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An integrated management strategy for the control of purple loosestrife *Lythrum salicaria* L. (Lythraceae) in the Netley-Libau Marsh, southern Manitoba

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Abstract

We evaluated the effectiveness of an integrated vegetation management strategy (IVM) for the management of purple loosestrife (*Lythrum salicaria*) by comparing the performance of herbicides alone (glyphosate and triclopyr amine), a biological control agent (*Galerucella calmariensis*) alone, and herbicides integrated with the biological control agent. The study was conducted from 1996 to 1998 within field-cages placed in a 2-ha stand of purple loosestrife located in southern Manitoba. Using a randomized complete block design, each treatment was replicated three times. Herbicides were applied in the summer of 1996 while *G. calmariensis* were released at either low-densities (12 adults) or high-densities (24 adults) in 1997. We measured performance using data on final stem heights, terminal spike length, the number of flowering spikes, and the number of seed capsules in each year and the total number of all stems at the end of each year. Integrated treatments were found to cause significant reductions in the mean number of purple loosestrife stems. The *G. calmariensis* alone treatments resulted in no significant change in mean stem densities; however, herbivory reduced mean stem heights by almost 70%. Mean stem densities of purple loosestrife increased in the herbicide alone treatments when compared to pretreatment levels. Results indicated that an IVM strategy using herbicides integrated with *G. calmariensis* outperformed herbicide alone treatments and *G. calmariensis* alone. While further open-field investigations are required, these results have important management implications.

Keywords: Integrated vegetation management; *Lythrum salicaria*; *Galerucella calmariensis*; Glyphosate; Triclopyr amine; Weed biological control

1. Introduction

Purple loosestrife (*Lythrum salicaria* L.; Lythraceae) is a Eurasian wetland perennial accidentally introduced to North America in the early 1800s (Thompson et al., 1987). This species rapidly forms monospecific stands, displacing native plant species that provide food, cover, and breeding areas for a number of wildlife species (Malecki and Rawinski, 1979). Mal et al. (1992) suggested that where purple loosestrife is increasing, wildlife species are on the decline. In Manitoba, purple loosestrife is recognized as an invasive alien plant covering an estimated 5575 ha of habitat (Lindgren, 2003a).

Management of purple loosestrife has involved four general control approaches: cultural, mechanical, chemical, and biological. Cultural and mechanical control methods have been largely unsuccessful (Malecki and Rawinski, 1979; McKeon, 1959; Rawinski, 1982). Mowing or cutting mature purple loosestrife plants has been

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shown to decrease plant vigor and retard seed production (Malecki and Rawinski, 1979), but does not destroy the perennial rootstock. Cutting can 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). Malecki et al. (1993) noted that these control methods are costly, localized, and short-term.

Numerous studies have investigated the effectiveness of chemical herbicides for purple loosestrife control (Balogh, 1986; Gabor et al., 1995; McKeon, 1959; Rawinski, 1982; Reinartz et al., 1986; Skinner and Hollenhorst, 1989; Smith, 1964). Research has examined triclopyr amine (Gabor et al., 1995) and glyphosate (Skinner and Hollenhorst, 1989) as potential herbicides for purple loosestrife control. However, it has been found that in years following herbicide applications, treated areas were dominated by purple loosestrife seedlings recruited from the seed bank. A mature purple loosestrife plant can produce an estimated 2.7 million seeds per year (Thompson et al., 1987); therefore, large seed banks exist where purple loosestrife is well established (Welling and Becker, 1990). Thus, an effective method of controlling purple loosestrife seedling emergence after a herbicide application is required.

