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A PROPOSED WEED CONTROL PROGRAM FOR F. E. WARREN AIR FORCE BASE

Prepared for the
U.S. Air Force,
90 CES/CEVN
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I. OVERVIEW

INTRODUCTION

The riparian zones on F.E. Warren Air Force Base support a population of the rare Colorado butterfly plant (Gaura neomexicana ssp. coloradensis), a candidate for listing as a threatened species under the Endangered Species Act. Given this status, Base resource managers have been careful to avoid activities that might harm the butterfly plant's population. Unfortunately, the riparian zones also support large populations of two noxious weeds, Canada thistle (Cirsium arvense) and leafy spurge (Euphorbia esula). The Base resource managers are required by Wyoming state law (and principles of good resource management) to control the populations of these weeds. Careful control of the weeds may also benefit the butterfly plant by reducing competition from the dense weeds for space and light.

The Colorado butterfly plant grows in patches in the riparian zones of three creeks on the Base. Individuals of the butterfly plant grow as low rosettes for several years, then bolt and flower, and die. Annual census of flowering butterfly plants on the Base has given resource managers good knowledge of the distribution of the plant and of annual fluctuations in the numbers of flowering plants. Although the habitat of the butterfly plant is slightly wetter than the area supporting much of the thistle and leafy spurge infestations, the distributions of the butterfly plant, the thistle, and the leafy spurge overlap. Consequently, weed control efforts on the Base must be done cautiously to avoid harm to the butterfly plants.

The problem for the Base resource managers is implementing a weed control program that satisfies the following criteria:

(1) The populations of leafy spurge and Canada thistle should be reduced, both in the density of plants and the area they occupy.

(2) The weed control practices should not decrease the number of Colorado butterfly plants or the area they cover and should, if possible, increase the number of plants or the area they cover (or both).

(3) The weed control program can rely on mechanical and chemical control practices in the short term but should, to the extent possible, rely primarily on biological control in the long term.

WEED CONTROL CONSIDERATIONS

Canada thistle infestations in rangeland can be effectively controlled with several herbicides (Whitson and Miller 1989, Lym and Zollinger 1995a), especially formulations of glyphosate applied in the fall and clopyralid (Stinger) applied in the summer (Whitson and Miller 1989). No effective, practical biological control agents have been found for Canada thistle (Lym and Zollinger 1995a).

Control of leafy spurge has proven more difficult than control of Canada thistle. The herbicides that suppress leafy spurge, especially picloram (Tordon) and glyphosate + 2,4-D (Landmaster BW) (Hollingsworth 1995, Lym and others 1993) cannot be used in riparian areas like those at Warren Air Force Base. Biological control of leafy spurge by flea beetles (Aphthona spp., especially A. nigriscutis) has proven effective in several states, including Wyoming (Hollingsworth 1996). Flea beetles (Aphthona spp.) and the leafy spurge gall midge (Spurgia esulae) have been released on the Base in the past several years, but apparently the flea beetles have failed to establish a population (George Hittle, personal communication to Charla Hollingsworth). Unfortunately, weed control efforts directed at Canada thistle, such as herbicide application and cultural practices like burning, suppress the establishment of flea beetle populations (Lym and Zollinger 1995a) and thus reduce the effectiveness of biological control of spurge.

PROPOSED STUDIES

We propose a program comprising three studies and aimed at control of Canada thistle and leafy spurge, the two weeds that grow in close association with the Colorado butterfly plant on the Base. Study #1 is an experiment to see what effect a herbicide control effort for Canada thistle has on the butterfly plant. Although several widely-accepted, herbicide-based methods have proven effective in controlling Canada thistle (Whitson and Miller 1989, Lym and Zollinger 1995a), we recommend an experimental approach to make sure that those control methods have no adverse effects on the butterfly plants. In the experiment that we propose, part of the thistle infestation growing in the wettest parts of the riparian areas on the Base will go untreated until the Base resource managers determine that the control strategy can be safely applied throughout the distribution of the butterfly plant.

In study #2, we propose that one of the widely-accepted thistle control strategies be applied to the part of the thistle infestation growing without the butterfly plants. During preliminary work on this proposal, we discussed various experiments to test different thistle control strategies, but we have changed our recommendation in this final report for two reasons. First, the part of the thistle infestation growing in the drier riparian areas, without butterfly plants, presents a straightforward problem in weed control for which successful strategies are known. Second, we feel that most of the effort and expense in the weed control program should be directed at controlling the weeds growing with the butterfly plant. Consequently, although the large thistle infestation growing on the Base presents an opportunity for testing new control strategies, we suggest that the Air Force direct most of its efforts toward protecting the butterfly plant. If the Base resource managers would like to experiment with different thistle control strategies, we can help them design an experiment.

For the third study, we are proposing that flea beetles be released to control the leafy spurge infestation on the Base. This study also will apply a well-known, effective strategy, and we see no need for an experiment, although we strongly recommend that the flea beetle control effort be monitored.

The three studies are described below.

II. STUDY #1: AN EXPERIMENT TO DETERMINE THE EFFECTS ON COLORADO BUTTERFLY PLANT AND ON CANADA THISTLE OF HERBICIDES APPLIED IN AREAS WHERE THE COLORADO BUTTERFLY PLANT GROWS.

A. Designing the study.

1. What is the problem? How did the problem arise? What are the hypotheses?

<u>The Problem</u>

Study #1 seeks to test the effects on the butterfly plants of herbicide treatments for Canada thistle in weed patches containing butterfly plants or growing in potential butterfly plant habitat. This study will address a major question facing resource managers at WAFB: can Canada thistle be controlled without harm to the population of Colorado butterfly plant? The experiment will be conducted in the wetter riparian areas on the Base, where butterfly plant and Canada thistle grow together with little leafy spurge. The effectiveness of the treatments in controlling Canada thistle in the weed patches that contain no butterfly plants is considered in study #2.

Assumptions: (1) Throughout most of the riparian zone where the butterfly plant grows, the proximity of open water or the presence of a high water table preclude the use of any herbicide other than Rodeo (a formulation of glyphosate). (2) The treatment used in this study will have little effect on leafy spurge, and may suppress the populations of flea beetles released to control leafy spurge (study #3), so we will restrict this study to areas with little leafy spurge.

<u>The Hypotheses:</u> (1) The change in the number of Colorado butterfly plant individuals (rosettes and flowering plants) in areas treated for weeds will not be different than the change in the number of Colorado butterfly plant individuals on untreated areas. (2) The changes in the amount of canopy cover of Canada thistle in areas treated for weeds will not be different than the

changes in the amount of thistle canopy cover in untreated areas.

2. What are the experimental units?

The experimental units will be patches of Canada thistle with butterfly plants in the riparian zones of Diamond Creek and Crow Creek (Figure 1). Each experimental unit (treatment unit or control unit) will be an area covering ca. 1000 square meters (30 m x 30 m) within a thistle patch. The experimental units will be delineated to be as similar to one another in the number of butterfly plants and in the amount of canopy cover of Canada thistle and leafy spurge canopy cover as possible, according to visual estimate.

3. What will be measured in each experimental unit?

The measurements in each experimental unit will be the estimated number of butterfly plant rosettes (in three size classes), the estimated amount of canopy cover of Canada thistle, the estimated amount of canopy cover of all other plant species (perhaps by life-form), and the estimated amount of litter and bare ground.

We will test hypothesis 1 by comparing the change over the life of the experiment in the estimated number of butterfly plant rosettes and flowering plants in the treatment units with the change in the estimated number of rosettes and flowering plants in the control units. We will test hypothesis 2 by comparing the change over the life of the experiment in the estimated amount of canopy cover of Canada thistle in the treatment units with the change in the estimated thistle canopy cover in the control units. The estimates of canopy cover of all other species, and the estimates of litter and bare ground, will be used to see if reduction of Canada thistle canopy produces an increase in cover of other plants that then compete with the butterfly plant.

4. How are the measurements to be made? Will the measurements be correlated? Specifically, are repeated measurements being made on experimental units?

