but can be expensive and require long-term management (Blossey et al. 1994). More recently, several species of insects that are monophagous feeders on purple loosestrife have been introduced into North America from Europe in an attempt to reduce purple loosestrife infestations using biological control.

2.5.1 BIOLOGICAL CONTROL

2.5.1.1 Introduction

The concept of classical biological control arose from observations that some of the most damaging plant and insect pests in North America were aliens that were native to the Eurasian continent and were spread worldwide by marine exploration and commerce (Thompson et al. 1987). In their native areas most species are prevented from becoming too abundant by competition from other species and by control by a suite of natural enemies (Malecki et al. 1993). These interactions usually provide a balanced, self-sustaining system. The term classical biological control involves the selection of one or more natural enemies of a pest species from its native area. These natural enemies are then introduced to the invaded region in an effort to reduce the population density of the pest to an environmentally or economically acceptable level (Hight and Drea 1991). Weed biological control (or biocontrol) is the manipulation of a plant's natural enemies to reduce populations to an acceptable level (Malecki et al. 1993). Historically, biocontrol organisms have not been used in IWM systems on the scale that biocontrol techniques have been applied for use against pest insects and diseases (McWhorter and Shaw 1982). Range, pasture, and aquatic areas are not usually disturbed to the same extent as annual cropping systems. Thus, they may be more conducive for development of biological control techniques that tend to work best under conditions of minimal disturbance (Zimdahl 1991). Control methods such as drainage, discing, and repeated mowing are commonly employed in agricultural land. These would not be compatible with the

maintenance of natural areas or with most wildlife habitat objectives, where the need to maintain community integrity requires minimum impact methods (Thompson et al. 1987).

2.5.1.2 Insects

Batra et al. (1986) found 120 species of phytophagous insects associated with purple loosestrife in Europe, including 14 species apparently restricted to this plant. Five species of natural enemies of purple loosestrife were approved by USDA-APHIS for introduction into North America in 1992 (Malecki et al. 1993, Hight et al. 1995), i.e. (leaf-feeding beetles *Galerucella calmariensis* L., *G. pusilla* Duftschmidt (Coleoptera: Chrysomelidae), *Hylobius transversovittatus* (Goeze), *Nanophyes marmoratus* Goeze, and *N. brevis* Boheman (Coleoptera: Curculionidae). *Hylobius transversovittatus* is a root-boring weevil and is also highly host-specific on purple loosestrife (Blossey et al. 1994). Pre-release quarantine tests showed that both *Galerucella* species avoided the taxonomically related host plant, *Decodon verticulatus* L., Ell. and *Lythrum alatum* (Malecki et al. 1993). Both plant species are native to North America. In Canada, *D. verticulatus* is found only in southern Ontario and *L. alatum* is found in Ontario and British Columbia (Cody 1978). Both species of *Galerucella* are host-specific on purple loosestrife, are primarily leaf-feeders in both the larval and adult stages, and are capable of completely defoliating the plant (Malecki et al. 1993). These beetles prefer to consume the young buds and leaves on shoot tips (Blossey 1992). Feeding on stem tissue and inflorescences has also been observed in the field. These host-specific insects are expected to reduce the competitive ability of purple loosestrife, thus favoring the

currently suppressed North American flora (Blossey 1993). Blossey (1992) demonstrated that both introduced *Galerucella* spp. are key factors in the population dynamics of purple loosestrife in Europe. Adult *G. calmariensis* are approximately 5 mm long and emerge from the soil from overwintering at about the same time that emergent purple loosestrife shoots are appearing. Both *Galerucella* species overwinter in leaf litter at or close to a purple loosestrife site (Blossey, 1995). *Galerucella* females require 3-4 weeks to reach full oviposition capacity of 10-12 eggs/day (Blossey 1995). According to Blossey (1995), eggs are distributed evenly over entire plants and adult feeding and oviposition last for approximately 2 months. Development from egg to adult is 20-40 days (Blossey and Schat 1997). Young larvae feed primarily on developing leaf and flower buds while older larvae feed on all plant parts (Blossey and Schat 1997). New generation F_1 adults appear in midsummer and feed for a short while before overwintering. A small second generation can be produced by early emerging F_1 adults (Blossey 1995). Both *Galerucella* species have minor oviposition periods before they overwinter but individuals that emerge early can have oviposition rates that are comparable to peak levels of overwintered adults (Blossey 1995).

Galerucella calmariensis negatively affects purple loosestrife plant performance, even at low densities (Blossey and Schat 1997). Herbivory reduces the amount of biomass allocated to shoots and roots, resulting in a decrease in total dry biomass (Blossey and Schat 1997). The largest negative impact of herbivory by *G. calmariensis* was biomass allocation to long-term storage in the rootstock (Blossey and Schat, 1997). Larvae readily

leave defoliated plants and seek out unattacked plants (Hight and Drea 1991). At lower populations, adult and early larval feeding prevents the normal growth of purple loosestrife by destroying terminal meristematic regions (Hight and Drea 1991). The continued larval feeding throughout the period of maximum plant growth delays and often prevents the production of flower spikes (Hight and Drea 1991). In outbreak densities the beetles kill seedlings, completely defoliate mature plants, and destroy or prevent the formation of flower spikes (Hight and Drea 1991). At high larval densities (200 larvae/plant) plants were completely stripped of all green tissue, leaving only whitish skeletons, thereby preventing seed production (Hight and Drea 1991). None of these insects are integrated yet with any other purple loosestrife control method and little effort in general has been directed toward integrating biological control with other more conventional weed practices. This may be due to the limited number of proven weed-control insects for use against weeds, combined with the observation that other techniques are more economically feasible (Andres 1982). Insects used for weed control often take several years to reach effective population densities, or they may be only marginally effective (Messersmith and Adkins 1995). Integrating herbicides with weed biocontrol insects may provide the most satisfactory control of loosestrife and other weed problems. The rationale is that weed densities could be reduced below the economic threshold more quickly than through the use of biocontrol insects alone. Another rationale is that biocontrol success could be increased where they would otherwise be marginally effective alone (Messersmith and Adkins 1995, Gabor et al. 1996).

In order to determine if biological control insects with herbicides are compatible as an integrated strategy, an evaluation of the effects of herbicides on biocontrol agent fecundity, behavior, mortality, etc. must first be carried out. Such an evaluation was carried out for the purple loosestrife biocontrol agent, *G. calmariensis* by Lindgren et al. (1998). They reported that the herbicide, triclopyr amine, used at a rate similar to that used on loosestrife has no significant effects on adult oviposition, mortality or viability. Similarly, no adverse effects were detected with larvae exposed to similar rates of triclopyr amine

2.5.1.3 Mycoherbicides

A leaf spot fungus, *Septoria lythrina* Pk. has been isolated from purple loosestrife (Mal et al. 1992), but no mycoherbicides have been developed from pathogens of purple loosestrife either in North America or Europe (Nyvall 1995). Nyvall and Hu (1993), however, isolated two promising candidates for mycoherbicidal control of purple loosestrife, *Alternaria alternata* (Fries) Keissler and *Botrytis cinerea* Pers.: Fr.. Plants exposed to these organisms were either killed, had numerous leaf spots, or were stunted due to necrosis of apical growing points. Nothing else has been reported in the literature regarding fungi and other pathogens attacking purple loosestrife.

2.5.2 HERBICIDES

2.5.2.1 Introduction

More than 80% of the weed science effort has been associated with the development of food production systems that involve herbicides as the key component for weed control (McWhorter and Shaw 1982). The current cost for registering a pesticide (including the cost of a production plant) can be as high as $200 million, with successful registrations often taking 7-10 years (Hamill et al. 1994). Materials manufactured for the control of weeds and other unwanted vegetation account for more than one-half of the pesticides used and produced in the United States (Flint and Van den Bosch 1981).

2.5.2.2 Purple loosestrife control with herbicides

The mode of action of herbicides encompasses the entire progression of events from the time a compound contacts a plant to the time at which the plant is dead or growth is suppressed (DiTomaso and Linscott 1991). The most commonly used herbicides against loosestrife are: glyphosate [N-(phosphonomethyl)glycine], 2,4-D (2,4-dichlorophenoxyacetic acid), and on an experimental basis, triclopyr {[3,5,6-trichloro-2-pyridinal)oxy]acetic acid}, (Blossey et al. 1994). Of the three, glyphosate is most commonly used for herbicidal control of purple loosestrife. Glyphosate is a systemic herbicide that is absorbed by leaf surfaces and translocated to the entire plant, including roots and underground stems. It is effective at relatively low concentrations, and has a low bioaccumulation potential (Thompson et al. 1987). Brust (1990) demonstrated that there was no toxic or repellent effect of glyphosate to five species of carabid beetles in the field. Forschler et al. (1990) found that soil nematodes exposed to glyphosate had reduced activity in aqueous suspensions. This herbicide however, failed to reduce the

ability of these nematodes to infect insect larvae when the nematodes were washed after they were removed from the herbicide solution. Herbicides routinely applied generally fail to provide effective long-term control of perennial weeds (Glenn et al. 1997). Results of field trials suggest that once purple loosestrife is established, it is virtually impossible to control over the long term with herbicides alone (Mal et al. 1992). Well-timed applications of glyphosate are effective against purple loosestrife but pose a risk to nontarget grasses (Rawinski 1982). Malecki and Rawinski (1985) found that glyphosate was effective in killing 100% of adult purple loosestrife plants when applied at a rate of 1.7 kilograms per hectare, during the bloom stage of purple loosestrife. These authors, however, did not discuss purple loosestrife recruitment from the seed bank. Triclopyr amine is an auxin-type systemic, broadleaf herbicide that breaks down quickly in water and soil (Dow Chemical Co. 1988). Gabor et al. (1995) evaluated the effects of triclopyr amine on purple loosestrife and non-target vegetation in a southern Ontario wetland. They found that application of triclopyr amine at a rate of 12.0 kg/ha effectively controlled adult purple loosestrife, but stressed that additional applications are likely required to prevent re-establishment from the seed bank in the soil. Using a selective herbicide such as triclopyr amine to manage purple loosestrife would result in reduction of the target with minimal disturbance to desirable monocot vegetation. Unlike the United States, neither of these herbicides are registered in Canada for use over water (DeClerck-Floate 1992). Smith (1964) recognized that treating purple loosestrife with various herbicides (including 2,4-D) did not result in eradication of all the loosestrife plants, evidenced by resprouting the following season. Moreover, a small percentage of

purple loosestrife rootcrowns appear to undergo summer dormancy, a fact which will further reduce the effectiveness of herbicides (Thompson et al. 1987, Mal et al. 1992). A similar situation exists for Canada thistle (*Cirsium arvense* (L.) Scop.) control with herbicides. Thomas et al. (1994), and Moore (1975) report that single herbicide applications do not provide long-term control due to the difficulty in killing the root system, which can survive even though the shoots are destroyed. Attempts to control leafy spurge (*Euphorbia esula* L., (Euphorbiaceae)) with herbicides have also met with limited success, due largely to the regenerative capabilities of the deep, extensive root system (Stougaard et al. 1994).

2.5.2.3 Resistance to herbicides

All biological species harbor genetic variation. Populations respond to environmental change by shifts in their genome (Holt and Hochberg 1997). The evolution of resistance by insect and weed pests to chemical pesticides is a problem of increasing importance in applied ecology (Holt and Hochberg 1997). Shaner (1995) argues that the weed science community has not been successful in preventing herbicide resistance. Intensive use of specific and selective herbicides has led to the evolution of resistance in weed species common to many cropping systems (Maxwell et al. 1990). Reasons for the increase in resistance to pesticides in general include the difficulty in predicting the spread of resistance, and the lack of economically feasible alternatives to pesticides (Shaner 1995). Resistance to pesticides is reported in at least 447 species of insects and mites, 100 species of plant pathogens, 48 species of weeds, five species of rodents, two species of

nematode parasitic on plants and five species of nematodes parasitic on animals (Rousch 1991). Weed resistance to herbicides has followed the same pattern of resistance by insects to insecticides and fungi to fungicides. The evolution of resistance has taken longer to occur in weeds. One reason is thought to be primarily because of the limited number of generations produced per year as compared to other pests (Slife 1991). As older herbicides are being removed from the market because of lack of re-registration or regulatory activity, weed control is more often being practiced with herbicides with a single mode of action (Elmore 1996). Resistance develops through the continuous use of a herbicide or herbicides sharing the same mode of action as the primary method of weed control (Shaner 1995). For example, populations of weed species resistant to sulfonylurea herbicides have developed rapidly since the introduction of this herbicide family. Tank mixing or rotating herbicides may be one effective way in preventing development of populations of weed-resistant biotypes (Durgan et al. 1997). There are at least two reported cases of glyphosate resistance in rigid ryegrass, *Lolium rigidum* Gaudin (Graminaceae: Hordeae) in Australia (Robert and Baumann 1998). There are no reported instances of purple loosestrife resistance to herbicides in the literature.

Herbicides have also been of concern because of detection of residues in some surface and groundwater sources, leading many agriculturists to reevaluate current practices and policies (Mulder and Doll 1993). Herbicides are frequently lost to sensitive areas because of leaching and runoff (Schweizer 1988). An important aspect of reduction of herbicide use is the integration of chemical, mechanical, and cultural weed management methods,

and to a greater knowledge of weed populations and seed bank dynamics (Mulugeta and Stoltenberg 1997).

2.5.3 MECHANICAL CONTROL

2.5.3.1 Mowing and cutting

According to Klingman (1991), and Slife (1991), mowing controls weeds in two ways. If properly timed, mowing prevents seed production, and repeated mowing will also aid in the control of some perennial plants by depleting the underground food supply. MacDonald et al. (1994) studied the effectiveness of integrating herbicides with mowing to control dogfennel (*Eupatorium capillifolium* (Lam.) Small (Compositae)), a perennial, broadleaf weed that infests pastures, fallow fields, rangeland, roadsides, and ditchbanks. Mowing alone reduced dogfennel regrowth by 81%, but when combined with dicamba + 2,4-D or triclopyr + 2,4-D, regrowth was reduced by over 94%.

Linde et al. (1976) suggested mowing of plants should be done when below-ground reserves of the species under consideration are at their lowest. Malecki and Rawinski (1985) found that late summer cutting of purple loosestrife resulted in a more significant reduction in the number of shoots than did midsummer cutting. This is presumably because purple loosestrife plants cut later in the season are less able to replenish the carbohydrate reserves that are necessary for vigorous growth the following year. The same authors also point out, however, that cutting alone does not control re-establishment

of purple loosestrife and should not be relied upon unless accompanied by some other stress such as flooding or possibly burning. Moreover, vegetative regeneration and clonal spread of purple loosestrife can occur from cut stems following late summer cutting (Keddy 1988, Mal et al. 1992). Gabor and Murkin (1990) found that most purple loosestrife seedlings, when clipped at more than 21 days of age, were able to sprout two new shoots. Haworth-Brockman et al. (1991) found that purple loosestrife seedlings clipped underwater were able to quickly produce new stems which then grew through the water column.

2.5.3.2 Tillage

There has been concern expressed by some (e.g. Buhler 1997) that exposure to light breaks seed dormancy in many plant species, including several important weeds. A major source of light for buried weed seeds is the short-term exposure to a light flash received during tillage. Buhler (1997) investigated the effect of tillage conducted during daylight and at night on the emergence of 13 summer annual weed species. Emergence of small-seeded, broadleaf species (like purple loosestrife) was often lower when tillage was conducted at night.

Coffman and Frank (1991), state that reducing or eliminating tillage is known to favor the establishment and spread of many perennial weeds because their underground reproductive structures are minimally disturbed. The perennial growth pattern of purple loosestrife makes it susceptible to control by plowing or discing, wherever these methods

are feasible and cost-effective (Thompson et al. 1987). The most practical application of tillage for the control of purple loosestrife will be in field crops, summerfallow fields, and certain pastures where it has become established and where such machinery can successfully operate.

2.5.4 CULTURAL CONTROL

2.5.4.1 Competition

The rationale here is that if purple loosestrife is a very competitive plant then, perhaps another competitive plant could be introduced to reduce purple loosestrife infestations. Malecki and Rawinski (1985), reported that Japanese millet (*Echinochloa crusgalli* var. *frumentacea* (Roxb.) Wight (Graminaceae)) can successfully outcompete purple loosestrife. Although high densities of purple loosestrife seedlings were present at the beginning of their research, Japanese millet completely eliminated them by the end of the growing season. These authors also point out that Japanese millet could also be used as a food source by wildlife. However, they do not discuss the implications of introducing another very competitive plant. Presumably the introduction of Japanese millet into wetlands could have serious impacts on native vegetation far worse than purple loosestrife. In contrast, Welling and Becker (1993) did not find that seeding Japanese Millet reduced purple loosestrife seedling densities on a consistent basis, indicating that this is not a reliable strategy.

2.5.4.2 Hand pulling

According to Klingman (1991), and Slife (1991), hand pulling is probably the oldest method of controlling weeds and can be effective against annual and biennial plants, provided the root system is extracted. With perennials, hand pulling is effective on the seedling, but less effective on most established plants, particularly if small pieces of root material resprout. Hand weeding can greatly reduce the invasion of a weed species into new areas by the removal of scattered plants (Slife 1991). In the primary infestation period (i.e. the initial establishment of a species in a new area), hand pulling is an effective measure in controlling purple loosestrife, but care must be exercised to ensure that the perennial rootstock is removed along with the aboveground portion of the plant (Mal et al. 1992).

In most areas where purple loosestrife is well established, hand pulling would not be an effective control strategy. Welling and Becker (1990) found that purple loosestrife seeds did not germinate when buried at a depth of >2 cm, but small disturbances in the soil surface led to further recruitment. A result similar to that which is observed with herbicide applications would likely result, that is, recruitment from the seed bank would occur. The result would be re-establishment of purple loosestrife within a few years.

2.5.4.3 Fire

Fire has been used for many decades to control weeds in rangelands, ditchbanks, roadsides and other waste areas (McWhorter and Shaw 1982). Controlled burning is used

as a cultural practice in forest management (Klingman 1991). In particular, controlled burning is important in certain coniferous forests to keep the forest free of undesirable understory vegetation, thus reducing the threat of forest fires (Slife 1991). Controlled burning of rangeland in the western states is perhaps the single most important practice used to improve or increase livestock forage by eliminating competitive plants or by reducing litter and stimulating growth of desirable forage plants (Slife 1991). Flame weeding, using a flame-thrower, is widely used in organic farming for pre-emergence weed control in carrots and other slow-germinating row crops (Ascard 1995) and to control weeds in alfalfa (*Medicago sativa* L.) and in cotton (McWhorter and Shaw 1982). Propane and butane are the most commonly used sources of energy used in flame weeders (McWhorter and Shaw 1982, Ascard 1995). Flame weeding poses problems such as high energy consumption, low driving speed and irregular weed control (Ascard 1995). The overwintering growing points on the rootstock of purple loosestrife lie about 2 cm below the soil surface, where they are fairly well insulated from the heat of a surface fire (Thompson et al. 1987). Impacts of fire on desirable vegetation may be severe, further enhancing purple loosestrife spread (Keddy 1988).

2.5.4.4 Mulches

Most perennial weeds are not well controlled by the use of mulches since they have enough food reserve in their root systems to emerge through the mulch (Slife 1991). The literature does not contain any examples regarding the use of mulches to control purple

loosestrife. Purple loosestrife also has a very large rootstock that is capable of sending forth many shoots, most of which would have little difficulty in penetrating a mulch layer.

