Design and Importance of Post-Release Monitoring

BERND BLOSSEY¹ and LUKE SKINNER²

¹Department of Natural Resources, Fernow Hall, Cornell University,
Ithaca, New York 14853, USA
²Minnesota Department of Natural Resources, DNR Building,
500 Lafayette Road, St. Paul, Minnesota 55155-4025, USA

Abstract
Standardized monitoring protocols that evaluate the impact of biocontrol agents on
target weed and associated plant and animal communities are important but underutilized
tools to improve the scientific basis of biological weed control. We need to understand
interactions of control agents, target weeds, and native plant communities, the contribu-
tion of species attacking different plant parts, the impact of single and multiple species on
plant performance and weed populations, to improve our success rate and increase the pre-
dictability of biocontrol programs. The responsibility for developing monitoring protocols
rests with biocontrol practitioners most familiar with the target plant and its response to
target agent feeding. To allow widespread adoption of protocols and participation by
non-academic personnel, monitoring protocols should balance scientific sophistication
with ease of application. We have developed a standardized protocol incorporating meas-
ures of control agent abundance (number of adults, eggs and larvae) and impact on the
host plant (number of stems, plant height, seed output, etc.) and assessing the performance
of associated plant communities to monitor the impact of insects released to control purple
loosestrife (Lythrum salicaria L.) in North America. National and local workshops
introduced personnel in the use of the protocol, and instructions and forms are available
on the internet. This has allowed widespread adoption and participation by natural
resource managers and students. We present data from 3 years of monitoring depicting the
decline of purple loosestrife as a result of insect feeding pressure at sites in Minnesota and
New York.

Introduction
Much of the emphasis in biological control programs has been on finding, screening,
releasing and distributing control organisms, and little effort has been expended on post-
release monitoring (McEvoy and Coombs 1999). Although biocontrol practitioners clearly
identified the need for long-term follow-up work decades ago (Schroeder 1983), little
progress has been made in collecting quantitative data on the effect of biocontrol agents
on target plant performance or in documenting the response of associated plant commu-
nities. Recent reviews of biocontrol programs found that few evaluations provided objec-
tive measures of the depression in weed abundance (Crawley 1989a) and when monitor-
ing programs were implemented they generally focused on presence and spread of control
agents (McClay 1995). This lack of data on the effects of control organisms on target host
plants and their associated fauna and flora is a severe handicap for improving the scien-
tific basis of biological weed control. Without long-term evaluations it is impossible to
increase success rates, gain predictability of outcomes or move away from trial-and-error
approaches that still dominate weed biocontrol programs (Lawton 1990, McFadyen 1998, McEvoy and Coombs 1999). In addition, growing public and scientific demand for data on impact of invasive plants (to justify control programs, see Blossey 1999), and for quantitative measures of safety and effectiveness of control organisms will require new priorities in biological control programs.

Emphasis (and programs) on targeting non-indigenous plants with biological control has accelerated over the last two decades (Julien and Griffiths 1998). Associated with these programs are excellent large scale experimental opportunities (see Malecki et al. 1993 and Blossey et al. 1996 for examples for purple loosestrife) usually unachievable by individual researchers (Crawley 1989b). Despite a significant lack of data on the effects of herbivores on plant population dynamics, biocontrol practitioners and other members of the scientific community have rarely taken advantage of biocontrol programs to help gather data in any rigorous fashion (Crawley 1989a, McClay 1995, McFadyen 1998). The puzzling question is: Why Not? McFadyen (1998) in the most recent review of weed biocontrol programs cites the long duration and logistical difficulties in addition to resistance by sponsors to fund such long-term investigations. Although the funding culture of sponsoring agencies may be changing (in the US the USDA now requires the development of long-term areawide monitoring programs before biocontrol programs are considered for funding; E.S. Delfosse, pers. comm.) it is still uncertain whether funding can be maintained for extended periods. However, other scientists/ecologists have been able to develop model systems and long-term funding within the (restrictive) short-term framework of funding agencies. We believe that (a) lack of expertise and training, (b) lack of enthusiasm (increased workload), (c) attitude problems (is it really necessary?), and (d) lack of standardized protocols are at least equally responsible factors explaining the lack of follow-up studies.

