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Integration of biological control agents with other weed management technologies: Successes from the leafy spurge (*Euphorbia esula*) IPM program

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Abstract

An invasive weed can occupy a variety of environments and ecological niches and generally no single control method can be used across all areas the weed is found. Biological control agents integrated with other methods can increase and/or improve site-specific weed control, but such combinatorial approaches have not been widely utilized. The successful leafy spurge (*Euphorbia esula* L.) control program provides examples for future integrated weed programs that utilize biological control agents with traditional methods. Weed control methods can be used separately, such as when the leafy spurge gall midge (*Spurgia esulae* Gagné) reduced seed production in wooded areas while herbicides prevented further spread outside the tree line. Traditional methods also can be used directly with biological control agents. Incorporation of *Aphthona* spp. with herbicides has resulted in more rapid and complete leafy spurge control than either method used alone. Also, the insect population often increased rapidly following herbicide treatment, especially in areas where *Aphthona* spp. were established for several years but had been ineffective. Incorporation of *Aphthona* spp. with sheep or goat grazing has resulted in a larger decline in leafy spurge production than insects alone and in weed density than grazing alone. Controlled burns can aid establishment of biological control agents in marginally suitable environments, but timing of the fire must be coordinated to the insect's life-cycle to ensure survival. Integration of biological control agents with revegetation programs required the agent to be the last method introduced because the cultivation and herbicide treatments necessary to establish desirable grasses and forbs were destructive to the insect. In a practical application, herbicides were combined with *Aphthona* spp. to help the insect establish and control leafy spurge in the habitat of the western prairie fringed orchid (*Platanthera praeclara* Sheviak and Bowles), an endangered species. Several experimental designs can be used to evaluate biological control agents with cultural, mechanical, and chemical control methods or with additional biological agents.

Keywords: *Aphthona czwalinae*; *Aphthona flava*; *Aphthona lacertosa*; *Aphthona nigriscutis*; Leafy spurge flea beetles; *Euphorbia esula*; *Spurgia esulae*; Biological control; IPM; Invasive weeds; Endangered species

1. Introduction

Invasive weeds are one of the greatest threats to croplands, rangelands, and wildlands world-wide (Mullin et al., 2000). In 2003, 16 invasive weeds were estimated to occupy 49 million ha of the United States, which resulted in tremendous losses in production and disruption of native habitat (Duncan et al., 2004). One or more of these 16 weeds occupied nearly every ecological habitat available from the Pacific Northwest and Northern Great Plains to the desert Southwest and semi-tropical Southeast.

An invasive weed can occur on a wide variety of terrain and in many diverse environments including flood plains, woodlands, prairies, and mountain slopes (Duncan et al., 2004). Initially, control of invasive weeds

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has been attempted with herbicides. While herbicides can provide effective control, chemical and application costs required to control weeds on large infestations can quickly become unrealistic, herbicides cannot be used in all environments where a weed may be found, and the need for frequent retreatments becomes disheartening. The use of biological control agents is considered more cost-effective than herbicides for large-scale weed control programs. For instance, Bangsund et al. (1996) estimated that herbicide treatments for leafy spurge (*Euphorbia esula* L.) control provided a positive return for cow–calf operations only if the leafy spurge patch was 0.5 ha or less in size. In contrast, potential economic benefits from biological control of leafy spurge were estimated to be nearly $59 million per year if the biological control agents eliminated 65% of the leafy spurge infestation by the year 2025 (Bangsund et al., 1999).

Classical biological control of invasive weeds has been attempted on many species and there are examples where both single- and multiple-agent introductions successfully controlled the target species (Briese, 1997; Hosking et al., 1988; Pemberton and Turner, 1990). Despite these and other successes, the use of biological agents alone to control weeds has been effective in only about 30% of the attempts (McFadyen, 1998; Meyers, 1984). Even when biological control agent(s) are successful, it may take 20 years or more for the weed to be reduced to a manageable level (McFadyen, 2000). With the average annual spread of invasive weeds ranging from 8 to over 30% (Duncan et al., 2004), such a long time-line for control would be unacceptable.

It seems intuitive that long-term invasive weed control programs would be most successful if all available methods were used. No one would expect to fight a raging wild fire with only one method (Dewey, 1996; Dewey et al., 1995). Air-drops of water and retardants (chemicals) are needed for rapid suppression, fire-breaks (mechanical) and back-fires (controlled burns) are needed to stop the fire from spreading. How can one expect to fight the invasion of weeds in all environments where they occur with only one method and often one tool (single insect)?