For all of the measurements, the estimate for each experimental unit will be made by recording data from sampling plots and calculating the mean from those data.

Specifically, the estimate of the number of butterfly plant rosettes and flowering plants in the experimental unit will be made by counting the butterfly plants in the sampling plots and calculating the mean number of rosettes (in each of three size classes) and flowering plants from the counts in the sampling plots. Similarly, the estimate of the thistle canopy cover in each experimental unit will be made by recording (either from visual estimate or from point sampling frames) the canopy cover within the sampling plots and calculating the mean cover from those data. The estimated canopy cover of all other species in each experimental unit will be made in the same way: canopy cover will be recorded in the sampling plots (either by visual estimate or from point sampling frames) and the mean cover calculated for the experimental unit.

The estimated amount of litter cover and bare ground in each experimental unit will also be made by recording the amount of litter and bare ground in the sampling plots, and calculating the means.

If possible, we will use the same sampling plots for counting butterfly plants, estimating Canada thistle canopy cover, and estimating canopy cover of other species. Whether we can do so depends on the variance in the measurements of butterfly plant and canopy cover. The sampling plots will measure ca. 0.5 meter x 2 meters and will be oriented with the long axis perpendicular to the nearest stream channel.

Data will be collected from the sampling plots after mid-summer, when the canopy cover of Canada thistle and other plants has reached its maximum extent and the Colorado butterfly plants that are going to flower have bolted.

All of the measurements will be made in the same sampling plots in the same experimental units each year through the life of the experiment, to examine the effect of weed treatment over time. Hence, the measurements will be correlated.

5. Are the <u>target</u> population and the population to be <u>sampled</u> the same? The target population and the sampled population of Colorado butterfly plant are the same. The target population and sampled populations of Canada thistle are also the same.

6. What method of sampling is to be used? (Simple, stratified, etc.) Sampling will be simple.

7. What treatments (factors) are to be compared? What levels (ranges) of the factors are deemed necessary and sufficient?

Treatment: The plant overstory in the treatment units will be burned in the early spring and herbicide applied to some units afterward. There will be three levels of treatment: no treatment = 0, burning only = 1, burning plus herbicide = 2.

Details: Glyphosate (Rodeo) will be used as the herbicide, and it probably will be applied by wick in the spring when the Canada thistle is taller than the Colorado butterfly plants (and, perhaps, other species). Glyphosate is most effective in controlling Canada thistle if applied during late summer, after August 20th but before killing frost (Whitson and Ferell (1989);

Steve Miller, personal communication to Charla Hollingsworth). Nevertheless, we feel constrained to use spring application in this case because glyphosate is a non-selective herbicide that will kill all plants that it contacts (including butterfly plants), so it should be used in the spring before the butterfly plants bolt. During the first year of the study, we will observe the vegetation to see if Canada thistle is enough taller than the flowering butterfly plants and other plants in late summer that glyphosate can be applied then, when it will be more effective.

Formulations of the herbicide clopyralid act selectively on thistle and hence would have a smaller effect on the non-target plants, but these herbicides leach easily and cannot be used in areas with high water tables (Whitson et al. 1989), so they are unsuitable for this experiment.

The surfactant (if any) to be used with Rodeo, the concentration of herbicide to be used in the wick applicator, and the type of applicator to be used must still be determined.

8. What <u>covariates</u> (quantitative factors) are to be measured?

The amount of canopy cover of plants other than Canada thistle is a covariate. Reduction in the cover contributed by thistle may allow the cover of other plants to increase and suppress the germination or growth of butterfly plants.

9. Are homogeneous units available in sufficient quantities to allow blocking on the qualitative factors?

Individual thistle stands on the insides of meanders on Diamond Creek and in meadows on Crow Creek (Figure 1) may be large enough that we can divide each stand into three experimental areas, and thus block the treatments. If each experimental area is to measure ca. 30 m x 30 m with a 5 meter-wide buffer on each side, then a thistle patch must measure ca. 120 m x 120 m to contain three experimental units. If we cannot find thistle patches large enough, then we will place the experimental units in different patches and the treatments will not be blocked.

10. What are the budget limitations in time and money? Are estimates of the cost per observation and time per observation available?

<u>Time per unit</u>

Following are estimates of the amount of time required for various steps in the experiment.

- In one year -

(1) Choosing the experimental units: 12 units, 4 units/day for a 2-person crew, 3 days total, (1/2 person-day/unit, 6 person-days total)
(2) Locating and reading the sampling plots for numbers of Colorado butterfly plants, cover of Canada thistle and other weeds, and cover of litter and bare ground, in all experimental units: 12 units, 1 unit/day for a 2-person crew, 12 days total for a 2-person crew; (2 person-days/unit, 24 person-days total)
(3) Burning the treatment units: 8 units, 2 units/day for a 4-person crew, 4 days total for a 4-person crew; (2 person-days/unit, 16 person-days total)
(4) Applying herbicide: 4 units, 2 units/day for a 2-person crew, 2 days total (1 person-day/unit, 4 person-days total)

- Entire experiment _

-- Control unit (treatment level 0): 8.5 person-days/unit.
-- Burn only units (treatment level 1): 14.5 person-days/unit.
-- Herbicide units (treatment level 3): 17.5 person-days/unit.

These are the assumptions we are using in estimating the total time per experimental unit through the life of the experiment: the experiment will run for four years (see part C. below); step 1 must be done only during the first year; step 2 must be done during all four years; and steps 3 and 4 must be done during years two through four.

Cost per unit

The costs per experimental unit are being calculated.

11. What precision is desired? Are prior estimates (guesses) of the variance available?

Precision

For all of our estimates (number of Colorado butterfly plant rosettes in each of three size classes, number of flowering butterfly plants, cover of Canada thistle, cover of other plants, cover of litter, and amount of bare ground), we want a level of precision, expressed in terms of the standard error of the mean of each measure for each experimental unit, of

(Std error of the mean/mean) ≤ 0.1.

Variance

The following ranges in numbers of flowering butterfly plants in each census area are from annual censuses from 1989 through 1995. The original data are in Fertig (1996). Figure 1 shows the census areas.

Crow Creek census areas

Area	Range	Area	Range
1	0	17	0 to 15
2	0	18	1 to 145
3	4 to 33	19	10 to 112
4	1 to 29	20	7 to 78
5	0 to 31	21	2 to 48
6	4 to 90	22	16 to 129
7	59 to 304	23	52 to 236
8	0 to 42	24	0 to 28
9	4 to 366	25	0 to 27
10	14 to 378	26	0 to 26
11	0 to 15	27	0 to 4
12	14 to 194	28	0 to 37
13	16 to 586	29	0 to 4
14	10 to 423	30	0 to 28
15	0 to 26	31	0 to 8
16	29 to 566	32	0 to 10

Diamond Creek census areas

From 1989 through 1993, the numbers of flowering plants from the north side and south side of the creek in each Diamond Creek census area were reported together. In 1994 and 1995, the numbers were reported separately for the north side and south side of the creek in each area.

Area	Range
1	Total 207 to 1499
	South 322 to 1093
	North 406 to 976
2	Total 405 to 1267
	South 209 to 601
	North 145 to 1058
3	Total 561 to 2359
	South 263 to 1922
	North 437 to 760

4	Total 275 to 786 South 138 to 557 North 229 to 390
5	Total 3 to 37 South 0 to 12 North 0 to 11

Unnamed drainage census areas

Area	Range
1	84 to 855
2	650 to 1027

We have no estimates of the variance in the number of butterfly plant rosettes.

The following estimates of Canada thistle density (plants/m^2) and 95% confidence intervals were calculated by Floyd (1995) for preliminary thistle control experiments in the area. Each estimate is the mean from thirty-two 1-m^2 plots.

Treatment	Mean Density (stems/m^2)	Approx. 95% C.I.
1	55.31	48 to 67
2	51.31	47 to 55
3	55.86	50 to 62
4	48.34	41 to 55

We have no estimates of the variances in canopy cover of other species, litter cover, or bare ground.

