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Compatibility of Glyphosate with *Galerucella calmariensis;* a Biological Control Agent for Purple Loosestrife (*Lythrum salicaria*)

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ABSTRACT

By integrating *Galerucella calmariensis* with glyphosate there is potential to achieve both immediate and sustained control of purple loosestrife (*Lythrum salicaria*). The objective of this study was to determine the compatibility of glyphosate on the oviposition and survival of adult *G. calmariensis* and on the ability of *G. calmariensis* third instar larvae to pupate to teneral adults. Our results revealed glyphosate (formulated as Roundup®) at a concentration of 2% (2.43 L/acre) and 4% solution (4.86 L/acre) had no impact on the ability of *G. calmariensis* third instar larvae to pupate to new generation adults. To examine the effect of a 2% solution of glyphosate on adult *G. calmariensis* oviposition and survival, adults were randomly divided between a direct contact group (adults sprayed directly), an indirect contact group (host plants with adults were sprayed), and a control group. Our results revealed that glyphosate does not impact *G. calmariensis* oviposition or adult survival. The results of this study indicate that *G. calmariensis* is compatible with glyphosate indicating that further field studies examining integrated control strategies for purple loosestrife are warranted.

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INTRODUCTION

The negative impact of exotic species has attributed to the decline of 42% of threatened or endangered species in the United States causing an estimated $97 billion in direct economic losses (Stein and Flack 1996). Purple loosestrife (*Lythrum salicaria* L., Lythraceae) is a Eurasian wetland perennial introduced into North America in the early 1800s (Thompson et al. 1987). It is an aggressive plant that quickly forms monospecific stands thereby displacing native vegetation that provide food, cover, and breeding areas for wildlife. Purple loosestrife degrades natural habitats such as wetlands and riparian areas reducing overall ecosystem biological diversity and threatening endangered species. Cultivated varieties of purple loosestrife widely used by gardeners and landscapers across North America further contribute to the spread of purple loosestrife (Lindgren and Clay 1993). Subsequently, purple loosestrife has been elevated to noxious weed status in a number of Canadian provinces and in several states in the U.S.

Biological control has been identified as a potential long-term management strategy for the control of purple loosestrife in North America (Malecki et al. 1993, Hight et al. 1995). While a classical biological weed control strategy may potentially provide long-term, sustainable control of purple loosestrife, it may also take several years before an agent has an impact on an established population of purple loosestrife. The aggressive nature of purple loosestrife in concert with its prolific reproductive abilities may not allow resource managers to wait several years for measurable results from a classical biological control strategy. Economic and environmental losses between introduction of biological control agents and the suppression of target weed populations may necessitate research toward integrating control strategies (Kok and Kok 1982). Integrating classical biological weed control with herbicide applications may have potential to achieve both immediate as well as long-term sustainable control of purple loosestrife.

However, herbicidal control strategies can be costly and require long-term application (Skinner et al. 1994). Researchers have found that in the years following a herbicide application, treated areas were dominated by purple loosestrife seedlings (Skinner et al. 1994, Gabor et al. 1996). A long-term herbicide control strategy is suggested to control established purple loosestrife plants, seedlings, as well as second and third generation seedlings[4]. Numerous applications of a herbicide to control purple loosestrife within sensitive natural areas such as wetland ecosystems may not be a desired management strategy.

Galerucella calmariensis L. (Coleoptera: Chrysomelidae) is a host specific phytophagous beetle initially released in North America in 1992 as a biological control agent against purple loosestrife (Hight et al. 1995). *Galerucella calmariensis* has established at a number of sites across North America and adapted to local plant phenology (Hight et al. 1995). European studies reveal that when population levels reach high densities *G. calmariensis* is capable of defoliating whole stands of purple loosestrife (Blossey and Schroeder 1991). Life-history studies (Blossey et al. 1994) suggest that *G. calmariensis* may be compatible with glyphosate, providing an ideal candidate for inclusion in an integrated vegetation management (IVM) strategy for purple loosestrife.

