ELSEVIER

Aquatic Botany 76 (2003) 259–265

Aquatic botany

www.elsevier.com/locate/aquabot

Short communication

The effect of a native biological control agent for Eurasian watermilfoil on six North American watermilfoils

Sallie P. Sheldon[a,*], Robert P. Creed, Jr[b]

[a] *Department of Biology, Middlebury College, Middlebury, VT 05753, USA*
[b] *Department of Biology, Appalachian State University, Boone, NC 28608, USA*

Received 14 August 2001; received in revised form 23 January 2003; accepted 2 April 2003

Abstract

There is increasing concern that introduced, classical biological control agents can have significant negative effects on non-target species. One alternative to classical biological control is the use of native species to control exotic pests. A North American weevil, *Euhrychiopsis lecontei*, is being used as a biological control agent for the introduced aquatic plant Eurasian watermilfoil (*Myriophyllum spicatum*) in the United States. Previously, we determined that *E. lecontei* did not have a significant effect on several unrelated species of native North American aquatic plants. Here, we examine the effects of this weevil on six native North American watermilfoils. In six separate experiments, individual shoots of each native watermilfoil species were exposed to zero, two or four weevil adults. Changes in length and final dry mass were determined for each shoot at the end of the experiments. We also recorded the number of weevil eggs and larvae on these native watermilfoil species at the end of the experiment. In treatments with two weevils per plant there were no significant impacts of weevils on the native watermilfoils. However, in treatments with four weevils per plant, final length of *M. verticillatum* after 11 days was 13% shorter than controls, and with four weevils final dry mass of *M. alterniflorum* was 65% less than controls and *M. humile* 43% less. Weevils laid fewer eggs on all native watermilfoil species than on *M. spicatum* controls. Few of the eggs laid on the native watermilfoils hatched. Our results suggest that when its density is high, *E. lecontei* can have impacts on some native watermilfoil species. However, due to reduced fecundity on native watermilfoils, *E. lecontei* will probably have little impact on the native species. *E. lecontei* appears to be an example of a native biological control agent that can reduce the abundance of an exotic species without a significant negative impact on closely related, non-target species.

Keywords: Myriophyllum spicatum; Aquatic plants; Herbivory; Invasive species

* Corresponding author. Tel.: +1-802-443-2072; fax: +1-802-443-2072.
E-mail address: sheldon@middlebury.edu (S.P. Sheldon).

0304-3770/03/$ – see front matter
doi:10.1016/S0304-3770(03)00055-X

1. Introduction

The accidental or intentional introduction of exotic species, many of which can become widespread pests, is a serious environmental problem (Carlton and Geller, 1993; Vitousek et al., 1996). The two main options for controlling nuisance exotic species are the application of pesticides and biological control. Of the two options, biological control, the use of one organism to control another, has been considered by some to have fewer impacts on non-target, native organisms (DeBach and Rosen, 1991; Frank, 1998). In classical biological control, natural enemies of a pest in its native range are identified, evaluated and then released in the invaded habitat (DeBach and Rosen, 1991; Harley and Forno, 1992). Appropriate classical biological control agents should be specific to the pest species, and should not have a negative impact on non-target species. While there have been notable successes using biological control agents, e.g. the control of prickly pear cactus (*Opuntia*) in Australia by a moth (*Cactoblastis cactorum*), and the control of giant waterfern (*Salvinia*) by a weevil (*Cyrtobagous salvinae*), there have also been some disasters. These disasters include the impacts of generalist consumers, e.g. cane toads and mongoose, as well as some more specialized classical biological control agents, e.g. *C. cactorum* and *Rhinocyllus conicus*, on non-target species (e.g. Harris, 1988; Howarth, 1991; Simberloff, 1992; Simberloff and Stiling, 1996a,b; Louda et al., 1997; Hamilton, 2000). Since even fairly specialized classical biological control agents may have negative impacts on non-target species, it is important to examine alternative approaches. One possible alternative is the use of control agents that are native to the habitat invaded by a pest species.