Theoretically, integration of other weed control methods with biological control agents can reduce a weed below the economic threshold more quickly than the insects alone and may also increase the effectiveness of marginally successful agents (Fig. 1). The shaded area in Fig. 1. is the theoretical potential gain from biological control agents combined with herbicides compared to the either method alone (Messersmith and Adkins, 1995). The number of herbicide applications required is dependent on the weed, the biological control agent, economics, and the level of control required.

Several attempts have been made to combine biological agents with herbicides for invasive weed control in the field (Ainsworth, 2003; Messersmith and Adkins, 1995). Sometimes, herbicides were used to rapidly reduce an infestation and contain further spread while the biological control agent had time to become established (DiTomaso and Enloe, 2000). Less often herbicides have been applied directly on weed infestations where biocontrol agent were established (Paynter and Flanagan, 2004). Despite these successes, there are few examples of biological control agents combined with other methods to control weeds when herbicides cannot be used or are ineffective.

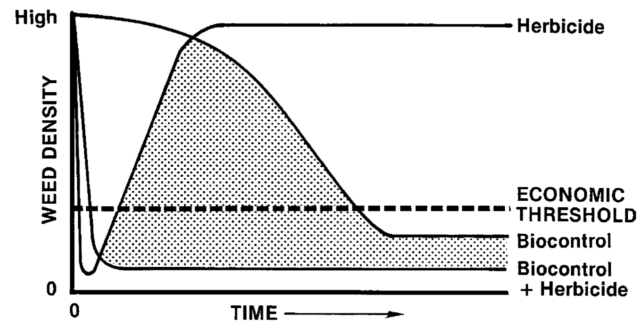

Fig. 1. Relative efficacy of a herbicide, biocontrol agent, and herbicide plus biocontrol agent for weed control over time. Shaded area presents the potential gain in control from integration of biological control agent with an herbicide. In general, weed control is more rapid and at a higher level when the two methods are combined compared to either alone. From Messersmith and Adkins (1995). Reprinted with permission.

The leafy spurge control program is an example of integrated weed management that combined several traditional methods with biological control agents (Lym, 1997) to control the weed in a variety of environments. Since leafy spurge occurred across the Great Plains and Mountain West from Canada to New Mexico, researchers recognized from the start that several control options would be needed and that no one tool could be used in all areas where the weed occurred. Land managers required cost-effective control methods, especially for large infestations, but were also under social restraints such as working in wilderness areas, near waterways, and on public lands.

Herbicide treatments would be too costly for widespread use and often could not be used in sensitive environments where leafy spurge occurred (Lym, 1998). Grazing would reduce leafy spurge top-growth and slow the spread but would not reduce the root system. Also, grazing is not always compatible with the land management programs in many leafy spurge-infested areas. Reseeding of native species likely would not succeed unless the weed was controlled first. A dedicated program to control this aggressive invasive weed involved many government and research agencies in North America and led to a widely diverse integrated research and control program (Messersmith and Lym, 1990). The vast scale of this program can provide guidance for future weed control programs that integrate biological control agents with chemical, mechanical, and cultural methods.

1.1. Integrated leafy spurge biological control

Leafy spurge became a major problem in the United States and Canada beginning in the mid-1920s (Lym, 1997). The infestation doubled in acreage in North Dakota alone every 10 years from 1940 to the mid-1990s when an integrated program started to reduce the spread (Lym and Messersmith, 2003; Messersmith and Lym, 1983). Since leafy spurge infested millions of hectares before the program began, the ultimate goal was to reduce the weed to a manageable level where it occurred and to keep the plant from spreading to uninfested areas. Initially, biological control was unavailable and a major program to evaluate and introduce agents was initiated in the 1980s. To date, 12 biological control agents have been released (Carlson and Mundal, 1990; Julien and Griffiths, 1998; Lym, 1998). Once the biological control agents became established, the insects were incorporated with other methods commonly used to control leafy spurge, which included herbicides, grazing management, burning, and seeding of competitive native replacement species.

1.1.1. Integration with herbicides

Incorporation of herbicides with biological control agents for leafy spurge control began soon after the first insect species was introduced. The leafy spurge hawkmoth (*Hyles euphorbiae* L.) (Lepidoptera: Sphingidae), a foliar feeder, was introduced in the 1970s. The insect became widespread but sparse and herbicides were still needed to keep leafy spurge from spreading. Leafy spurge hawkmoth larvae were over-sprayed and/or fed leafy spurge plants sprayed with 2,4-D or picloram without affecting the insect (Reese and Fay, 1989). A combination of picloram plus 2,4-D was the most commonly used herbicide treatment for leafy spurge control (Lym, 1998) and was successfully incorporated with the hawkmoth as long as the herbicides were applied in the fall when hawkmoth larvae had reached the fourth or fifth instar stage (Reese and Fay, 1989).