Since 1992, control efforts have focused on the introduction and establishment of classical biological control agents for purple loosestrife (Blossey and Schat, 1997; Diehl, 1999; Landis et al., 2003; Lindgren, 2003b; McAvoy et al., 1997, 2002). Biological control programs against purple loosestrife began across North America with releases approved by the USDA-APHIS and by the Plant Protection Division of Food, Production, and Inspection Branch of Agriculture Canada (Blossey et al., 1994; Hight et al., 1995). Biological control agents approved for release in Canada include two phytophagous beetles, *Galerucella calmariensis* L. and *Galerucella pusilla* Duftschmidt (Coleoptera: Chrysomelidae), a root-mining weevil *Hylobius transversovittatus* (Goeze) (Coleoptera: Curculionidae), and flower- and seed-feeding weevils *Nanophyes marmoratus* (Goeze) and *Neolamprologus brevis* (Boheman) (Coleoptera: Curculionidae), (Lindgren et al., 2002).

Herbicides and biological control have been the most widely used management strategies against purple loosestrife in North America. Classical biological control may represent a long-term solution as it may take several years for agents to reach population densities sufficient to control established stands of purple loosestrife. Conversely, herbicidal control strategies may provide immediate control, but may be costly and require repeated applications (Skinner et al., 1994). A management solution may be in the integration of these two approaches. An integration of herbicides with biological control may provide immediate as well as long-term control of purple loosestrife. There is a need to evaluate the potential benefits derived from combining herbicide and biological control strategies for purple loosestrife management in North America.

The objective of this study was to evaluate an integrated vegetation management (IVM) strategy for the management of purple loosestrife by comparing the results of IVM treatments (the biological control agent *G. calmariensis* combined with either glyphosate or triclopyr amine) against single herbicide and *G. calmariensis* treatments. We hypothesized that IVM treatments would result in a greater suppression of purple loosestrife stems.

2. Materials and methods

2.1. Study area

The study area was the Netley-Libau Marsh, located in the delta region of the south basin of Lake Winnipeg (50°20′73″N, 96°41′29″W). Situated approximately 65 km north of Winnipeg, Manitoba, it is 24,381 ha of upland and wetland habitats. This marsh is a complex of lakes and channels whose water levels are influenced by Lake Winnipeg water regulation. The Netley-Libau Marsh is a candidate Manitoba Heritage Marsh and a Canadian Important Bird Area. The invasion of purple loosestrife into the marsh has been documented in several vegetation surveys. A vegetation survey carried out in 1936 reported no purple loosestrife in the marsh (Hinks and Fryer, 1936). In 1944, purple loosestrife was discovered upstream of the marsh near Lockport (on the Red River) and by the 1970s it was a plant of common occurrence throughout the marsh. Surveys conducted in the early 1990s have indicated that the Netley-Libau Marsh contained 26% of all purple loosestrife in Manitoba (Lindgren, 2003a).

2.2. Biological control agent

We selected the biological control agent *G. calmariensis*, as it is easily reared, has high reproductive potential, has established and over-wintered successfully in Manitoba, and controlled studies have determined it to be compatible with the herbicides triclopyr amine and glyphosate (Lindgren et al., 1998, 1999). Blossey et al. (1994) has described the life history of *G. calmariensis*. Adult beetles emerge from winter diapause in late spring, feeding on young leaves and meristematic tissues of purple loosestrife. Females begin oviposition 7–10 days after emergence/feeding. Young-instar larvae are found on the host plant's shoot tips while older instars feed on all plant tissue. Mature larvae pupate in the soil and emerge as teneral adults in 7–10 days. New-generation beetles may have a short oviposition period prior to entering the soil to overwinter. In Manitoba, adult beetles emerge from

winter diapause in late May through early June, oviposition begins in early June, and peak larval densities are found in late June through early July (Lindgren, 2000).

2.3. Lumite field cages

Trials were conducted inside walk-in screen cages to reduce variability potentially arising from dispersal and predation of *G. calmariensis*. Each screen cage was 8 m^3 and covered with screening material (Lumite, style 50090000, 20 × 20 mesh, 15% shade, 1629 cfm porosity; Synthetic Industries Performance Fabrics Division, 2100A, P.O. Box 977, Atlanta Highway, Gainesville, GA) on all sides plus the top, with a zipper on one end to enable access to the interior of the cages. Cage frames were constructed using 6 cm × 6 cm kiln-dried spruce wood. Frames were reinforced with wooden cross-braces from corner to corner on all four sides to prevent wind-induced lateral movement. The cage frames were anchored to the ground using 60 cm long by 10 mm diameter steel rods. Lumite 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. Lumite screening was placed over wooden frames in early May of each year prior to purple loosestrife shoot emergence and removed in early October of each year to allow snowfall accumulation on the research plots, thereby providing adequate thermal insulation for the overwintering beetles.