Many biocontrol practitioners and scientists are recruited from the fields of entomology, botany, and plant pathology or belong to “user groups” such as ranchers, farmers, or natural areas managers. The need for scientifically rigorous, quantitative data collection and evaluation requires expertise in and knowledge of different taxa (at least insects and plants) and scientific methodologies. It is our experience (we have worked extensively with natural areas managers and federal and state agency personnel in North America) that taxonomic expertise is often restricted to either plant or animal taxa (rarely insects). Without significant training or the recruitment of additional personnel, practitioners or agencies are unable to engage in long-term monitoring. This leaves the monitoring to the biocontrol scientists, who often lack enthusiasm for such studies. In times of shrinking budgets and ever increasing workloads, follow-up studies have had low priority. However, workload and shortage of personnel and expertise may only partially explain the lack of enthusiasm. We have also encountered “attitude problems” questioning the need for monitoring protocols, often on grounds that success will be obvious, long-term monitoring tedious, “before” and “after” photographs will be sufficient and the “excuse” that funding is hard to come by. We believe that, at least in part, these reasons are a cheap excuse - how often have we as biocontrol scientists asked for money to conduct such studies and made absolutely clear how important this support is? Every biocontrol program needs to better balance overseas research and importation, release and distribution of control agents with long-term follow-up work. It is clear that biocontrol programs will become more expensive, but with the additional knowledge obtained we will be able to increase predictability of biocontrol systems (which will decrease costs wasted on unsuc-
cessful agents in the long run) and satisfy the concerns over lack of evaluation and potential non-target impacts.

We also believe that it is the responsibility of the biocontrol scientists to develop such long-term monitoring protocols, if necessary with the help of appropriate taxonomic experts. We can not (and should not) expect that agencies or new participants interested in the monitoring of biocontrol agents will develop their own protocols. The knowledge of life-cycles and impacts on the host plant necessary to develop meaningful monitoring protocols rests with those who have studied the organisms in their native range and evaluated the impact of control agents on the target plant (and ideally on plant populations). This information should be incorporated into any protocol developed in the introduced range (under much higher abundance of the host plant). This highlights the need for increased cooperation beyond funding and shipping of control agents and is another indication of the value of pre-release studies (in the native and introduced range). Standardized protocols will reduce redundancy in developing protocols at multiple locations, and increase the compatibility and likelihood that results can be compared among different regions and researchers.

**Development of a monitoring protocol for purple loosestrife**

Program overviews, history and preliminary results of the purple loosestrife biocontrol program are provided by Malecki *et al.* (1993), Hight *et al.* (1995) and Blossey *et al.* (1996). Briefly, purple loosestrife is a European wetland perennial introduced to North America in the early 19th century. The species has spread to all lower 48 states except Florida and large, monotypic stands reduce the value of infested wetlands for native biota. Conventional control measures are ineffective, and a biological control program was started in 1985. In 1992 two species of leaf beetles (*Galerucella calmariensis* and *G. pusilla*) and a root feeding weevil (*Hylobius transversovittatus*) were introduced followed by the release of a flower feeding weevil (*Nanophyes marmoratus*) in 1994. All species are successfully established and mass production of leaf feeders and root feeder have resulted in their widespread distribution (>30 states). The combined release of insects attacking separate plant parts occurred under the assumption that simultaneous attack would accelerate control and be superior to the impact of any single species (Malecki *et al.* 1993). These hypotheses are currently under investigation in North America.

A standardized monitoring protocol was developed to document changes in target weed populations, control agent abundance, and changes in plant communities. Monitoring guidelines and techniques were simple enough to allow participation by non-specialists, yet sophisticated enough to allow scientific evaluation. The suggested procedures represented the minimum effort; more detailed investigations (especially on ecosystem effects) were encouraged. Preliminary versions of the protocol were tested in 1994 and 1995 and a final version was distributed to interested parties by 1996. (Instructions and forms are available at: [www.dnr.cornell.edu/bcontrol/weeds.htm](http://www.dnr.cornell.edu/bcontrol/weeds.htm)). Standardization of data collection facilitates comparison of data obtained by different people/agencies in different regions across North America. The long-term nature of such investigations (5-10 years) makes it of overriding importance that changes in personnel do not put the continuation of the monitoring program at risk. Therefore, the protocol asks initially for documentation regarding site location, physical characteristics, GPS locations, landownership etc. (Table 1) as well as insect release history. Photographic evidence of changes in vegetation over time can be a powerful tool for presentations and to re-
enforce quantitative data.

The control agents are expected to reduce the number of stems and the number of plants per area, sometimes to very low levels (Blossey 1995), therefore our design had to incorporate these anticipated changes. During preliminary investigations in 1994 it became clear that the spatial variability of communities at field sites made the assessment of changes in purple loosestrife abundance and in plant communities extremely time consuming. Completely randomized or even transect sampling designs would therefore discourage widespread participation. We decided to collect data in randomly placed, permanently marked quadrats (1m²) instead. This requires non-destructive sampling which reduces interference with insect population build-up. Quadrats of less than 1m² in size have a high potential of loosing all purple loosestrife plants which in turn would require a change in the sample unit size. To avoid this, the recommended quadrat size is 1 m² and a minimum of 5 quadrats should be placed at any site.