Although the combination of the hawkmoth with herbicides could be used together, leafy spurge control was not improved compared to herbicides alone (Lym, 1998). The leafy spurge hawkmoth suffered from an internal virus that often prevented the insect from maintaining a high enough population for this combination treatment to be effective.

The leafy spurge gall midge *Spurgia esulae* Gagné (Diptera: Cecidomyiidae), introduced in 1986, causes stem tip galls thereby decreasing seed production (Carlson and Mundal, 1990; Lym and Carlson, 1994). The gall midge reproduced most successfully near wooded areas and was widely distributed. However, a second method of control was needed because only a portion of a leafy spurge infestation was affected by the insect, so seed production was reduced but not eliminated. Also, reducing seed production of a perennial weed will not decrease density within already infested areas nor decrease the spread by roots.

Three herbicides, imazethapyr, picloram, and 2,4-D, were successfully incorporated with *Spurgia esulae* as long as 15–20% of the leafy spurge remained untreated (Fig. 2) (Lym and Carlson, 1994). Integration of herbicides with the gall midge prevented leafy spurge spread from wooded areas and reduced seed production under the tree canopy where herbicides could not be used.

The flea beetles *Aphthona nigriscutis* Foudras (Coleoptera: Chrysomelidae) and mixed populations of *A. czwalinae* Weise and *A. lacertosa* Rosenhauer have established in the highest population densities and reduced stand densities more than any other agent released for leafy spurge control (Hansen et al., 1997; Kirby et al., 2000). All three species are univoltine (Gassmann et al., 1996; Maw, 1981). *Aphthona* spp. adults emerge from the soil from late May to early July and feed on leafy spurge foliage. Females deposit eggs just below the soil surface near leafy spurge crowns. Larvae emerge in approximately 8 days and feed on leafy spurge root tissue. Larvae overwinter in the soil, resume feeding when the soil temperature reaches approximately 15 °C the following spring, and then emerge as adults in late-spring to early summer.

Leafy spurge control with *Aphthona* can be variable, ranging from zero to >95% stem reduction (Kirby et al., 2000). Thus, additional methods were needed to control leafy spurge in all the environments where it occurs (Bangsund et al., 1997). A common denominator among release sites with poor leafy spurge control was very low *Aphthona* densities even 5–7 years after release. Low *Aphthona* populations often occurred when the agents were released in very dense leafy spurge stands (>320 stems/m^2) (Lym, 1998).

Initially, all *Aphthona* spp. releases were in isolated areas not subjected to herbicides or other control methods (Carlson and Mundal, 1990). In 1991, a population of *A. nigriscutis* established near Minot, ND, was accidentally over-sprayed in the fall with picloram plus 2,4-D. Although *A. nigriscutis* had established 2 years prior to the herbicide application, the population was low and had not noticeably reduced the leafy spurge infestation. In the spring following herbicide treatment, the leafy spurge density was reduced by over 90% and more than 1 million *A. nigriscutis* adults were collected for redistribution (R. Carlson, personal communication).

The initial (and accidental) combination of biological and chemical control for leafy spurge management increased both the *A. nigriscutis* population and leafy spurge control. This event stimulated additional research that combined *Aphthona* spp. with herbicides (Lym and Nelson, 2002). A series of experiments were conducted with caged and open-releases of *Aphthona* spp. over-sprayed with herbicides in a variety of environments. Leafy spurge control generally was achieved more