12. What differences between control areas and treatment areas will be of practical importance (as opposed to statistical significance)?

Two results will be considered of practical importance. (1) If the numbers of Colorado butterfly plants decline more in the treatment areas than they do in the control areas, we will consider that to be an effect of the weed control treatments of practical importance. (2) We will consider a 25% reduction in Canada thistle cover in the treatment units by the end of the experiment as being of practical importance.

13. What sample size is to be taken within the bounds of cost, time, and desired precision? Our desired sample size is four experimental units for each of three levels of the treatment (control, burning, burning + herbicide), for a total of twelve experimental units. Estimates of costs may require that we reduce the sample size to three experimental units for each treatment level.

14. What probes (checks, pilot studies, preliminary analysis) can be included in the study to provide advance

a. information on unforeseen problems with employees, instructions, coding, sources of variation, etc? Should the study be aborted or continued?

Annual census of Colorado flowering butterfly plants (Fertig 1995) has shown that observers can be trained readily to recognize and count flowering butterfly plants. Training of field crews early in the study will be necessary to assure consistency in: (1) counting of butterfly plant rosettes in each of three size classes, (2) estimating canopy cover of Canada thistle stems and other plants, and (3) estimating cover of litter and bare ground. Double sampling (i.e., having different people take the same measurements in the same sampling plots) can be used early in the experiment to reveal inconsistencies between people and to correct those inconsistencies.

b. estimates of variance, cost per unit, time per unit, and hence adjustments in sample sizes? The work during the first year of the study will show the variance in the data and how much time and expense are required to collect data, and the sample size can be adjusted accordingly.

B. Advance Analysis of the Study

1. What mathematical "model" is to be used? Can the assumptions be justified? If not, how badly are they violated and how robust are the procedures?

Hypothesis 1: The change in the number of Colorado butterfly plant individuals (rosettes and flowering plants) in areas treated for weeds will not be different than the change in the number of Colorado butterfly plant individuals on untreated areas.

This hypothesis will be tested every year of the experiment with an analysis of covariance of the form:

$$y_{ijk} = u + L_i + W_j + U_k + B(x_{ijk} - x_{...}) + (LW)_{ij} + (LU)_{ik} + (WU)_{jk} + (LWU)_{ijk} + e_{ijk}$$

where:

y_{ijk} = the estimated number of butterfly plants of life-form i, under weed treatment j, in experimental unit k

u = the overall mean number of butterfly plants per experimental unit

L_i = the life-form of butterfly plant (i = 0 for small rosette, i = 1 for medium-sized rosette, i = 2 for large rosette, i = 3 for flowering plant)

W_j = the effect of weed treatment i (i = 0 for no treatment, i = 1 for burning only, i = 2 for burning + herbicide)

U_k = the effect of census area j (j = 1 for area 1, 2 for area 2, etc.)

B = the coefficient of linear regression of the density of butterfly plants on the canopy cover of other plants (i.e., the covariate)

x_{ijk} = the measurement of the canopy cover of other plants (i.e., the covariate) corresponding to y_{ijk}

$x_{...}$ = the overall mean cover of other species in all the sampling plots

$(LW)_{ij}$ = the interaction between life form i and weed treatment j

$(LU)_{ik}$ = the interaction between life-form i and experimental area k

$(WU)_{jk}$ = the interaction between weed treatment j and census area k

$(LWU)_{ijk}$ = the interaction between life-form i, weed treatment j, and census area k.

e_{ijk} = random error.

The analysis of variance will be most straightforward if the same number of experimental units is assigned to each of the three treatment levels, and the same number of sampling plots is used in each experimental unit (that is, if the design is balanced).

Because the effect of time cannot be included in the analysis of variance model, the effect of the weed treatments on butterfly plant numbers over time will be investigated with a linear regression analysis of the change in butterfly plant numbers with time. The form of the model is being worked out.

<u>Hypothesis 2:</u> The changes in the amount of canopy cover of Canada thistle in areas treated for weeds will not be different than the changes in the amount of thistle canopy cover in untreated areas.

This hypothesis will be tested every year of the experiment with an analysis of variance of the form:

$$y_{ij} = u + W_i + U_j + (WU)_{ij} + e_{ij}$$

where:

y_{ij} = the canopy cover of Canada thistle in experimental unit j with the i^{th} weed treatment

u = the overall mean thistle canopy cover per experimental unit

W_i = the effect of weed treatment i (i = 0 for no treatment, i = 1 for burning only, i = 2 for burning + herbicide)
U_j = the effect of experimental unit j (j = 1 for area 1, 2 for area 2, etc.)
$(WU)_{ij}$ = the interaction between weed treatment i and experimental unit j
e_{ij} = random error.

Because the effect of time cannot be included in the analysis of variance model, the effect of the weed treatments on Canada thistle canopy cover over time will be investigated with a linear regression analysis of the change in thistle cover on time.

C. Schedule for study #1

We assume that the experiment will run for four years, as follows:

Year 1

Choose the 12 experimental units: 3 days for a 2-person crew, in early summer after the butterfly plants are out.

Locate and read the sampling plots: 12 days for a 2-person crew, in late summer.

Preparation for field work (procuring supplies, making data forms, etc.): 3 days for one person.

Post-field work (data analysis, report writing): 7 days for one person

Years 2 and 3, each

Burn the treatment units: 2 days for a 4-person crew, in the spring.

Apply herbicide to 4 experimental units: 2 days for a 2-person crew.

Locate and read the sampling plots: 12 days for a 2-person crew, in late summer.

Preparation for field work (procuring supplies, making data forms, etc.): 3 days for one person.

Post-field work (data analysis, report writing): 7 days for one person.

Year 4

Burn the treatment units: 2 days for a 4-person crew, in the spring.

Apply herbicide to 4 experimental units: 2 days for a 2-person crew.

Locate and read the sampling plots: 12 days for a 2-person crew, in late summer.

Preparation for field work (procuring supplies, making data forms, etc.): 3 days for one person.

Post-field work (data analysis, report writing): 14 days for one person.

III. STUDY #2: MONITORING THE EFFECTIVENESS OF CANADA THISTLE CONTROL IN AREAS FREE OF COLORADO BUTTERFLY PLANT.

A. Designing the study.

1. What is the problem? How did the problem arise? What hypotheses does he or she have in mind?

The Problem

Canada thistle (Cirsium arvense) and leafy spurge (Euphorbia esula), two noxious weeds, grow on F. E. Warren Air Force Base, in the riparian zones of three creeks. Those riparian zones also support a population of the rare Colorado butterfly plant (Gaura neomexicana ssp. coloradensis), a candidate for listing as a threatened species under the Endangered Species Act. Given this candidate status, the resource managers on the Base have been careful to avoid activities that might harm the butterfly plants, so weed control efforts have been suspended for several years, during which time the weeds have flourished.

Although the distributions of the butterfly plant, Canada thistle, and leafy spurge overlap, large patches of both weeds grow with no butterfly plants and so can be treated using standard, accepted weed control practices and monitoring, with little risk to the butterfly plants. The point of study #2, under consideration here, is to monitor the results of a widely-accepted method for controlling Canada thistle -- fall application of glyphosate -- in those weed patches that contain no butterfly plant, to see if the method provides acceptable control or if another method should be tried. The effects of Canada thistle control in areas containing butterfly plant is the subject of the separate study #1.

The following alternative weed treatments have been suggested by weed scientists Steve Miller (University of Wyoming Department of Plant, Soil, and Insect Sciences) and John Randall (The Nature Conservancy's Wildland Weed Control Program) as possibilities for an experiment to test the effectiveness of different treatments. They are mentioned here in case the Base resource managers want to try several treatments, and in case fall application of glyphosate proves ineffective.

-- Fall burning versus spring burning to remove the dead plant overstory, followed by late-summer application of glyphosate
-- Spring application of glyphosate followed by application of triclopyr (Garlon)
-- Burning versus mowing to remove the dead plant overstory, followed by spot application of clopyralid plus 2,4-D (Curtail, a proven thistle control herbicide)

<u>The Hypothesis</u>: The change in the amount of Canada thistle canopy cover in areas subject to the weed control treatment will not differ from the change in canopy cover in untreated areas.