In addition to *G. calmariensis*, *G. pusilla* Duftschmid (Coleoptera: Chrysomelidae) a leaf-eating beetle; *Hylobius transversovittatus* Goeze (Coleoptera: Curculionidae), a root-mining weevil that attacks the main storage tissues of loosestrife; and *Nanophyes marmoratus* Goeze, a flower-feeding beetle capable of reducing seed production (Malecki et al. 1993, Hight et al. 1995) have been released in North America against purple loosestrife. *Hylobius transversovittatus* and *N. marmoratus* may not be compatible with a herbicide control strategy in that root systems and flowers of established plants would be destroyed leaving no host plant material available to sustain insect populations. Blossey (1995) reports that *G. pusilla* has a similar life-history and occupies a similar ecological niche to that of *G. calmariensis*. While our study focused on *G. calmariensis*, similarities between the two *Galerucella* species suggest that *G. pusilla* may also be compatible with a herbicide strategy.

Numerous studies have investigated the integration and/or compatibility of herbicide and biological control strategies (Trumble and Kok 1979, 1980, Haag 1986, Story et al. 1988). Leafy spurge (*Euphorbia esula* Gagne) is similar to purple loosestrife in that it is an aggressive exotic weed species that displaces native vegetation. Lym and Carlson (1994), found the gall midge *Spurgia esulae* compatible with 2,4-D (2,4-dichlorophenoxyacetic acid) and picloram as long as 15 to 25% of the leafy spurge population was left untreated to sustain biological control agents. Trumble and Kok (1980) found that the herbicide 2,4-D can be used in an integrated control strategy with the weevil *Rhinocyllus conicus* Froelich for the control of *Carduus* thistles. Lindgren et al. (1998) concluded that *G. calmariensis* is compatible with the dicot selective herbicide triclopyr amine([(3,4,6-trichloro-2-pyridinyl) oxy] acetic acid]). Research on the impacts of glyphosate (N-(phosphonometryl) glycine) on terrestrial invertebrates by Burst (1990) indicated glyphosate had no direct acute or chronic toxic effect on five species of carabid beetles. Yokoyama and Pritchard (1984) found that glyphosate did not impact the western bigeyed bug *Geocortis pallens* Stal. and that exposure to glyphosate resulted in females ovipositing more viable eggs. No information is available regarding the compatibility of *G. calmariensis* and glyphosate for purple loosestrife control.

The recommended glyphosate application window for purple loosestrife control is at or beyond bloom stage, applied using a spray-to-wet technique[5]. In southern Manitoba, purple loosestrife begins to bloom in mid-July and initiates seed production in mid-August. Seed production should mark the end of the glyphosate application window. Within the glyphosate application window, *G. calmariensis* may be dominantly present as either late instar larvae or ovi-

[4]Monsanto Company. 1997. Label: Roundup liquid herbicide by Monsanto. Monsanto Canada, Inc. Mississauga, Ontario.

[5]Monsanto Company. 1997. Label: Roundup liquid herbicide by Monsanto. Monsanto Canada, Inc. Mississauga, Ontario.

positing adults (all life stages will be found but at lower densities). Therefore, late instar larvae and ovipositing adults could be impacted by a glyphosate application in southern Manitoba.

Classical biological control and herbicides represent the most promising management techniques currently available for the control of purple loosestrife in North America. Integrating a biological weed control strategy with a herbicide weed control strategy may not only provide effective weed management (DeLoach 1991), but accelerate purple loosestrife management efforts in North America. The objective of this study was to determine the compatibility of glyphosate on the oviposition of adult *G. calmariensis* and on the ability of *G. calmariensis* third instar larvae to successfully emerge as new generation adults.