2.5.4.5 Rotations

Alfalfa has long been used in rotations with other crops to reduce the stand density of Canada thistle (Klingman 1991). Blackshaw et al. (1994) conducted a long-term study at Lethbridge, Alberta to evaluate weed population responses to various crop rotations and tillage treatments. In all rotations more weeds were present in zero tillage plots than in either minimum or conventional tillage plots. Furthermore, dandelion (*Taraxicum officinale* Weber) and perennial sow thistle (*Sonchus arvensis* L.) densities increased slightly over time in the minimum and zero tillage treatments. Thus, the presence of purple loosestrife in minimum or zero-till fields may become an increasing problem over time unless zero-till is integrated with biological control and/or herbicides.

2.5.4.6 Flooding and drawdowns

Periodic flooding is a natural aspect of wetland ecosystems. Therefore, wetland plants have evolved mechanisms for tolerating such conditions. Irreversible negative impacts of flooding are unlikely (Mal et al. 1992). Malecki and Rawinski (1985) argue however, that in many impoundment's, purple loosestrife grows along the edges or in shallow water zones where deep flooding can be difficult or impossible to accomplish, and that maintaining flooded conditions on a sustained basis is detrimental to most forms of emergent vegetation. Conversely, Gabor and Murkin (1990) argue that drawdowns of

wetlands to reestablish desirable plant species and improve productivity may actually increase purple loosestrife densities. Malecki and Rawinski (1985) found that flooding purple loosestrife to depths ranging between 0 and 50 centimeters for two years had little effect on the stature and reproductive output (i.e. number of flowers). Purple loosestrife can develop stem roots and new layers of aerenchyma within days of submergence, allowing it to withstand periodic flooding (Rawinski 1982). Balogh (1986) was able to kill purple loosestrife seedlings up to 15 cm in height by submergence in 30 cm or more of water for five weeks. Haworth-Brockman et al. (1993) found that flooding up to 30 cm did not significantly affect the survival of purple loosestrife seedlings over the growing season. Smith (1964) suggests that purple loosestrife control in small marshes that are drawn down should involve herbicide treatment to kill the seedlings and mature plants. When the marsh is re-flooded, further treatments (cutting, hand-pulling) would be required to control scattered plants that resprout. Flooding impoundment's is expensive and the range of possible water depths are limited (Haworth-Brockman et al. 1993).

3. DISCUSSION

This literature review of purple loosestrife control methods has revealed many strategies for purple loosestrife control, and why many of them have failed. The discipline of weed science has focused until recently on the integration of herbicides with mechanical control methods. The problem in developing a control strategy for purple loosestrife is its plasticity and presence in a wide variety of habitats. This plant can adapt to almost any

environment, however, it is the occurrence of purple loosestrife in wetlands that is of greatest concern to wetland managers.

Clearly, the use of mechanical control methods such as discing, mowing or tillage is not normally practical for use in wetlands. Chopping up purple loosestrife plants and/or rootstock in a moist environment will only exacerbate the problem, as purple loosestrife is able to regenerate from pieces of leaves, stems, etc. The use of burning has also proven to be ineffective in a wetland environment, largely because the rootstock is well protected from the heat generated by fire. Fungicides are not yet available for purple loosestrife control, but could be very useful in a purple loosestrife control program. Herbicide restrictions prevent their use over open bodies of water. They would be very useful in an integrated program if a herbicide could be developed that would have minimal effects on wetland ecosystems. Biological control of purple loosestrife seems to be the most promising agent that we have at our disposal at this time.

Using herbicides with classical biological control offers another promising management strategy for purple loosestrife control (Lindgren et al. 1998). Removing established stands of adult purple loosestrife with a herbicide prior to the release of biocontrol agents used in biological control may decrease the time required to control established stands of this plant (Gabor et al. 1996). The rationale for this strategy is that a herbicide would be applied to kill adult purple loosestrife plants. Biological control agents then are released to control the seedlings which would be recruited from the seed bank. These seedlings

would be prevented from setting seed. It may also accelerate management of current infestations. This strategy has several advantages: 1) it minimizes the use of herbicides for purple loosestrife control, 2) once the biological control agents are released, they will self-perpetuate within the infestation, and 3) the integration of 1 and 2 above allows native vegetation to reestablish, thereby increasing the competitive pressure on purple loosestrife. As Lindgren et al. (1998) point out, an integrated approach for the control of purple loosestrife using biocontrol and herbicides must first ensure that both approaches are compatible.

There is still much research that needs to be done about purple loosestrife and its control. For example, there is a need to quantify the impact of purple loosestrife on wetland ecosystems. Much of the literature (e.g. Thompson et al. 1987) sensationalizes purple loosestrife as the primary agent that is responsible for the reduction of wetland biodiversity that has been observed over the last several decades. However, little quantitative data were offered to support this claim. It seems that this area of research has been largely ignored, with most alleged effects lacking scientific proof or based on anecdotal evidence. Anderson (1995) offers a devil's advocate approach in critically evaluating the purple loosestrife literature. He found numerous instances of contradictory or ignored evidence about the impact of purple loosestrife on North American wetlands. Anderson suggests that the percent cover of purple loosestrife has indeed increased over time and that purple loosestrife may be competing with native species for limited space, light, or other resources. However, other species may remain constant in biomass, or they

have increased at a slower rate than purple loosestrife. Anderson goes further to assert that the type of biased information does little to help in the solution of an ecological problem.

There is also a need to quantify the specific impacts of herbicides such as glyphosate and triclopyr amine on wetland ecosystems. Efforts should be undertaken to search for evidence of pathogens on purple loosestrife and exploit them as mycoherbicides. Little is known about purple loosestrife seed viability, longevity, and seed bank dynamics. These are all-important considerations that need to be addressed if an integrated weed management program for purple loosestrife is to be successful.

Because purple loosestrife is so well established, widespread and prolific, it is clear that it will be impossible to completely eliminate it from the North American flora (White et al. 1993). Diehl et al. (1997) found that native generalist herbivores of purple loosestrife in southern Manitoba does not limit the amount of purple loosestrife available for the introduced biological control agents. Instead the herbivore stress on purple loosestrife could be increased by the native insect fauna.

In conclusion, purple loosestrife management requires the development of novel new approaches. Purple loosestrife is a permanent addition to the North American flora and we must learn to accept its presence. The objectives of modern, and future, purple

loosestrife control strategies should seek to allow this exotic plant to coexist with native plants, but at lower densities.

CHAPTER 3. EVALUATION OF AN INTEGRATED MANAGEMENT APPROACH FOR THE CONTROL OF PURPLE LOOSESTRIFE, *Lythrum salicaria* L. IN SOUTHERN MANITOBA: BIOLOGICAL CONTROL AND HERBICIDES.[1]

Abstract

Purple loosestrife (*Lythrum salicaria* L.) is a European wetland perennial that was introduced to North America in the early 1800s. It forms large monodominant stands that are thought to adversely affect ecosystem dynamics wherever it occurs. Field cage experiments were conducted in a 2 ha stand of purple loosestrife at Netley-Libau marsh in southern Manitoba from 1996 to 1998 to evaluate the effectiveness of an Integrated Vegetation Management (IVM) strategy using single techniques (herbicides (glyphosate and triclopyr amine) and classical biological control (*Galerucella calmariensis* L.)) and combinations of techniques (herbicides combined with classical biological control). Trials were conducted inside 8 m^3 screened walk-in cages between May and September of each year. Results indicated that: 1) Defoliation by *G. calmariensis* reduced purple loosestrife stem densities, vertical growth, flower production and seed production, 2) where herbicides were used alone, purple loosestrife recovered rapidly through recruitment from the seed bank, and purple loosestrife stem densities were eventually two-to-three times higher than pretreatment levels, 3) the integration of herbicides with *G. calmariensis* resulted in a more sustained reduction of purple loosestrife stem densities than either method used alone, 4) where herbicides were integrated with *G. calmariensis*, reduced competition from purple loosestrife allowed native non-target dicotyledonous

[1] This chapter is intended to be a separate manuscript. It repeats some information from the general introduction.

flora to recover to densities that were higher than pretreatment levels, two years after treatments were made, 5) *G. calmariensis* fed on and oviposited on seedlings recruited from the seed bank. 6) where purple loosestrife was removed by herbicides and then seedlings were subsequently controlled by *G. calmariensis*, a succession to native broad leaf plant taxa appeared to occur. Results of this study indicate that herbicides and classical biological control are compatible strategies for purple loosestrife management. Triclopyr amine would be effective in areas where native vegetation still persists while glyphosate may be effective for purple loosestrife control in areas where monospecific stands of adult purple loosestrife dominate. Based upon the results of this study, it is best to treat an area infested with purple loosestrife with a herbicide, leaving areas of refugia for biocontrol agents. Release of *G. calmariensis* can be done at about the same time herbicides are applied or the year after herbicides are applied.

3.2 INTRODUCTION

Purple loosestrife (*Lythrum salicaria* L.) is a European wetland perennial that was accidentally introduced to North America in the early 1800s (Thompson et al. 1987). It is an aggressive plant that quickly forms monospecific stands that are thought to replace native plant species that provide food, cover, and breeding areas for a number of wildlife species (Malecki and Rawinski 1979). Purple loosestrife is also said to degrade natural habitats such as wetlands and riparian areas, reducing overall biodiversity. Purple loosestrife has existed in North America for nearly two centuries and is now a permanent addition to our flora. The problem with purple loosestrife is that it has become extremely abundant in many areas and appears to be disrupting ecosystems. In essence, purple loosestrife is a weed that needs to be controlled.

Management of purple loosestrife involves four general control approaches: cultural, mechanical, chemical, and biological. Cultural and mechanical control methods have been largely unsuccessful (McKeon 1959, Malecki and Rawinski 1979, Rawinski 1982). Small and isolated populations of purple loosestrife plants can be removed by hand pulling. However, this is not an effective technique for removing older plants or large numbers of plants. Mowing or cutting of mature plants has been shown to decrease plant vigour and retard seed production (Malecki and Rawinski 1979). However, this technique does not destroy the perennial rootstock. Cutting does reduce stem densities, but many repeated cuts are necessary and purple loosestrife may never be eliminated from a site using this technique (Haworth-Brockman et al. 1991).

Numerous studies have investigated the effectiveness of herbicides for controlling purple loosestrife (McKeon 1959, Smith 1964, Rawinski 1982, Balogh 1986, Reinartz et al. 1986, Skinner and Hollenhorst 1989, Gabor et al 1995). Most recently, research has focused on triclopyr amine and glyphosate as potential herbicides for purple loosestrife control. Skinner and Hollenhorst (1989), and Gabor et al. (1995) reported triclopyr amine to be effective for purple loosestrife control. Triclopyr amine is a systemic herbicide selective for broad-leaved weeds. It has a number of other characteristics that make it a potential tool for purple loosestrife control. It is easily absorbed and translocated to the root system and breaks down quickly in soil and water (Dow Chemical 1988). Research indicates that triclopyr amine has minimal effects on fish (Mayes et al. 1984, Barron et al. 1991, Janz et al. 1991) and other aquatic organisms (Gersich et al. 1984).

Skinner and Hollenhorst (1989) reported glyphosate can provide 95% control of purple loosestrife. In addition, Rawinski (1982) found that glyphosate applied to purple loosestrife during its peak bloom provided greater than 90% control, and desirable indigenous vegetation established. Glyphosate is a non-selective herbicide that is quickly absorbed by leafy surfaces and translocated to the entire plant (Thompson et al. 1987). Moreover, it has relatively low toxicity to birds (Batt et al. 1980), mammals (Thompson et al. 1987), and aquatic invertebrates and fish (Folmar et al 1979).

Studies by Skinner and Hollenhorst (1989), and Gabor et al. (1995) indicate that herbicide applications and subsequent canopy removal resulted in purple loosestrife recruitment from the seed bank. In both studies high densities of purple loosestrife seedlings were reported following herbicide applications. Removal of the canopy created favourable conditions for purple loosestrife seed germination and growth. Mature purple loosestrife plants can produce an estimated 2.7 million seeds per year (Thompson et al. 1987) and therefore large seed banks exist in areas where purple loosestrife is well established (Welling and Becker 1990). An effective method of controlling seedling emergence after herbicide application is required.

Recommended protocols for both triclopyr amine and glyphosate herbicides are available depending on site-specific requirements. Triclopyr amine may be effective in areas where native vegetation still persists while glyphosate may be effective for purple loosestrife control in areas where monospecific stands of adult purple loosestrife dominate. However, neither herbicide will control purple loosestrife re-establishment from the seed bank, unless repeated applications of herbicides are employed.

Current purple loosestrife control efforts include the introduction and establishment of biological control agents as a long-term management option. Biological control of weeds is an attempt to reunite an introduced plant with its natural enemies. Biological control programs for purple loosestrife began across North America in 1992 with release approvals from the Animal and Plant Health Inspection Service, USDA, and by the Plant

Protection Division of Food, Production and Inspection Branch of Agriculture Canada (Hight et al. 1995). Species approved for release in Canada are two leaf-eating beetles, *Galerucella calmariensis* L. and *G. pusilla* Duftschmidt, (Coleoptera: Chrysomelidae) which defoliate the plant; *Hylobius transversovittatus* Gauze, a root-mining weevil (Coleoptera: Chrysomelidae) that attacks the root system and *Nanophyes marmoratus* and *N. brevis* Boheman, flower-feeding weevils that attack the seed pods. Both species of *Galerucella* and *H. transversovittatus* were released in Manitoba in 1992 as part of a biological control program. All three species have successfully overwintered and established in Manitoba.

RATIONALE AND OBJECTIVES

Herbicides and biological control are the most promising techniques available for controlling purple loosestrife. Biological control is a long-term solution because it may take many years for the insects to increase population size and density to sufficient levels to control established stands of adult purple loosestrife plants. Although herbicides can be effective for killing adult plants, results are short-lived due to recruitment of purple loosestrife from the seed bank. Repeated sprays may be necessary to prevent subsequent purple loosestrife establishment. An integration of the two techniques may be effective and thus provide immediate, as well as long-term, control of this invasive species.

The rationale behind combining the two techniques is that the process of biological control could be accelerated with the aid of herbicide application. Chemical control is often avoided with biocontrol agent releases. This decision is usually made with little or no knowledge of the effects of a herbicide on a biocontrol agent (Rees and Fay 1989). Studies examining the compatibility of biocontrol agents with herbicides report that herbicides either do not affect biocontrol agent survival (Haag 1986, Kok 1980, Tipping 1991), herbicides reduce biological control agent survival (Haag 1986, Tipping 1991, Rees and Fay 1989), or that proper timing of herbicide application is crucial to ensure biocontrol agent survival (Story et al. 1988, Trumble and Kok 1980 a, b). There is a need to investigate the potential effects of combining control strategies for purple loosestrife.

Information on effective purple loosestrife control techniques is extremely important to wetland and wildlife managers faced with purple loosestrife infestations. Using biological control agents in partnership with herbicides may potentially accelerate purple loosestrife management efforts. Experimental studies, both in the laboratory and under field conditions, are needed to develop an effective and efficient Integrated Vegetation Management (IVM) approach and develop an effective national purple loosestrife control strategy.

There are a number of important considerations regarding the biology of the insects and the timing of the herbicide application. Two general combinations can be envisioned. First, mature purple loosestrife plants could be removed using a selective herbicide for

broadleaf plants, such as triclopyr amine. Following mature purple loosestrife removal, biological control agents would be released to control purple loosestrife re-establishment from the seed bank. A second approach would involve releasing insects, allowing them to establish, and then using triclopyr amine or glyphosate to eliminate the adult plants.

The objective of this study was to investigate an IVM approach for purple loosestrife control using biological control agents and herbicides. Field experiments (1996-98) were conducted in an established stand of purple loosestrife at Netley-Libau Marsh to determine effectiveness of single techniques and combinations of techniques. The biological control insect used was *G. calmariensis*. Adult *G. calmariensis* are approximately 5 mm long and emerge from the soil from overwintering at about the same time that emergent purple loosestrife shoots are appearing (mid-late May). Adult feeding and oviposition last for approximately two months. New generation F_1 adults appear in midsummer and feed for a short while before overwintering. This species was chosen because it is easily reared, has high reproductive potential and has established and over-wintered successfully in Manitoba. The closely-related *G. pusilla* has also established and over-wintered successfully in Manitoba and could have been used in this study as well. The remaining biological control insects (*H. transversovittatus*, *N. marmoratus*, *N. brevis*) would not be suitable for an IVM approach. Herbicides kill the adult purple loosestrife plants and therefore eliminate the food source for the root boring and flower-feeding weevils. Adult *G. calmariensis* can move from adult purple loosestrife plants to

untreated areas ("refugia") and then to the target plants, the emerging purple loosestrife seedlings.

OBJECTIVES

The objectives associated with this study were: 1) to compare purple loosestrife densities between single treatments (herbicides or insects), combinations of treatments (herbicides and insects) and untreated plots; 2) to compare non-target plant densities between single treatments (herbicides or insects), combinations of treatments (herbicides and insects) and untreated plots; 3) to evaluate impact of *G. calmariensis* on purple loosestrife under caged, field conditions; 4) to develop an IVM approach for purple loosestrife control by integrating biological control with herbicides.

3.3 MATERIALS AND METHODS

Field experiments were conducted from 1996-1998 in a seasonally flooded area (i.e. receives water during spring runoff and heavy rainfall) of Netley-Libau marsh located in the delta region south of Lake Winnipeg (50° 20′ 73″ N, 96° 41′ 29″ W). The 1996 field season (mid June to September) was allocated to construction and placement of 24 field cages, initial baseline plant sampling and herbicide applications. In 1997, the biological control agent, *G. calmariensis* was introduced into 15 field cages.

Field cages

All treatments were conducted inside field cages (Figure 1) to reduce variability resulting from dispersal, predation, etc. of biological control insects. Each cage was 8 m^3 and covered with screening material [Lumite ®, style 50090000, 20 X 20 mesh (15% shade, 1629 cfm porosity)-Synthetic Industries Performance Fabrics Division. 2100A, Atlanta Highway, Gainesville, GA, P.O. Box 977]. Cage frames were constructed using 6 cm x 6 cm kiln-dried spruce wood. Cages were reinforced with wooden cross-braces from corner to corner on all four sides to prevent wind-induced lateral movement. The cages were also anchored to the ground using 60 cm long by 10-mm diameter steel rods. The insect screening was secured to the wood frames along the bottom portion of the cage frame using 25 mm roofing nails in combination with 10-mm washers. Insect screening was removed in late September or early October to allow normal snowfall accumulation on the research plots, thereby providing adequate thermal insulation for the overwintering

beetles. Screens were reinstalled in early May of 1997 and 1998 before purple loosestrife shoots began to appear.

Experimental design

The cages were organized into three blocks of eight cages (Figure 1). Treatments (described below) were assigned within each block according to a randomized complete block design. Each treatment was replicated 3 times.

Treatments

The following eight treatments were employed: Herbicide **alone**, where the two herbicides glyphosate and triclopyr amine were used separately; Insect **alone**, where the biological control agents (released in spring 1997) were evaluated alone and were not integrated with herbicides; **High-density insect release integrated with herbicides**, where glyphosate and triclopyr amine applications to separate cages in 1996 were followed by release of biological control agents at a high density in spring 1997; **Low-density insect release integrated with herbicides**, where glyphosate and triclopyr amine applications to separate cages in 1996 were followed by release of biological control agents at a low density in spring 1997; a **Control**, where neither herbicides were applied nor biological control agents were released.

1) Herbicide alone

Herbicides were applied using a 15-L pump action backpack sprayer. All vegetation in herbicide treated cages was sprayed with herbicides until the plant surface was wet.

a) Triclopyr amine, the triethylamine salt formulation of triclopyr [[(3,4,6-trichloro-2-pyridinyl)oxy]acetic acid], was applied 26 July 1996 at a rate of 1.5% (V/V), (12 kg/ha active ingredient) when purple loosestrife was in the bud to early bloom stage. Triclopyr amine is a broadleaf specific herbicide. This treatment was denoted as **TA**.

b) Glyphosate [N-(phosphonomethyl) glycine] was applied 28 August 1996 at a rate of 2% (V/V) during the late bloom stage of purple loosestrife. Glyphosate is a non-selective herbicide. This treatment was denoted as **GL**.