2.4. Sampling design

Twenty-four Lumite field cages were set out in the study area in three blocks of eight cages. Treatments (described below) were assigned within each block into a randomized complete block design to account for any variance within the study area. Each treatment was replicated three times. In treatments with herbicides, herbicides were applied using a 15 L, pump-action, backpack sprayer. All vegetation in herbicide-treated cages was sprayed-to-wetness. The following treatments were employed.

2.4.1. Herbicides alone

Triclopyr amine, a broadleaf-specific herbicide, 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. Glyphosate [*N*-(phosphonomethyl) glycine], a nonselective herbicide, was applied 28 August 1996 at a rate of 2% (V/V) during the late bloom stage of purple loosestrife.

2.4.2. Galerucella calmariensis alone

Adult *G. calmariensis* were collected and translocated from established populations in the Delta and Netley-Libau Marshes as well as from populations established in Minnesota, USA. Adult beetles were sexed (Manguin et al., 1993), sorted, and counted in the laboratory prior release in field cages. Six pairs of adults ($n = 12$) were released into the insect alone treatment cages on 18 June 1997.

2.4.3. Herbicides integrated with high densities of G. calmariensis

Triclopyr amine and glyphosate were applied as in the herbicide alone trials. Twelve pairs ($n = 24$) of adult *G. calmariensis* were released into each of the three triclopyr-amine-treated and three glyphosate-treated cages on 18 June 1997. In all integrated treatments, four potted purple loosestrife plants were placed in each cage the year following the herbicide application to sustain adult *G. calmariensis* until purple loosestrife seeds germinated at which time the potted plants were removed from the cages.

2.4.4. Herbicides integrated with low densities G. calmariensis

Triclopyr amine and glyphosate were applied as in the above herbicide alone trials. Six pairs of adult *G. calmariensis* ($n = 12$) were released into three triclopyr-amine-treated cages and three glyphosate-treated cages on 18 June 1997. This density is equivalent to the *G. calmariensis* alone density.

2.4.5. Control

Control Lumite cages received no herbicides or *G. calmariensis* introductions.

2.4.6. Sampling purple loosestrife performance

Within each treatment cage, 15 purple loosestrife stems ($n = 45$ stems/treatment) were randomly selected, tagged at the base of the plant with orange flagging tape, and numbered for identification. Stems were selected as the sampling unit as it is difficult to define an individual purple loosestrife plant without digging up the root system. Data on stem heights, main spike length (the flowering spike forming the apex of the plant), the number of flowering spikes, and seed capsules were recorded. In 1996, data were collected on 31 July (prior to herbicide applications), 14 August, and 9–10 September. In 1997, data were collected on 26 June, 29 July, and 15–19 September. In 1998, data were collected on 9 July, 28 August, and 30 September.

2.5. Data analysis

Analyses of variance (ANOVA) were performed on the data using JMP IN 3.1 for Windows (Sall and Lehman, 1996). Differences between treatment means were analyzed using ANOVA (F and t tests) and Tukey–Kramer Honest Significant Difference (HSD) test. Tests were considered significant at $\alpha = 0.05$ level of probability.

Stem density data were transformed [$\log_{10}(X+1)$] to reduce the heterogeneity of treatment variances (Zar, 1974).

3. Results and discussion

3.1. Purple loosestrife stem densities and height

By September 1997 (the year following herbicide application), the mean number of purple loosestrife stems in the integrated treatments had increased as much as seven times vs. pretreatment levels. A similar increase in stem densities occurred in the herbicide alone treatments. As a result of observed beetle herbivory, purple loosestrife stem densities in the integrated treatments were significantly lower when compared to the herbicide alone treatments and the control [Table 1, Tukey–Kramer HSD: 1997 $df = 7,16$ ($F = 1.5$); 1998 $df = 7,16$ ($F = 13.1$)] by September 1998. All purple loosestrife stems in the integrated treatments with high density of *G. calmariensis* were totally defoliated and considered dead, while only a few stems (mean = 9.0) survived in the integrated treatments that received low densities of *G. calmariensis* (Fig. 1).