### Table 1.
General variables to be recorded for long-term monitoring in the purple loosestrife biocontrol program

- Site Location
- Contact Person and Legal Landowner
- Site Characteristics (habitat type etc.)
- Road Map
- Site and Vegetation Map
- Photographic evidence

Assessment of insects and plants occurs twice during the season. The first visit coincides with peak insect abundance in spring, the second assesses plant performance at the end of the growing season in the fall (before complete senescence). The exact dates for spring visits vary with latitude and in accordance with local weather conditions, therefore no specific guidelines can be provided. The fall visit occurs between late August and late September. All insect species introduced to control purple loosestrife overwinter as adults and re-appear in the spring and can be counted once the host plant is 20-30cm tall. This allows for easy assessment of individuals of all species at the same time. For the leaf-feeders it is possible to count adults, eggs and larvae since they are visible without dissections. Root and flower feeders lay eggs into the soil or into flower buds and their population assessment has to rely on counting adults. To avoid biasing data according to variation in search efforts by different investigators, search time was standardized. For each species released and for each visible life stage search time is 1 minute. For example, at a site where only the Galerucella species are present, 3 minutes total are spent searching for eggs, larvae, and adults (species are inseparable in the field). At a site where only Hylobius is present, 1 minute total search time is spent looking for adults. Where both Galerucella and Hylobius are present, 3 minutes are spent for Galerucella and 1 minute for Hylobius, etc. We recommend that 3 people observe the sample quadrat from different sides. Total search time then has to be divided by the number of observers. In addition to insect abundance, impact on the host plant (leaf area removed), % cover, height (5 tallest),
number of stems of purple loosestrife and other associated plants are recorded (Table 2) during spring visits. In the fall, assessments include % cover, number of stems and height of purple loosestrife and other associated species, as well as an assessment of the reproductive effort of purple loosestrife (Table 3). To assess the result of an increase in control agent abundance on other herbivores or predators, the occurrence and abundance of other insect species is recorded during each visit.

After initial test runs in 1994 and 1995, a final version of the monitoring protocol was developed and interested parties introduced to it’s use at regional and national workshops. The same protocol is in use since 1996 and a wide variety of organizations and people participate in data collections.

**Table 2.**
**Variables recorded to assess insect abundance and their damage during spring sampling for long-term monitoring in the purple loosestrife biocontrol program**

- Number of adults, eggs, larvae (time counts; abundance categories)
- Feeding damage on purple loosestrife (leaf area removed; damage categories)
- % cover, height (5 tallest), and number of stems of purple loosestrife
- % cover, height (5 tallest), and number of stems of *Typha* sp. or other common associated species (site specific)
- presence and abundance of other insect herbivores or predators

**Table 3.**
**Variables recorded to assess impact of insect biocontrol agents on purple loosestrife and associated wetland plants during fall sampling for long-term monitoring in the purple loosestrife biocontrol program**

- % cover, number of stems and height (5 tallest) of purple loosestrife
- % cover, height (5 tallest), and number of stems of *Typha* sp. or other common associated species (site specific)
- number of inflorescences of the 5 tallest *L. salicaria* plants
- total number of inflorescences per quadrat
- length of terminal inflorescence on each of the 5 tallest stems
- number of flower buds per 5 cm inflorescence (center piece) of the 5 tallest stems
- identity and % cover of other plant species (cover classes)
- presence of other insect herbivores or predators

