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The effect of *Aphthona* spp. flea beetle (Coleoptera: Chrysomelidae) larval feeding on Leafy Spurge, *Euphorbia esula* L., Root Systems and Stem Density in North Dakota: 1986-96

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Abstract

Leafy spurge, *Euphorbia esula* L., is a perennial plant native to Eurasia that has become a widespread weed in North Dakota. Since 1986, North Dakota State University, Entomology Department, has conducted research on leafy spurge roots to determine what impact larval feeding has on the root mass and stem density. Five *Aphthona* spp. flea beetles were used in the leafy spurge research project, *A. flava* Guillebeau, *A. cyparissiae* (Koch), *A. nigriscutis* Foudras, *A. czwalinae* Weise, and *A. lacertosa* Rosenheim.

Aphthona czwalinae, A. lacertosa and *A. nigriscutis* reduced the stem density by 95.2% and the root mass by 85 - 95% through 1996. *A. flava* reduced the root system and stem densities 40 to 60% within five years in study areas where a population level of 85-100 adults per m^2 was constant. The impact on the leafy spurge by *A. cyparissiae*, at one release site, was a 18% reduction in root dry weight, a 36% reduction in root buds, and the stem density was reduced 18%.

The impact on leafy spurge by *Aphthona* spp. flea beetles is more beneficial where the habitat characteristics meet the requirements of the *Aphthona* spp. used and there is good overwinter survival.

Introduction

Leafy Spurge, *Euphorbia esula* L. (Euphorbiaceae), is a perennial herbaceous plant that is native to Europe and Asia. Since its introduction into North America in the late 19th century (Dunn 1985), leafy spurge has infested uncultivated and cultivated lands with rangeland the most impacted in the northern United States and the southern half of Canada. Leafy spurge infestations in pastures and rangelands that are utilized for cattle or horse production can have a negative economic impact on those industries. Leafy spurge is rarely eaten by cattle or horses and some livestock avoid infested areas within the pastures despite the presence of palatable grasses, (Hein & Miller 1992, Lym & Kirby 1987). Significant (>50%) reduction in forage utilization by cattle result when leafy spurge achieves 10% or more of the plant cover (Hein & Miller 1992). The avoidance by cattle and horses is due to irritation caused by latex present in all portions of the plant structure.

Leafy spurge is an aggressive plant out competing both grasses and other forbs for available water, nutrients, and light made possible by its expansive root system and dense stem growth. This aggressiveness can cause the disappearance of some plants species (Belcher & Wilson 1989, Norwierski & Harvey 1987).

Leafy spurge reproduction is generated by seed and root production. Three seeds are produced by each spurge flower and are a source for new plant growth within an established infestation or a source that can be transported by birds, livestock, vehicles, wind, and water to generate new infestations of leafy spurge. The root system is responsible for most of the density increase within established leafy spurge infestations. A small root segment with one root node can also be transported, usually by mechanical means, from its origin to start a new leafy spurge infestation.

Classical biological control has become a valuable management tool against leafy spurge infestations in North America. Nine leafy spurge host specific, Eurasian insect species have been approved for release in the United States. The species used in North Dakota include the gall midge, *Spurgia esulae* Gagne, (Diptera: Cecidomyiidae); a stem and root boring beetle,

Oberea erythrocephala (Schrank), (Coleoptera: Cerambycidae); and five flea beetle species (Coleoptera: Chrysomelidae): *Aphthona flava* Guillebeau, *A. cyparissiae* (Koch), *A. nigriscutis* Foudras, *A. czwalinae* Weise, and *A. lacertosa* Rosenheim.

The habitat necessary for *Aphthona* spp. establishment in the United States had not yet been determined to be the same as their native habitat in Eurasia. Because leafy spurge infests a range of environmentally different habitats in North Dakota, several diverse locations were selected for initial release sites for the *Aphthona* spp. These locations were different not only geographically but also in topography, soil type, surrounding vegetation, and climatic conditions. The leafy spurge vegetative growth patterns (density and plant height) were also included in site selections.

The objectives of this study were to determine the impact of *Aphthona* spp. larval activity on leafy spurge root systems, and to determine the levels to which *Aphthona* flea beetles reduce the density of leafy spurge stands, at selected sites across North Dakota.

Materials and Methods

Aphthona spp. obtained from the USDA-ARS-WR and APHIS-PPQ agencies were released into different locations in North Dakota (ND) as they became available (Fig. 5). Site selections began in 1985 with *A. flava* and *A. cyparissiae* and continued through 1991 for these two species. In 1988, *A. czwalinae* and *A. lacertosa* were added to the study, and *A. nigriscutis* was added in 1989. The duration of monitoring of each study site varied depending on the success of that particular insect colony. Data collections continued through 1996 for all study sites where the insects maintained at least low population levels (40 adults per m^2), and where increased population levels were observed.