Assumptions: (1) Large stands of Canada thistle on the Base contain no butterfly plant. (2) The presence of leafy spurge will prevent us from using fire to remove the dead plant overstory in some areas.

2. What are the experimental units, and what <u>exactly</u> is to be measured within each experimental unit?

The experimental units will be areas of Canada thistle, each unit relatively homogenous for thistle density and containing as little leafy spurge as possible. Each unit will cover several hundred square meters. Our main interest in this study is the control of Canada thistle, so we will look primarily for Canada thistle patches with relatively little leafy spurge as our experimental units. The control of leafy spurge is the subject of study #3.

The measurement on each experimental unit will be an estimate of the amount of canopy cover of Canada thistle.

3. How are the measurements to be made? Will the measurements be correlated? Specifically, are repeated measurements being made on experimental units?

For each experimental unit, the estimate of the amount of Canada thistle canopy cover will be made by recording (either from visual estimate or from point sampling frames) the canopy cover within sampling plots and calculating the mean cover from those data. The hypothesis will be tested by comparing the change in the amount of canopy cover in the treatment units to the change in the control units.

The sampling plots will measure ca. 0.5 meter x 2 meters. Data will be collected from the sampling plots after mid-summer, when the canopy cover of Canada thistle has reached its maximum extent. The measurements will be made on the same sampling plots in the same experimental units each year through the life of the experiment, to examine the effect of weed treatment over time. Hence, the measurements will be correlated.

4. Are the <u>target</u> population and the population to be <u>sampled</u> the same? The target population and the sampled population of Canada thistle are the same.

5. What method of sampling is to be used? (Simple, stratified, etc.) Sampling will be simple.

6. What treatments are to be compared?

<u>Treatment:</u> The plant overstory in the treatment units will be burned in the early spring and herbicide applied to some units afterward. There will be two levels of treatment: no treatment = 0, burning plus herbicide = 1.

Details: Because the herbicide will be applied with a wick, the dead overstory must be removed from the treatment units for the herbicide to reach the thistles. For the herbicide, we suggest a glyphosate formulation (probably Rodeo), applied during late summer, after August 20th but before killing frost (24°F) (Whitson and Ferell (1989). We suggest Rodeo because it is approved for use in areas near water (Whitson et al. 1989). We assume that the Canada thistle plants will be taller than the associated species then. The type of wick applicator and the concentration of herbicide to be used must still be determined.

7. What <u>covariates</u> (quantitative factors) are to be measured?
None

8. Are homogeneous units available in sufficient quantities to be able to block on the qualitative factors? Patches of Canada thistle may be large enough to allow the placement of at least two experimental units in a patch. If so, the treatments will be blocked.

9. What are the budget limitations in time and money? Are estimates of the cost per observation and time per observation available?

<u>Time per unit</u>

Following are estimates of the amount of time required for various steps in the study.

- In one year -

(1) Choosing the experimental units: 8 units, 2 units/day for a 2-person crew, 4 days total, (1 person-day/unit, 8 person-days total)
(2) Locating and reading the sampling plots for cover of Canada thistle in all experimental units: 8 units, 2 units/day for a 2-person crew, 4 days total for a 2-person crew; (1 person-day/unit, 8 person-days total)
(3) Burning the treatment units: 4 units, 2 units/day for a 4-person crew, 2 days total for a 4-person crew; (2 person-days/unit, 8 person-days total)
(4) Applying herbicide to the treatment units: 4 units, 4 units/day for a 2-person crew, 1 day total (0.5 person-day/unit, 2 person-days total)

- Entire study -

-- Control unit (treatment level 0): 4 person-days/unit.
-- Treatment units (treatment level 3): 11.5 person-days/unit.

These are the assumptions we are using in estimating the total time per experimental unit through the life of the study: the study will run for three years (see part C. below); step 1 must be done only during the first year; step 2 must be done during all three years; and steps 3 and 4 must be done during years two and three.

Cost per unit

The costs per experimental unit are being calculated.

10. What precision is desired? Are prior estimates (guesses) of the variance available?

Precision

The level of precision we want, expressed in terms of the mean thistle canopy cover per census area (estimated as the mean from the sampling plots), is:

(Std error of the mean/mean) $\leq$ 0.2.

Variance

No estimates of the variance in Canada thistle canopy cover are available, but Floyd (1995) presented the following estimates of thistle density and 95% confidence intervals. Each estimate is the mean from thirty-two 1-m^2 plots.

Treatment	Mean Density (stems/m^2)	Approx. 95% C.I.
1	55.31	48 to 67
2	51.31	47 to 55
3	55.86	50 to 62
4	48.34	41 to 55

11. **What "differences" will be of practical value (as opposed to statistical significance)?** A reduction of 25% in weed density by the end of the study will be considered of practical value.

12. **What sample size is to be taken within the bounds of cost, time, and desired precision?** Our desired sample size is four control units and four treatment unit, for a total of six experimental units. Estimates of costs may show that we can increase the sample size to four control units and four treatment units.

13. **What probes (checks, pilot studies, preliminary analysis) can be included in the study to provide advance**

a. information on unforeseen problems with employees, instructions, coding, sources of variation, etc? Should the study be aborted or continued? The study design and the methods can be changed at any time.

b. estimates of variance, cost per unit, time per unit, and hence adjustments in sample sizes? The work during the first year should tell us whether we need to adjust the sample size.

B. Advance Analysis of the Study

1. **What mathematical model is to be used? Can the assumptions be justified? If not, how badly are they violated and how robust are the procedures?**

The hypothesis will be tested with the two-factor analysis of variance of the form

$$y_{ij} = u + W_i + U_j + e_{ij}$$

where

y_{ij} = the thistle canopy cover in experimental unit j with the i^{th} weed treatment

u = the overall mean thistle canopy cover per experimental unit

W_i = the effect of weed treatment i (i = 0 for no treatment, i = 2 for burning + herbicide)

U_j = the effect of experimental unit j (j = 1 for area 1, 2 for area 2, etc.)

e_{ij} = random error.

2. Is the analysis available? If not, is someone able and willing to provide it? The analysis is readily available.

C. Schedule for study #2

We assume that the experiment will run for three years, as follows:

Year 1

Choose the 8 experimental units: 2 days for a 2-person crew, in early summer after the butterfly plants are out.

Locate and read the sampling plots: 4 days for a 2-person crew, in late summer.

Preparation for field work (procuring supplies, making data forms, etc.): 3 days for one person.

Post-field work (data analysis, report writing): 7 days for one person

Year 2

Burn the treatment units: 2 days for a 4-person crew, in the spring.

Apply herbicide to the treatment units: 1 day for a 2-person crew.

Locate and read the sampling plots: 4 days for a 2-person crew, in late summer.

Preparation for field work (procuring supplies, making data forms, etc.): 3 days for one person.

Post-field work (data analysis, report writing): 7 days for one person.

Year 3

Burn the treatment units: 2 days for a 4-person crew, in the spring.

Apply herbicide to the treatment units: 1 day for a 2-person crew.

Locate and read the sampling plots: 4 days for a 2-person crew, in late summer.

Preparation for field work (procuring supplies, making data forms, etc.): 3 days for one person.

Post-field work (data analysis, report writing): 14 days for one person.

IV. STUDY #3: A PROGRAM TO MONITOR THE CONTROL OF LEAFY SPURGE BY FLEA BEETLES.

A. Designing the study.

1. What is the problem? How did the problem arise?

Flea beetles (Aphthona spp.) and the leafy spurge gall midge (Spurgia esulae) have been released on F. E. Warren Air Force Base in the past several years to control leafy spurge, but apparently the flea beetles have failed to establish a population on the Base, and no formal monitoring has been done to document the effects the insects have had on leafy spurge (George Hittle, personal communication to Charla Hollingsworth). The Base resource managers need information from a systematic monitoring plan to judge whether flea beetles are an effective spurge control method on the Base.