**Methods and Materials**

Permanent quadrats were established at two field sites in western New York State (between Rochester and Buffalo), the Tonawanda and Braddock Bay Wildlife Management Areas (WMA) and one site in south central Minnesota, Circle Lake. The Tonawanda WMA consists of a series of impoundments with water control structures that are primarily managed for waterfowl and other marsh birds. The study area, Paddy 5, consists of a 20 ha artificial impoundment dominated by purple loosestrife. To allow better
establishment of the biocontrol agents water levels were lowered to avoid standing water during the activity periods of the insects. The leaf beetles (3,000 adults) and the root feeder (400 adults) were introduced to the impoundment in 1992 (Hight et al. 1995). In spring 1996, seven 1m² quadrats were established along a permanent transect (each 50m apart) and corners marked with metal pipes driven into the ground. Field visits occurred twice annually. In fall 1997, 3 of the seven quadrats were accidentally mowed and fall data lost, however, quadrats were relocated and sampling resumed in spring 1998. Braddock Bay WMA is a 100x100 m marsh adjacent to a bay of Lake Ontario. The vegetation was dominated by purple loosestrife with an understory of *Typha latifolia*. Five permanent quadrats were established (each 20 m apart) in spring 1996 along a permanent transect and marked as described above. Insects (10,000 lab reared *Galerucella*) were released in 2 field cages in July 1995. Cages were removed in spring 1996 and sampling occurred twice annually as outlined in the monitoring protocol. Circle Lake is a highly fertile lake located 50km south of Minneapolis. A 25 ha emergent and palustrine wetland at the west end of the lake was 50% dominated by purple loosestrife with a rich background vegetation of *Typha* spp. *Scirpus fluviatilis, Polygonum* spp. *Sparganium* spp. and other species. *L. salicaria* was established at this site for over 20 years and had spread through the entire wetland complex. In June 1994 500 *Galerucella* spp. were released in the center of the wetland. In 1995, six 50m long transects were established up to 400m from the release point. On each transect five permanent 1m² quadrats were established (each 12.5 m apart, see above). Monitoring occurred twice during the growing season, similar to other sites.

### Results and Discussion

**Minnesota.** Population build-up of the *Galerucella* species resulted in widespread damage to purple loosestrife throughout the wetland complex. Approximately 95% reduction in flowering was observed, the number of stems of purple loosestrife at Circle Lake remained constant, however after insects reached high abundances in 1997 stem height declined to less than 50% of the original height (Table 4). That not more dramatic results could be reported is most likely associated with (1) a design flaw in our monitoring protocol and (2) a function of the insect distribution at the site(s). First, to reduce the time necessary to measure plant height (stem densities of well over 100 stems per m² are not uncommon) and to avoid biasing the selection of stems to tall ones we decided to meas-

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<td>Number of stems</td>
<td>14.4±3.08</td>
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<td>Stem height (cm)</td>
<td>174±3.46</td>
<td>170.9±4.29</td>
<td>177.5±3.04</td>
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Fig. 1. Number of adult *Galerucella* spp. (A), number of *Galerucella* spp. eggs and larvae (B) and defoliation status (% leaf area removed) (C) of purple loosestrife plants in permanent 1m² quadrats at Tonawanda WMA, New York.
ure the 5 tallest shoots per quadrat (expecting a somewhat homogenous reduction in stem height over time). After several years of observation it is clear that even in a 1 m² sampling area, stem heights vary significantly. Our measurements reveal only the most conservative reduction in height and not the average decline of purple loosestrife performance at the release site. To obtain a more realistic assessment of insect feeding impact we should measure all live stems. Second, the distribution of insects at a site is not homogenous. Beetles are highly aggregated (Blossey 1995) and damage spreads out like a front from the original release site.

**New York.** Both sites experienced a dramatic population build-up of the *Galerucella* species and thousands of beetles have been distributed to adjacent purple loosestrife infestations. Very little information is available for the root feeder at Tonawanda because quantitative sampling of rootstocks was avoided and regular monitoring did not detect any adults. Occasional excavations revealed, however, that *H. transversovittatus* was present and has spread from the release area.

Between 1996 and 1999 adults spread through the entire marsh. This is reflected in the
Fig. 3. Number of adult *Galerucella* spp. (A), number of *Galerucella* spp. eggs and larvae (B) and defoliation status (% leaf area removed) (C) of purple loosestrife plants in permanent 1m² quadrats at Braddock Bay WMA, New York.
number of adults found during spring monitoring in our permanent quadrats (Fig. 1a). In 1996 beetles had spread less than 200 meters from the original release area (around quadrat 1). By 1999 we could no longer detect adult beetles, eggs or larvae in the quadrats near the release area (Fig. 1a, b). The distribution of beetles, eggs and larvae was concentrated in certain areas and overall spread occurred as an advancing front with the damage, measured as percentage of leaf area removed, spreading from the release area through the site (Fig. 1c). In 1999, damage was highest furthest from the release area. Plants in the release area declined to very low numbers; stem numbers in quadrat 1 for example dropped from 50 in 1996 to one in 1998 (Fig. 2a). This decline in stem numbers was found throughout the site but overall reduction was highest closest to the release area (Fig. 2a). Stem height in 1996 showed a peak in the center of the transect, but stem height ultimately declined in all quadrats to less than 30% of the original level. We do expect further declines in stem numbers and stem height. Gaps in the data for quadrats 3, 4, and 5 in 1997 represent accidental mowing, gaps in the data for quadrat 7 reflects the inability to find
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this sampling quadrat in 1997 and 1998 (an indication how difficult maneuvering in over 2m tall old purple loosestrife stands is).