A North American aquatic weevil, *Euhrychiopsis lecontei* (Dietz), has recently been identified as a biological control agent for Eurasian watermilfoil (*Myriophyllum spicatum* L.), a nuisance aquatic weed that has spread throughout North America (Couch and Nelson, 1986; Creed, 1998). *E. lecontei* has been associated with declining Eurasian watermilfoil populations (Sheldon, 1990; Creed and Sheldon, 1995; Sheldon, 1997; Newman and Biesboer, 2000). In aquarium, pool and field experiments, weevils had a significant negative effect on *M. spicatum* growth and buoyancy (Creed et al., 1992; Creed and Sheldon, 1993, 1994b, 1995; Sheldon and Creed, 1995; Newman et al., 1996). Based on this research, weevils are now being released for the purpose of controlling *M. spicatum* in North America (Madsen et al., 2000).

Although *E. lecontei* is native to North America, it is still important to determine the potential effects of this native weevil on native plants. Previously, we determined that the weevil did not have significant negative effects on several unrelated species of aquatic plants (Sheldon and Creed, 1995). While we examined the impact of the weevil on one native watermilfoil (northern watermilfoil (*Myriophyllum sibiricum*)) in those experiments, there are several other species of watermilfoil that are native to North America. In this study, we investigated the possible effects of weevils on several common native North American watermilfoils. As these species are more closely related to *M. spicatum*, it is possible that the weevil could have negative impacts on these non-target species.

2. Methods

We ran six separate experiments with six species of *Myriophyllum*. These included *M. alterniflorum* DC, *M. heterophyllum* Michx., *M. humile* (Raf.) Morong, *M. laxum* Schuttlw. ex Chapm., *M. sibiricum*, and *M. verticillatum* L. *M. alterniflorum* and *M. sibiricum* were collected from ponds in Vermont. *M. heterophyllum* and *M. verticillatum* were collected from ponds in New Hampshire. *M. humile* was collected from a pond in Maine. *M. laxum* was collected in Florida. Plants were examined under a dissecting microscope and all invertebrates and eggs were removed. Only plants with intact apical meristems were used. The initial shoot length was recorded. Individual plants were rooted in $\sim$100 cm^3 pots of lake sediment, and then placed in clear (30 cm tall, 6 cm inside diameter) plastic chambers, capped with a lid of 202 μm Nitex. For each species, 18 chambers were placed in six rows, with three chambers per row, in a wading pool (375 l, mean water depth: approximately 30 cm) filled with well water (conductivity: 165 μS/cm; alkalinity: 50.8 mg/l) (Vermont Department of Environmental Conservation). Once in the pool, each chamber was individually aerated. The experimental design for each species was a randomized block design with three treatments (the addition of zero, two, and four adult weevils per chamber) and six replicates. Chambers for each of the six species were in different wading pools. Three additional chambers, each with one Eurasian watermilfoil stem and four adult weevils, were placed in each of the six wading pools. The purpose of these three additional chambers was to make sure that the pool environment did not adversely affect the weevils (i.e. procedural controls) and to allow for a comparison of weevil egg and larval abundance on the native watermilfoils versus *M. spicatum*. The weevils used in these experiments were collected from lakes and ponds in the vicinity of Middlebury, Vermont, USA. All six wading pools were in a single greenhouse. The duration of the experiments for each species are as follows: *M. humile*, *M. sibiricum*—9 days; *M. alterniflorum*, *M. heterophyllum* and *M. laxum*—10 days; *M. verticillatum*—11 days. The experiments with *M. alterniflorum*, *M. heterophyllum*, *M. humile* and *M. verticillatum* were conducted in July 1993. The experiments with *M. laxum* and *M. sibiricum* were conducted in July and August 1994. Water temperature in the pools during these experiments ranged from 16.1 to 25.6 °C for *M. heterophyllum* and *M. humile*, and from 18.9 to 32.2 °C for *M. verticillatum* and *M. alterniflorum*. For *M. sibiricum* and *M. laxum* water temperatures ranged from 19.2 to 31.1 °C.