2) Insect alone

Adult *G. calmariensis* were obtained from a variety of sources to ensure genetic variability and to reduce effects of inbreeding. The sources of adult *G. calmariensis* were: Delta and Libau marshes, Winnipeg, and Minnesota. The adults were identified according to Manguin et al. (1993) to ensure that all were *G. calmariensis* and not the closely related *G. pusilla* Duftschmidt. The adults were sexed, sorted and counted in the laboratory prior to the introductions. Six pairs of adults (n=12, 6 males and 6 females) were released into the insect alone treatment cages on 18 June, 1997 to coincide with the

natural early summer presence of established *Galerucella* populations in other areas of Manitoba. This treatment was denoted as **GC**.

3) High-density insect release integrated with herbicide application

In all integrated treatments, four potted purple loosestrife plants were placed in each cage the year following the herbicide application as refugia to sustain adult *G. calmariensis* until purple loosestrife seeds in the soil germinated.

a) Triclopyr amine was applied as discussed above in 1(a). Twelve pairs of both sexes of adult *G. calmariensis* (n=24) were released into three of the triclopyr amine treated cages on 18 June 1997. This number was chosen to determine if higher insect numbers would result in faster purple loosestrife seed bank depletion. This treatment was denoted as **HT**.

b) Glyphosate was applied as discussed above; 1(b). Twelve pairs of both sexes of adult *G. calmariensis* (n=24) were released into three glyphosate treated cages on 18 June 1997. This treatment was denoted as **HG**.

4) Low-density insect release integrated with herbicide application

a) Triclopyr amine was applied as specified above; 1(a). Six pairs of both sexes of adult *G. calmariensis* (n=12) were released into three triclopyr amine treated cages on 18 June 1997. This treatment was denoted as **LT**.

b) Glyphosate was applied as specified above; 1(b). Six pairs of both sexes of adult *G. calmariensis* (n=12) were released into three triclopyr amine treated cages on 18 June 1997. This treatment was denoted as **LG**.

5) Control- no treatment

The cages designated as controls were not treated with any herbicides nor were any biological control agents introduced.

6) Open cage plot-no treatment

On 18 June, 1997 it was noted that purple loosestrife inside cages appeared to be taller, had thicker stems and had larger leaves than purple loosestrife outside of cages. On 26 June 1997 one 4 m^2 plot was randomly selected from each treatment block. These uncaged plots were selected for similar purple loosestrife composition and proximity to each of the caged controls. This was done to evaluate cage effects on purple loosestrife biometrics. As with other treatments, 15 purple loosestrife plants were randomly selected early in the season, tagged, and monitored at various intervals throughout the growing season. Stem heights, main spike length, number of flowering spikes, and number of flowers on main spike were recorded.

Effect of treatments

a) Purple loosestrife morphometrics

Within each cage, 15 purple loosestrife plants (total of 45 plants/treatment) were randomly selected, tagged and monitored at various intervals. Stem heights, main spike length, number of flowering spikes, and presence of biocontrol agents were recorded. In 1996, measurements were taken 31 July, 14 August, and 9-10 September. In 1997, measurements were taken 26 June, 29 July, and 15 to 19 September. In 1998, measurements were taken 9 July, 28 August, and 30 September. At the end of each sampling season, the tagged plants were harvested and brought to the laboratory for dissection. Stem heights, main spike length, number of flowering spikes, number of flowers on main spike and lengths of all lateral stems originating from the main stem were recorded. Haworth-Brockman (1993) also used mean stem height as an indication of the growth and health of purple loosestrife plants over the growing season. In addition, harvested purple loosestrife plants were dried thoroughly and dry weights were recorded.

b) Vegetation sampling

Each cage was separated into four, $1m^2$ quadrats to facilitate vegetation sampling. A complete plant inventory (composition and numbers) was conducted for each cage. Stems were considered dead if no green portions were observed. High-density species such as combined grass and combined sedge species were counted from within one randomly selected m^2 plot per treatment. Surveys were conducted 15-25 July 1996 (prior to triclopyr amine application), 22-26 August 1996 (one month post-triclopyr application

and prior to glyphosate application), and 25 September 1996 (one month post-glyphosate application). In 1997, surveys were conducted 16 May, 18-20 June, 15-21 July, and 26-30 August. In 1998, surveys were conducted 28 May, 22 June, 25 July to 11 August, and 26 to 28 September.

c) Determination of seed bank depletion

At the termination of field studies in 1998, four soil cores were randomly extracted from each treatment cage using an 15 cm deep X 7.5 cm diameter corer. Soil cores were cut to a depth of at least 10 cm. Soil cores were bagged in groups of four per cage tagged, and returned to the laboratory. Individual soil cores were labeled as to originating treatment and treatment block. Soil cores were frozen in darkness at -8° C for 60 days. After 60 days, soil cores were removed from the freezer and allowed to thaw for two days so that the cores could be separated without damaging them. The soil cores were then placed into plant pots. Care was taken not to disturb soil cores during this process as this could affect results by stirring up buried purple loosestrife seeds. The pots containing the soil cores were then placed into a walk-in growth chamber where they were then randomly assigned among three flats within the chamber. Once per week, one-third of the pots would be reassigned to different areas amongst the three flats. This was done to ensure that every pot had an equal chance of being exposed to possible differing microclimatic conditions inside the growth chamber. Soil cores were watered every two days to ensure the soil was kept moist. The cores were not watered directly but rather the trays in which they were maintained were flooded to a depth of 2.5 cm. Capillary uptake of water by the

soil cores allowed the soil to remain moist without disturbing the purple loosestrife seeds. The environmental conditions inside the chamber were 16:8 light-dark cycle, 24° C temperature and ~60% R.H. After approximately four weeks, composition and numbers of all plant taxa were recorded. Soil cores then were returned to -8° C for another freeze cycle of 60 days. Methods described for the first trial were repeated again for the second trial.

d) Monitoring of biological control insects

Non-destructive sampling techniques (direct observation) were attempted in order to determine the number of adults, larvae, and egg masses of *G. calmariensis* inside field cages. This strategy was abandoned as being far too labour intensive and time-consuming. Adult *Galerucella* detach and fall from plants upon the slightest disturbance, making it very difficult to count them in cages. Instead, frequent observations were made as to the relative abundance of the biological control insects inside cages (i.e. tens, hundreds, thousands). Observations were also made with regard to biological and ecological information.

Laboratory experiment

a) Oviposition on seedlings

A test was done to determine if *G. calmariensis* females confined inside cages would oviposit on purple loosestrife seedlings when mature (i.e. 1+ year-old) purple loosestrife was removed, or when seedlings were available in addition to mature plants. On June 4,

1998 purple loosestrife shoots of similar size (~15 cm) were collected with roots intact at the Libau marsh study site and placed inside ten, 10 cm diameter plastic pots to which potting soil was added. Five pots were filled with potting soil that contained no purple loosestrife shoots or seedlings. To these pots and five pots that contained purple loosestrife shoots, 0.01 g of purple loosestrife seed was scattered and allowed to germinate for two weeks before *G. calmariensis* adults were introduced. This seed was obtained from purple loosestrife seed capsules at the Netley-Libau marsh study site in September 1997. Fifteen copulating pairs of *G. calmariensis* were collected at the study site on June 18, 1998 and brought to the laboratory. Individual pairs were placed into each container. Each pot was covered with a 20 cm X 10 cm clear plastic cage to prevent *G. calmariensis* from escaping. Each cage had several holes cut, over which screen was placed to allow air movement. All pots were placed in an incubator at 26° C (day) 13° C (night) and a 16:8 photoperiod for two weeks. Adults were then removed from the pots and killed in a freezer. Numbers of egg masses and numbers of eggs per mass were recorded.

DATA ANALYSES

Analyses of variance were performed on the data using JMP IN 3.1 for Windows® (Sall and Lehman 1996). Differences between treatment means were analyzed using Analysis of Variance (F and t-tests) and Tukey-Kramer Honestly Significant Difference (HSD). Tests were considered significant at the α=0.05 level of probability. Stem density data

were transformed (log (X+1)) to reduce the heterogeneity of treatment variances (Zar 1974). Stem height data did not require transformation.

3.4 RESULTS

The results of this study are organized into the following major sections: 1) Evaluation of cage effect on purple loosestrife biometrics, 2) Flower production-estimating numbers of seed capsules on main flowering spike, 3) Response to treatments-purple loosestrife morphometrics of tagged plants, 4) Plant taxa response to treatments-mean stem densities, 5) Purple loosestrife seedling densities inside treatment cages, 6) Determination of soil seed bank depletion, and 7) Oviposition by *G. calmariensis* females on purple loosestrife.

1) Evaluation of cage effect on purple loosestrife morphometrics

By September 1997 mean purple loosestrife stem heights in the no-cage plots were significantly lower than the control. The mean number of flowering spikes produced by individual purple loosestrife plants within the no-cage treatment plots was not significantly different than the control (Table 3). Mean purple loosestrife dry weights in the no-cage plots were not significantly different than the control (Table 4). The mean number of lateral stems originating from the main stem of purple loosestrife plants in the no-cage treatment plots was not significantly different than the control (Table 5). The mean total length of all lateral stems originating from the main stem of purple loosestrife plants within the no-cage plots were not significantly different than the control (Table 5). There was no correlation ($P>0.05$, $r=0.02$: $df=1, 43$ ($F=o.8$)) between stem heights and number of lateral stems arising from the main stem.

2) Flower production-estimating numbers of seed capsules on main flowering spike

In this study, there was a fairly strong correlation between the length of the main flowering spike and the number of seed capsules produced on purple loosestrife flowering spikes measured from plants in the no-cage plots. These plants were chosen for the development of a regression equation because they were not exposed to any treatment effects and therefore would provide an ideal model for potential reproduction of the purple loosestrife plants in the treatment cages. The regression equation obtained (df=1, 38 (F=193.6)) was:

 # Seed capsules= 41.2 + 5.49 (spike length (cm)), r=0.84

3) Response to treatments: Purple loosestrife morphometrics-tagged plants

a) INSECT ALONE vs. CONTROL-1996

Pre-release baseline data was obtained for the insect alone treatment cages in 1996. The data obtained from these plots were included in the analysis, although no *G. calmariensis* were actually present in these cages in 1996. It was thought that potential differences between the insect alone treatment and the control should be followed throughout the study in order to better ascertain the impact of *G. calmariensis* on purple loosestrife vegetative growth. There were no significant differences in the mean dry weights, mean number of flowers, or mean stem heights of the tagged plants between the control and insect alone treatment in 1996. Results are summarized in Table 1.

b) Stem heights (refer to Table 2)

Mean purple loosestrife stem heights in the insect alone treatment cages were significantly lower than the control and were significantly higher than the herbicide alone and all integrated treatments by September 1997. By September 1998 mean stem heights were significantly lower than the herbicide alone, the high-density biocontrol agent+herbicide treatment cages as well as the control. Mean purple loosestrife stem heights in all other treatments as well as the control increased from 1997 to 1998. Mean stem heights in the glyphosate alone treatments were significantly lower than the control by September 1997, and by September 1998. Mean stem heights in the glyphosate alone treatment cages were significantly higher than for the insect alone and all integrated treatments by September 1998. Mean stem heights in the triclopyr amine alone treatment cages were significantly lower than the insect alone treatments and the control by September 1997, and were significantly lower than the control by September 1998. Mean purple loosestrife stem heights in the high-density biocontrol agent+glyphosate treatment were significantly lower than all other treatments and the control by September 1997, and were significantly lower than the control by September 1998. Mean purple loosestrife stem heights in the high-density biocontrol agent+triclopyr amine treatment cages were significantly lower than the insect alone treatment and the control by September 1997, and were significantly lower than the control by September 1998. Mean purple loosestrife stem heights in the low-density biocontrol agent+glyphosate treatment were significantly lower than the insect alone treatment and the control by September 1997, and were significantly lower than the herbicide alone treatments and the control by

September 1998. Mean purple loosestrife stem heights in the low-density biocontrol+triclopyr amine treatment were significantly lower than the insect alone treatment and the control by September 1997, and were significantly lower than the herbicide alone treatments and the control by September 1998.

c) Mean number of flowering spikes (Refer to Table 3)

The mean number of flowering spikes produced by individual purple loosestrife plants within the insect alone treatment cages was significantly higher than all treatments and the control by September 1997. Purple loosestrife plants in the insect alone and the high-density biocontrol agent+herbicides treatment cages produced no flowering spikes in 1998 and flower spike production was significantly lower than the herbicide alone treatments and the control. Mean number of flowering spikes in the glyphosate alone treatment was significantly lower than the control by September 1997 and was not significantly different than the control but were significantly higher than the insect alone and all other treatment cages by September 1998. The mean number of flowering spikes produced by individual purple loosestrife plants within the triclopyr amine alone treatment cages was significantly lower than the insect alone treatments and the control by September 1997, but was not significantly different than the control by September 1998. Individual purple loosestrife plants in the high-density biocontrol agent+glyphosate and the high-density biocontrol agent+triclopyr amine treatment cages produced no flowering spikes in 1997 and 1998. Individual purple loosestrife plants in the low-density biocontrol agent+glyphosate and the low-density biocontrol

agent+triclopyr amine cages produced no flowering spikes in 1997. The mean number of flowering spikes produced by purple loosestrife plants in the low-density biocontrol agent+glyphosate treatment cages was significantly lower than the herbicide alone treatments and the control by September 1998. The mean number of flowering spikes produced by individual purple loosestrife plants in the low-density biocontrol agent+triclopyr amine cages treatment cages was significantly lower than the herbicide alone treatments and the control by September 1998.

d) Mean number of seed capsules (refer to Table 3)

Purple loosestrife plants inside the insect alone cages produced significantly fewer mean numbers of seed capsules on the main flowering spike than the control by September 1997. The mean number of seed capsules produced by purple loosestrife plants in the glyphosate alone treatment cages were not significantly different than the control but significantly higher than the insect alone treatment by September 1997. The mean number of seed capsules produced by purple loosestrife plants in the triclopyr amine alone treatment cages was significantly lower than the control by September 1997. Since no flowering spikes were produced in any of the biocontrol+herbicide treatment cages, purple loosestrife plants inside these treatments had significantly fewer mean numbers of seed capsules on the main flowering spike than the control in 1997. By September 1998, the mean number of seed capsules in the control was significantly higher than all other treatments. The mean number of seed capsules in the herbicide alone treatments was significantly higher than the insect alone and all integrated treatments.

e) Mean dry weights (refer to Table 4)

Mean purple loosestrife dry weights in the insect alone treatment cages were not significantly different from the control but were significantly higher than all other treatments by September 1997, but were significantly lower than the control by September 1998. Mean purple loosestrife dry weights from the glyphosate alone treatment cages were significantly lower than the control by September 1997, but were not significantly different from the control by September 1998. Mean purple loosestrife dry weights from the triclopyr amine alone treatment cages were significantly lower than the insect alone treatment and the control by September 1997, and were not significantly different from the control by September 1998. Mean purple loosestrife dry weights from the high-density biocontrol agent+glyphosate, low-density biocontrol+glyphosate and high-density biocontrol agent+triclopyr amine treatment cages were significantly lower than the insect alone treatment and the control by September 1997, and were significantly lower than the control by September 1998. Mean purple loosestrife dry weights from the low-density biocontrol agent+triclopyr amine treatment cages were significantly lower than the insect alone treatment and the control by September 1997, and were significantly lower than the control by September 1998.

f) Mean number of lateral stems (refer to Table 5)

The mean number of lateral stems originating from the main stem of purple loosestrife plants in the insect alone treatment plots was not significantly different than the control, but was significantly higher than the herbicide alone and all integrated treatments by

September 1997, and was significantly lower than the herbicide alone treatments, the high-density biocontrol agent+glyphosate, and the control by September 1998. The mean number of lateral stems originating from the main stem of purple loosestrife plants in the glyphosate alone treatment cages was significantly lower than the insect alone treatment and the control by September 1997, but was not significantly different than the control by September 1998. The mean number of lateral stems originating from the main stem of purple loosestrife plants in the triclopyr amine alone treatment cages was significantly lower than the insect alone treatment and the control by September 1997, and was not significantly different than the control but significantly higher than the insect alone treatment by September 1998. The mean number of lateral stems originating from the main stem of purple loosestrife plants in the high-density biocontrol agent+glyphosate treatment cages was significantly lower than the insect alone treatment and the control by September 1997, and was not significantly different than the control but significantly higher than the insect alone treatment by September 1998. The mean number of lateral stems originating from the main stem of purple loosestrife plants in the high-density biocontrol agent+triclopyr amine treatment cages was significantly lower than the insect alone treatment and the control by September 1997, and was significantly lower than the control but significantly higher than the insect alone treatment by September 1998. The mean number of lateral stems originating from the main stem of purple loosestrife plants in the low-density biocontrol agent+glyphosate and the low-density biocontrol agent+triclopyr amine treatment cages was significantly lower than the insect alone treatment and the control by September 1997, and was not significantly different than the

herbicide alone and high-density biocontrol agent+glyphosate treatments as well as the control by September, 1998.

g) Mean total lateral stem lengths (refer to Table 6)

The mean total length of all lateral stems originating from the main stem of purple loosestrife plants within the insect alone treatment cages were not significantly different than the control but were significantly higher than all other treatments by September 1997, and by September 1998. The mean total length of all lateral stems originating from the main stem of purple loosestrife plants in the glyphosate alone treatment cages was significantly lower than the insect alone treatment and the control by September 1997, and was not significantly different than the control but was significantly higher than the insect alone and all integrated treatments by September 1998. The mean total length of all lateral stems originating from the main stem of purple loosestrife plants in the triclopyr amine alone treatment cages were significantly lower than the insect alone treatment and the control by September 1997, and was not significantly different than the control but was significantly higher than the insect alone treatment by September 1998. The mean total length of all lateral stems originating from the main stem of purple loosestrife plants in the high-density biocontrol agent+glyphosate treatment cages were significantly lower than the insect alone treatment and the control by September 1997, and were significantly lower than the glyphosate alone treatment and the control by September 1998. The mean total length of all lateral stems originating from the main stem of purple loosestrife plants in the high-density biocontrol agent+triclopyr amine

alone treatment cages were significantly lower than the insect alone treatment and the control by September 1997, and was significantly lower than the herbicide alone and high-density biocontrol agent+glyphosate treatments as well as the control by September 1998. The mean total length of all lateral stems originating from the main stem of purple loosestrife plants in the low-density biocontrol agent+glyphosate and the low-density biocontrol agent+triclopyr amine treatment cages were significantly lower than the insect alone treatment and the control by September 1997, and were significantly lower than the glyphosate alone treatment and the control by September 1998. A fairly strong correlation was found between the number of lateral stems and the total lateral stem lengths. In 1997, total lateral stem lengths plotted against number of lateral stems produced an r-value of 0.70 (df=1, 403 (F=941.5)). In 1998, an r-value of 0.66 (df=1, 140 (=283.1)) was produced.