At Braddock Bay no adults were detected in 1996 in the permanent quadrats (Fig. 3a), although they were present outside and eggs and larvae were located throughout the site (Fig. 3b). With increasing insect numbers in 1998 (Fig. 3b) widespread defoliation occurred through the site (Fig. 3c). Most plants were completely stripped of their entire photosynthetic tissue by late June (B. Blossey, pers. obs.). Surprisingly, most plants recovered from this attack and produced an abundance of shorter stems (Fig. 4a) that did not flower. Actual stem counts were higher at the end of 1997 then the previous year in 3 of the 5 permanent quadrats (Fig. 4a). Stem height declined from 1996 to 1997 in all quadrats (Fig. 4b), however, plants somewhat recovered in 1998, particularly in the center of the site and at the end of the growing season were taller than in 1997 (Fig. 4b). This can be explained, in part, by our measuring only the five tallest plants in each quadrat, which is a very conservative assessment of the impact of insect feeding on plant performance. In

**Fig. 5.** Number of stems (A) and height (B) of *Typha latifolia* in permanent 1m² quadrats at Braddock Bay WMA, New York.
addition it reflects the compensatory ability of well established mature purple loosestrife plants even after seemingly devastating defoliation (75-100% leaf area loss). The results at Tonawanda demonstrate that several years of heavy feeding will be necessary to weaken purple loosestrife and deplete large rootstock reserves sufficiently to result in plant death.

The response of *T. latifolia* to feeding of leaf beetles at Braddock Bay was a general increase in the number of stems per quadrat (Fig. 5a), however, overall plant height remained largely unchanged (Fig. 5b). Again, the apparent lack of change of plant height can be partially explained by our measurements of the 5 tallest cattail stems instead of taken a random sample or measuring all stems in a quadrat. Observations not reflected in the measurements include the complete change in aspect of the site; from a purple loosestrife dominated wetland with a cattail understory to a cattail marsh with a purple loosestrife understory. Cattails produced flowers in 1997 for the first time since monitoring began. The water table rose and the central part of the wetland became permanently flooded (probably a result of reduced evapotranspiration rates with reduced purple loosestrife foliage, J. Yavitt, pers. comm.) and muskrats colonized the area. In 1998 2 muskrat houses were found in the central part of the wetland and their feeding most likely reduced the number of *T. latifolia* stems.

**Outlook**

Despite an excellent safety record (Crawley 1989a) skepticism concerning the safety and effectiveness of exotic insect introductions for weed control remains high among the general public, administrators and even scientists. But despite an increase in the number of programs initiated, the ability to select and to establish control agents has not progressed to a point where the rate of success has improved (Crawley 1989a). Basic questions about the kind of herbivore species to introduce, impact of single and multiple species herbivory, and release strategies remain unanswered. The development of long-term monitoring programs incorporating control agents, target weed and plant communities is one of the most important tools for improving the scientific basis of biological control. Biocontrol practitioners and scientists need to better balance priorities of funding for study, detection and importation with follow-up work. Well executed long-term monitoring programs offer exciting opportunities for ecological and applied work. Monitoring programs should include multiple taxa to increase knowledge about the effect of biological control agents on native biota and ecosystem processes. Credibility for the science of biological control will significantly increase among fellow ecologists and with the public if we can demonstrate that the release of biological control agents is beneficial and not an ecological disaster (as some fear, see Louda *et al.* 1997). Without being out in the field and collecting the necessary long-term data, our claims that biocontrol of weeds is safe will always be looked at with suspicion.

The development of a standardized long-term monitoring protocol for purple loosestrife has allowed widespread participation in the follow-up work across North America. All indications provide for a very optimistic scenario for the overall success of the biocontrol program. Our results from 3-4 years of monitoring show that declines of purple loosestrife are visible (occasionally spectacular) but that parts of the monitoring protocol should be improved (such as the height measurements). It is also clear that studying the invasion of unoccupied space by biocontrol agents makes for some challenging study designs. Assessing performance across a site when insects spread as an advancing front...
will not reveal the full impact on host plants or associated plant communities. Our per-
manent plot design is well suited to follow spread and document changes in insect abun-
dance and plant communities. Different control agents and target plants will require dif-
f erent monitoring protocols but we believe that quantitative designs can be and should be
developed for all weed biocontrol programs by those most knowledgeable with the sys-
tem, i.e. the biocontrol scientists and practitioners.

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