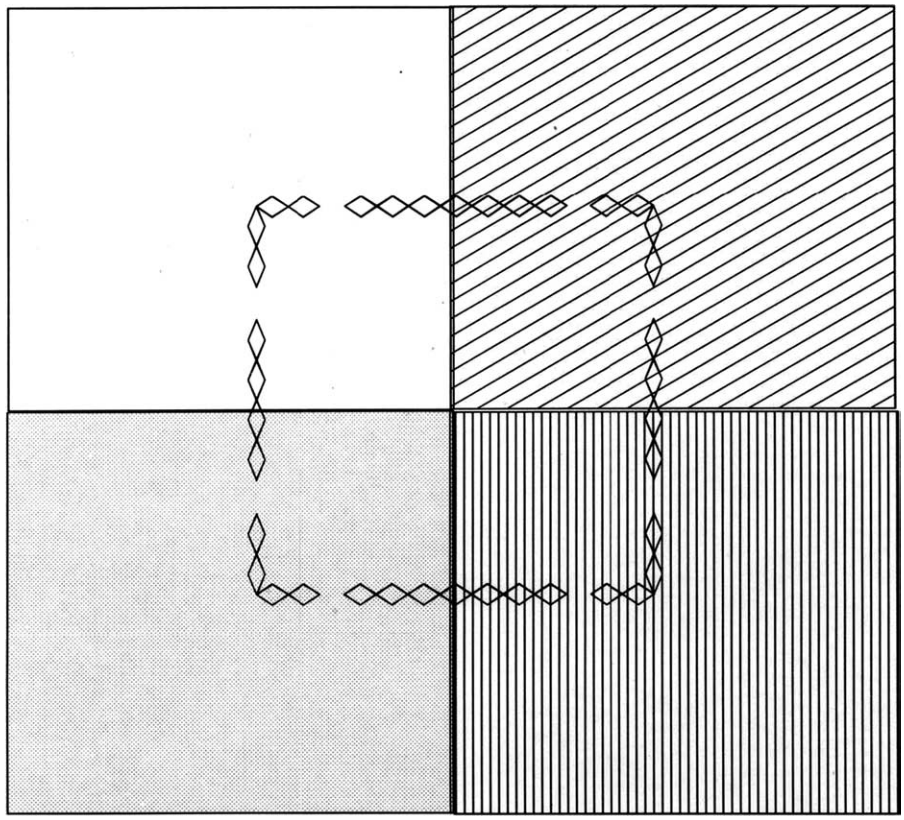

Fig. 2. Plot design to evaluate incorporation of the leafy spurge gall midge (*Spurgia esulae*) with herbicides. A 6 × 6-m square was divided into four 3 × 3-m plots for each replication of the experiment with a 1.8 × 1.8-m cage (◇◇◇) placed in the center to inhibit *S. esulae* movement. This design allows herbicides to be applied in various quadrats and to evaluate the effect of the gall midge and herbicides alone or combined on leafy spurge. From Lym and Carlson (1994). Reprinted with permission.

rapidly in these studies when herbicides and *Aphthona* were combined compared to either method used alone. Often only a single herbicide application was required, which reduced the cost of leafy spurge control threefold or more because land managers previously reapplied herbicides annually for 3–5 years.

Leafy spurge control was generally more rapid when herbicides were applied after the *Aphthona* spp. had become established compared to application the same season the insects were released (Lym and Nelson, 2002). Besides increased control, the combination treatment sometimes resulted in a rapid increase in agent population. The increase in *Aphthona* spp. population was likely due to a reduction in leafy spurge stem density which increased successful egg laying on the soil surface rather than on litter and increased movement of adults to outside the initial release zone. Once leafy spurge stem density had been reduced, the biocontrol agents maintained control for at least 7 years. Such long-term control of leafy spurge with herbicides could only be maintained with periodic herbicide reapplication.

A practical application of integrating herbicides with *Aphthona* spp. is when *Aphthona* have been established for several years but have not or only slightly reduced the leafy spurge infestation. For instance, *A. nigriscutis* had established and reduced leafy spurge stem density along a railroad right-of-way by 65% 3 years after release, but neither leafy spurge control nor the *Apwhthona* population increased in the following 5 years (Lym and Nelson, 2000). A fall-applied herbicide treatment rapidly decreased the leafy spurge density compared to the untreated (biological agent only) control.

The use of herbicides with biological control agents can be targeted separately or combined for weed control depending on the agent and the location. In these examples, the leafy spurge gall midge was used to reduce seed production in wooded areas where herbicides generally cannot be used and in turn herbicides used outside the tree line prevented further weed spread. The combined method of herbicides applied directly to leafy spurge infested with *Aphthona* spp. provided increased weed control compared to either method alone and often aided in agent establishment.

1.1.2. Integration with grazing

Grazing with sheep or goats has been a cost-effective option to control leafy spurge top growth when the plant infests large areas (Olson and Lacey, 1994; Sedivec and Maine, 1993). Grazing animals can reduce leafy spurge cover 55–85% in about 3 years and the land owner can recover some control costs from sale of the animals (Helgeson and Longwell, 1942; Olson and Lacey, 1994; Walker et al., 1994). However, even after 8 years of intensive grazing by sheep that nearly eliminated the leafy spurge top growth, the plant began regrowing from roots 1 year following removal of the sheep and quickly reestablished to stand densities and cover found prior to the study (Bowes and Thomas, 1978). Since grazing alone will not kill leafy spurge roots (Bowes and Thomas, 1978; Olson and Lacey, 1994), a second control method was needed to prevent rapid reinfestation.