At the end of the experiment, plants were removed from the chambers and the number of weevil eggs and larvae were recorded. Egg and larval numbers were standardized as the number per weevil instead of number per plant in order to compare treatments with two and four weevil adults. Change in plant shoot length and final dry mass were determined. In some cases, plants broke when they were removed from the chambers, making measurement of shoot length difficult. Change in stem length is based on intact plants only. Mean dry mass data are for all plants. Data for each species were analyzed using analysis of variance. Treatment effects (weevil density) were assessed using Tukey's HSD test. Fecundity for each native species was compared to the *M. spicatum* controls was analyzed with a one-way analysis of variance for each single species.

3. Results

Weevils fed on stems, leaves and apical meristems of the native *Myriophyllum* species. Weevils significantly reduced stem elongation of only *M. verticillatum* (Table 1). Weevils significantly reduced final dry mass of only *M. alterniflorum* and *M. humile*. Significantly, fewer eggs and larvae were recovered from the native watermilfoils than from the *M. spicatum* controls that were in the same pools (Table 2). No eggs were recovered from *M.*

Table 1
The effect of weevils on shoot length change and final dry mass of six native watermilfoils after 9–11 days

	Number of weevils			P
	0	2	4	
Change in length (cm)				
M. alterniflorum	3.85 ± 1.20	2.67 ± 5.11	0.50 ± 2.67	0.125
M. humile	1.90 ± 0.77	−0.67 ± 0.82	−1.03 ± 1.01	0.068
M. sibiricum	3.43 ± 0.70	1.80 ± 0.64	1.52 ± 0.74	0.157
M. laxum	1.76 ± 0.85	0.13 ± 0.18	−0.37 ± 0.69	0.062
M. heterophyllum	4.83 ± 4.17	−0.20 ± 2.24	0.00 ± 2.67	0.629
M. verticillatum	2.82 ± 1.04[a]	0.22 ± 0.31[b]	0.02 ± 0.48[b]	0.001
Final dry mass (g)				
M. alterniflorum	0.11 ± 0.02[a]	0.07 ± 0.03[a]	0.04 ± 0.01[b]	0.001
M. humile	0.07 ± 0.02[a]	0.06 ± 0.02[a]	0.03 ± 0.01[b]	0.015
M. sibiricum	0.08 ± 0.02	0.10 ± 0.02	0.09 ± 0.02	0.829
M. laxum	0.17 ± 0.03	0.13 ± 0.03	0.21 ± 0.07	0.084
M. heterophyllum	0.28 ± 0.07	0.30 ± 0.08	0.23 ± 0.12	0.584
M. verticillatum	0.17 ± 0.03	0.16 ± 0.06	0.17 ± 0.06	0.991

Values are means (±1 S.E.). Treatments with different letters (a, b) are significantly different (Tukey's Test). The P-values in the far right-hand column of the table are for the overall treatment effect in the one-way analysis of variance. See Section 2 for the number of replicates for each species.

Table 2
The mean (±1 S.E.) number of eggs and larvae per weevil found on the native watermilfoil species and the respective Eurasian watermilfoil controls for each of the six experiments

Watermilfoil species	Eggs			Larvae		
	Native	*M. spicatum*	P	Native	*M. spicatum*	P
M. alterniflorum	0.00 ± 0.00	0.80 ± 0.11	0.000	0.00 ± 0.00	0.33 ± 0.28	0.000
M. humile	0.14 ± 0.22	1.25 ± 1.89	0.051	0.03 ± 0.08	0.83 ± 1.17	0.005
M. sibiricum	0.42 ± 0.24	1.42 ± 0.28	0.001	0.25 ± 0.53	1.08 ± 0.22	0.022
M. laxum	0.06 ± 0.15	1.00 ± 0.50	0.002	0.00 ± 0.00	0.33 ± 0.81	0.006
M. heterophyllum	0.11 ± 0.22	2.83 ± 1.89	0.001	0.00 ± 0.00	3.00 ± 1.17	0.000
M. verticillatum	0.00 ± 0.00	0.25[a]	0.000	0.00 ± 0.00	1.25[a]	0.000

Values are expressed as number of either eggs or larvae per adult weevil to pooled over treatments with two or four weevil adults; P-values are from one-way ANOVA. Replication was $n = 12$ for native watermilfoil and $n = 3$ for *M. spicatum*.