The initial releases of *Aphthona* flea beetles were made on Army Corp of Engineer property, identified as the Katie Olson Wildlife Management area (Township 142N - Range 58W - Section 16 - SE1/4), along Lake Ashtabula in the Sheyenne River basin in Barnes county (Co.) ND. This location was selected because of the wide range of different habitats all within a 64.77 hectare tract of land.

The number of flea beetle adults released varied between 50 and 250 specimens at each location for the first three years of the project (1985 -1988). The first releases of *A. flava* and *A. cyparissiae* were made inside 1.8 m^3, 0.16 cm^2 nylon mesh enclosures because of the small numbers of adults released. The same type of enclosure was used in 1988 for the *A. czwalinae* and *A. lacertosa* release location. The *A. czwalinae* and *A. lacertosa* release was a mixed population of the two spices totaling 80 specimens. The enclosures were removed in the fall of each year for three years after initial releases to allow snow, an insulation factor, to cover the release points. The enclosures were replaced each spring before adult emergence began. Collections of *A. nigriscutis*, made in Canada by USDA-APHIS-PPQ and shipped to North Dakota State University Entomology department were of large enough quantities that no cage (open) release sites were established. The number of adults at each release site ranged between 200 to 1000, and all of the insects were released at one point which was marked with a stake for identification. The latitude and longitude readings were taken with a Magellan 5000 DXL GPS

unit (Magellan Systems Corp. San Dimas, California) at each release site.

Sweep net (standard) samples were taken yearly at peak adult emergence at each release site to determine the overwintering success of the *Aphthona* spp. One sweep was an 180 degree horizontal swing motion, and five sweeps equaled 1 m^2. Five, five sweep samples, were collected for a total of twenty-five sweeps at each release site. At the sites where enclosures were used, one, five sweep sample, was collected inside the enclosure and four, five sweep samples, collected outside the enclosure; one, five sweep sample, on each side of the enclosure. At the open release sites one, five sweep sample, was collected at the point of the release and four, five sweep samples, collected five m away from the release point in four different directions. All of the sweep samples were taken to the lab and the number of adult flea beetles were counted, individually for numbers below five hundred and volumetrically for numbers over five hundred. The volumetric counts were made by using a graduated vial with predetermined quantity marks of five hundred, one thousand, fifteen hundred and two thousand adults. The counts were recorded and a mean was calculated for each release site.

Soil samples were collected each fall between the middle of September and the last week of October to measure the impact of *Aphthona* flea beetle larvae on leafy spurge root systems. All soil samples were taken with a 10.16 cm diameter golf cup cutter to a depth of 15.24 cm. Five random samples were collected inside the enclosures, and five random samples were collected around the point of release at the open release sites each year the site was evaluated after the initial insect release year. Individual soil samples were placed in 3 mil plastic bags and labeled. The soil samples were kept refrigerated at - 4^o C, 0:24 L:D until removed for examination.

The number of leafy spurge root segments, root buds, and the number of stems were recorded for each soil sample. Each sample is approximately 0.01 of a m^2. The stem is the above ground vegetative portion of the leafy spurge plant. The root buds are either vegetative (producing a vegetative shoot) or root (producing roots) of each root segment in the sample. The root segments included any separate piece of leafy spurge root from an adjacent leafy spurge

clone that was cut off during sampling, lateral roots (primary and secondary), and leafy spurge tap roots in each sample. Dry weights were recorded for root segments dried at room temperature for 30 days.

Five random soil cores were collected at each site location release point for soil analysis. The analysis was conducted by North Dakota State University, Soils Department (Table 9).

The annual precipitation amounts and ambient temperatures were acquired from data supervised by the National Oceanic and Atmospheric Administration. It was compiled using information from weather observing sites in North Dakota and published in the Climatological Data Annual Summary North Dakota.

The percentage of direct sunlight at each evaluation site was an estimate derived from personal observations of topography and shading vegetation.

Data Analysis

Each release site's yearly data was compared only to its original data collected in the fall of the first year of insect release because of the differences, geographically and environmentally, among locations. This base line data represents a zero percent impact by *Aphthona* flea beetle larvae on the leafy spurge root system and stem densities. A percent decrease/increase in the leafy spurge root system was calculated for each sample year. For each plant data variable, sample year data was divided by first year base-line data and subtracted from 100% to equal a percent change in the root variable amounts between the initial release year and sample year.