Sheep grazing combined with *Aphthona* spp. flea beetles reduced leafy spurge stem density and cover more than either grazing or biological control agents used alone (Beck and Rittenhouse, 1999, 2000; Hansen, 1993; Samuel et al., 2004). In a multi-species grazing trial *Aphthona* species alone gradually reduced leafy spurge stem density but did not reduce leafy spurge herbage production because the vigor and production of surviving plants compensated for the loss in stems (Samuel, 2003). Integration of sheep with *Aphthona* spp. provided a more rapid reduction of stem density than the biological control agents alone, and the leafy spurge biomass was reduced by grazing. This combination allowed the pasture to support a greater number of cattle than either rotational grazing with sheep or with *Aphthona* spp. used alone. Also, the combination of sheep with *A. flava* effectively managed leafy spurge in riparian areas and shelter belts where herbicide use was very limited (Beck and Rittenhouse, 2000).

Similar to the biocontrol–herbicide combinations, grazing can be combined with biocontrol agents to provide better control and in wider variety of habitats than either method had achieved when used alone. Grazing has also been combined with herbicides for increased leafy spurge control (Lym et al., 1997). The next logical step would be incorporation of biological control agents with grazing and herbicides in areas where all three are viable methods.

1.1.3. Incorporation with fire

Fire is often used for management of plant communities in North America (Wright and Bailey, 1982), including management of some invasive species (Masters and Sheley, 2001). Fire does not directly reduce the density of leafy spurge or lower seed viability (Wolters et al., 1994). Fire has been used to remove thatch and open the leafy spurge canopy to increase herbicide spray coverage (Lym, 1998). However, leafy spurge quickly reestablished dominance and prevented desirable species from establishing if herbicides or other control methods were not incorporated with the burn (Richardson, 2004).

Biological control agents must be able to survive controlled burns that are used to aid in returning leafy spurge-infested areas to natural habitat. Often, the timing of the controlled burn will determine if an agent survives (Briese, 1996). For instance, established populations of *A. nigriscutis* were unaffected by fire as long as the burns were conducted in May or October when the agent was in the larval growth stage below the soil surface (Fellows and Newton, 1999). *Aphthona* adults feeding on stem tissue would not survive a fire. The timing of a controlled burn would have to be adjusted for other leafy spurge control agents such as *S. esulae*, which has multiple generations.

Controlled burns have aided in establishment of leafy spurge biological control agents in marginally suitable environments. For instance, establishment of *A. nigriscutis* was higher if sites had been burned prior to release of the agents compared to non-burned sites (Fellows and Newton, 1999). However, most colonies established with the aid of fire did not survive unless the habitat was otherwise suitable.

Controlled burns may also be useful to increase recruitment of *Aphthona* spp. during the first few generations. Release of flea beetles in very dense leafy spurge infestations (>320 stems/m^2) reduced the probability of establishment and subsequent weed control (Lym, 1998). A controlled burn in these dense stands prior to *Aphthona* release often led to an increase in larval survival because eggs were laid on the soil surface rather than on several centimeters of plant thatch.

Fire alone generally does not control an invasive weed, but often is necessary in wildlands to promote regeneration of desirable species (Kruger, 1983). If properly timed, fire could help a biological control agent establish and aid in the successful control of the weed.

1.1.4. Incorporation with reseeding

Leafy spurge control programs that include establishment of introduced and native perennial grasses have resulted in increased forage production and improved long-term weed control compared to single method programs (Ferrell et al., 1998; Lym and Tober, 1997; Masters and Nissen, 1998). However, incorporation of biological control agents with reseeding has been difficult, primarily due to the cultural methods such as cultivation and top-growth control of all plant material required to establish a productive stand of competitive species. To establish a suitable site for seeding in Wyoming, glyphosate (which kills leafy spurge top growth) had to be applied twice in mid-summer 30 days apart and then the site was tilled (Ferrell et al., 1998), while in North Dakota, repeated tillage was required over a 2-month period to obtain a suitable seedbed (Lym and Tober, 1997). These methods resulted in the establishment of competitive grass species in both states, but also effectively reduced leafy spurge for several years which would likely eliminate an established population of biological control agents or prevent newly introduced agents such as *Aphthona* spp. from becoming established.