[a] Eggs and larvae were inadvertently combined for the three Eurasian watermilfoil controls in this trial.

alterniflorum and *M. verticillatum*. Eggs were recovered from the other four native species. The greatest number of eggs (0.42 per weevil) was recovered from northern watermilfoil. The mean number of eggs recovered from the Eurasian watermilfoil controls ranged from 0.8 to 2.83 per weevil. Across all six experiments, the mean number of eggs on native watermilfoils was 0.17 per weevil versus 2.40 per weevil on Eurasian watermilfoil. Larvae were only recovered from *M. sibiricum* and *M. humile*. Larval abundance on these two species was significantly lower than on *M. spicatum*.

4. Discussion

Weevils did feed and survive on the native watermilfoils, in contrast to previous experiments with native plants from other families (Sheldon and Creed, 1995). In these "no choice" trials, in treatments with four weevils per plant, weevils had a significant negative effect on three native watermilfoil species. However, in no case were both length and mass negatively affected for any of the native species. Weevil densities used in our experiments were considerably higher than we have observed in the field on native watermilfoils. We have routinely collected *E. lecontei* from *M. sibiricum* in much of New England and elsewhere, as have other researchers (Creed and Sheldon, 1994a; Newman and Maher, 1995). Adult *E. lecontei* abundance on *M. sibiricum* is frequently one adult per 50–100 plants (Sheldon and Creed, personal observation). Of the other four watermilfoil species from northern North America, we have only collected *E. lecontei* from *M. alterniflorum* on one occasion (Creed, personal observation). In a survey of *M. alterniflorum* plants growing adjacent to an *M. spicatum* plant in a lake containing weevils, no eggs were observed on 30 *M. alterniflorum* plants. The mean (± 1 S.E.) number of eggs on the adjacent *M. spicatum* plants was 0.40 ± 0.61 (Sheldon, unpublished data).

Five of the native watermilfoil species that we tested occur in lakes and ponds in the northern United States and southern Canada. This appears to be the native range of *E. lecontei* and its native host, *M. sibiricum* (Creed, 1998). Thus, these five species already co-occur with the weevil. Granted, these five species have probably never been exposed to weevil densities as high as those likely to occur in a lake with an *M. spicatum* invasion. However, our experiments simulated such high densities and our results suggest that these five watermilfoil species should be able to tolerate such high weevil densities. Therefore, the weevil does not appear to pose a significant threat to these five species if it is used in a control project in their range. *M. laxum*, which is rare in Florida, was included in this study since *E. lecontei* might be used in control projects in Florida. While it too was able to tolerate high weevil densities, we have not studied the interaction between this watermilfoil species and weevils in the field. Without further studies, we therefore suggest that it is premature to suggest the use of the weevil for control projects in the southern United States where *M. laxum* occurs.

While adult weevils had some impact on three of the six native watermilfoil species they laid fewer eggs on them. Few of these eggs hatched and larval numbers were also low. Since larvae appear to have the greatest impact on watermilfoils (Creed and Sheldon, 1993, 1994b) this result also suggests that this specialist herbivore will have little impact on the survival or growth of these native watermilfoils.