The task in this study is to design and implement a program to monitor the establishment of a population of black-dot flea beetles (Aphthona nigriscutis) on Base and their effectiveness in reducing the population of leafy spurge.

The monitoring of flea beetles is being done as a separate study because the overstory removal treatments and the herbicides used in the other studies may suppress the flea beetles used in this study (Lym and Zollinger 1995b).

2. What are the study units, and what exactly is to be measured within each study unit?

The study units will be areas of dense leafy spurge, each covering several hundred square meters, within which the flea beetles are released. These study units will contain little Canada thistle and hence will be excluded from studies #1 and #2.

The measurements made within each study unit will be an estimate of the canopy cover of leafy spurge and an estimate of the numbers of flea beetles. Photographs will be taken in each study area to show the leafy spurge stand.

3. How are the measurements to be made? Will the measurements be correlated? Specifically, are repeated measurements being made on experimental units?

Within each study unit, the number of flea beetles will be estimated by the sweep-net collection procedure described by Hollingsworth (1996, p. 7-8). This procedure will allow semiquantitative comparisons of the size of the flea beetle population from year to year.

The canopy cover of leafy spurge in each study unit will be estimated by recording the canopy cover within sampling plots (either from visual estimate or from point sampling frames) and calculating the mean cover from those data. The sampling plots will measure ca. 0.5 meter x 2 meters. Data will be collected from the sampling plots after mid-summer, when the canopy cover of leafy spurge has reached its maximum extent. The measurements will be made on the same sampling plots in the same experimental units each year through the life of the study, to examine the effects of the flea beetles over time. Hence, the measurements will be correlated.

The photographs of each study unit will be made according to the procedure described by Hollingsworth (1996, p. 7).

4. Are the target population and the population to be sampled the same? The target population and the sampled population of leafy spurge are the same.

5. What method of sampling is to be used? (Simple, stratified, etc.) Sampling will be simple.

6. What treatments (factors) are to be compared? What levels (ranges) of the factors are deemed necessary and sufficient?

The only treatment will be the release of black-dot flea beetles (Aphthona nigriscutis) in leafy spurge patches. We assume that the flea beetles will spread from the points of release into the rest of the leafy spurge patches on the Base, so we doubt that we can designate untreated areas to use as controls in testing the effectiveness of the beetles. Furthermore, beetles (and leafy spurge gall midges) have already been released on the Base, and we can't be sure which leafy spurge patches are free from beetles.

7. What covariates are to be measured? None.

8. Are homogeneous units available in sufficient quantities to be able to block on the qualitative factors? There will be no blocks.

9. What are the budget limitations in time and money? Are estimates of the cost per observation and time per observation available?

Time per unit

Following are estimates of the amount of time required for various steps in the study.

- In one year -

(1) Choosing the study units: 6 units, 3 units/day for a 2-person crew, 2 days total, (0.67 person-day/unit, 4 person-days total)
(2) Trapping the flea beetles: The closest trapping location for flea beetles probably is Fremont County. Trapping enough beetles to release in all 6 unites will require ca. 2.5 days of travel for one person.
(3) Releasing the flea beetles: 6 units, 6 units/day for one person, 1 day total, (0.17 person-day/unit, 6 person-days total)
(4) Locating and reading the sampling plots for cover of leafy spurge: 6 units, 2 units/day for a 2-person crew, 3 days total for a 2-person crew; (1 person-day/unit, 12 person-days total)

- Entire study: 4.2 person-days/study unit plus 2.5 days of travel for one person.

These are the assumptions we are using in estimating the total time per experimental unit through the life of the study: the study will run for three years (see part C. below); step 1 must be done only during the first year, and steps 2, 3, and 4 must be done during all three years.

10. What precision is desired? Are prior estimates (guesses) of the variance available?

In terms of the standard error of the mean canopy cover of leafy spurge per study unit, we would like a precision of:

(Std error of the mean)/(mean) $\leq$ 0.25.

Calculating the precision of the flea beetle population size is impractical, and probably unnecessary in this case.

11. What "differences" will be of practical value (as opposed to statistical significance)? A 25% reduction in leafy spurge density within 3 years will be of practical value.

12. What sample size is to be taken within the bounds of cost, time, and desired precision? We hope to establish six study units in which to release the beetles and monitor the beetle population and the leafy spurge canopy cover.

13. What probes (checks, pilot studies, preliminary analysis) can be included in the study to provide advance
a. information on unforeseen problems with employees, instructions, coding, sources of variation, etc? Should the study be aborted or continued? The results from the first year's work should indicate whether the study needs to be changed.

b. estimates of variance, cost per unit, time per unit, and hence adjustments in sample sizes? We have no information to suggest the variance of leafy spurge canopy cover, or the cost or time per sampling unit needed to estimate leafy spurge density. The results of the first year's work should indicate whether the number of sample sizes should be changed.

B. Advance Analysis of the Study

1. What mathematical "model" is to be used? Can the assumptions be justified? If not, how badly are they violated and how robust are the procedures?

We will do no statistical analysis of data. Instead, we will simply graph the canopy cover of leafy spurge and the number of flea beetles in each study unit in each year of the study, and use the photographs of each study unit to judge qualitatively the change in leafy spurge canopy cover over time.

C. Schedule for study #3

We assume that the study will run for three years, as follows:

<u>Year 1</u>

Choose the 6 study units: 2 days for a 2-person crew, in early summer.

Trap the flea beetles: 2.5 days for one person, in late June

Release the flea beetles: 1 day for one person, late June

Locate and read the sampling plots for cover of leafy spurge: 3 days total for a 2-person crew in late summer.

Preparation for field work (procuring supplies, arranging for beetle collections, making data forms, etc.): 3 days for one person.

Post-field work (data analysis, report writing): 7 days for one person

<u>Year 2</u>

Trap the flea beetles: 2.5 days for one person, in late June

Release the flea beetles: 1 day for one person, late June

Locate and read the sampling plots for cover of leafy spurge: 3 days total for a 2-person crew in late summer.

Preparation for field work (procuring supplies, arranging for beetle collections, making data forms, etc.): 3 days for one person.

Post-field work (data analysis, report writing): 7 days for one person

Year 3

Trap the flea beetles: 2.5 days for one person, in late June

Release the flea beetles: 1 day for one person, late June

Locate and read the sampling plots for cover of leafy spurge: 3 days total for a 2-person crew in late summer.

Preparation for field work (procuring supplies, arranging for beetle collections, making data forms, etc.): 3 days for one person.

Post-field work (data analysis, report writing): 14 days for one person.

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Canada thistle (*Cirsium arvense*) and leafy spurge (*Euphorbia esula*) biological control program as proposed for the Colorado butterfly plant Research Natural Area on F.E. Warren Air Force Base.

A report to the Wyoming Nature Conservancy

by

Charla Hollingsworth

April 1996

INTRODUCTION

The implementation of biological control techniques allows disturbed habitats the luxury of quietly and efficiently containing the spread and proliferation of the habitat's exotic species. While often a useful tool, nature's solutions occur in a time frame often divorced from the rigors of the ecosystem manger's fiscal year or project schedules. Stated simply, this approach takes longer than a "quick technological fix".

Even with time-related drawbacks, biological control organisms can usually out-perform technologically advanced weed control programs in the long-term by their permanent, self-perpetuating characteristics which do not require close managerial supervision.

Leafy spurge (*Euphorbia esula*) has become established at a high population density on F. E. Warren Air Force Base (WAFB). This noxious weed has been found to have many biological predators, of which flea beetles (*Aphthona* spp.) hold the most promise for control. The impact of flea beetle feeding will usually be visible three to five years after insect establishment. The most common symptom of declining plant vigor from feeding pressure is a reduced plant stem density. Unfortunately, roots that are not attacked send up fresh shoots to resupply the plant with sugars for new root reserves. Visibly weakened spurge plants are encouraging to see, but do not preclude possible recovery. The plants will quickly rally if they are not exposed to dependable, perennial feeding pressures. For this reason, it is critical that the habitat have a thriving biological control population and receive yearly monitoring.