4) Plant response to treatments-mean stem densities

All stems of plant taxa that were present inside field cages were counted. This was done in order to ascertain changes in floral composition in treatment cages after treatments were made. In addition to purple loosestrife, other vegetation present within the treatment cages were: tufted loosestrife ***Lysimachia thyrsiflora*** L. (Primulaceae), swamp milkweed ***Asclepias incarnata*** L. (Asclepiadaceae), water smartweed, ***Polygonum punctatum*** Ell. var. ***confertiflorum*** (Meisn.) Fassett (Polygonaceae), field mint ***Mentha arvensis*** L. var. ***villosa*** (Benth.) S.R. Stewart (Labiatae), sedges ***Carex spp.*** (Cyperaceae), and grasses (Gramineae). Pre-treatment baseline data were obtained in early July 1996

from cages to be treated with herbicides. Mean stem densities of all plant taxa in all treatment cages were not significantly different from the control in early July 1996, before herbicide applications were done.

In general, there were few significant effects of treatments on the number of stems of any of the common plant taxa, either in 1997 or 1998 samples. Stem densities of tufted loosestrife (Table 7), smartweed (Table 8), swamp milkweed (Table 9) and grasses (Table 12) never showed significant treatment effects. In 1998 samples only, the stem densities of purple loosestrife were lower in integrated treatments than in the herbicide alone treatments as well as the control (Table 6), the stem density in the insect alone treatment did not differ from that in any other treatment. Mint stem densities in 1997 were lower than the control in all treatments, but this tendency was not significant for the glyphosate alone or the high-density biocontrol agent+triclopyr amine treatment (Table 10). In contrast, in 1998, stem densities of mint were higher than the control in all treatments, although this was not significant for the two herbicide alone treatments. Sedges showed treatment effects only in 1997 (Table 11), when densities were highest in the insect alone treatment and this density was significantly different from all treatments with glyphosate and also from the low-density biocontrol agent+triclopyr amine treatment. No treatments differed from the control, although all treatments that included herbicides had low stem densities of sedges in 1997. By 1998, there were no treatment effects on stem densities of sedges.

5) Purple loosestrife seedlings-densities inside treatment cages (refer to Table 13)

As expected, purple loosestrife recovered quickly because of its extensive seed bank in the soil. A count of numbers of seedlings present in the treatment cages during early August 1998 revealed that significantly higher numbers of seedlings were present in the herbicides alone and all integrated treatment cages than the control. Mean seedling densities in the insect alone treatment cages were not significantly different than the control. The highest numbers of seedlings were found in the triclopyr amine alone treatment and the lowest numbers of seedlings were found in the control.

6) Determination of soil seed bank depletion (refer to Table 14)

The first soil core seed bank depletion trial showed that some degree of depletion of the purple loosestrife seed bank had occurred in all treatment cages. However, only the Low-density biocontrol agent plus herbicides treatments (LG and LT) had significantly lower numbers of seedlings that germinated from the soil core than the control. The results of the second soil core trial were somewhat different from the first trial. Only the herbicides alone as well as the high-density biocontrol agent+triclopyr amine and low-density biocontrol agent+glyphosate had significantly lower numbers of seedlings that germinated from the soil cores than the control. The control had the highest numbers of seedlings in both trials.

7) Oviposition by *G. calmariensis* females on purple loosestrife (refer to Table 15)

Laboratory tests of *G. calmariensis* female oviposition preference for purple loosestrife seedlings, one-year old purple loosestrife plants and combinations of seedlings and one-year old plants showed that the mean number of ova in an egg mass did not differ significantly among the three treatments. However, the total numbers of eggs oviposited differed significantly among the treatments. On 26 June 1997 81 egg masses were collected from loosestrife plants outside of the treatment cages at the Netley-Libau marsh study site. The mean number of eggs per egg mass was 4.99 ± 0.38. The maximum number of eggs per egg mass was 17 and the minimum was 1. The number of eggs per egg mass laid by *G. calmariensis* females near the study site (4.99 ± 0.38) is very close to the mean batch size of 5.3 ± 0.2 eggs reported by Blossey (1995) for *G. calmariensis*.

3.5 DISCUSSION

1) Cage effect on purple loosestrife biometrics

By September 1997 mean purple loosestrife stem heights in the control cages were significantly higher than mean purple loosestrife stem heights in the no-cage treatment cages. This suggests that there was a strong cage effect on purple loosestrife vegetative growth. In fact, purple loosestrife stem heights in the control were an average 30 cm higher than the previous year (September 1997 vs. September 1996). In 1996, cages were set up at a time when purple loosestrife plants were already nearly fully-grown. Survival of purple loosestrife in the sun is much higher than in the shade (Shamsi and Whitehead (1977). Purple loosestrife responds to a decline in light intensity by increasing leaf area and decreasing leaf pubescence but increases dry weight production of chlorophyll per unit leaf area (Shamsi and Whitehead 1974). Thompson et al. (1987) report that purple loosestrife can survive in 50% of full sun but declines in vigor and fails to reproduce at lower light levels. At 40% of full sun there is a significant decrease in mean dry weight of seed produced and a marked delay and reduction in number of flowers as well as a corresponding delay and reduction in the number of seeds and seed capsules (Shamsi and Whitehead, 1974). This is confirmed by our study which indicated that although mean purple loosestrife spike lengths in the no-cage plots were shorter than those produced by purple loosestrife plants in the control plots, the mean number of seed capsules produced by purple loosestrife spikes in the control plots was actually less than those in the no-cage plots.

2) Response to treatments: Purple loosestrife biometrics-tagged plants

The fact that mean purple loosestrife stem heights in the integrated treatment cages were found to be consistently lower than stem heights in the herbicide alone treatments suggests that *G. calmariensis* was effective in suppressing purple loosestrife vegetative growth. This result is supported by the fact that mean purple loosestrife stem heights in the insect alone treatment cages were significantly lower than the control in both 1997 and 1998. By September 1998 mean purple loosestrife stem heights were also found to be significantly lower in the insect alone and integrated treatment cages as compared to mean purple loosestrife stem heights in the herbicide alone treatment cages. An unusual result was that purple loosestrife stem heights were higher in the high-density biocontrol agent+herbicide cages than in the low-density biocontrol agent+herbicide cages. An opposite result was expected. Presumably, purple loosestrife plants in the high-density biocontrol agent+herbicide treatments had overcompensated stem growth in response to the greater herbivore pressure.

Insect herbivory affects flower production directly by destroying flowers and flower buds and indirectly through other types of damage (defoliation) that reduce bud production and bud burst (Crawley 1989). Purple loosestrife plants in the insect alone treatment cages produced significantly more flowering spikes than the control in 1997. Many of these flowering spikes were, on average, shorter than flowering spikes produced by purple loosestrife plants in the control cages. Feeding by *G. calmariensis* adults and larvae on the terminal ends of purple loosestrife plants resulted in the growth of 'bushy' plants (i.e.

purple loosestrife plants had produced many terminal branches in response to herbivory of the terminal portions of branches). As a result, purple loosestrife plants in the insect alone treatments had produced significantly more flowering spikes than purple loosestrife plants in the control. No flowering spikes were produced by purple loosestrife plants in any of the integrated treatments in 1997. By September 1998, no flowering spikes were produced by purple loosestrife plants in the high-density biocontrol agent+herbicide treatments. A few flowering spikes were produced by purple loosestrife plants in the low-density biocontrol agent+herbicide treatments. Many purple loosestrife plants in the herbicide alone cages produced flowering spikes in 1997, but they produced significantly fewer flowering spikes than the control. This was likely due to the fact that these were first-year growth purple loosestrife plants (i.e. plants that had germinated from seed earlier in 1997) and had not achieved their full reproductive potential. By September 1998, purple loosestrife plants in the herbicide alone treatments produced comparable numbers of flowering spikes to the control. Results from 1997 and 1998 suggest that defoliation by *G. calmariensis* adults and larvae was effective in suppressing purple loosestrife flower production in these treatment cages.

Flies in the families Syrphidae, Stratiomyidae, and to a lesser degree, Culicidae appeared to have been responsible for some pollination of purple loosestrife flowers inside cages. However, full pollination was severely limited because most pollinators such as bees were excluded from the cages. Many flowering spikes did not produce a full complement of seed capsules. Evidence suggests that fruit set tends to be resource limited rather than

pollination limited, so a reduction in pollination may not have a significant impact on plant population dynamics (Crawley, 1989). However, because each unpollinated flower left a scar when it fell off, the number of seed capsules could still be counted and an estimate of seed production was achieved.

The mean number of seed capsules produced by purple loosestrife main flowering spikes was found to be significantly lower in the insect alone, triclopyr amine alone, and glyphosate alone treatment cages than the control and no-cage plots in 1997. Flowering spikes in the herbicide alone treatments were shorter because, again these were plants that were flowering in the same year that they germinated. Flowering spikes in the insect alone treatment were shorter due to herbivory by *G. calmariensis*. Feeding by *G. calmariensis* larvae on developing flowers was commonly observed. Cooper (1996) reported that *G. calmariensis* had a negative impact on relative fitness (i.e. reproductive potential) of purple loosestrife. In Cooper's study the beetles reduced flower production directly by feeding on the flowers and indirectly by defoliation. Defoliation effectively reduces the amount of available carbohydrate available to the plant for flower production (Crawley, 1983). In addition, feeding on ripening fruits and on seeds prior to dispersal causes enormous reductions in the reproductive potential of many plant species (Crawley, 1989). Insect herbivory also reduces the numbers of seeds per fruit and may subsequently lead to reduced seed sizes and subsequent seedling competitive ability (Crawley, 1989).

By September 1997 purple loosestrife stem densities in the herbicide alone treatment cages were much higher than the control, but the dry weights from the herbicide alone treatment cages were significantly lower than the control. The purple loosestrife plants in the herbicide alone treatments were in their first year of growth following germination earlier in 1997 and did not attain the aboveground biomass of plants in the control cages that were growing from established rootstocks. By September 1998, dry weights of purple loosestrife plants from the insect alone treatment cages were significantly lower than purple loosestrife dry weights from the integrated treatment cages.

Purple loosestrife plants in the insect alone treatment cages were intensely defoliated during early July 1998. New shoots were observed growing from the rootstocks as well as defoliated plants, but these were subsequently suppressed as well. These observations are in agreement with those of Katovich et al. (1999) who also observed that purple loosestrife plants that were intensely defoliated by *Galerucella* spp. would produce new shoots from the rootstock and defoliated stems during the growing season, and suffered an average reduction of 81% of the aboveground biomass compared to the control. Purple loosestrife plants in the insect alone treatment plots were also completely defoliated during late August and early September 1998. This was during the time that a large population of second-generation *G. calmariensis* was present. Approximately 10,000 adults were estimated to be in each of the insect alone treatment cages during this time. Although not significantly different, mean dry weights in the integrated treatments were lower than those of the herbicide alone treatments.

Purple loosestrife plants in the insect alone treatment cages produced significantly more lateral stems than the herbicide alone treatments in 1997. This was presumably due to pruning of the terminal portions of purple loosestrife plants by *G. calmariensis* in the insect alone treatment cages. Cooper (1996) also reported that feeding by *G. calmariensis* on purple loosestrife plants resulted in shorter plants with many, short axial stems. Somewhat fewer lateral stems grew from purple loosestrife main stems in the integrated treatments than in the herbicide alone treatments. As might be expected, purple loosestrife plants that had higher numbers of lateral stems also had higher mean total lateral stem lengths.

3) Flower production-estimated number of seed capsules on main flowering spike

According to Cooper (1996) the impact of herbivory on seed production can be extrapolated from purple loosestrife flower production data. The regression equation that was developed could be applied to estimate the number of seed capsules that are being produced per flowering spike and thus, an estimate of the number of seeds being produced per plant. The average number of seeds produced per seed capsule is reported to be between 90 and 100 (Mal et al. 1992). For example, the mean number of seed capsules on the main flowering spike in the no-cage plots was approximately 308 and the mean number of flowering spikes per plant was approximately 4.77. This translates into approximately 4.77 [308(100)]= 146,916 seeds produced per plant. Cooper (1996) suggests that the number of flowers per spike or the length of each flower spike can be used as a low-effort herbivore-damage indicator in the field.

4) Plant taxa response to treatments-mean stem densities

a) Plant response to herbicide treatments-1996

The complete removal of purple loosestrife (Fig. 2) in cages that were treated with triclopyr amine in 1996 is consistent with results of Gabor et al. (1995). These authors found that triclopyr amine applied at a rate of 12 kg/ha in a shallow southern Ontario wetland resulted in complete removal of the aboveground portion of adult purple loosestrife plants during the first year of treatment. Complete suppression of purple loosestrife in cages that were treated with glyphosate in 1996 is consistent with results of Rawinski (1982), Balogh (1986) and Skinner and Hollenhorst (1989) who found that glyphosate effectively controlled the aboveground portion of purple loosestrife during the first year of treatment.

By 12 June 1997 many purple loosestrife plants exceeded 50 cm in height in the control and insect alone treatment cages. No purple loosestrife plants were growing inside any of the herbicide treated cages at this time, demonstrating that herbicide applications done in 1996 were effective in killing all purple loosestrife rootstocks inside these treatment cages. Dicotyledonous plant reestablishment in general was much slower in cages where triclopyr amine was applied. This phenomenon may indicate that triclopyr amine offers some degree of long-term control of dicotyledonous plant species. Triclopyr amine is reported to degrade rapidly (Dow Chemical 1988), so this phenomenon of slower reestablishment of broadleaf plants in the triclopyr amine treated plots than broadleaf

plants in the glyphosate treated plots is not understood. It is possible that the conditions in a Manitoba wetland do not result in herbicide breakdown at a rate claimed by Dow Chemical. Studies of triclopyr amine degradation rates under wetland conditions in southern Manitoba should be conducted in order to determine if indeed there is long-term suppression of broadleaf plant species.

The decline in stem densities of tufted loosestrife and smartweed after exposure to triclopyr amine (Figs. 3 and 4) were also consistent with the effects of triclopyr amine on loosestrife stem densities. Tufted loosestrife and smartweed are broadleaf plants and therefore, the resultant decline of these species was expected. Natural senescence of tufted loosestrife between the second and third sampling periods may account for the apparent similarity in decline between the insect alone, all glyphosate treated plots and the control.

The decline in *Carex* spp. stem densities (Fig. 5) after exposure to triclopyr amine are in agreement with results of Gabor et al. (1995), who found that triclopyr amine applied at a rate of 12 kg/ha resulted in a decline in *Carex* spp. stem densities during the first year of treatment. These authors also found that sedges were able to recover from this level of application and there did not appear to be any long-term negative impacts of triclopyr amine on these species. In our study, grass species were relatively unaffected by exposure to triclopyr amine (Fig. 6). Gabor et al. (1995) also found that triclopyr amine applied at a rate of 12 kg/ha did not affect grass species during the first year of treatment.

Mullin (1998) does mention that triclopyr amine damage to grasses and grass-like plants can occur under some conditions. High dosages of triclopyr amine such as those used in our study resulted in some foliar damage of grasses and sedges. In a study by Nelson et al. (1996) some monocotyledonous species exposed to triclopyr amine exhibited injury symptoms (e.g. leaf burn and chlorosis) and reduced plant vigor may have resulted. The reasons for this impact of a broadleaf specific weed on a monocot are not well understood. The elimination of all grass species and *Carex* spp. after exposure to glyphosate was expected because glyphosate is a non-selective herbicide, effective at eliminating all plant species (Skinner and Hollenhorst, 1989).

In summary, applications of triclopyr amine and glyphosate in 1996 resulted in the removal of all purple loosestrife stems, as well as most other non-target species (except grasses in the triclopyr amine treated plots) by the end of the 1996-growing season. Non-target plant species responses to purple loosestrife removal by herbicides (i.e. changes in non-target densities in response to purple loosestrife removal) were not observed in 1996.

b) Effect of treatments on plant stem densities-1997 and 1998

Variation in purple loosestrife stem densities between treatment blocks was quite high. Differences in the amount of plant litter present inside cages were noted. Cages with a thick layer of plant litter apparently had fewer purple loosestrife seedlings and fewer non-target plant taxa than cages with less and/or thinner layers of plant litter. Standing water inside some cages that persisted longer than inside others may have also contributed to

some variation in purple loosestrife stem densities within and between treatment blocks. The fact that purple loosestrife stem densities in all treatment cages were not significantly different from the control by September 1997 could be explained by the fact that recruitment of purple loosestrife from the seed bank eventually resulted in purple loosestrife stem densities that were similar to densities in the control. In addition, feeding by *G. calmariensis* did not effectively suppress purple loosestrife stem densities in any of the treatment cages in which they were used in 1997. This was probably because they had not built large enough populations inside the field cages to affect purple loosestrife stem densities (i.e. kill purple loosestrife stems). However, they did have a measurable impact on loosestrife growth and development in that stem heights and flower production were suppressed in 1997. Purple loosestrife dominated the broadleaf plant fauna in the control and herbicide alone treatment cages throughout the course of the experiment (Table 16). At the end of the experiment, mean purple loosestrife stem densities in the herbicide alone treatment cages were four to nine times higher than pretreatment levels (Table 17). Purple loosestrife stems in the high-density biocontrol agent+herbicide treatment cages were completely suppressed, while only a few purple loosestrife stems remained in the low-density integrated treatment cages at the end of the experiment. Purple loosestrife stem densities were only moderately suppressed in the insect alone treatment cages as compared to the control, but the stem heights of purple loosestrife plants in this treatment were much lower than the control. Insect herbivores do not usually cause mortality to established perennial plants (Crawley, 1989). Cooper (1996) states that *G. calmariensis* has a greater impact on younger purple loosestrife plants than

older and larger plants. According to Katovich et al. (1999), extensive carbohydrate reserves are present in the large woody crowns of purple loosestrife plants. Several years of consistent severe damage to mature purple loosestrife plants may be required to stress the rootstock sufficiently to kill the plant.

Mean purple loosestrife stem densities within all treatment cages declined over the course of the growing seasons (Table 18). They were highest in June and lowest in September. Shamsi and Whitehead (1974) and Haworth-Brockman (1993) also reported declines in purple loosestrife stem densities throughout the growing season. Lower purple loosestrife stem densities during the last sampling periods of each year are due to a general decline in numbers over the growing season (i.e. self-thinning) rather than to seasonal senescence (Haworth-Brockman 1993).

In general, the triclopyr amine alone, low-density biocontrol agent+triclopyr amine and the high-density biocontrol agent+triclopyr amine treatments had lower tufted loosestrife stem densities than the other treatments. It is possible that the higher density of monocotyledonous plants in the cages that were treated with triclopyr amine in 1996 may have suppressed tufted loosestrife reestablishment through competition.

Grasses and sedges did not recover in glyphosate treated cages in 1997. This was expected because glyphosate was applied to these plots on August 28, 1996. Grass stems were entirely absent from the biocontrol agent+glyphosate treatment cages. Grass and

sedge stem densities were also low in the treatments in which triclopyr amine was applied in 1996. Variation in numbers of grass and sedge stems between treatment blocks was so high that meaningful analysis of grass and sedge stem densities was not possible.

5) Recovery of non-target plant taxa

One aspect of importance for this study was to evaluate the recovery of native vegetation following the application of herbicides and/or biocontrol agents. If purple loosestrife is a pest due to its monodominant stands then an important consequence of its control that is desirable is the recovery of a more diverse native flora. The stem densities over time for selected plant taxa are given in tables 18-23.

By the end of the experiment, mean tufted loosestrife stem densities in the high-density biocontrol agent+glyphosate, low-density biocontrol agent+glyphosate and glyphosate alone treatment cages were higher than pretreatment levels, when stem densities of August, 1998 were compared to stem densities of July 1996 (Table 18). Tufted loosestrife stem densities in the control and insect alone treatment cages declined over the course of the experiment. Tufted loosestrife stem densities in plots that were treated with triclopyr amine never recovered to pretreatment levels.

Mean smartweed stem densities in all treatment cages recovered to greater than pretreatment levels (July 1996 vs. August 1998), (Table 19). The highest number of smartweed stems was found within the integrated treatment cages. The lowest number of

smartweed stems was found in the glyphosate alone, low-density biocontrol agent+glyphosate and the control.