All purple loosestrife stems in the herbicide alone treatments were eliminated after herbicide applications in 1996 (Fig. 2). Rawinski (1982), Balogh (1986), and Skinner and Hollenhorst (1989) similarly reported that glyphosate applications controlled the aboveground portion of purple loosestrife while Gabor et al. (1995) found that triclopyr amine applied to purple loosestrife in a wetland in southern Ontario also eliminated all purple loosestrife stems. However, by September 1998 purple loosestrife stem densities were almost nine times higher in the triclopyr amine alone treatment and 4.5 times higher in the glyphosate alone treatment than pretreatment levels. These results indicate that one application of either herbicide will not provide long-term control of purple loosestrife and will in fact, increase the number of purple loosestrife stems within two growing seasons.

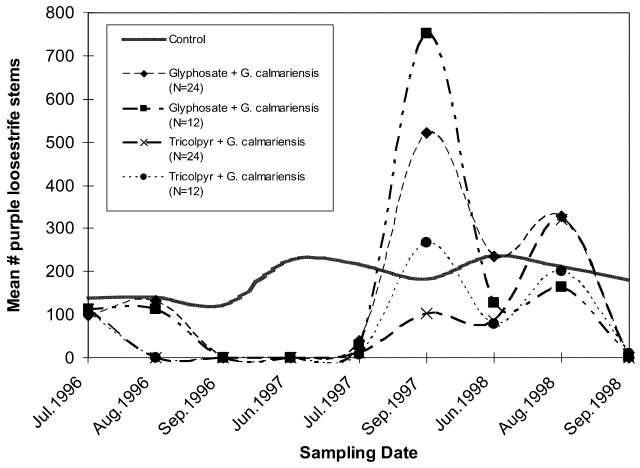

Fig. 1. A time-series graph showing mean purple loosestrife stem densities in response to treatments: 1996–1998 at Netley-Libau Marsh, Manitoba. Treatments where herbicides were integrated with *G. calmariensis* are shown, compared with the untreated control. Herbicides were applied in 1996 and *G. calmariensis* were released into cages in 1997.

By September 1997 and 1998, mean stem heights of purple loosestrife in all treatments were significantly lower when compared to the control [Table 1, Tukey–Kramer HSD: 1997 $df = 8,396$ ($F = 427.7$); 1998 $df = 7,352$ ($F = 57.3$)]. Mean stem heights significantly increased in the herbicide alone treatments and in the integrated treatments from 1997 to 1998. By September 1998, the low-density integrated treatments had significantly lower mean stem heights than the herbicide alone treatments.

The biological control agent *G. calmariensis* established in all treatments where released in this study. It was not the intent of this study to measure *G. calmariensis*

Table 1
Effects of treatments on purple loosestrife morphometry[a]