Canada thistle (*Cirsium arvense*) has also become established at a high population density on F. E. Warren Air Force Base (WAFB). Research on effective biological control organisms of the noxious weed species is not complete. Initial control of Canada thistle through physical and chemical methods has proven most successful for many land managers which subsequently reduces biological control research momentum. WAFB's Canada thistle infestations should receive biological control organism releases following traditional physical and chemical agricultural weed control methods. Insect releases at this time will help to establish long-term habitat sustainability.

The release of the following natural insect predators for leafy spurge infestations at WAFB are an important part of controlling the noxious weed species.

I. LEAFY SPURGE

APHTHONA CYPARISSIAE

This tan flea beetle has been released by the United States Department of Agriculture, Animal and Plant Health Inspection Services, Plant Protection and Quarantine (USDA-APHIS-PPQ) in Crook, Campbell, Johnson, and Laramie counties, Wyoming between 1989 and 1993. The populations grew as well as *A.*

nigriscutis during the first two years following release, but after reaching a certain point, population growth leveled off and the beetles became less abundant. *A. cyparissiae* failed to recover from its population depression and did not reestablish prior population densities (USDA-APHIS-PPQ, 1995). Two hypotheses exist for this abrupt stall in positive population growth. The first could be due to a quicker and more efficient dispersal pattern than evidenced by other *Aphthona* spp. when exposed to increasing levels of intraspecific competition. The second hypothesis addresses the possibility of sluggish and/or inefficient reproduction.

Adults feed on spurge leaves and flowers while larvae feed in the primary and secondary roots. Reduction of root tissues decreases the plant's ability to absorb water and nutrients. This flea beetle prefers alluvial fans with sandy loam soils higher in moisture and organic matter than tolerated by *A. nigriscutis*, but less than that preferred by *A. flava* (Rees and Spencer, 1993a).

One particular drawback to *A. cyparissiae* is the fact that the beetle's life cycle includes long developmental periods which may reduce its usefulness in climates such as Cheyenne that have inherently short growing seasons (Rees and Spencer, 1993a). Refer to Table 1, at the end of this report, for a brief summary of this insect's life history characteristics.

This particular insect was collected at the Devil's Tower Insectary in June 1995. Insectary sites around the state have now been turned over to respective biological control cooperators for continuing management. The USDA-APHIS-PPQ will no longer be monitoring *A. cyparissiae* sites, but will be available, by request, for assistance on collection days (USDA-APHIS-PPQ, 1995).

<u>*APHTHONA FLAVA*</u>

This copper flea beetle was released recently in Crook and Laramie counties by USDA-APHIS-PPQ, but populations are not increasing as hoped. Monitoring will continue until such time as Wyoming populations stabilize. Collections will not be allowed in 1996, since doing so would place struggling insectaries under additional small population stress (USDA-APHIS-PPQ, 1995). This species, along with other *Aphthona* spp., is currently available through a Bozeman, MT company named BioControl of Weeds. Noah Poritz, owner of the company, has supplied a current price list of the biological controls he offers for sale.

Adult *A. flava* feeding takes place on the leaves and flowers while larval feeding occurs in the primary and secondary roots. This insect favors south facing slopes, eighteen to twenty inches of moisture each year, and sunny locations (Rees, 1993a). Refer to Table 1, at the end of this report, for a brief summary of this insect's life history characteristics.

A. flava has been released annually in Montana since 1985. Consequently, some "Big Sky" areas have been demonstrating large reductions in leafy spurge densities. Unfortunately, Wyoming has not had the same level of success, and is, in fact, still struggling to get this flea beetle to establish successful insectaries.

Indicator plant species for favorable *A. flava* sites include aspen, cottonwood, poplar, willow, and chokecherry (S. Lewis, correspondence).

<u>*APHTHONA LACERTOSA*</u> / <u>*APHTHONA CZWALINAE*</u>

Together these black flea beetles were collected from an insectary in North Dakota for release at new sites. Both species were released in Crook County in 1993 and 1994 with Sheridan, Johnson, Campbell, and Fremont Counties receiving insects for release in 1995 (USDA-APHIS-PPQ, 1995). Populations at the earliest sites (1993 and 1994) are increasing more rapidly than the heretofore successful *A. nigriscutis*. *A. lacertosa* has been determined to be the most populous of the two insects in the black flea beetle mix. The 1993 Cedar Hill insectary in Crook County supported a small 1995 collection and it is hoped that the Crook County insectary will support a substantial increase in collections in 1996 (USDA-APHIS-PPQ, 1995). Beetles should be collected for transport to new sites immediately upon spring emergence.

In a few short years North Dakota populations of the two beetle species have grown and expanded over a three square mile radius. One assumption for the apparent success of the black flea beetles is that they appear to do better than *A. nigriscutis* on slightly wetter sites (USDA-APHIS-PPQ, 1995).

- <u>*APHTHONA LACERTOSA*</u>

Adults have a relatively wide host range in the subgenus *Esula* (Rees and Spencer, 1993c). The mature beetles feed on spurge leaves and flowers while the larvae feed on and in primary and secondary roots. The beetles tolerate high levels of intraspecific competition, so concentrated feeding occurs before the beetles move to another site. As a result of slow relocation tendencies, localized plant growth stunting and foliar chlorosis occur when large insect populations are present.

A. lacertosa prefers mesic-dry to wet sites with well-developed vegetation such as thick grasses. It does not like dry or flooded areas (Rees and Spencer, 1993c). Refer to Table 1, at the end of this report, for a brief summary of this insect's life history characteristics.

- <u>*APHTHONA CZWALINAE*</u>

Adults feed on spurge foliage while larvae feed on and in primary and secondary roots of the plant. Larvae feeding reduces the plant's intake of water and nutrients which lessens its vigor.

This flea beetle prefers moist habitats with high relative humidity where the spurge plants are growing in a densely intermixed plant community with other vegetation (Rees and Spencer, 1993b). Accumulation of deep plant matter and shaded sites do not disturb *A. czwalinae's* feeding and life cycle - much unlike other *Aphthona* species. However, the insects do not respond well when they are either placed directly under trees or in sites of dense, tall stands that are primarily dominated by leafy spurge. *A. czwalinae* is also sensitive to spring flooding (S. Lewis correspondence). Refer to Table 1, at the end of this report, for a brief summary of this insect's life history characteristics.

<u>*APHTHONA NIGRISCUTIS*</u> (Black Dot Leafy Spurge Flea Beetle)

Aphthona nigriscutis has been released in Wyoming annually by the USDA-APHIS-PPQ and other cooperators since 1989. Most of the documented release sites have established large populations today. The first collection conducted in 1993 netted 120,000 insects; in 1994, 350,000 mature beetles were captured; and in 1995 over 700,000 hungry spurge consuming biological control units were distributed from Wyoming collection sites. The general consensus around the state is that over-collection of *A. nigriscutis* from established insectaries is highly improbable in light of rapidly increasing insect populations, and may, in fact, stimulate the productivity of the collection site (B. Shoemaker, personal communication). Beetles should be collected for transport to new sites immediately upon spring emergence.

These insects are currently abundant in Wyoming. This state is supplying flea beetles to South Dakota, Idaho, Washington, and Nebraska. Insectary populations continue to expand rapidly even as thousands of *A. nigriscutis* are annually removed from collection sites.

As local *A. nigriscutis* populations start to decline from limiting food resources, the insects have been documented to fly approximately one mile in search of new leafy spurge patches (USDA-APHIS-PPQ, 1995).

Adult beetle feeding is limited to species in the *Euphorbia esula* complex. Attack of plants by mature beetles is strictly on the leaf and flowers while larvae feed on and in primary and secondary roots. Constant larval feeding pressures reduce the plant's ability to make sugars for root reserves by slowing accumulation of essential nutrients. The subsequent effects on the plant are a delay in flowering, a decrease in height, and a reduced aboveground plant density (S. Lewis, correspondence).