Mean milkweed stem densities in all treatment cages other than the control was much higher than 1996 levels (September 1998 vs. September 1996), (Table 20). At the end of the experiment, mean milkweed stem densities in the control cages were the same as pretreatment levels. The most significant recovery occurred in cages that were treated with triclopyr amine in 1996, both with the herbicide alone and in combination with *G. calmariensis*. These treatment cages had higher milkweed stem densities than the plots that were treated with glyphosate in 1996. Overall, the highest milkweed stem densities were found within the integrated treatment cages.

Mint stem densities in all treatment cages other than the control were much higher than pretreatment levels by the end of the study (Table 21). Mint stem densities in the control cages declined to well below pretreatment levels. The highest mint stem densities were found in the integrated treatments by the end of September 1998.

From Table 22 it is clear that mean sedge stem densities tended to decline over the course of the experiment in the control and insect alone treatments. This decline may have occurred because of reduced light levels inside field cages. Sedge stem densities were higher than pretreatment levels in only the high-density biocontrol agent+triclopyr amine treatment cages. Sedge stem densities were close to pretreatment levels in the high-

density biocontrol agent+glyphosate treatment cages. However, sedge stem densities had recovered considerably during the 1998 season and had higher densities than the control in the insect alone, triclopyr amine alone and high-density biocontrol agent+triclopyr amine treatments.

Grass stem densities recovered to higher than pretreatment levels in the triclopyr amine alone and the low-density biocontrol agent+triclopyr amine treatment cages (Table 23). In this study, very few monocot plant stems were recorded from glyphosate treated cages after this herbicide was applied. It was hypothesized that native grasses and sedges would quickly re-establish in areas where purple loosestrife was removed by herbicides and subsequently controlled by *G. calmariensis*. This was not the case. In plots that were treated with the combination of triclopyr amine and *G. calmariensis*, reestablishment of native sedges occurred faster than in cages treated with glyphosate. To reiterate, glyphosate had killed all grasses and sedges when it was applied in 1996. By the end of the 1998 field season, however, grass and stem densities were so variable among the treatment blocks that meaningful analysis was not possible. One possible reason for this could be that grasses and sedges are later successional species of a habitat, such as the one where this study took place. Alternatively, the grasses and sedges were unable to recover quickly under caged field conditions whereby reduced light levels may have hindered reestablishment. Nevertheless, grasses and sedges in the insect alone plots did increase, possibly in response to the stress placed upon loosestrife plants by *G. calmariensis*.

In summary, recovery of non-target plant taxa did not occur as quickly as expected. However, it was clear that relaxed competition from purple loosestrife plants that were being defoliated by *G. calmariensis* allowed many native broad-leaved plant taxa to increase in numbers by the end of the experiment that were eventually higher than pretreatment levels. In a loosely similar study, on a different system, Lym et al. (1996) reported that an unexpected finding in their study was that native plant production did not return to normal, even after leafy spurge density was reduced by *Aphthona spp.* (Coleoptera: Chrysomelidae-a biological control agent of leafy spurge) feeding for several years.

6) Purple loosestrife seedlings

As expected, purple loosestrife recovered quickly because of its extensive seed bank in the soil. Purple loosestrife seedling densities in the insect alone treatment cages were not significantly different from the control in August 1998. This likely occurred because removal of purple loosestrife in the insect alone treatment cages was recent and seedlings had not germinated by the time the seedling counts were made. Defoliation by *G. calmariensis* adults and larvae in the integrated treatment cages likely resulted in favorable conditions (more light, higher temperatures) for further purple loosestrife germination. Gabor et al. (1995) found that removal of adult purple loosestrife allowed more light to reach the substrate, creating ideal conditions for seed germination and plant growth. Nelson et al. (1996) found that seedlings were most abundant in bare ground

areas adjacent to dead purple loosestrife plants. Purple loosestrife seedlings were first observed in the herbicide alone cages around 24 June 1997 but remained under water for nearly three weeks during which time they grew very little. Water levels eventually receded and these seedlings grew rapidly through July and August. Peak seedling emergence was noted to be around the middle of August. Seedlings in cages that were treated with triclopyr amine in 1996 appeared to have germinated later in the season than in cages that were treated with glyphosate. As mentioned above, purple loosestrife plants in cages treated with herbicides alone grew much taller than purple loosestrife in cages treated with the herbicide-plus-insect integrations. Shading by reestablishing purple loosestrife plants likely suppressed further purple loosestrife germination from the seed bank in the herbicide alone treatment cages. According to Shamsi and Whitehead (1977), purple loosestrife seeds contain very little reserve material and must germinate in conditions where photosynthesis can soon occur if seedlings are to survive. Furthermore, many purple loosestrife plants in the herbicide alone treatments were able to produce flowering spikes in 1997 and 1998. Shamsi and Whitehead (1977) reported that purple loosestrife seedlings are able to flower and set seeds in the first year. During the 1997 and 1998 study years, potted purple loosestrife plants were placed in the biocontrol agent+herbicide treatment cages as refugia to sustain adult and larval *G. calmariensis* until seeds from the seed bank could germinate. Purple loosestrife seedlings within herbicide-treated cages were first noted near the end of June of both years. Water 15-30 cm deep submerged most purple loosestrife seedlings during June and much of July of both years. Water did not recede until the end of July of both years, at which time purple

loosestrife seedlings continued to grow. Further germination occurred through the following weeks, peaking around mid-August. Casual observations of *G. calmariensis* activity indicated that the adults were able to locate purple loosestrife seedlings, feed, and lay eggs on them. Based on personal observations of seedling defoliation within the field cages, defoliation of loosestrife seedlings by *G. calmariensis* adults and larvae was 50-90%. Mortality of many seedlings occurred while others suffered little or no damage. Most suffered 50% defoliation. Herbivores may kill seedlings outright or reduce their growth and subsequent ability to compete with other plants (Crawley, 1989).

7) Soil core trials

The results of the second soil core trial were somewhat different from the first trial. It is possible that some purple loosestrife seeds may have been brought to the surface by disturbance of soil cores, perhaps by watering and handling them. Nevertheless, this experiment demonstrated that *G. calmariensis* appeared to have enhanced the recruitment of purple loosestrife from the seed bank inside the field cages. Evidence for this was seen in the reduced number of seedlings that germinated in the treatments that included *G. calmariensis*, after the experiment was terminated. This was especially evident when compared with the herbicide alone and control treatments. A number of Canada thistle (*Cirsium arvense* (L.) Scop.) (Asteraceae) plants also grew from some of the soil cores during both soil core trials. The likely reason for this could be that many Canada thistle plants were observed near the study area in 1997 and 1998. Canada thistle seeds may have been blown in from surrounding areas during the period of time between late fall

1997 and early spring 1998, when the cage sleeves were removed from the cage frames. An alternative explanation is that Canada thistle seeds were already present in the soil seed bank in a dormant state for several years. Conditions were ideal for germination of Canada thistle seeds, since competition from purple loosestrife and other plants was minimal. This has important implications for an IWM strategy. According to Randall, (1996) there is the risk that when one pest is eliminated another may take its place, i.e., the infestation is merely the symptom of a greater problem. Plants such as purple loosestrife and Canada thistle are primary colonizers of disturbed areas. According to Begon et al. (1990), when an r-selected species (such as purple loosestrife) is removed, a bare patch of soil results. This creates an ideal habitat for another opportunistic, colonizing, r-type species.

8) Oviposition on seedlings and one-year-old plants

In the laboratory oviposition tests, considerably more eggs were laid on purple loosestrife plants in the seedlings-plus-year old purple loosestrife treatment. It should be noted also that the number of eggs laid on seedlings alone (594) plus the number of eggs laid on year-old purple loosestrife plants (658) is very close to the total number of eggs laid on seedlings plus year-old purple loosestrife (1,386). The reason for this anomaly is unknown but may be due to an ability of females to detect the higher amount of purple loosestrife biomass present in this treatment and thus lay more eggs. Oviposition by *G. calmariensis* females on both established purple loosestrife plants and seedlings were observed inside the field cages. In addition, fewer *G. calmariensis* adults were present

inside the integrated treatment cages as compared with the insect alone treatment. This may have been the result of less purple loosestrife biomass available for feeding and oviposition inside the integrated treatment cages than inside the insect alone treatment cages.

9) ***G. calmariensis* activity**

Observations made at the Netley-Libau marsh study site provided some evidence in 1998 that populations of *G. calmariensis* underwent two generations that year. Overwintered adults were first observed in the field on 13 May 1998. By 28 May, adults were still copulating and laying eggs and first-instar larvae were also seen on this date. By late June over-wintered adults could not be found at the site. First generation adults began emerging around 16 July and were observed copulating and laying eggs by 21 July. Third-instar larvae (from eggs laid by over-wintered adults in mid-June) were observed during late July as well. A massive emergence of second generation adults began around 28 August. Emergence appeared to peak around 12 September. Throughout this period adults were feeding on loosestrife but copulating pairs and egg masses were never observed. McAvoy et al. (1997) reported that *G. calmariensis* adults were present in early July during a study in Virginia but no adults, eggs, or larvae were found on subsequent sampling dates. These F_1 adults had gone into diapause and no adults or immature stages were detected for the remainder of the year, indicating the presence of one generation per year in Virginia. Daylength in Manitoba is considerably longer than in

Virginia during the summer months. The differing and regional number of generations per season may indicate a facultative life cycle for *G. calmariensis*.

By late September 1998, all purple loosestrife plants outside of the field cages at the 2 ha Netley-Libau marsh study site were 100% defoliated. These plants resembled those killed by herbicides. The massive population increase of *G. calmariensis* that was mentioned earlier occurred at the study site during late August and persisted throughout October 1998, both inside and outside of the treatment cages. It was common to observe >100 *G. calmariensis* adults on a single purple loosestrife leaf. As of mid-June, 1999 neither *G. calmariensis* over-wintered adults nor emergent purple loosestrife stems could be located at the Netley-Libau marsh study site (C.J. Lindgren, Pers. Comm.). By August 16 2000, purple loosestrife plants at the Netley-Libau marsh study site had stunted bushy growth. Very few flowering spikes were observed at this time. There was no evidence that any rootstock mortality had occurred.

Although desirable, an effort to ascertain the number of eggs being laid by *G. calmariensis* on tagged purple loosestrife plants was abandoned as being far too labor-intensive. Egg masses were often so densely packed that it was impossible to reliably identify individual egg masses. In addition, since *G. calmariensis* adults were being confined inside field cages they were prevented from dispersing naturally and were also being protected from natural predation. Any population estimates for *G. calmariensis*

inside field cages may not be applicable to open field conditions, since the caged environment may have been different from open field conditions.

General Conclusions

1. Mature purple loosestrife plants produced new shoots from rootstocks and defoliated stems during the growing season when defoliation by *G. calmariensis* was 90-100%.

2. Defoliation by *G. calmariensis* reduced purple loosestrife stem densities, vertical growth, flower production and seed production.

3. Where herbicides were used alone, purple loosestrife recovered rapidly through recruitment from the seed bank. Purple loosestrife stem densities in these treatments were two-to-three times higher than pretreatment levels.

4. Integrating *G. calmariensis* with herbicides (triclopyr amine and glyphosate) resulted in a more sustained reduction of purple loosestrife stem densities than either method used alone.

5. Where herbicides were integrated with *G. calmariensis*, reduced competition from purple loosestrife in the integrated treatments allowed native non-target broad leaf

plant taxa stem densities to recover to levels that were higher than pre-treatment levels and the control, two years after treatments were made.

6. *G. calmariensis* fed on and oviposited on purple loosestrife seedlings that were recruited from the seed bank.

7. Where purple loosestrife was removed by herbicides and then seedlings were subsequently defoliated by *G. calmariensis*, a succession to native broad leaf plant taxa appeared to occur.

Management of purple loosestrife by integrating biological control with herbicides:

1) Large monodominant stands of purple loosestrife:

In areas where native vegetation has been largely displaced by large infestations of purple loosestrife, a broad-spectrum herbicide such as glyphosate could be used. Based on previous studies, glyphosate is most effective when applied during the late bloom stage of purple loosestrife.

2) Smaller stands of purple loosestrife interspersed with areas of monocotyledonous plant species:

In areas where purple loosestrife has not completely displaced native monocotyledonous plant species, a broadleaf herbicide such as triclopyr amine could be used. Based on previous studies, triclopyr amine is most effective when applied during the bud-to-early bloom stage of purple loosestrife.

In both 1) and 2) above, it would be best to leave an untreated area that will serve as a refugium for the biocontrol agents. The biocontrol agents could be released inside these refugia just before or at the same time the herbicides are applied, or they can be released the following season.

Management Considerations

1. If possible, areas to be treated with herbicides could be re-seeded with native grass and/or sedge species that are characteristic of the treated area(s) to compete with re-establishing loosestrife seedlings.
2. When treating with herbicides, care should be taken to avoid spraying all of the loosestrife plants in the area. This strategy would provide refugia for the biocontrol agents until seedlings germinate from the seed bank.

3. Minimize exposure of non-target flora to herbicide exposure. This will allow non-target flora to recover more quickly and compete better with loosestrife seedlings recruited from the seed bank.

Suggestions for future research

1) Test integration of herbicides with *G. calmariensis* under open field conditions.

2) Investigate long-term suppression of broadleaf flora by triclopyr amine.

3) Investigate long-term population dynamics between *L. salicaria* and *G. calmariensis*.

3.6 GENERAL DISCUSSION

Figure 7 illustrates some of the many concepts and disciplines that should be addressed in an integrated vegetation management program for purple loosestrife. For example, it is important to consider such aspects as the biology, ecology and taxonomy of the biocontrol agent as well as target and non-target flora. The herbicides to be applied should be thoroughly evaluated and understood for their potential effects on the target plant, the non-target flora and the biocontrol agents to be used.

Lym et al. (1996) suggest that weed control programs should be designed for specific situations. Lindgren et al. (1998) suggest that when developing a long-term sustainable weed control program by integrating a classical biological control agent with a herbicide, allowances must be made to protect a sufficient portion of the target weed required to sustain the field colony of the biological control agent. For example, triclopyr amine does not have a direct toxic effect on *G. calmariensis* but would have an indirect effect by eliminating the food source (Lindgren et al. 1998). These authors found that by fourteen days post-treatment, sprayed host plant quality had deteriorated (i.e. purple loosestrife had a browned-out or dried down appearance) necessitating that a healthy, unsprayed purple loosestrife plant be placed in cages to sustain *G. calmariensis* adults. Maintenance of resident *G. calmariensis* could be achieved through seedlings emerging from the established seed bank, and/or by protecting small areas of purple loosestrife during herbicide applications (Lindgren et al. 1998). Mullin (1998) recommends spot spraying

and suggests that competitive surrounding vegetation should be preserved to fill in where purple loosestrife is controlled. This could be achieved by using low pressures, large droplets, and narrow spray patterns. Re-vegetation should be done with native non-invasive perennial grasses, cattails, or rushes that are adapted to aquatic or moist conditions (Mullin 1998). Gabor et al. (1996) found that after an application of triclopyr amine, native monocotyledonous plant species increased and were able to slow the rate of purple loosestrife reestablishment and suggested native plant competition could offer further control.

A biocontrol agent release in year 1 followed by herbicide application in year 2 may not be as successful as a strategy where both are done during the same year, or if biocontrol agents are released the year following a herbicide application. It would seem to be a counterproductive management strategy to release a biocontrol agent into an area, allow their populations to increase, and then apply a herbicide to remove their food source. Even if refugia are left in place, there is the risk that the population of biocontrol agents would suddenly exceed the carrying capacity of their food source and starve on what remained, perhaps before they could successfully reproduce. It is also possible that too many biocontrol agents (larvae and adults) feeding on a limited number of loosestrife plants may result in disease, increased predation and reduced adult fitness of the biocontrol agents. However, conditions in cage studies are different from field studies. In studies using cages, the biocontrol agents cannot actively disperse under adverse conditions (e.g. poor food quality and/or unusually dense herbivore populations). Under

natural conditions, biocontrol agents are not going to remain in a herbicide treated area and wait for loosestrife seeds to germinate. They will likely disperse from depleted sources of food in search of other patches of purple loosestrife. This phenomenon was observed on September 18 1998 at the Netley-Libau marsh study area. Purple loosestrife was 100% defoliated and thousands of adult *Galerucella* were observed flying at altitudes of 5-10 m, for distances of up to several tens of metres at a time. Densities of *G. calmariensis* used in our study would not necessarily be the same as those that would be released in the field under natural conditions. Biocontrol insect densities released into the low-density insect treatments in our study would equal 20,000 adults/ha. Densities released into the high-density insect treatments would equal 85,000 adults released/ha. However, in this study, satisfactory control of purple loosestrife was achieved with the low-density release.

According to Gabor et al. (1996) very little is known about long-term responses of native plant communities to complete removal of an r-selected species such as purple loosestrife or the impact of native species on purple loosestrife recruitment from the seed bank. An alternative strategy would be to treat purple loosestrife infestations at a lower rate of application. In regards to triclopyr amine, this may result in less than 100% adult purple loosestrife removal but damage to monocotyledonous plants would likely be minimized, thereby allowing these species to more rapidly fill in areas where purple loosestrife once dominated. Conversely, using a non-selective herbicide such as glyphosate on mature purple loosestrife stands only gives short-term management, since complete vegetation

removal enhances the accessibility of the treated area to recruitment from the seed bank (Nelson et al. 1996) and other r-selected plant species. Thompson et al. (1987) stress that application of a broad-leaf herbicide to a natural wetland that may support a complex of more than 100 plant species occupying several photosynthetic levels, and supporting a fauna living at many trophic levels, would result in a drastic blow to a wetland community. Not treating all purple loosestrife plants with a herbicide does have several other advantages as well, such as 1) food sources for the biocontrol agents until seedlings emerge. 2) allowing gradual recovery of native species, 3) less area available for colonization by other r-selected species.

What is the economic benefit of expediting purple loosestrife control by integrating herbicides with biocontrol agents, i.e., what will be gained by controlling purple loosestrife at an earlier stage with the use of herbicides, factoring short-term losses from the herbicide treatment? One answer would be the preservation of rare flora. At the Libau marsh study site, only a few yellow lady slipper orchids *Cypripedium calceolus* L. (Orchidaceae) could be found in areas where purple loosestrife had not yet spread, suggesting that this species and other wetland orchids could be threatened by the spread of purple loosestrife unless intervention is considered. Other applications would include the clearing of waterways, irrigation canals and retention ponds that have become clogged with purple loosestrife.

A 75% reduction in purple loosestrife densities would probably allow wetland managers to cope with the species (Thompson et al. 1987). Surviving purple loosestrife would provide a substantial base of pollen and nectar forage for pollinators such as honey bees amidst a greater variety of native plants. Agren (1996) found that a positive correlation exists between population size and seed production and between seed produced per flower and seed produced per plant in purple loosestrife populations in Europe. He also found that seed production is a function of insufficient pollen transfer in small populations. Large purple loosestrife populations are more attractive to pollinators than small populations. In small purple loosestrife populations the low seed produced could potentially limit population growth and may threaten local persistence of purple loosestrife in particular. As Cooper (1996) points out, the rate of decline of purple loosestrife populations will also be dependent on the extent and longevity of the seed bank, coupled with the rate of defoliation, and the impact of defoliation on competition with other wetland plants.

Herbicides have been successfully combined with biological control agents in at least one other study. Lym (1998) reported that treatments using the biological control agents *Aphthona* spp. (Coleoptera: Chrysomelidae) and *Spurgia esulae* Gagne (Diptera: Cecidiomyidae) combined with herbicides resulted in a greater leafy spurge density reduction than either method used alone. Large increases in biological control agent populations and increased subsequent leafy spurge control were observed in the field when herbicides were combined with *Aphthona* spp. flea beetles (Lym et al. 1996).