MATERIALS AND METHODS

Insects. Adult *G. calmariensis* were obtained from Cornell University, Department of Natural Resources, Ithaca, New York. To calibrate insect phenology within the glyphosate application window, *G. calmariensis* used in this study were near the end of their oviposition period. Third instar larvae used in this study were obtained from an outdoor breeding colony maintained by the Manitoba Purple Loosestrife Project in Winnipeg, Manitoba.

Herbicide. Glyphosate (N-(phosphonomethyl) glycine) herbicides have been used to provide effective control of purple loosestrife (Rawinski 1982, Malecki and Rawinski 1985, Balogh 1986). Glyphosate is a non-selective, broad spectrum, post emergent herbicide with systemic activity in plants. Roundup® is a glyphosate formulation registered for terrestrial plant control[6] and is not registered for direct application to bodies of water due to the ionic surfactant present in the formulation (Balogh 1986). Roundup® is registered for the terrestrial control of purple loosestrife in Canada at a recommended volume application of 2% solution (2.43 L/acre).

Experiment 1: Survival and Pupation of *G. calmariensis* Third Instar Larvae. Sixty *G. calmariensis* third instar larvae were randomly divided between a control group and a glyphosate exposure group. Third instar larvae were selected for this experiment for they would most likely be present in the field during the recommended glyphosate application window. Each treatment was replicated three times (n = 10 larvae/treatment) within a greenhouse setting. Within the greenhouse, larvae were exposed to natural photoperiods and ambient greenhouse temperatures.

Two separate studies were conducted. On 4 July 1996 the study was conducted with a 2% Roundup® solution (2.43 L/acre) and on 22 July 1996 the study was repeated with a 4% Roundup® solution (4.86 L/acre). A hand-held Continental E-Z sprayer (625 ml; Continental Industries, Brampton, Ontario) was used to expose *G. calmariensis* third instar larvae to Roundup® herbicide. The sprayer was set to a fine mist and larvae were sprayed-to-wet. Using an artist's paintbrush (size 00), the larvae were immediately removed from the petri dish and placed into pupation chambers on purple loosestrife shoots (in floral pics containing water). Control group larvae were placed into pupation chambers untreated. Purple loosestrife shoots were watered as necessary in order to ensure a fresh food supply for the larvae and emergent teneral adults.

Pupation chambers consisted of Rubbermaid Clearboxes (18.5 L; 40.6 by 27.9 by 22.9 cm; Rubbermaid Canada Inc., Mississauga, Ontario) with a section of the lid (28 by 15 cm) cut away and a mesh screening attached with silicone to provide ventilation. The bottom of the pupation chambers contained a layer of approximately 5 cm of moistened Sunshine Mix peat moss as pupation substrate. The pupation chambers were checked daily for newly emerged adults. As new generation adults were found they were removed from the pupation chamber. Similar methods were previously used to test the compatibility of triclopyr amine and *G. calmariensis* (Lindgren et al. 1998).

Experiment 2: Survival and Oviposition of *G. calmariensis* Adults. Adult *G. calmariensis* were randomly divided into three treatments, (1) a direct contact group (2) an indirect contact group and (3) a control group. Each treatment was replicated three times, containing 20 adults (ca. 1:1 male female ratio) each. On 31 July 1996, adult *G. calmariensis* were sprayed-to-wet with a 2% solution of glyphosate. Adults were placed into outdoor oviposition cages and monitored over a 14-day period.

Oviposition cage frames (45 by 45 by 91 cm) were constructed of 6 cm by 6 cm spruce wood. Screening was attached to the wood frame with silicone and wood staples. The top and bottom sections of each cage consisted of a piece of 45 by 45 cm plywood (0.63 cm in thickness). A plywood door (0.63 cm thick), approximately 45 cm in height, was hinged to the front of each cage. Weather stripping was placed around the door to create a tight seal between the door and the cage. Once insects were placed into a cage, the door was sealed with duct tape. Oviposition cages were placed outside to expose biocontrol agents to ambient environmental conditions. Each cage contained one potted purple loosestrife plant. All potted plants were at the same phenological stage, approximately 70 cm in height, nonblooming, and had an average of 12 stems per plant.