Seeding of competitive species using a no-till planter to avoid cultivation would be less detrimental to an established leafy spurge biological control agent than conventional seeding techniques. Unfortunately, no-till seeding of desirable species has not been successful except when the site was mowed or burned prior to seeding and an herbicide was applied to control competitive cool-season grasses (Masters and Nissen, 1998; Masters et al., 2001). The thick thatch of leafy spurge canes often found in old stands reduced grass seedling establishment and the undesirable cool-season grass species competitively replaced the remaining seeded grasses. Thus, the intensive management required to seed competitive species precludes the use of biological control agents until the seeded species have become established and the weed has begun to regrow. Research in progress has shown initial leafy spurge control with *Aphthona* spp. combined with no-till reseeding and herbicides provided better control than any single method or biocontrol agent plus herbicides used alone (Juricek et al., 2005) and may be a valuable treatment option in the future, especially where native plant stands are desired.

1.2. IPM methods to save a threatened species: a case study

How traditional weed control methods can be used with biological agents to increase control of an invasive weed is demonstrated by the effort to save an endangered orchid species when leafy spurge invaded the plants remaining habitat. The western prairie fringed orchid (*Platanthera praeclara* Sheviak and Bowles) (WPFO) is a native of the tallgrass prairie that once was distributed throughout areas west of the Mississippi River (Sheviak and Bowles, 1986). With conversion of prairie to cropland and urban development, the orchids have become rare and were placed on the federal threatened species list in 1989 (U.S. Fish and Wildlife Service, 1989). Various threats to survival of the western prairie fringed orchid remain, with habitat invasion by leafy spurge one of the most critical (Sieg and Bjugstad, 1994; U.S. Fish and Wildlife Service, 1996; Wolken et al., 2001), especially on the Sheyenne National Grassland (SNG) which has one of the largest remaining WPFO populations (Sieg and Wolken, 1999).

The use of *Aphthona* spp. flea beetles would be an ecologically favorable control method for leafy spurge and seemed the logical choice for leafy spurge control in WPFO habitat. However, *Aphthona* spp. have not survived in the sandy, mesic habitat of the WPFO. In fact, prior to this study, no releases made in the orchid habitat have effectively established (Lym, 1998).

An experiment to evaluate herbicides for leafy spurge control near orchid habitat in the early 1990s had to be discontinued 2 years after establishment because the WPFO appeared in areas treated with fall-applied herbicides (Kirby et al., 2003). By law, herbicides could not be used on or near the WPFO.

As leafy spurge continued to invade and spread in the remnant WPFO habitat, herbicides to control the weed seemed the only viable option. A permit to screen various leafy spurge control herbicides on the orchid was obtained from the U.S. Fish and Wildlife Service in 1999. Subsequent research found that the herbicides imazapic and quinclorac provided good leafy spurge control with little or no injury to the orchid (Erickson and Lym, 2002; Kirby et al., 2003). However, to provide consistent long-term leafy spurge control and reduce pesticide use in WPFO habitat, additional control methods were needed. Therefore, a study was initiated to evaluate the interaction of imazapic and quinclorac with a mixed population of *Aphthona* spp. flea beetles for leafy spurge control in the habitat of the WFPO (Erickson, 2003).

2. Materials and methods

The experiment was established within in the SNG in June 2001. WPFO orchids were located immediately adjacent to but not within the study. A mixture of *A. czawalinae* and *A. lacertosa* was collected from an established population near Lisbon, North Dakota, approximately 29 km from the experiment location. Approximately 24 h after collectoin, 350 adult *A. czawalinae* and *A. lacertosa* were released into insect cages on June 27, 2001, and 100 additional flea beetles were released on July 17, 2001, to ensure appropriate sex ratios (Olson and Mundal, 1999). Cages were $1.8 \times 1.8 \times 1.8$ m with a PVC frame covered by a plastic screen (32×32 Lumite) (Lym and Nelson, 2002; Nelson and Lym, 2003). Imazapic and quinclorac were applied on September 20, 2001, using a CO_2-pressurized backpack sprayer delivering 80 liter/ha at 240 kPa with four flat-fan 8001 nozzles. Cages were removed from the plots for the winter prior to herbicide application (Lym and Nelson, 2002; Nelson and Lym, 2003).

Aphthona spp. population was estimated in late June from 2001 through 2004. Vegetation in the subplots was swept for adults with a sweep net having a 38-cm diameter hoop. Quarters of each subplot and portions of the subplot border, totaling 1 m^2 each, were swept in five sweeps, and adults captured were counted and returned. The cages were removed in September 2002 and *Aphthona* moved among all treatments thereafter. Leafy spurge control was monitored by counting leafy spurge stems in four 0.25-m^2 areas in each subplot both before and after treatment (Lym and Nelson, 2002).