These authors also found that the leafy spurge density gradually declined when only insects were present and took three years longer to reduce the infestation to the same level achieved in one year by the herbicide-plus-insect treatment. According to Lym et al. (1996) an integrated control program combining two or more methods would provide a more successful and cost-effective long-term solution to the leafy spurge problem than a single method used alone.

In the Netley-Libau marsh IVM study, the least disruptive strategy was found to be the insect alone treatment. Native grasses increased in this treatment to levels that were higher than pretreatment levels. Native sedges on the other hand were nearly the same as pretreatment levels. In Manitoba, *G. calmariensis* can effect satisfactory control of purple loosestrife within three to four years post-release (Lindgren, in press). Treating large-scale infestations of purple loosestrife with herbicides could drastically disrupt wetland ecosystems. Thus, careful consideration should be given to the nature of the infestation before applying herbicides to large-scale purple loosestrife infestations.

Table 1: Mean dry weights (g ± standard error (SE)), mean number of seed capsules (± SE) and mean stem heights (cm ± SE) of purple loosestrife plants from the control and insect alone experimental plots (n=45 plants/ treatment) at Netley-Libau marsh, Manitoba, 19 September 1996. Measurements were taken before *G. calmariensis* was released into the Insect alone treatment cages. Treatments with identical letters are not significantly different at α=0.05 (t-test).

Treatment	**Mean dry weight (g ± SE)**	**Mean # seed capsules (± SE)**	**Mean stem heights (cm ± SE)**
Control	202.99 ± 44.04 **a**	357.51 ± 23.82 **a**	145.58 ± 3.17 **a**
Insect alone	221.19 ± 31.97 **a**	361.47 ± 25.70 **a**	144.58 ± 2.64 **a**

Table 2: Mean stem heights (cm ± standard error (SE)) of purple loosestrife plants-all experimental plots (n=45 plants/treatment) at Netley-Libau marsh, Manitoba, 19 September 1997 and 26 September 1998. Treatments with identical letters are not significantly different at α=0.05 (Tukey-Kramer HSD; (1997 df=8, 396; F=427.7; 1998 df=7, 352; F=57.3). HG=high-density biocontrol agent+glyphosate, HT=high-density biocontrol agent+triclopyr amine, LG=low-density biocontrol agent+glyphosate, LT= low-density biocontrol agent+triclopyr amine.

Treatment	Mean stem height (cm ± SE)	
	1997	1998
Control	176.1 ± 3.7 **a**	183.2 ± 4.3 **a**
No-cage	146.7 ± 2.8 **b**	-
Insect alone	145.5 ± 4.5 **b**	41.6 ± 3.3 **e**
Glyphosate alone	37.9 ± 4.6 **c**	120.3 ± 8.2 **b**
Triclopyr alone	34.6 ± 3.3 **c**	100.7 ± 9.2 **b,c**
HG	18.4 ± 1.4 **d**	87.1 ± 4.9 **c,d**
HT	27.7 ± 1.9 **d**	69.9 ± 4.1 **c,d**
LG	28.9 ± 2.5 **d**	59.5 ± 5.1 **d,e**
LT	24.2 ± 1.5 **d**	61.8 ± 5.9 **d,e**

Table 3: Mean number of flowering spikes/purple loosestrife plant and mean number of seed capsules/spike (± standard error (SE)-all experimental plots (n=45 plants/treatment) at Netley-Libau marsh, Manitoba, 19 September 1997 and 26 September 1998. Treatments with identical letters are not significantly different at α=0.05. (Tukey-Kramer HSD #flowering spikes 1997 df=8, 219 (F=17.09); 1998 df=7, 352 (F=8.2); # seed capsules 1997 df=8, 109 (F=4.6); 1998 df= 7, 352 (F=77.8)). HG=high-density biocontrol agent+glyphosate, HT=high-density biocontrol agent+triclopyr amine, LG=low-density biocontrol agent+glyphosate, LT=low-density biocontrol agent+triclopyr amine

Treatment	Mean # flowering spikes (± SE)		Mean # seed capsules (± SE)	
	1997	1998	1997	1998
Control	4.42 ± 0.43 **b**	6.1 ± 0.7 **a**	298.77 ± 19.04 **a**	253.5 ± 15.8 **a**
No-Cage	4.77 ± 0.43 **b**	-	308.22 ± 18.61 **a**	-
Insect alone	7.62 ± 1.35 **a**	0.0 ± 0.0 **c**	191.65 ± 27.91 **b**	0.0 ± 0.0 **d**
Glyphosate alone	1.16 ± 0.57 **c**	5.0 ± 0.9 **a,b**	206.00 ± 62.41 **a,b**	159.2 ± 21.9 **b**
Triclopyr alone	0.38 ± 0.27 **c**	6.4 ± 2.5 **a**	108.00 ± 88.27 **a,b**	57.2 ± 14.3 **c**
HG	0.0 ± 0.0 **d**	0.0 ± 0.0 **c**	0.0 ± 0.0 **c**	0.0 ± 0.0 **d**
HT	0.0 ± 0.0 **d**	0.0 ± 0.0 **c**	0.0 ± 0.0 **c**	0.0 ± 0.0 **d**
LG	0.0 ± 0.0 **d**	0.1 ± 0.1 **c**	0.0 ± 0.0 **c**	1.2 ± 1.2 **d**
LT	0.0 ± 0.0 **d**	1.1 ± 0.8 **b,c**	0.0 ± 0.0 **c**	5.4 ± 3.8 **d**

Table 4: Mean dry weights (g ± standard error (SE)) of purple loosestrife plants-all experimental plots (n=45 plants/treatment) at Netley-Libau marsh, Manitoba, 19 September 1997 and 26 September 1998. Treatments with identical letters are not significantly different at α=0.05. (Tukey-Kramer HSD (1997 df=8, 18 (F=21.06); 1998 df=7, 16 (F=3.8)). HG=high-density biocontrol agent+glyphosate, HT=high-density biocontrol agent+triclopyr amine, LG=low-density biocontrol agent+glyphosate, LT=low-density biocontrol agent+triclopyr amine.

Treatment	Mean dry weight (g ± SE)	
	1997	1998
Control	318.4 ± 47.4 **a**	181.3 ± 42.4 **a**
No-Cage	282.3 ± 13.6 **a**	-
Insect alone	332.8 ± 77.3 **a**	3.5 ± 0.8 **b**
Glyphosate alone	60.5 ± 20.3 **b**	123.7 ± 44.7 **a,b**
Triclopyr alone	41.9 ± 6.0 **b**	78.7 ± 44.5 **a,b**
HG	15.3 ± 11.4 **b**	51.8 ± 22.7 **a,b**
HT	7.4 ± 2.8 **b**	14.4 ± 6.1 **b**
LG	3.8 ± 1.9 **b**	40.4 ± 25.9 **a,b**
LT	14.8 ± 5.2 **b**	32.6 ± 25.0 **a,b**

Table 5: Mean number of lateral stems (l.s.) and mean total l.s. lengths (± standard error (SE)) of all l.s. originating from the main stem of purple loosestrife plants-all experimental plots (n=45 plants/ treatment) at Netley-Libau marsh, Manitoba, 19 September 1997 and 26 September 1998. Treatments with identical letters are not significantly different at α=0.05 (Tukey-Kramer HSD: # l.s 1997 df=8, 396 (F=37.1), 1998 df=8, 396 (F=60.9); total l.s. lengths 1997 df=8, 396 (F=60.9), 1998 df=7, 142 (F=9.9)). HG=high-density biocontrol agent+glyphosate, HT=high-density biocontrol agent+triclopyr amine, LG= low-density biocontrol agent+glyphosate, LT=low-density biocontrol agent+triclopyr amine

Treatment	Mean # l.s.(±SE)		Mean total l.s. lengths (cm±SE)	
	1997	1998	1997	1998
Control	17.1 ± 1.3 **a**	14.6 ± 1.3 **a**	400.2 ± 37.7 **a,b**	322.4 ± 36.5 **a**
No-Cage	14.9 ± 1.0 a	-	324.9 ± 27.0 **b**	-
Insect alone	19.3 ± 1.2 **a**	3.4 ± 0.6 **b,c**	443.5 ± 36.6 **a**	27.6 ± 7.5 **c**
Glyphosate alone	7.2 ± 1.3 **b**	14.2 ± 1.5 **a**	79.3 ± 27.1 **c**	357.3 ± 56.1 **a**
Triclopyr alone	6.9 ± 1.1 **b**	14.0 ± 2.9 **a**	60.4 ± 15.9 **c**	291.0 ± 102.7 **a,b**
HG	2.8 ± 0.6 **b**	13.8 ± 1.9 **a**	11.2 ± 2.5 **c**	172.9 ± 33.5 **b,c**
HT	4.4 ± 0.8 **b**	8.4 ± 1.6 **a,b**	29.9 ± 8.3 **c**	72.7 ± 15.9 **b,c**
LG	5.1 ± 1.1 **b**	9.7 ± 1.4 **a,b**	42.9 ± 12.4 **c**	102.3 ± 17.2 **b,c**
LT	3.0 ± 0.7 **b**	8.9 ± 1.9 **a,b**	14.2 ± 4.2 **c**	93.6 ± 24.2 **b,c**

Table 6: Transformed ($log_{10}(X+1)$ mean number of purple loosestrife stems/$4m^2$ (± standard error (SE))-all experimental plots at Netley-Libau marsh, Manitoba, 19 September 1997 and 27 September 1998. Treatments with identical letters are not significantly different at $\alpha=0.05$ (Tukey-Kramer HSD: 1997 df=7, 16 (F=1.5); 1998 df=7, 16 (F=13.1)). HG=high-density biocontrol agent+glyphosate, HT=high-density biocontrol agent+triclopyr amine, LG=low-density biocontrol agent+glyphosate, LT=low-density biocontrol agent+triclopyr amine

Treatment	Mean # stems (± SE)	
	1997	1998
Control	2.25 ± 0.09 **a**	2.23 ± 0.12 **a**
Insect alone	2.09 ± 0.07 **a**	1.34 ± 0.74 **a,b**
Glyphosate alone	2.50 ± 0.26 **a**	2.81 ± 0.21 **a**
Triclopyr alone	1.90 ± 0.30 **a**	2.95 ± 0.04 **a**
HG	2.45 ± 0.35 **a**	0 **b**
HT	1.95 ± 0.17 **a**	0 **b**
LG	2.75 ± 0.25 **a**	0.32 ± 0.32 **b**
LT	2.19 ± 0.37 **a**	0.48 ± 0.48 **b**

Table 7: Transformed ($log_{10}(X+1)$ mean number of tufted loosestrife stems/$4m^2$ (± standard error (SE))-all experimental plots at Netley-Libau marsh, Manitoba, 21 July 1997 and 11 August 1998. Treatments with identical letters are not significantly different at $\alpha=0.05$ (Tukey-Kramer HSD: 1997 df=7, 16 (F=2.9); 1998 df=7, 16 (F=1.5)). HG=high-density biocontrol agent+glyphosate, HT=high-density biocontrol agent+triclopyr amine, LG=low-density biocontrol agent+glyphosate, LT=low-density biocontrol agent+triclopyr amine.

Treatment	Mean # stems (± SE)	
	1997	1998
Control	1.69 ± 0.17 **a**	1.08 ± 0.19 **a**
Insect alone	1.41 ± 0.32 **a**	1.07 ± 0.30 **a**
Glyphosate alone	1.25 ± 0.21 **a**	0.98 ± 0.50 **a**
Triclopyr alone	0.61 ± 0.31 **a**	0.32 ± 0.32 **a**
HG	1.38 ± 0.17 **a**	1.45 ± 0.13 **a**
HT	0.63 ± 0.07 **a**	0.87 ± 0.29 **a**
LG	1.13 ± 0.34 **a**	1.49 ± 0.38 **a**
LT	0.70 ± 0.20 **a**	0.77 ± 0.23 **a**

Table 8: Transformed ($log_{10}(X+1)$ mean number of smartweed stems/$4m^2$ (± standard error (SE))-all experimental plots at Netley-Libau marsh, Manitoba, 19 September 1997 and 27 September 1998. Treatments with identical letters are not significantly different at $\alpha=0.05$ (Tukey-Kramer HSD: 1997 df=7, 16 (F=0.9); 1998 df=7,16 (F=1.5)). HG=high-density biocontrol agent+glyphosate, HT=high-density biocontrol agent+triclopyr amine, LG=low-density biocontrol agent+glyphosate, LT=low-density biocontrol agent+triclopyr amine.

Treatment	Mean # stems (± SE)	
	1997	1998
Control	1.19 ± 0.21 **a**	0.58 ± 0.28 **a**
Insect alone	1.55 ± 0.15 **a**	1.07 ± 0.22 **a**
Glyphosate alone	1.09 ± 0.43 **a**	0.72 ± 0.28 **a**
Triclopyr alone	1.05 ± 0.13 **a**	0.92 ± 0.47 **a**
HG	1.70 ± 0.17 **a**	1.51 ± 0.04 **a**
HT	1.05 ± 0.13 **a**	1.20 ± 0.20 **a**
LG	1.32 ± 0.38 **a**	0.84 ± 0.20 **a**
LT	1.09 ± 0.33 **a**	1.34 ± 0.18 **a**

Table 9: Transformed ($log_{10}(X+1)$ mean number of swamp milkweed stems/$4m^2$ (± standard error (SE))- all experimental plots at Netley-Libau marsh, Manitoba, 19 September 1997 and 27 September 1998. Treatments with identical letters are not significantly different at $\alpha=0.05$ (Tukey-Kramer HSD: 1997 df=7, 16 (F=0.4); 1998 df=7, 16 (F=1.5)). HG=high-density biocontrol agent+glyphosate, HT=high-density biocontrol agent+triclopyr amine, LG=low-density biocontrol agent+glyphosate, LT=low-density biocontrol agent+triclopyr amine

Treatment	Mean # stems (± SE)	
	1997	1998
Control	1.01 ± 0.27 **a**	0.69 ± 0.12 **a**
Insect alone	0.82 ± 0.45 **a**	1.17 ± 0.24 **a**
Glyphosate alone	0.98 ± 0.24 **a**	0.84 ± 0.12 **a**
Triclopyr alone	0.88 ± 0.23 **a**	1.14 ± 0.07 **a**
HG	0.75 ± 0.36 **a**	1.66 ± 0.48 **a**
HT	0.75 ± 0.32 **a**	1.45 ± 0.31 **a**
LG	0.68 ± 0.46 **a**	1.25 ± 0.23 **a**
LT	0.36 ± 0.23 **a**	1.63 ± 0.40 **a**

Table 10: Transformed ($log_{10}(X+1)$ mean number of mint stems/$4m^2$ (± standard error (SE))-all experimental plots at Netley-Libau marsh, Manitoba, 19 September 1997 and 27 September 1998. Treatments with identical letters are not significantly different at $\alpha=0.05$ (Tukey-Kramer HSD: 1997 df=7, 16 (F=5.5); 1998 df=7, 16 (F=4.5)). HG=high-density biocontrol agent+glyphosate, HT=high-density biocontrol agent+triclopyr amine, LG=low-density biocontrol agent+glyphosate, LT=low-density biocontrol agent+triclopyr amine.

Treatment	Mean # stems (± SE)	
	1997	1998
Control	1.16 ± 0.24 **a**	0.36 ± 0.18 **b**
Insect alone	0.36 ± 0.06 **b**	1.60 ± 0.08 **a**
Glyphosate alone	0.64 ± 0.17 **a,b**	1.55 ± 0.13 **a,b**
Triclopyr alone	0.16 ± 0.16 **b**	1.13 ± 0.57 **a,b**
HG	0.36 ± 0.18 **b**	1.87 ± 0.12 **a**
HT	0.52 ± 0.14 **a,b**	1.98 ± 0.22 **a**
LG	0.16 ± 0.16 **b**	1.63 ± 0.22 **a**
LT	0.00 ± 0.00 **b**	1.87 ± 0.11 **a**

Table 11: Transformed ($log_{10}(X+1)$ mean number of sedge stems/m^2 (± standard error (SE))-all experimental plots at Netley-Libau marsh, Manitoba, 19 September 1997 and 27 September 1998. Treatments with identical letters are not significantly different at $\alpha=0.05$ (Tukey-Kramer HSD: 1997 df=7, 16 (F=5.0); 1998 df=7, 16 (F=1.5)). HG=high-density biocontrol agent+glyphosate, HT=high-density biocontrol agent+triclopyr amine, LG=low-density biocontrol agent+glyphosate, LT=low-density biocontrol agent+triclopyr amine.

Treatment	Mean # stems (± SE)	
	1997	1998
Control	1.17 ± 0.42 **a,b**	0.84 ± 0.49 **a**
Insect alone	1.40 ± 0.30 **a**	1.39 ± 0.39 **a**
Glyphosate alone	0.00 ± 0.00 **b**	0.30 ± 0.17 **a**
Triclopyr alone	0.26 ± 0.26 **a,b**	1.40 ± 0.36 **a**
HG	0.00 ± 0.00 **b**	1.45 ± 0.16 **a**
HT	0.84 ± 0.44 **a,b**	1.55 ± 0.48 **a**
LG	0.00 ± 0.00 **b**	0.63 ± 0.48 **a**
LT	0.10 ± 0.10 **b**	1.05 ± 0.20 **a**

Table 12: Transformed ($log_{10}(X+1)$ mean number of grass stems/m^2 (± standard error (SE))-all experimental plots at Netley-Libau marsh, Manitoba, 19 September 1997 and 27 September 1998. Treatments with identical letters are not significantly different at $\alpha=0.05$ (Tukey-Kramer HSD: 1997 df=7, 16 (F=1.1); 1998 df=7, 16 (F=1.3)). HG=high-density biocontrol agent+glyphosate, HT=high-density biocontrol agent+triclopyr amine, LG=low-density biocontrol agent+glyphosate, LT=low-density biocontrol agent+triclopyr amine.

Treatment	Mean # stems (± SE)	
	1997	1998
Control	0.97 ± 0.69 **a**	0.63 ± 0.63 **a**
Insect alone	0.26 ± 0.26 **a**	1.05 ± 0.55 **a**
Glyphosate alone	0.44 ± 0.44 **a**	0.36 ± 0.18 **a**
Triclopyr alone	0.37 ± 0.37 **a**	0.69 ± 0.69 **a**
HG	0.00 ± 0.00 **a**	0.75 ± 0.15 **a**
HT	0.28 ± 0.28 **a**	0.30 ± 0.30 **a**
LG	0.00 ± 0.00 **a**	0.36 ± 0.23 **a**
LT	0.91 ± 0.10 **a**	1.72 ± 0.06 **a**

Table 13: Mean $\log_{10}(X+1)$ transformed number of purple loosestrife seedlings/0.01m^2 (± standard error (SE), n=30 samples/treatment) in the experimental plots at Netley-Libau marsh, Manitoba. Counts were made on 7 August 1998. Treatments with identical letters are are not significantly different at α=0.05 (Tukey-Kramer HSD: df=7, 112 (F=23.9)). HG=high-density biocontrol agent+glyphosate, HT=high-density biocontrol agent+triclopyr amine, LG=low-density biocontrol agent+glyphosate, LT=low-density biocontrol agent+ triclopyr amine.