In the direct contact group, adults were placed on a 0.2 m^2 of mesh screening. A Continental E-Z sprayer was set to a fine mist and adults were sprayed-to-wet with a 2% solution of glyphosate. Adults were removed from the mesh screening with an artist's paintbrush (size 00), placed into a petri dish and transferred onto a purple loosestrife plant in the outdoor oviposition cages.

In the indirect contact group, adults were placed in the oviposition cages and allowed to acclimatize for 24 h. This group closely simulated field conditions where insects would be naturally distributed on the host plant. Potted purple loosestrife plants were placed inside oviposition cages and then sprayed with a 2% solution of glyphosate using a hand-held Continental E-Z sprayer set to a fine mist. In the control group, adults were placed into the outdoor oviposition cages.

At 14-days post treatment (DPT), the number of egg masses and the number of eggs per egg batch oviposited on purple loosestrife were recorded using a dissecting micro-

[6]Monsanto Company. 1997. Label: Roundup liquid herbicide by Monsanto. Monsanto Canada, Inc. Mississauga, Ontario.

scope. The data were analyzed using SAS procedures (SAS Institute, 1985). A one-way analysis of variance was used to analyze oviposition data. Independent groups t-tests were used to analyze third instar larvae pupation data. Tests were considered significant at $P = 0.05$.

RESULTS AND DISCUSSION

Experiment 1: Survival and Pupation of *G. calmariensis* Third Instar Larvae. Four DPT, no larvae were observed on the loosestrife shoots as larvae had moved into the soil substrate to pupate. On 17 July (13 DPT) new generation adults began to emerge. Pupation chambers were monitoring daily until 29 July 1996 (25 DPT) when it was determined that no additional beetles would emerge.

All larvae were alive and robust immediately following exposure to 2% glyphosate. Larvae found dead were immediately removed from pupation chambers to reduce the spread of disease/fungi. In the control group, two larvae were found dead as a result of the entomophagous fungus *Beauveria bassiana* (Balsamo) Vuillemin (for statistical purposes, data were converted to a percentage of the total number of third instar larvae that entered pupation and then re-scaled to reflect a total of ten). The mean number of DPT until teneral adults emerged from herbicide treated larvae was 10.56 ± 0.13 (mean ± standard error) days and 10.88 ± 0.11 for control larvae. The mean number of third instar larvae treated with glyphosate that pupated to teneral adults was 8.33 ± 0.66 compared with 9.23 ± 0.76 for the control group; differences were not significant (T-test, $P = 0.426$). These results indicated that a 2% solution of glyphosate did not affect the ability of third instar larvae to develop into adult beetles.

The experiment was repeated on 22 July 1996 using a 4% glyphosate solution. Larvae began pupation on 25 July 1996 (3 DPT) and teneral adults first began to emerge 6 August 1996 (15 DPT). The chambers were monitored daily until 13 August 1996 (22 DPT) when it was determined no additional beetles would emerge.

All larvae were alive and robust immediately following exposure to the 4% glyphosate solution. One larvae was found dead inside a floral water pic (assumed drowned) while a second was found dead as a result of *Beauveria bassiana*; each were in separate treatment group replications. The mean number of DPT until teneral adults emerged for herbicide treated larvae was 12.14 ± 0.06 days and 12.40 ± 0.14 for control larvae. The mean number of third instar larvae treated with glyphosate that pupated through to teneral adults was 9.62 ± 0.37 compared with 10.00 ± 0.00 in the control group; differences were not significant (T-test, $P = 0.374$). The results of this study indicated that glyphosate at double the recommended field application rate (4%), had no deleterious impact on the ability of third instar *G. calmariensis* larvae to develop into adult beetles.