The experiment was arranged as a randomized complete block-design with a split-plot arrangement and four replicates. Whole plots were 3.05 m wide and 9.15 m long. Whole plots consisted of herbicides alone, and subplots consisted of insects plus herbicides, insects alone, and an untreated control (no insects or herbicides). Measures were compared to an untreated check using an ANOVA, and individual treatment means were separated using Fischer's protected LSDs calculated at the 95% levels of confidence.

3. Results

Aphthona czwalinaellacertosa adults were collected 1 year after release in the field and the population was higher in the insect-only subplots than in the herbicide plus *Aphthona* subplots (Table 1). *Aphthona* spp. averaged five adults/m^2 in 2002 which increased to an average of 17 adults/m^2 in the insect-only treatment 2 and 3 years after release. By 2004, *Aphthona* generally increased in all treatments except imazapic. *Aphthona* were also found in the control plots because the cages had been removed in September 2002.

Imazapic or quinclorac applied alone or with *Aphthona* spp. flea beetles reduced leafy spurge density more than flea beetles alone in 2002, 1 year after release (Table 2). Leafy spurge stem density was reduced from 150 to 41 stems/m^2 (53% control) with *A. czwalinaellacertosa* alone compared to a reduction from an average of 114 to 4 stems/m^2 (96% control) with herbicides alone, and from an average of 126 to 1 stem/m^2 (99% control) with herbicides plus flea beetles. Imazapic and quinclorac provided similar leafy spurge control 1 year following treatment regardless of rate applied.

Table 1
Effect of herbicides on *Aphthona* spp. flea beetle population collected annually in mid-July from 2002 to 2004 following insect release and herbicide application in 2001 in the habitat of the western prairie fringed orchid within the Sheyenne National Grassland near Lisbon, North Dakota

Treatment[a]	Rate (g/ha)	Adults (No./m^2)[b]		
		2002	2003	2004
Imazapic	140	<1c	3c	3c
Imazapic + *Aphthona*	140	1bc	17a	7c
Imazapic	210	0c	6c	6c
Imazapic + *Aphthona*	210	2bc	6c	5c
Quinclorac	840	<1c	7bc	19ab
Quinclorac + *Aphthona*	840	1bc	18a	16ab
Quinclorac	1120	<1c	16a	23a
Quinclorac + *Aphthona*	1120	1bc	22a	16ab
Aphthona	—	5a	17a	18ab
Control	—	1bc	15ab	16b
		$P = 0.0005$	$P = 0.0001$	$P = 0.0001$
		$F = 3.05$	$F = 4.52$	$F = 8.52$

[a] Three hundred fifty *Aphthona* spp. flea beetles per subplot were added on June 27, 2001, and an additional 100 *Aphthona* spp. flea beetles per subplot were added on July 17, 2001. Herbicides were applied on September 15, 2001. The insects were caged until September 2002.
[b] Means followed by the same letter(s) are not significantly different at the 5% level according to Fisher's protected LSD test. $df = 146$.

Table 2
The effect of *Aphthona* spp. flea beetles, herbicide application, or both on leafy spurge density following insect release and herbicide application in 2001 in the habitat of the western prairie fringed orchid within the Sheyenne National Grassland near Lisbon, North Dakota

Treatment[a]	Rate (g/ha)	Leafy spurge density (Stems/m^2)[b]			
		2001	2002	2003	2004
Imazapic	140	104c	7c	28b	35b
Imazapic + *Aphthona*	140	115abc	<1c	23b	34b
Imazapic	210	105bc	1c	9b	38b
Imazapic + *Aphthona*	210	150a	0c	6b	19b
Quinclorac	840	96c	4c	70a	68a
Quinclorac + *Aphthona*	840	132abc	0c	25b	27b
Quinclorac	1120	149ab	3c	54a	60a
Quinclorac + *Aphthona*	1120	107abc	2c	63a	28b
Aphthona	—	150a	41b	28b	37b
Control	—	99c	80a	61a	39b
		$P = 0.0069$	$P = 0.0001$	$P = 0.0001$	$P = 0.0001$
		$F = 2.36$	$F = 29.66$	$F = 7.22$	$F = 4.74$

[a] Three hundred fifty *Aphthona* spp. flea beetles per subplot were added on June 27, 2001, and an additional 100 *Aphthona* spp. flea beetles per subplot were added on July 17, 2001. The insects were caged until September 2002. Herbicides were applied on September 15, 2001 and leafy spurge stem density evaluated in July of each year.
[b] Means followed by the same letter(s) are not significantly different at the 5% level according to Fisher's protected LSD test. $df = 146$.