	Stem height		No. inflorescences/tagged stem		No. of seed capsules on main flowering spike		No. of stems	
	1997	1998	1997	1998	1997	1998	1997	1998
Single treatments								
Control	176.1 ± 3.7 a	183.2 ± 4.3 a	4.4 ± 0.4 b	6.1 ± 0.7 a	298.8 ± 19.0 a	253.5 ± 15.8 a	184.7 ± 37.5 a	181.3 ± 43.2 a
G. calmariensis	145.5 ± 4.5 b	41.6 ± 3.3 e	7.3 ± 1.4 a	0.0 ± 0.0 c	191.7 ± 27.9 b	0.0 ± 0.0 d	125.0 ± 20.6 a	133.3 ± 120.6 ab
Glyphosate	38.0 ± 4.6 c	120.3 ± 8.2 b	1.2 ± 0.6 c	5.0 ± 0.9 ab	206.0 ± 62.4 a	159.2 ± 21.9 b	451.3 ± 266.9 a	771.3 ± 279.8 a
Triclopyr amine	34.6 ± 3.3 c	100.7 ± 9.2 bc	0.4 ± 0.3 c	6.4 ± 2.6 a	108.0 ± 88.3 a	57.2 ± 14.3 c	93.3 ± 40.4 a	897.7 ± 82.0 a
Integrated treatments								
24 *G. cal* & Glyp[b]	18.4 ± 1.4 d	87.1 ± 4.9 cd	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 c	0.0 ± 0.0 d	522.0 ± 374.7 a	0.0 ± 0.0 b
24 *G. cal* & Tric	27.7 ± 2.0 d	69.9 ± 4.1 cd	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 c	0.0 ± 0.0 d	103.3 ± 43.3 a	0.0 ± 0.0 b
12 *G. cal* & Glyp	28.9 ± 2.5 d	59.5 ± 5.1 de	0.0 ± 0.0 d	0.1 ± 0.1 d	0.0 ± 0.0 c	1.2 ± 1.2 d	750.0 ± 372.3 a	2.7 ± 2.7 b
12 *G. cal* & Tric	24.2 ± 1.5 d	61.8 ± 5.9 de	0.0 ± 0.0 d	1.1 ± 0.8 bc	0.0 ± 0.0 c	5.4 ± 3.3 d	267 ± 152.1 a	9.0 ± 9.0 b

[a] Data are mean values followed by standard error. Represents data collected in September of each year except mean stem height, which is highest seasonal value. Means followed by the same letter are not significantly different at $\alpha = 0.05$.

[b] *Abbreviations: cal*, *calmariensis*; Glyp, glyphosate; and Tric, triclopyr amine.

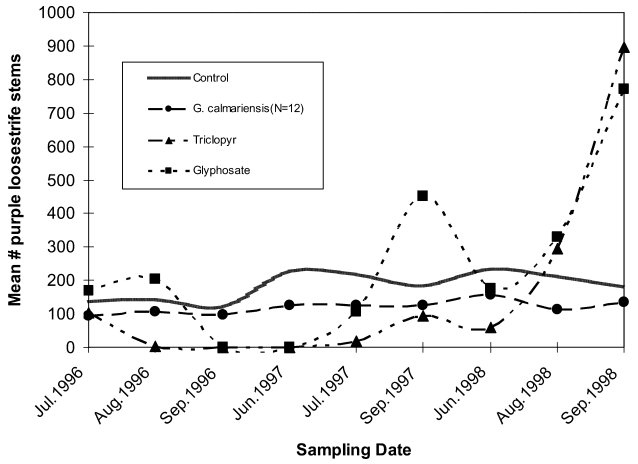

Fig. 2. A time-series graph showing mean purple loosestrife stem densities in response to treatments: 1996–1998 at Netley-Libau Marsh, Manitoba. Treatments where herbicides were used alone are shown, compared with the untreated control. Herbicides were applied in 1996 and *G. calmariensis* were released into cages in 1997.

establishment but to measure the impact of the selected management strategies. In the *G. calmariensis* alone treatments, mean stem heights were reduced by as much as 70% (Table 1) by beetle herbivory. Recent studies have indicated that *G. calmariensis* alone may provide measurable control of purple loosestrife. Lindgren (2003b) reported that within four years *G. calmariensis* herbivory reduced stem heights by 30% and eliminated all flowering stems, and that within six years all purple loosestrife stems were eliminated. Landis et al. (2003) also reported 3–5 years were required until *Galerucella* caused reductions in plant height or flowering. In the Delta Marsh, Diehl (1999) documented complete control of purple loosestrife three years after *Galerucella* release. Following the general *L. salicaria–G. calmariensis* interaction model presented by Lindgren (2000), we speculate that if our study were to continue, *G. calmariensis* would have also significantly reduced, if not eliminated, all purple loosestrife.