Experiment 2: Survival and Oviposition of *G. calmariensis* Adults. All adults were alive and robust immediately following exposure to 2% glyphosate solution. Results indicated no statistically significant differences (ANOVA, $P = 0.483$) between the mean number of adults that survived between the control group 10.33 ± 0.66, direct exposure group 11.66 ± 3.33 and indirect exposure group 14.00 ± 1.00. In general, adult mortality was similar across groups suggesting glyphosate had minimal impact on *G. calmariensis* adults.

There were no statistically significant differences in the mean number of eggs oviposited per plant (ANOVA, $P = 0.610$), the mean number of egg batches oviposited per plant (ANOVA, $P = 0.169$), the mean number of new generation adults produced (ANOVA, $P = 0.216$) or the mean egg batch size per plant (ANOVA, $P = 0.076$) among each of the three treatment groups (Table 1). *Galerucella calmariensis* were found to oviposite egg batches of variable sizes ranging between 1 egg per egg batch to 16 eggs per egg batch. Mean egg mass sizes were similar to those reported by Blossey (1995) and Lindgren (1997). Adult *G. calmariensis* used in this study were near the end of their oviposition period and a subsequent decline in reproductive effort was observed. Viable eggs (portion viable not recorded) were produced as indicated by the production of new generation adults (Table 1). These results indicated that glyphosate did not significantly impact *G. calmariensis* oviposition.

Towards an IVM Strategy. Glyphosate can be used in terrestrial habitats (in Canada) where monospecific stands of purple loosestrife have established leaving little or no native vegetation to be impacted by a herbicide application. An application of glyphosate results in the removal of all vegetation which is typically followed by an emergence of purple loosestrife seedlings that out-competes native vegetation (Gabor et al. 1996). Subsequently, the end result is another dense monospecific loosestrife worse than the original stand (Skinner et al. 1994). At this point an introduction of *G. calmariensis* may be beneficial, when the preferred meristematic tissues of young seedlings are available, and the biocontrol agent may control purple loosestrife.

There are a number of potential ways to integrate glyphosate and *G. calmariensis*. In the event glyphosate was to be applied early in the field season, it may indirectly effect the biological control agent by destroying its food source (i.e. purple loosestrife). This would be of concern at sites where glyphosate is applied and *G. calmariensis* populations have already established and are actively feeding. To sustain *G. calmariensis,* steps may be necessary to protect a number of plants from glyphosate exposure, a strategy also suggested by Lym and Carson (1994) for the integration management of leafy spurge.

A late bloom glyphosate application may be more compatible with *G. calmariensis* in that most adults may have already entered into winter diapause. There may be potential for *G. calmariensis* to then control purple loosestrife seedlings in

TABLE 1. OVIPOSITION DATA COLLECTED 14 DAYS POST TREATMENT FOR ADULT *GALERUCELLA CALMARIENSIS* EXPOSED TO A 2.0% SOLUTION OF GLYPHOSATE (FORMULATED AS ROUNDUP®).

	Mean (± SE) per plant			
Treatment	No. eggs	No. egg masses	Egg mass size	No. of new generation adults
Control	33.00 (±27.20)	6.00 (±3.20)	5.50 (±1.05)	6.33 (±0.33)
Direct	41.30 (±23.10)	8.60 (±4.60)	4.76 (±0.54)	7.00 (±1.00)
Indirect	63.60 (±11.20)	17.00 (±3.00)	3.74 (±0.33)	10.33 (±2.40)

the following year allowing for re-establishment of the desired indigenous plant community. This approach is supported by Rawinski (1982) who reported that the application rate of glyphosate (formulated as Rodeo®) was not as important as the date of application, with close to 100% control achieved with applications when purple loosestrife was in late bloom. Rawinski (1982) also reported that late bloom applications reduced seed viability. However, a disadvantage of a late bloom application would be that purple loosestrife will have begun to produce seed further contributing to the seed bank. Further field research is warranted to determine the effectiveness of these techniques and develop an IVM strategy for purple loosestrife.

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