Treatment	Mean number of seedlings/0.01 m^2 (± SE)
Control	0.03 ± 0.03 **e**
Insect alone	0.41 ± 0.17 **d,e**
Glyphosate alone	1.04 ± 0.08 **b,c**
Triclopyr alone	1.65 ± 0.09 **a**
HG	0.66 ± 0.17 **c,d**
HT	1.33 ± 0.10 **a,b**
LG	1.07 ± 0.11 **b,c**
LT	1.28 ± 0.05 **a,b**

Table 14: Mean numbers of purple loosestrife seedlings (± standard error (SE)) produced from soil cores collected from experimental plots (n=12 cores/treatment) at Netley-Libau marsh, Manitoba, September 1998. Treatments with identical letters are not significantly different at α=0.05 (Tukey-Kramer HSD: Trial #1 df=7, 88 (F=2.5); Trial #2 df=7, 88 (F=2.8)). HG=high-density biocontrol agent+glyphosate, HT=high-density biocontrol agent+triclopyr amine, LG=low-density biocontrol agent+glyphosate, LT=low-density biocontrol agent+triclopyr amine

Treatment	Trial #1	Trial #2
Control	14.6 ± 5.5 **a**	51.9 ± 19.8 **a**
Insect alone	4.7 ± 1.5 **a,b**	20.2 ± 4.1 **a,b**
Glyphosate alone	6.4 ± 2.3 **a,b**	14.7 ± 2.8 **b**
Triclopyr alone	6.4 ± 2.1 **a,b**	14.5 ± 5.3 **b**
HG	4.7 ± 1.5 **a,b**	20.6 ± 3.8 **a,b**
HT	4.8 ± 1.8 **a,b**	9.9 ± 3.4 **b**
LG	3.2 ± 1.0 **b**	10.3 ± 2.9 **b**
LT	1.7 ± 0.7 **b**	22.9 ± 4.1 **a,b**

Table 15: Mean numbers of eggs per egg mass (± standard error (SE)) laid by single *G. calmariensis* females on purple loosestrife seedlings and one-year-old plants and total number of eggs laid by five females in each treatment. Treatments with identical letters are not significantly different (Tukey-Kramer HSD: #eggs/mass df=2, 748 (F=1.7); total #eggs df=2, 12 (F=4.5)).

Treatment	Mean number of eggs per egg mass (± SE)	Total # eggs laid (n=5 females)
Seedlings alone	3.19 ± 0.18 **a**	594 **a**
Year-old loosestrife alone	3.61 ± 0.2 **a**	658 **a**
Seedlings + year old loosestrife	3.61 ± 0.15*$_1$ **a**	1,386*$_2$ **b**

*$_1$ (seedlings 2.94 ± 0.19, year-old loosestrife 3.89 ± 0.18)

*$_2$ (1,063 eggs on year-old purple loosestrife, 323 eggs on seedlings)

Table 16: Purple loosestrife stem density expressed per $4m^2$ and as % of stem density of all broadleaf plants in combined treatment cages (n=3 cages/treatment) at Netley-Libau marsh, Manitoba 1996-1998. HG=high-density biocontrol agent+glyphosate, HT=high-density biocontrol agent+triclopyr amine, LG=low-density biocontrol agent+glyphosate, LT=low-density biocontrol agent+triclopyr amine.

Treatment	1996 (Pretreatment)	1996 (Postreatment)	September 1997	September 1998
Control	418/68%	366/89%	554/75%	544/94%
Insect alone	279/70%	294/85%	375/63%	400/65%
Triclopyr alone	307/68%	0/0%	280/80%	2,693/93%
Glyphosate alone	503/82%	0/0%	1,354/88%	2,319/94%
HG	297/60%	2/100%	1,566/85%	0/0%
HT	339/72%	0/0%	310/81%	0/0%
LG	340/70%	2/100%	2,250/92%	8/3%
LT	316/52%	0/0%	803/91%	27/5%

Table 17: Mean number of purple loosestrife stems/4m^2 area (standard errors omitted for clarity) all treatment cages (n=3 replicates/treatment) at Netley-Libau marsh, Manitoba 1996-1998. HG=high-density biocontrol agent+glyphosate, HT=high-density biocontrol agent+triclopyr amine, LG=low-density biocontrol agent+glyphosate, LT=low-density biocontrol agent+triclopyr amine.

	Treatment							
	Control	**Insect alone**	**Triclopyr alone**	**Glyphosate alone**	**HG**	**LG**	**HT**	**LT**
Sampling date								
vii.1996 (Pretreatment)	**139.3**	**93**	**102**	**168**	**99**	**113.3**	**113**	**105.3**
viii.1996	142.7	106	3	204.3	133	112	0.3	0.7
ix.1996	122	98	0	0	0.7	0.7	0	0
vi.1997	228.7	125.3	0	0	0	0	0.3	0
vii.1997	218.3	124.7	19	106.3	39.3	29.7	13.3	6.7
ix.1997	184.7	125	93.3	451.3	522	750	103.3	267.7
vi.1998	236.3	156	61	176.7	235.7	128	85	77.3
viii.1998	212	113.7	295.3	327.8	327.7	162.7	321.7	201.3
ix.1998 (end of study)	**181.3**	**133.3**	**897.7**	**771.3**	**0**	**2.7**	**0**	**9**

Table 18: Mean number of tufted loosestrife stems/4m^2 area (standard errors omitted for clarity) all treatment cages (n=3 replicates/treatment) at Netley-Libau marsh, Manitoba 1996-1998. HG=high-density biocontrol agent+glyphosate, HT=high-density biocontrol agent+triclopyr amine, LG=low-density biocontrol agent+glyphosate, LT=low-density biocontrol agent+triclopyr amine.

	Treatment							
	Control	**Insect alone**	**Triclopyr alone**	**Glyphosate alone**	**HG**	**LG**	**HT**	**LT**
Sampling date								
vii.1996 (Pretreatment)	**32**	**21.7**	**13.3**	**18.3**	**30.3**	**17.7**	**15.3**	**55.7**
viii.1996	22	27.3	0	17.7	33.3	18.3	0	0
ix.1996	2	4.3	0	0	0	0	0	0
vi.1997	34	51.2	0.3	6.3	13.7	12.3	2	4.3
vii.1997	55.7	37	5	21	26.3	21	3.3	5
ix.1997	10	18.7	2.3	20.7	23	13	3.7	2.7
vi.1998	10.7	12	5	27	22.3	46	8	5
viii.1998	13.3	16	2.7	21	30	54.3	9.3	6.3
ix.1998 (end of study)	**0**	**0**	**0**	**0**	**0.7**	**4.3**	**1.7**	**0.3**

Table 19: Mean number of smartweed stems/4m^2 area (standard errors omitted for clarity) all treatment cages (n=3 replicates/treatment) at Netley-Libau marsh, Manitoba 1996-1998. HG=high-density biocontrol agent+glyphosate, HT=high-density biocontrol agent+triclopyr amine, LG=low-density biocontrol agent+glyphosate, LT=low-density biocontrol agent+triclopyr amine.

	Treatment							
	Control	**Insect alone**	**Triclopyr alone**	**Glyphosate alone**	**HG**	**LG**	**HT**	**LT**
Sampling date								
vii.1996 (Pretreatment)	**19.7**	**13**	**16.7**	**13.7**	**17.7**	**20.3**	**15.7**	**40**
viii.1996	31.3	30	0.7	25.3	39.3	24.7	0.7	0
ix.1996	2.3	6.3	0.3	0	0	0	0.3	0.3
vi.1997	40	53.7	1.7	3.7	9.7	6	2.7	2.3
vii.1997	20.7	47	4	7	18	16	3.3	5
ix.1997	18.7	39.3	11.3	25	58.7	40.7	11.3	20.3
vi.1998	36.3	28.7	34.3	13.7	27.7	48.3	28.3	35.3
viii.1998	34	53	56.3	28.3	62.3	66	43.7	86.3
ix.1998 (end of study)	**5**	**14**	**16**	**7**	**31.3**	**7.3**	**19**	**24.3**

Table 20: Mean number of milkweed stems/4m^2 area (standard errors omitted for clarity) all treatment cages (n=3 replicates/treatment) at Netley-Libau marsh, Manitoba 1996-1998. HG=high-density biocontrol agent+glyphosate, HT=high-density biocontrol agent+triclopyr amine, LG=low-density biocontrol agent+glyphosate, LT=low-density biocontrol agent+triclopyr amine.

	Treatment							
	Control	**Insect alone**	**Triclopyr alone**	**Glyphosate alone**	**HG**	**LG**	**HT**	**LT**
Sampling date								
vii.1996 (Pretreatment)	**6.3**	**5**	**6**	**3.7**	**12**	**9.7**	**8**	**8.7**
viii.1996	4.3	10.3	0.7	3.3	12.7	12	1	0
ix.1996	4	7	0	0	0	0	0	0.3
vi.1997	10.3	12.3	0	2.3	0	0	0	0
vii.1997	9	11.3	2.3	1.7	4.7	4.7	5.3	0.3
ix.1997	14.3	14.3	9.3	12.3	10.3	12.7	8.7	2
vi.1998	5.7	15.3	3.7	1.3	0	4.3	2	0.3
viii.1998	5	37	6.7	3	0.7	8.7	10	1.7
ix.1998 (end of study)	**4.3**	**19**	**13.3**	**6.3**	**95.3**	**23**	**43.3**	**76.3**

Table 21: Mean number of mint stems/4m^2 area (standard errors omitted for clarity) all treatment cages (n=3 replicates/treatment) at Netley-Libau marsh, Manitoba 1996-1998. HG=high-density biocontrol agent+glyphosate, HT=high-density biocontrol agent+triclopyr amine, LG=low-density biocontrol agent+glyphosate, LT=low-density biocontrol agent+triclopyr amine.

	Treatment							
	Control	**Insect alone**	**Triclopyr alone**	**Glyphosate alone**	**HG**	**LG**	**HT**	**LT**
Sampling date								
vii.1996 (Pretreatment)	**6.7**	**0**	**11.3**	**1.7**	**0**	**0.3**	**10.7**	**0**
viii.1996	8	0.3	2.3	4.7	5	2	0	0
ix.1996	7.3	0	0	0	0	0	0	0
vi.1997	15.7	1	0	0.3	0	0	0	0
vii.1997	17	1	0.7	2	1.7	0.3	1.3	1
ix.1997	17.7	1.3	0.7	4	1.7	0.7	2.7	0
vi.1998	2	0	0	2	4.7	4	5	1
viii.1998	2	9.3	26	20.3	43.7	1	44.3	24
ix.1998 (end of study)	**1.7**	**40**	**34**	**37.7**	**80**	**51.3**	**122.7**	**78.3**

Table 22: Mean number of sedge stems/m^2 area (standard errors omitted for clarity) all treatment cages (n=3 replicates/treatment) at Netley-Libau marsh, Manitoba 1996-1998. HG=high-density biocontrol+glyphosate, HT=high-density biocontrol+triclopyr amine, LG=low-density biocontrol+glyphosate, LT=low-density biocontrol+triclopyr amine.

	Treatment							
	Control	**Insect alone**	**Triclopyr alone**	**Glyphosate alone**	**HG**	**LG**	**HT**	**LT**
Sampling date								
vii.1996 (Pretreatment)	**40.3**	**62.3**	**64.3**	**47.7**	**34.3**	**40.3**	**45.7**	**64.7**
viii.1996	49	86	16	57.7	54.7	58	24.3	18
ix.1996	32.7	66.3	12	0.7	1	5	12	8
vi.1997	37.3	53.7	0.3	0.7	2.3	0	6	1.3
vii.1997	33.7	40.3	1	0	0	0	7.7	0.3
ix.1997	32.3	41.3	3.7	0	0	0	9.7	0.3
vi.1998	33	39.3	17	0	5.3	2	42.3	4.3
viii.1998	24.3	7.3	24.3	5.7	12.7	6	56.3	13.3
ix.1998 end of study)	**17.7**	**56**	**48**	**1.3**	**31**	**12.7**	**72**	**13**

Table 23: Mean number of grass stems/m^2 area (standard errors omitted for clarity) all treatment cages (n=3 replicates/treatment) at Netley-Libau marsh, Manitoba 1996-1998. HG=high-density biocontrol+glyphosate, HT=high-density biocontrol+triclopyr amine, LG=low-density biocontrol+glyphosate, LT=low-density biocontrol+triclopyr amine.

	Treatment							
	Control	**Insect alone**	**Triclopyr alone**	**Glyphosate alone**	**HG**	**LG**	**HT**	**LT**
Sampling date								
vii.1996 (Pretreatment)	**72**	**1.3**	**29.3**	**41.7**	**64**	**170**	**12.3**	**20.3**
viii.1996	58.3	18	12.7	38.3	82	180.3	4.3	9
ix.1996	51.7	8	4.7	0	0	0	0.3	1.3
vi.1997	99.3	1	0	1	0	0	0	0.3
vii.1997	95.7	1.7	1.3	4.7	0	0	0.3	1.7
ix.1997	67.7	1.7	4.3	6.7	0	0	2	7.7
vi.1998	66	4.7	36.7	0	0	1	0.7	17
viii.1998	29	37.7	37	0	8.3	1	1.7	33.7
ix.1998 (end of study)	**25.3**	**29.7**	**38.3**	**1.7**	**5.3**	**2**	**2.3**	**52.7**

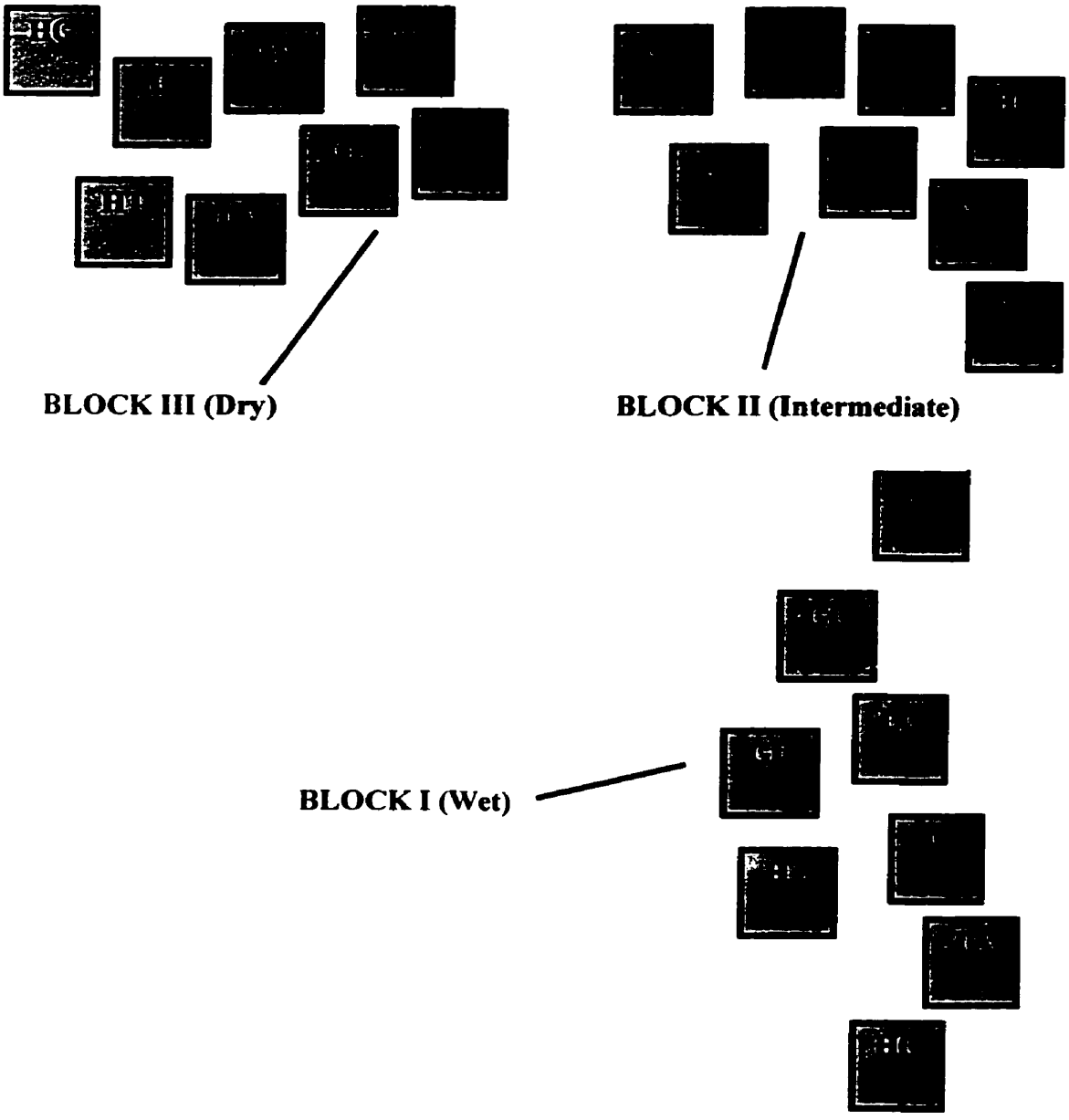

Figure 1: Experimental design layout (not to scale): IVM study area at Netley-Libau marsh, Manitoba 1996-1998. C=Control, GC=Insect alone, GL=Glyphosate alone, TA=Triclopyr amine alone, HG=High-density biocontrol agent+glyphosate, HT=High-density biocontrol agent+triclopyr amine, LG=Low-density biocontrol agent+glyphosate, LT=Low-density biocontrol agent+triclopyr amine

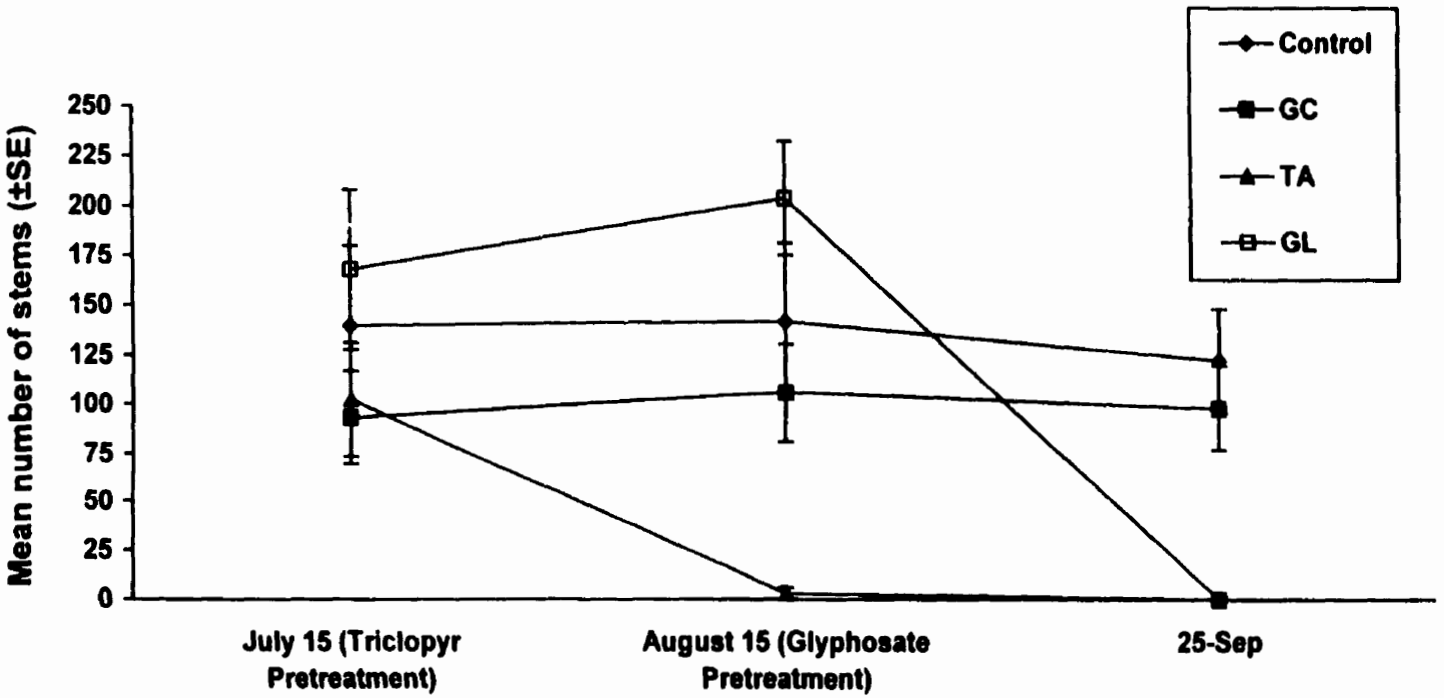

Figure 2: Mean number of purple loosestrife stems/4m² (± standard error (SE)) in the experimental plots, Netley-Libau marsh, Manitoba 1996. Plots were treated with triclopyr amine on 26 July 1996 and with glyphosate on 28 August 1996.