A. nigriscutis' favored habitats are dry, sandy areas free of vegetation shading to allow plenty of direct solar warming of the soil during the day. Peter Harris of Agriculture Canada recommends sites with needle-and-thread or porcupine grasses, flowering spurge stems less than 70 cm tall with fewer than 60 stems/m^2, and a well-drained soil type of 60% sand and less than 3% organic matter (S. Lewis, correspondence). Refer to Table 1, at the end of this report, for a brief summary of this insect's life history characteristics.

A. nigriscutis remains in original release site locations under tremendous levels of intraspecific competition. This unusual social characteristic has been attributed to a hormone which causes the insects to remain in a given location to feed, mate, and oviposit (Rees, Quimby, and Spencer, 1993). Normal emergence of *A. nigriscutis* adults occurs sometime around mid-June. The breeding period for this species begins ten days to two weeks after emergence and extends into the fall season. Early capture from insectaries and immediate release at new sites enable the relocated insects to lay a large number of eggs the first summer in their new habitat.

New *Aphthona* spp. releases, particularly those of *A. nigriscutis*, are susceptible to high levels of interspecific competition by other herbaceous insect species such as grasshoppers. Additionally, immature larvae serve as nutritious prey for large numbers of predatory ants. Therefore, release areas with large numbers of either species of insects should be avoided (S. Lewis, correspondence). It is extremely important that proper release sites be chosen for the most successful flea beetle establishment, since this insect tends to move very little after being released.

HYLES EUPHORBIA (Leafy Spurge Hawk Moth)

Prior to 1989, the leafy spurge hawk moth was the only established biological control in Wyoming for leafy spurge. It was released several times, but never became numerous enough to impact the plant (USDA-APHIS-PPQ, 1995). Because of its well documented history, much information has been gathered on the moth's feeding and life history. Refer to Table 1, at the end of this report, for a brief summary of this insect's life history characteristics.

Larvae feed on spurge bracts and leaves to the point of complete defoliation, but leaf loss, and subsequent regrowth, do not seem to negatively affect the plant's root reserves. If the spurge plant is under additional feeding pressure from other biological control insects, the added pressure of defoliation can be instrumental in reducing growth.

Known favorable habitats of the moth are those with heavy infestations of spurge and dense overgrowth. Unfortunately, habitats teeming with wildlife such as ground squirrels, birds, and other small animals reduce larvae populations significantly by predation (Nowierski and Rees, 1993). Several factors act to influence the moth's positive or negative population growth at any given time. Another such contributor is a host-specific "nuclear polyhedrosis virus" (Nowierski and Rees, 1993). Large moth populations are often followed by virus-induced population crashes, which cause the moth to be a very undependable long-term biological control agent.

OBEREA ERYTHROCEPHALA (Red Headed Stem Borer)

The red headed stem borer beetle is a new release in Wyoming. It was released in 1994 and 1995 in Crook County by USDA-APHIS-PPQ, but was seriously impacted through accidental herbicide applications shortly after release. Existing and new release sites will be monitored in 1996 by the USDA-APHIS-PPQ to determine future population establishment. Unfortunately, documentation from other states has revealed this biological control organism to possess a very slow population growth rate.

The red headed stem borer prefers large stemmed (>3 mm diameter) leafy spurge plants located in wet areas. This species tends to be host specific within the *Esula* complex and prefers certain biotypes over others (Rees, 1993d). Refer to Table 1, at the end of this report, for a brief summary of this insect's life history characteristics.

Adults feed on the leaves and stems, but actually do little observable damage to the plants. The female beetle cuts grooves and gnaws holes into selected stems in which she lays her eggs. In approximately seven to ten days, the eggs hatch and the larvae tunnel either up or down inside the stem (stem diameter determines direction). One larva survives to overwinter in each host stem, so a large plant with many stems may be attacked several times. Both the female's activity at the egg-laying stage and the larval feeding stage serve to weaken the affected stems, sometimes causing death. As the larvae eventually migrate downward to the crown and root portions of the plant, damage to, and reduction of, root reserves take place (Rees,1993d).

<u>*SPURGIA ESULAE*</u> (Shoot-tip Gall Midge)

The shoot-tip gall midge is a tiny fly imported from Italy. Larvae feed on leafy spurge shoots and stimulate the formation of a tumor-like gall on the plant's shoot tips. The gall, in addition to serving as a metabolic sink, reduces flower formation and lowers seed production (USDA-APHIS, 1992).

The gall fly has been released in Crook, Fremont, and Laramie counties. Population growth is currently being monitored by USDA-APHIS-PPQ and if sufficient progress in positive population growth occurs soon, redistribution from the larger Crook County site will be a possibility in 1996.

The female *Spurgia esulae* lays her eggs on the meristematic tips of the external leaves, while the newly hatched larvae migrate to the more protected inner leaves to feed and mature. Gall flies cause the abortion of growth on host shoots, which stimulates plants to grow more shoots. Ensuing shoots are attacked by a successive generation of gall flies. While three fly generations per season are not uncommon, insect abundance is largely dependent on shoot availability.

Spurgia esulae's known favorable habitats include south facing slopes occupied by dense stands of spurge. The insect overwinters in the mature larval stage in the soil, but during the spring-summer seasons uses the meristem galls as sites for maturing. Refer to Table 1, at the end of this report, for a brief summary of this insect's life history characteristics.

APTHONA SPP. RELEASE PROCEDURES

Instructions for flea beetle release are not complicated. Simply sprinkle the container of beetles over a small area of spurge plants or allow the beetles to exit the container on their own. The immediate release site should have an adequate number of healthy, green leafy spurge plants to support the released beetle population. Selected sites should be free from grazing and general traffic (Rees, 1993a).

Mark the site with a permanent fence post and attach either the release site information or a reference number to the post in some manner. Wyoming's weed and pest districts have many release sites that are in use, and have devised individual schemes for marking the posts with reference numbers. Each post is arbitrarily assigned a number and a label is affixed either by a weather-proof sleeve, or by attaching a cattle ear-tag to the post that directs the researcher to a reference file located in the district's office.

The next requirement for proper documentation of the release site includes photographs of the general area that show the release area, the leafy spurge infestation, and the fence post marker on the horizon. Mark the photo point so that the angle will be known, and repeated, each successive year (Rees, 1993a).

Insects should be released at the same site repeatedly until an insectary is established. Male to female ratios are uncertain, so it is best to provide a sizable pool of mates and genes from which the insects may draw to ensure reproductive success (S. Lewis, personal communication). Eventually, when the site becomes established, sweep-net collections may prove beneficial to spread actively feeding flea beetles to satellite sites.

MONITORING RECOMMENDATIONS

Yearly monitoring of release sites is a critical requirement of proper biological control documentation. Year-to-year comparisons of insect populations can determine the health and longevity of the monitored sites.

It is crucial for populations such as the *Aphthona* species to be monitored at the same point in their life cycle each year. As a general rule-of-thumb, Lars Baker, Fremont County Weed & Pest District, states that *Aphthona* populations reach a peak the second or third week in July with an immediate drop to an annual low population density by the second or third week of August. Mr Baker reports that *Aphthona* species have a primary flush of larvae maturing and emerging as adult beetles after approximately 865 growing degree days (L. Baker, personal communication). Normally, hatches take place during mid-June and extend to sometime in September (B. Shoemaker, personal communication).

Uniformly reliable field estimates of beetle population sizes can be attained by sweep-net capture on days with similar weather conditions each year. The insects should be collected on hot, dry, and sunny days. Optimum daily observation or sweeping periods are between noon and 3:00 PM (S. Lewis, personal communication). If inclement weather threatens, the beetles will remain too close to the ground for sweep net collection. Wind seems to be less of a factor than cold temperatures or moisture. The beetles are fairly active during a 10-15 mph breeze (B. Shoemaker, personal communication). It is not known at what point wind speed interrupts insect feeding enough to significantly alter population estimates.