Biological control has been thought to be less disruptive to native plants and animals than broad scale use of herbicides or insecticides, which in many cases are not selective to single species (DeBach and Rosen, 1991). Recently, however, there have been a series of papers detailing negative consequences that introduced biological control agents have had on native species (Harris, 1988; Howarth, 1991; McEvoy, 1996; Louda et al., 1997; Louda, 1998; Thomas and Willis, 1998; Ewel et al., 1999). Exotic species brought in as control agents have started feeding on non-target species, including rare natives (Harris, 1988; Howarth, 1991; Louda et al., 1997; Louda, 1998). Pathogens and parasites have also been introduced along with biological control agents (Hawkins and Marino, 1997; Guy et al., 1998). Not surprisingly, debate about the potential merits and costs of introducing species as biological control agents has increased (Simberloff and Stiling, 1996a,b, 1998; Jervis, 1997, 1998; Cowie and Howarth, 1998; Frank, 1998). Given the potential risks associated with the introduction of classical biological control agents, pest managers should look for possible native control agents in addition to searching for control agents in the original range of the pest species.

Acknowledgements

Our thanks to Linda O'Bryan and the many patient undergraduate students who helped on this project. Thanks to Ray Newman and two anonymous reviewers for helpful comments on previous versions of this paper. This research was funded by the EPA Clean Lakes Demonstration Program, the Vermont Department of Environmental Conservation, the US Army Corps of Engineer's Waterways Experiment Station, and Middlebury College.

References

Carlton, J.T., Geller, J.B., 1993. Ecological roulette—the global transport of non-indigenous marine organisms. Science 261, 78–82.

Couch, R., Nelson, E., 1986. Myriophyllum spicatum. In: Proceedings of the First International Symposium on Watermilfoil *Myriophyllum spicatum* and Related Haloragaceae Species. Aquatic Plant Management Society, Vicksburg, MS, USA, pp. 8–18.

Cowie, R.H., Howarth, F.G., 1998. Biological control: disputing the indisputable. Trends Ecol. Evol. 13, 110.

Creed Jr., R.P., 1998. A biogeographic perspective on Eurasian watermilfoil declines: additional evidence for the role of herbivorous weevils in promoting declines? J. Aquat. Plant Manage. 36, 16–22.

Creed Jr., R.P., Sheldon, S.P., 1993. The effect of feeding by a North American weevil, *Euhrychiopsis lecontei*, on Eurasian watermilfoil *Myriophyllum spicatum*. Aquat. Bot. 45, 245–256.

Creed Jr., R.P., Sheldon, S.P., 1994a. Aquatic weevils (Coleoptera, Curculionidae) associated with northern watermilfoil (*Myriophyllum sibiricum*) in Alberta Canada. Entomol. News 105, 98–102.

Creed Jr., R.P., Sheldon, S.P., 1994b. The effect of two herbivorous insect larvae on Eurasian watermilfoil. J. Aquat. Plant Manage. 32, 21–26.

Creed Jr., R.P., Sheldon, S.P., 1995. Weevils and watermilfoil: did a North American herbivore cause a decline of an exotic plant? Ecol. Appl. 5, 1113–1121.

Creed Jr., R.P., Sheldon, S.P., Cheek, D.M., 1992. The effect of herbivore feeding on the buoyancy of Eurasian watermilfoil. J. Aquat. Plant Manage. 30, 75–76.

DeBach, P., Rosen, D., 1991. Biological Control by Natural Enemies, second ed. Cambridge University Press, Cambridge.

Ewel, J.J., O'Dowd, D.J., Bergelson, J., Daehler, C.C., D'Antonio, C.M., Gomez, L.D., Gordon, D.R., Hobbs, R.J., Holt, A., Hopper, K.R., Hughes, C.E., LaHart, M., Leakey, R.R.B., Lee, W.G., Loope, L.L., Lorence, D.H., Louda, S.M., Lugo, A.E., McEvoy, P.B., Richardson, D.M., Vitousek, P.M., 1999. Deliberate introductions of species: research needs—benefits can be reaped, but risks are high. Bioscience 49, 619–630.

Frank, J.H., 1998. How risky is biological control?—comment. Ecology 79, 1829–1834.

Guy, P.L., Webster, D.E., Davis, L., Forster, R.L.S., 1998. Pests of non-indigenous organisms, hidden costs of introduction. Trends Ecol. Evol. 13, 111.