Leafy spurge control with *Aphthona* alone or combined with imazapic or quinclorac was similar 2 years after release (2003) and was better than quinclorac applied alone (Table 2). In general, leafy spurge stem density increased from an average of 7 stems/m^2 in 2002 to 34 stems/m^2 in 2003. *Aphthona* spp. alone or following imazapic or quinclorac treatment continued to reduce leafy spurge density in 2004, 4 years after the study was begun (Table 2). *Aphthona* were found feeding on leafy spurge in all plots (Table 1) with more adults in the untreated and quinclorac treated areas than those treated with imazapic.

This is the first reported establishment of *Aphthona* spp. flea beetles in the habitat of the WPFO. Although the evaluation of this study will continue for several more years, the results to date indicate the combination of herbicides with *Aphthona* could provide rapid leafy spurge reduction using herbicides and with the reduction enhanced and maintained using the biological agent. As depicted in Fig. 1, the herbicide treatment quickly controlled leafy spurge and temporarily prevented the weed from further invasion of orchid habitat.

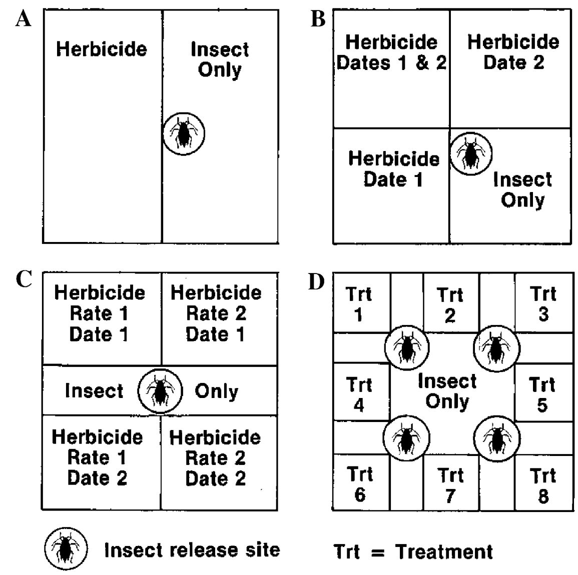

Fig. 3. Field designs for evaluating integration of herbicide treatments with biological control agents. Grazing or fire management, incorporation with revegetation or fertility programs, or with other biological agents could be adapted to these designs. From Messersmith and Adkins (1995). Reprinted with permission.

4. Summary and recommendations

Long-term leafy spurge control has been most successful when more than one method was used to control the weed. Incorporation of biological control agents with herbicides resulted in a more rapid and higher level of control compared to either method used alone. The *Aphthona* spp. population often has increased rapidly following application of herbicides. Herbicides also have been used to increase the usefulness of a control agent, such as when applied in conjunction with *S. esulae* to reduce stem density in concert with reduced seed production by the gall midge.

Grazing leafy spurge with sheep or goats will reduce leafy spurge production but not necessarily density unless *Aphthona* spp. flea beetles are also introduced. This combination has been used to control leafy spurge in riparian areas and shelter belts. Controlled burns are used to manage plant communities and can be used with biological control agents as long as the fire is properly timed with the insect life-cycle. Fire can also be used to increase the establishment of agents, especially in marginally suitable habitats.

The least successful integrated program has been incorporation of leafy spurge biological agents with revegetation programs. The intensive tillage and often repeated use of herbicides to ensure establishment of seeded grasses and forbs have not been compatible with biological agents. In this situation, insects may best be used after the desirable species are seeded and established and leafy spurge begins regrowth, although more work is needed to verify the effectiveness of insects in such settings.

Biological control agents used with other weed control methods such as herbicides may be additive, antagonistic, complementary, or synergistic. Experimental designs to evaluate the usefulness of integrated control methods with herbicides have been suggested by Messersmith and Adkins (1995). The authors described a series of experimental designs to incorporate insect biological control agents with herbicides (Fig. 3). These designs could easily be adapted for use in evaluation of integrating mechanical (revegetation, fertilization), cultural (grazing, fire) or other biological (insect or pathogen) control methods with previously established insects. A key feature of each design is the insect is released in an untreated area, which allows preservation of the agent if the treatment is antagonistic.

The overall goal of most weed control programs is a rapid and economical treatment that results in complete control of the weed with minimal effect on non-target species. An integrated approach of biological control with other methods adapted for a specific weed and environment has a much higher likely hood of long-term success than any single method used alone.

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