3.2. Purple loosestrife inflorescences

The mean number of inflorescences produced by tagged purple loosestrife plants in the *G. calmariensis* alone treatment was significantly higher than in all other treatments by September 1997 [Table 1, Tukey–Kramer HSD: 1997 $df = 8,219$ ($F = 17.09$); 1998 $df = 7,352$ ($F = 8.2$)]. By September 1997 and 1998, main inflorescences in the *G. calmariensis* alone treatment produced significantly fewer seed capsules when compared to the control treatments [Table 1, Tukey–Kramer HSD: 1997 $df = 8,109$ ($F = 4.6$); 1998 $df = 7,352$ ($F = 77.8$)]. By September 1998, the mean number of seed capsules found on the main inflorescences in the herbicide alone and the control treatments were significantly higher than in the integrated treatments.

Larvae feeding on developing flowers were commonly observed. In our study, purple loosestrife stems that responded to herbivory by *G. calmariensis* larvae produced more lateral branches resulting in 'bushy' plants with many, smaller flowering spikes. Crawley (1989) and Cooper (1996) reported that insect herbivory affects flower production by destroying flower buds and indirectly through other types of damage such as defoliation. Cooper (1996) also found that purple loosestrife responded to *G. calmariensis* herbivory by producing stems that were shorter with many short lateral stems.

3.3. Purple loosestrife seedlings

In August 1998, we found high densities (i.e., >200/0.01 m^2) of purple loosestrife seedlings present inside the herbicide alone and all integrated-treatment cages. This observation agrees with those by Gabor et al. (1995), Nelson et al. (1996), and Skinner and Hollenhorst (1989) who also found that herbicide treated areas are later dominated by purple loosestrife seedlings. Purple loosestrife seeds in the herbicide alone treatments germinated and produced flowering spikes in 1997 (one year after herbicide application) and in 1998. Shamsi and Whitehead (1977) also reported that purple loosestrife seedlings are able to flower and set seeds in the first year. *G. calmariensis* adults and larvae were observed feeding and ovipositing on emerged purple loosestrife seedlings indicating that seedlings emerging after a herbicide treatment were capable of supporting the biological control agent.

3.4. An integrated vegetation management strategy

Herbicides have been successfully combined with biological control agents in several studies. Lym et al. (1996) and Lym (1998) found that by integrating the biological control agents *Aphthona* spp. (Coleoptera: Chrysomelidae) and/or *Spurgia esulae* Gagne (Diptera: Cecidiomyidae) with herbicides, greater reductions in leafy spurge densities were achieved as opposed to using either control method alone. In this case, an integrated weed control program combining two or more methods provided a more successful and cost-effective long-term solution to the leafy spurge problem than a single method used alone (Lym et al., 1996). Paynter and Flanagan (2004) also achieved better control of mimosa in Australia by integrating biological control with other methods, including herbicides, than using any methods alone.

The results of our field study indicated that over a three-year period, an IVM strategy outperformed a single herbicide application and a single release of *G. calmariensis*. We suggest the following general IVM approach for the management of purple loosestrife. When purple loosestrife has formed a monodominant stand devoid of native vegetation, we suggest using the

broad-spectrum herbicide glyphosate. Glyphosate does pose a risk to nontarget vegetation (Rawinski, 1982) and would not be the herbicide of choice in sensitive areas such as wetlands, however, may be used when purple loosestrife has invaded habitats such as along railway lines. When desirable native vegetation is present, we suggest using the broadleaf-specific herbicide triclopyr amine. A broadleaf-specific herbicide has management advantages as Gabor et al. (1996) found that after an application of triclopyr amine, native monocotyledonous plant species increased and that these suppressed the rate of purple loosestrife reestablishment. In either case, managers can expect purple loosestrife seedlings to emerge from the seed bank in the following year or even within the year of herbicide application. These seedlings will become host plants for the biological control agent which can be either released in the following year (as done in our study) or in the year of herbicide application. If a small area of purple loosestrife is left untreated (preferably in the center of the infestation), *G. calmariensis* may be released in the same year as the herbicide application. The biological control agents should then establish and eliminate the need for further herbicide applications.

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