Hamilton, G., 2000. When good bugs turn bad. New Sci. 165, 30–33.

Harley, K.L.S., Forno, I.W., 1992. Biological Control of Weeds. Inkata Press, Melbourne.

Harris, P., 1988. Environmental impact of weed-control insects. Bioscience 38, 542–548.

Hawkins, B.A., Marino, P.C., 1997. The colonization of native phytophagous insects in North America by exotic parasitoids. Oecologia 112, 566–571.

Howarth, F.G., 1991. Environmental impacts of classical biological control. Annu. Rev. Entomol. 36, 485–509.

Jervis, M.A., 1997. Parasitoids as limiting and selective factors: can biological control be evolutionarily stable? Trends Ecol. Evol. 12, 378–380.

Jervis, M.A., 1998. Biological control: disputing the indisputable—reply. Trends Ecol. Evol. 13, 110.

Louda, S.M., 1998. Population growth of *Rhinocyllus conicus* (Coleoptera: Curculionidae) on two species of native thistles in prairie. Environ. Entomol. 27, 834–841.

Louda, S.M., Kendall, D., Connor, J., Simberloff, D., 1997. Ecological effects of an insect introduced for the biological control of weeds. Science 277, 1088–1090.

Madsen, J.D., Crosson, H.A., Hamel, K.S., Hilovsky, M.A., Welling, C.H., 2000. Management of Eurasian watermilfoil in the United States using native insects: state regulatory and management issues. J. Aquat. Plant Manage. 38, 121–124.

McEvoy, P.B., 1996. Host specificity and biological pest control, how well is research on host specificity addressing the potential risks of biological control? Bioscience 46, 401–409.

Newman, R.M., Maher, L., 1995. New records and distribution of aquatic insect herbivores of watermilfoils Haloragaceae, *Myriophyllum* spp. in Minnesota. Entomol. News 106, 6–12.

Newman, R.M., Holmberg, K.L., Biesboer, D.D., Penner, B.G., 1996. Effects of a potential biocontrol agent, *Euhrychiopsis lecontei*, on Eurasian watermilfoil in experimental tanks. Aquat. Bot. 53, 131–150.

Newman, R.M., Biesboer, D.D., 2000. A decline of Eurasian watermilfoil in Minnesota associated with the milfoil weevil, *Euhrychiopsis lecontei*. J. Aquat. Plant Manage. 38, 105–111.

Sheldon, S.P., 1990. A sudden decline of a Eurasian watermilfoil population in Brownington pond, VT. Army Corps of Engineers Technical Report A, vol. 90–93, pp.302–303.

Sheldon, S.P., 1997. Investigations on the potential use of an aquatic weevil to control Eurasian watermilfoil. Lake Reserv. Manage. 13, 79–86.

Sheldon, S.P., Creed Jr., R.P., 1995. Potential use of a native insect as a biological control for an introduced weed. Ecol. Appl. 5, 1122–1132.

Simberloff, D., 1992. Conservation of pristine habitats and unintended effects of biological control. In: Kauffman, W.C., Nechols, J.R. (Eds.), Selection Criteria and Ecological Consequences of Importing Natural Enemies. Thomas Say Publications in Entomology, Entomological Society of America, Springfield, pp. 103–177.

Simberloff, D., Stiling, P., 1996a. How risky is biological control? Ecology 77, 1965–1974.

Simberloff, D., Stiling, P., 1996b. Risks of species introduced for biological control. Biol. Cons. 78, 185–192.

Simberloff, D., Stiling, P., 1998. How risky is biological control?—reply. Ecology 79, 1834–1836.

Thomas, M.B., Willis, A.J., 1998. Biocontrol—risky but necessary? Trends Ecol. Evol. 13, 325–329.

Vitousek, P.M., D'Antonio, C.M., Loope, L.L., Westbrooks, R., 1996. Biological invasions as global environmental change. Am. Sci. 84, 468–478.