GC=Insect alone, TA=Triclopyr amine alone, GL=Glyphosate alone

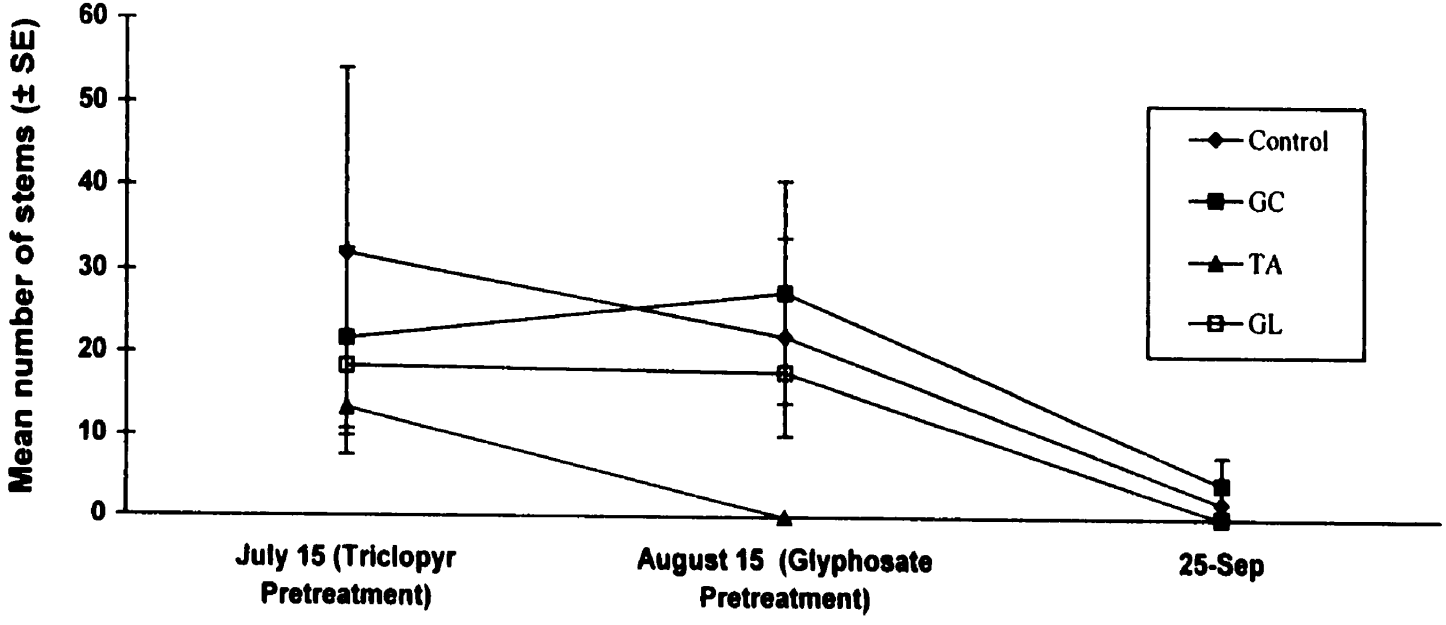

Figure 3: Mean number of tufted loosestrife stems/4m^2 (± standard error (SE)) in the experimental plots, Netley-Libau marsh, Manitoba 1996. Plots were treated with triclopyr amine on 26 July 1996 and with glyphosate on 28 August 1996.

GC=Insect alone, TA=Triclopyr amine alone, GL=Glyphosate alone

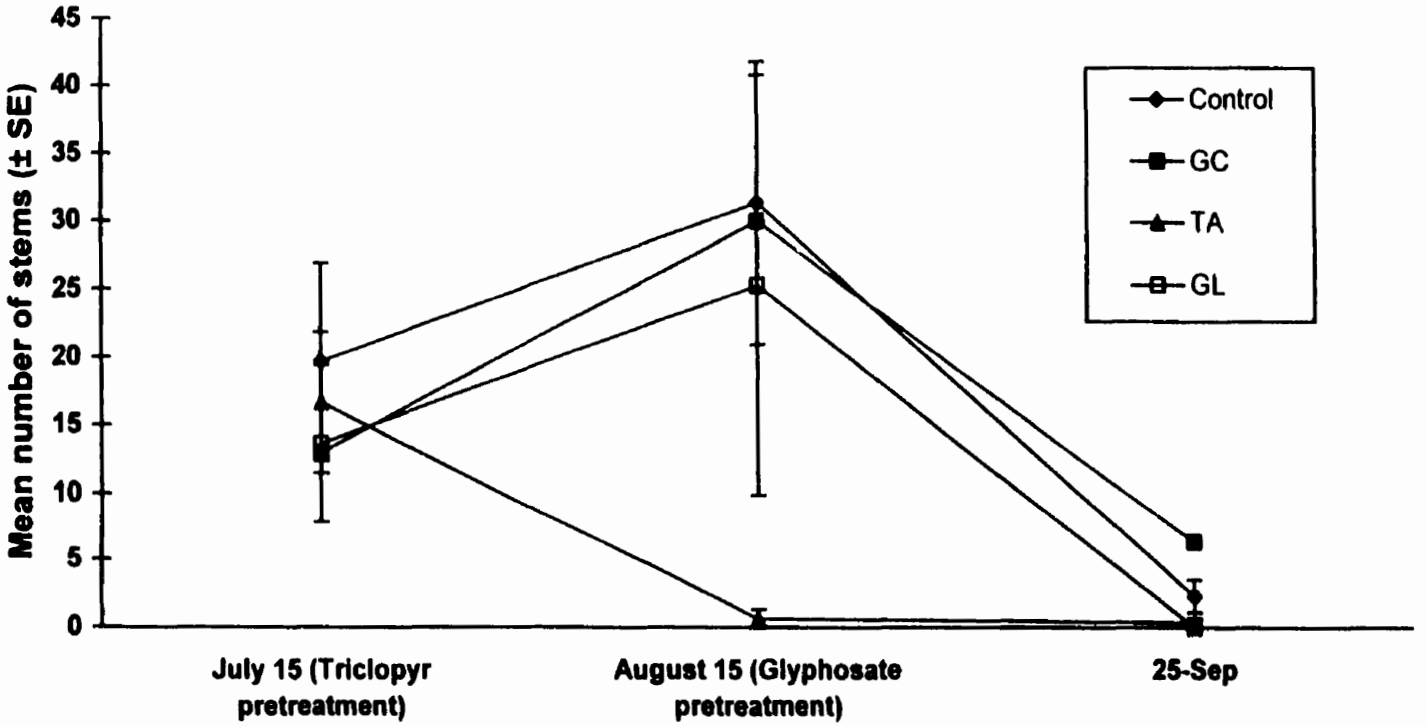

Figure 4: Mean number of smartweed stems/4m^2 (± standard error (SE)) in the experimental plots, Netley-Libau marsh, Manitoba 1996. Plots were treated with triclopyr amine on 26 July 1996 and with glyphosate on 28 August 1996.

GC=Insect alone, TA=Triclopyr amine alone, GL=Glyphosate alone

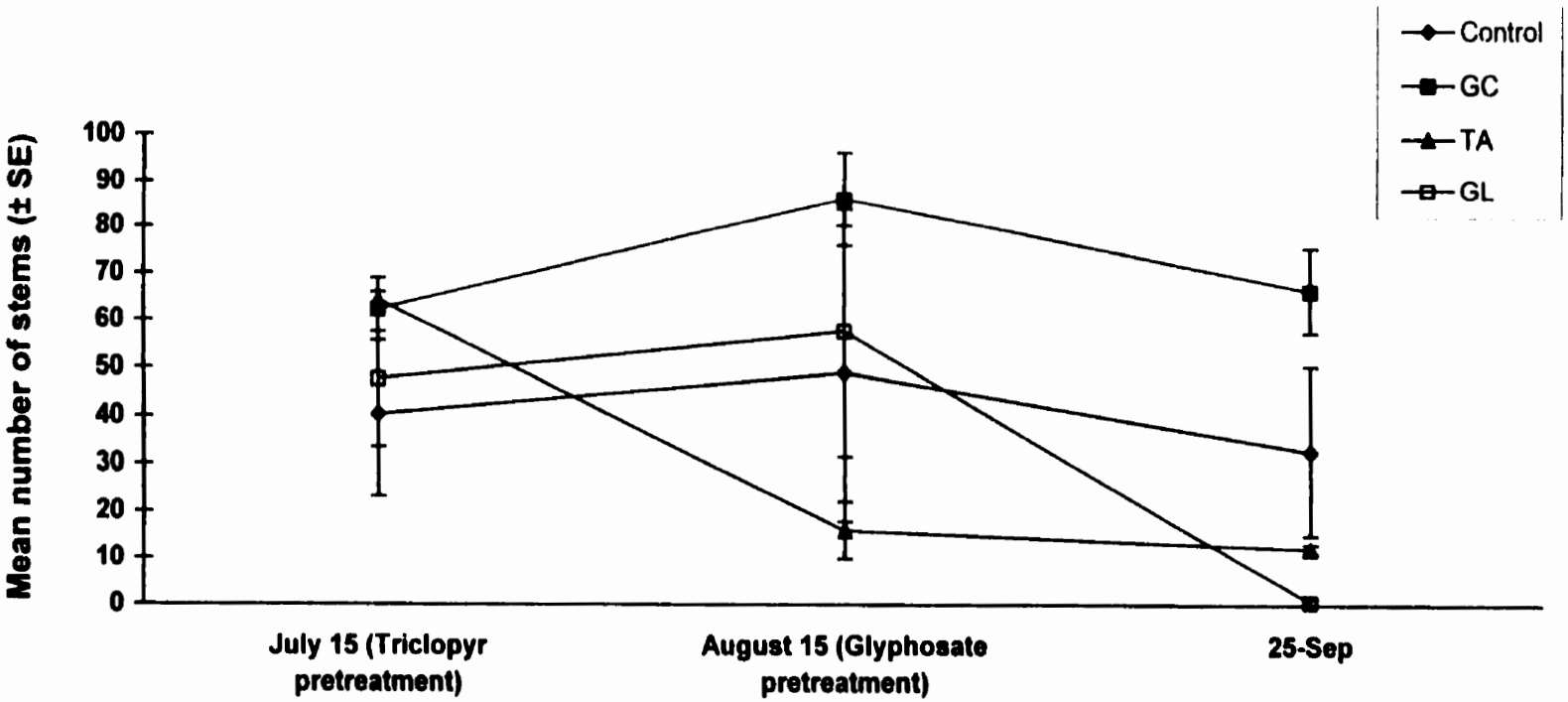

Figure 5: Mean number of sedge stems/m^2 (± standard error (SE)) in the experimental plots, Netley-Libau marsh, Manitoba, 1996. Plots were treated with triclopyr amine on 26 July 1996 and with glyphosate on 28 August 1996.

GC=Insect alone, TA=Triclopyr amine alone, GL=Glyphosate alone

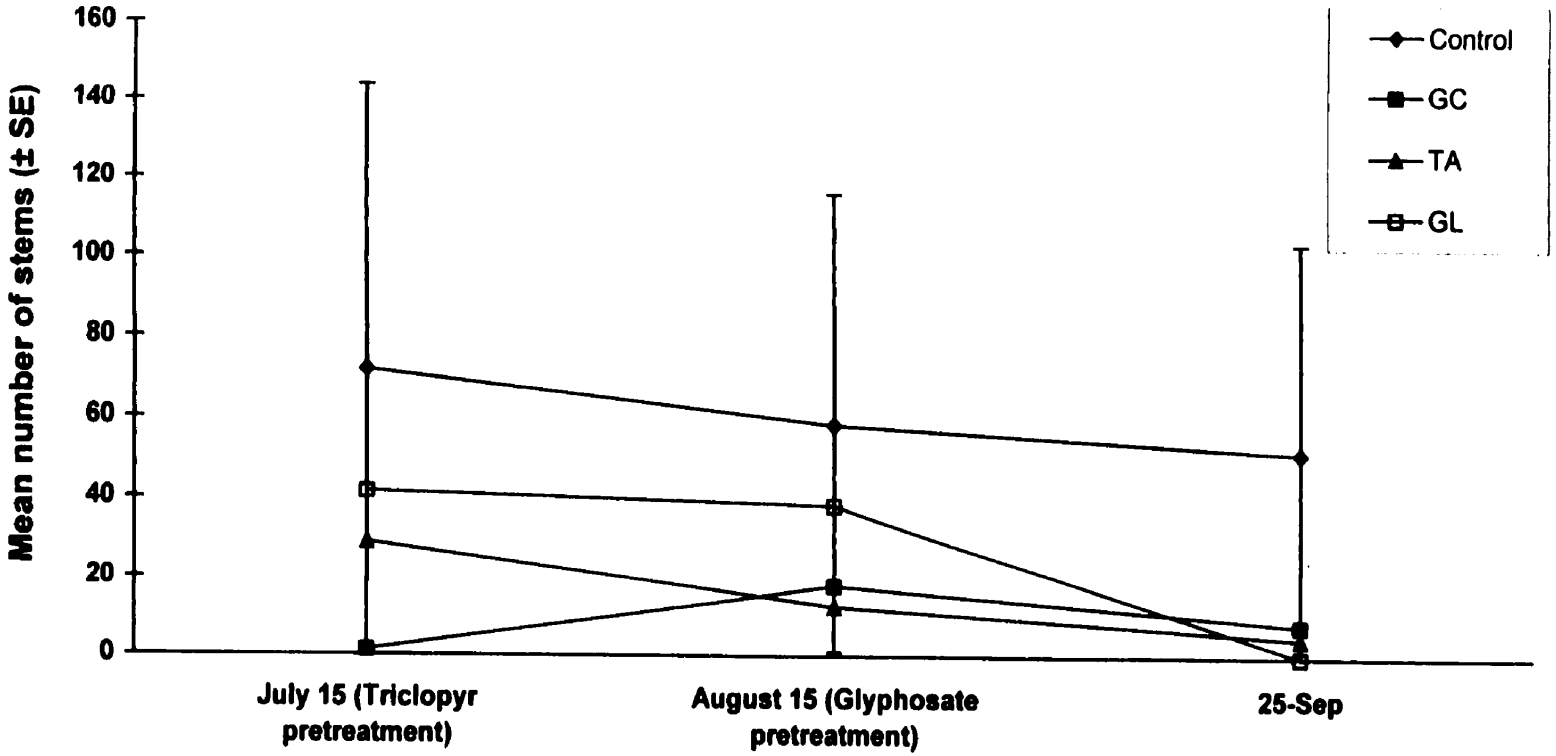

Figure 6: Mean number of grass stems/m^2 (± standard error (SE)) in the experimental plots, Netley-LibaumMarsh, Manitoba, 1996. Plots were treated with triclopyr amine on 26 July 1996 and with glyphosate on 28 August 1996.

GC=Insect alone, TA=Triclopyr amine alone, GL=Glyphosate alone

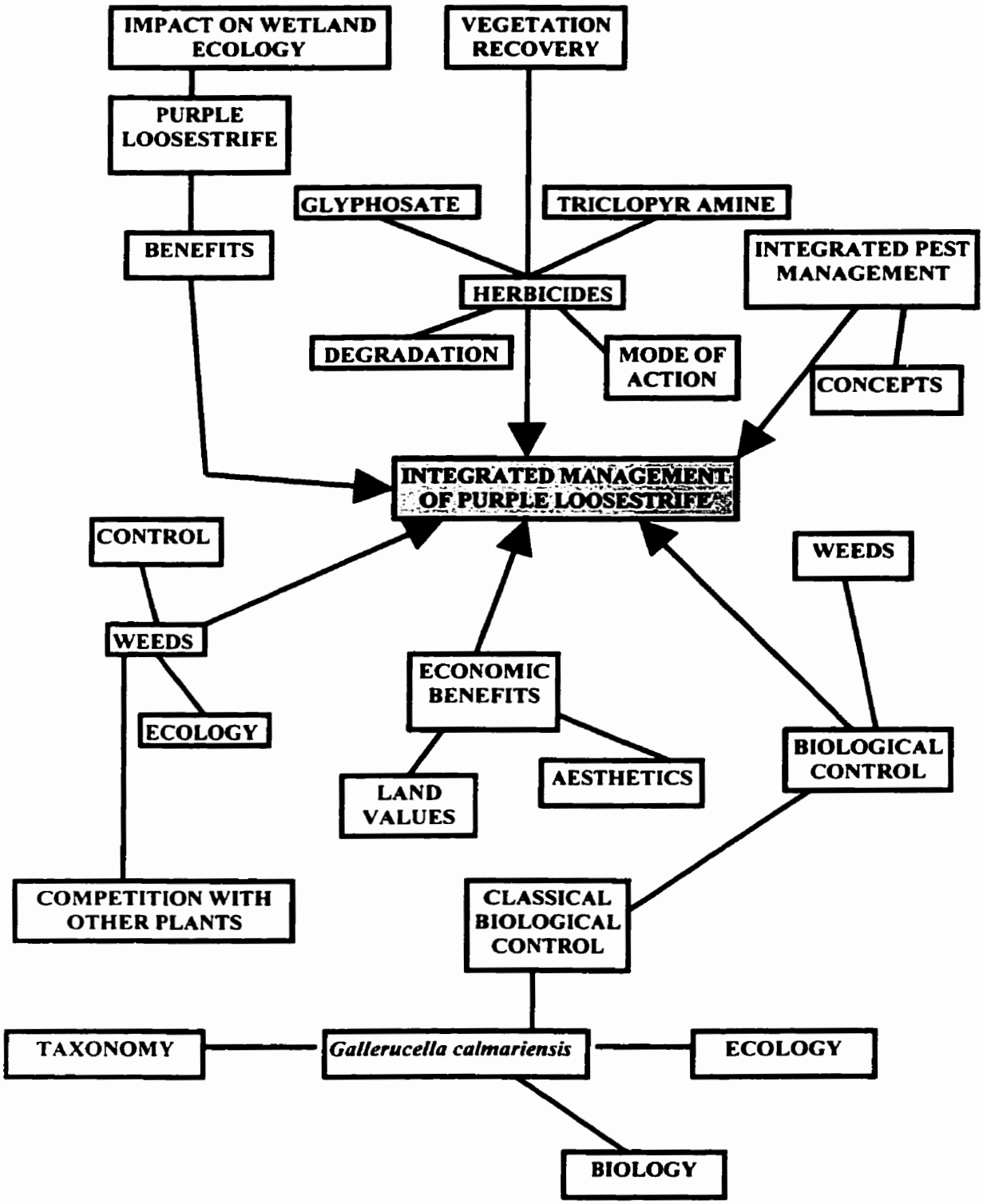

Figure 7: Flow diagram depicting the concepts and disciplines that should be considered in an integrated management program for *Lythrum salicaria* L.

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