The sweep-net should be cone-shaped, constructed of a light canvas or cotton material. The large, open end of the cone should be securely attached to a four foot handle. The insects are fairly small and will not be caught in the common gauze-type sweep net fabric. The sweep procedure consists of a full sweep with the net held approximately sixteen inches above the ground (this is dependent on height of the spurge plants). The collector should then advance fifteen to twenty steps before sweeping again. If the beetles are present, they will be in tight clumps, and unless visual spurge damage is noticed the insects will be difficult to catch randomly. As spurge growth is reduced in the immediate feeding area, the manager should sweep for the slowly dispersing insects on surrounding spurge plants.

The collector should estimate the captured population after each sweep prior to dumping the trash and insects at the site. The flea beetles are very active and can be spotted quickly when captured. The insects will immediately start migrating from the bottom of the net toward the open end, which should help with the population estimation.

LEAFY SPURGE SUMMARY

There are about forty species of *Aphthona* which are recorded as feeding on *Euphorbia* spp. in Europe and Asia (Rees, undated). Of these known taxa, only a few have received clearance for release in the United States by the USDA's biological control research labs. Of the species released, north and central Wyoming has had tremendous success with *A. lacertosa, A czwalinae,* and *A. nigriscutis*. The southeastern corner of Wyoming, as well as Colorado and a handful of other states, continue to look for the perfect biological control for leafy spurge.

Aphthona species have been released on WAFB, but the isolated populations lag behind the success of other Wyoming releases. Several contributing factors could be partially responsible for the *Aphthona* species' unremarkable population growth.

The first and foremost factor to be addressed is annual pesticide applications on or near the release sites. Past (and current?) applications of insecticide from the Base's mosquito reduction program would have detrimental effects on beneficial biological control insects. As noted in my first weed control program report, "(t)he use of broad spectrum insecticides will remove beneficial insects from the system much quicker than chronically recurring pests. There are new mosquito control insecticides available such as methoprene (Altocid) that are very target specific and won't threaten a population of introduced biological control agents" (Hollingsworth, 1995).

A second contributing factor (of which I am fairly sure), is the lack of release site monitoring and insectary management by associated governmental entities involved in insect releases at, or near, WAFB. Assorted federal and state agencies, as well as the local county weed and pest district, have all released different beneficial organisms on Base, but have failed to communicate sufficiently with each other concerning their efforts.

The release of the following natural insect predators for Canada thistle infestations at WAFB is an important part of managing the noxious weed in a sustainable manner, after initial herbicide treatments.

II. CANADA THISTLE

LARINUS PLANUS (Seed Head Weevil)

The seed head weevil derives its name from the female's reproductive activities of drilling her eggs into an unopened Canada thistle bud. She deposits only one egg per bud which assures that many buds are affected by each insect. Active larvae feeding on bud tissue prevent the buds from opening naturally, and reduce the number of viable seeds per bud. This weevil species prefers small buds (6 mm diameter) in which to deposit its eggs (Rees, 1993c).

This biological control agent was accidentally introduced into North America, and is the only known *Larinus* species. Because of its exotic nature, it is assumed that native parasitoids will not attack it, so the weevil could theoretically reach dense populations under the right climatic conditions (Rees, 1993c). Unfortunately, the seed head weevil's Canada thistle control efforts have been unremarkable to date.

UROPHORA CARDUI (Stem Gall Fly)

The stem gall fly larval stage is the only destructive life stage of the insect. The larvae mature inside the stems of Canada thistle plants and cause a gall to form. The only visible symptom of parasitism on the plant is the growth of a large, green, spherical gall on an otherwise healthy appearing stem. This gall serves as a metabolic sink by draining a portion of the plant's resources, which in turn makes the weakened plant more susceptible to secondary infections by pathogens or other insects (Rees, Coombs, and Story, 1993).

The stem gall fly's favored habitat areas are those with occasional flooding or grazing. Additionally, the insect's population growth is positively influenced in the presence of mown and chemically treated locations (Rees, Coombs, and Story, 1993). Unfortunately, according to Skip Lewis, Crook

County Weed & Pest District, this organism has been reported to avoid windy habitats (S. Lewis, personal communication).

CEUTORHYNCHUS LITURA (Stem and Root Mining Weevil)

The stem and root mining weevil is well established in Montana. The adults and larvae feed on Canada thistle in spring and summer, but do not exert much feeding pressure on the plant. Damage occurs to the weedy species when stem holes from the exiting weevils attract secondary infection from small insects and pathogens (Rees, 1993b). Secondary infections can cause the death of the stem or, at best, death of an isolated portion of the plant.

Favorable habitats include dense Canada thistle infestations where the population is not under stress from limited nutrients or unfavorable environmental conditions. The weevil will avoid areas with flooding, mowing, or chemical use (Rees, 1993b).

MONITORING RECOMMENDATIONS

Visual observation and sweep net collection will serve to successfully identify habitat occupied by *Larinus planus* and *Urophora cardui*. The seed head weevil will cause a sizable plot to have the unmistakably distorted bud symptom, while the stem gall fly's tell-tale gall symptoms are also highly visible. Unfortunately, *Ceutorhynchus litura* presents a problem for both monitoring and collecting. This insect's feeding causes no outward visual symptoms on the affected plants, and the stem and root mining weevil's presence cannot be verified until the plant's roots are closely examined and the larvae are removed by tweezers for identification.

CONCLUSION

Canada thistle is adequately controlled by several types of herbicides. Because of this, less research has gone into a biological control program. There are insects currently under quarantine in USDA labs that show promise, but projected releases of the organisms extend ten years into the future. The organisms currently available for use at WAFB are neither aggressive nor dependable enough to use in reducing this weedy species' population. They are, however, an excellent way to establish long-term habitat health in a sustainable manner. Therefore, Canada thistle biological control organisms should be released following the completion of other physical and chemical control strategies.

Biological control organisms for leafy spurge are working well in many parts of Wyoming, Montana, and South Dakota. It is my hope that we can build our own success story in Laramie County through proper flea beetle release timing, annual monitoring, and active management of the beetles' insectaries.

For years, weed control professionals at Wyoming's weed and pest districts have tried to find an adequate control for their leafy spurge infestations. These knowledgeable professionals have obtained a wealth of practical, "hands-on" experiences that they are eager to pass on. With their help, and the help of the *Aphthona* species, I believe we will accomplish our task at the Research Natural Area on Warren Air Force Base.

LEAFY SPURGE BIOLOGICAL CONTROL ORGANISMS

Species	Location of Attack	Known Favorable Habitat	Known Unfavorable Habitat	Availability
Aphthona cyparissiae	Beetles attack leaves and flowers/larvae feed on primary & secondary roots	Sandy loam soils, moist and a relatively high organic matter	Unknown	Biological Control of Weeds in MT
Aphthona flava	Beetles attack leaves and flowers/larvae feed on primary & secondary roots	South slopes, sunny locations, 18-20 inches of moisture per year	Clay soils, shaded areas, acidic soils	Biological Control of Weeds in MT
Aphthona lacertosa	Beetles attack leaves and flowers/larvae feed on primary & secondary roots	Mesic-dry to wet sites with loamy soils and well developed herbaceous vegetation	Very dry or flooded areas	Biological Control of Weeds and possibly APHIS in 1996
Aphthona czwalinae	Beetles attack leaves and flowers/larvae feed on primary & secondary roots	Moist areas with high relative humidity where spurge is intermixed with other vegetation	compact clay soils and open, dry sites	Biological Control of Weeds and possibly APHIS in 1996
Aphthona nigriscutis	Beetles attack leaves and flowers/larvae feed on primary & secondary roots	Sandy, dry knolls	Overgrown, moist sites	APHIS and Wyoming weed & pest districts
Hyles euphorbia	Leaves & bracts	Areas near trees with dense spurge	Areas with small wildlife that would prey on the pupae	APHIS
Oberea erythrocephala	Stem & crown	Plant stems greater than 3.0 mm in diameter	Unknown	Biological Control of Weeds in MT
Spurgia esulae	Meristems & growing points	South facing slopes with dense spurge	River locations supporting high predator densities	Biological Control of Weeds in MT

Table 1. Life history characteristics of biological controls for leafy spurge (Data compilation from the "Biocontrol Agent" information series authored by Rees et al., 1993).

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