Aphthona flava

Materials and Methods

The department of Entomology at North Dakota State University received fourteen shipments of *Aphthona flava* between 1986 and 1994 from the USDA-ARS-WR or USDA-APHIS-PPQ (Table 1). These fourteen shipments, of *A. flava,* provided material for twenty-eight release sites.

The first shipment of 200 adults was received July 8, 1986. The date originally field collected, on leafy spurge, *Euphorbia esula,* was June 12, 1986 at San Rossore (Pisa), Italy. The number of adults received alive totaled 187. Three sites within the Katie Olson Wildlife Management Area were selected for two caged releases of 50 (site FL1-09BA and site FL2-22BA, Site Description A), and one caged release of 87 (site FL3-23BA, Site Description A).

A second shipment, of 220 adult flea beetles, was received on July 23, 1986. The original collection date was July 3, 1986 at San Rossore (Pisa), Italy. The original host plant was *E. esula.* One open (no cage) point release of the entire shipment was made in the Katie Olson Wildlife Management study area (site FL4-25BA, Site Description A).

The third shipment, containing 250 female adults, was received July 20, 1988. The original collection date was June 25, 1988 in Vdine, Italy and the host plant was *E. cyparissus*. Two caged releases, one of 100 adults (site FL5-03LA, Site Description A) and the other of 92 adults (site FL6-04LA, Site Description A), were in Lamoure county on North Dakota State Fish and Wildlife waterfall production land. The remaining 58 were dead and some were kept as voucher specimens.

Shipments four (20 adult females) and five (82 adult females) both received in 1988; and

six (250 adult females), seven (186 adult females), and nine (86 adult females) received in 1990 were not sampled after the initial release year because the flea beetles did not appear to overwinter.

On July 30,1990, the eighth shipment of 353 adults was received. The insects were originally collected on June 19, in Yugoslavia. One open, (no cage), point release was made at site FL11-26BA (Site Description A) located in Barnes county.

The tenth, eleventh, and twelfth shipments received in 1993 totaled 13,200 flea beetles. The thirteenth and fourteenth shipments totaled 6,000 individuals. These shipments were collected at locations in the US and Canada. From shipments 10, 11 and 12 sixteen additional releases of *A. flava* were made in North Dakota. Each of the sixteen sites were ecologically different and in five geographically different locations.

The thirteenth and fourteenth shipments of *A. flava* were not used in this study.

Results

Root dry weight was reduced by 40%, root segments by 2%, root buds by 3%, but the number of stems increased at release site FL1-09BA after the first year of larval feeding (Fig. 1A). The reduction in the number of root buds continued until 1990 reaching a peak reduction of 37% before dropping to 20% in 1991. Root dry weight, root segments, and stem densities steadily increased from 1988 through 1991. In 1991 the root dry weight had increased by 78%, root segments increased by 47%, stem densities by 30%, and root buds decreased to a 20% reduction.

The results at release site FL2-22BA (Fig. 1B) were similar to that of site FL1-09BA (Fig. 1A). The impact (50-75% reduction) on the leafy spurge root system and stem density was substantial the first year after the initial release of adult beetles, but by the fifth year (1991) the percent change for all data variables showed a 19% increase of root dry weight, 19% increase in root segments, 10% in root buds, and a 17% increase in leafy spurge stem density.

The results at release site FL3-23BA were similar to that of site FL1-09BA and site FL2-22BA (Fig. 1C). The impact on the leafy spurge was substantial the first year after the initial insect release. But, by the fifth year (1991), the percent change for all data variables showed a 70% increase in root mass dry weight, 49% increase in root segments, 42% increase in root buds, and a 27% increase in leafy spurge stem density.

At site FL4-25BA, all variables increased the first year after the initial release year (Fig. 1D). By 1989, the root dry weight and root buds counts remained higher than the release year, but the change in number of stems and root segments had increased 20%. *Aphthona flava* larvae maintained a 40 to 50% reduction on the root mass and stem density of leafy spurge from 1990 to1994 compared to the initial release year data.

There was no reduction of the leafy spurge at site FL5-03LA during 1988 through 1991 (Fig. 1E). In 1991, the root dry weight was similar to the release year, and the number of root buds and stems had increased by 20%. The root segments increased by 62%.

Site FL6-04LA yielded more favorable results during 1988 through 1991 though the amount of reduction in the spurge root system was similar to its original base line data by 1991 (Fig. 1F). *Aphthona flava* larvae did have an impact on leafy spurge during 1989 and 1990. In 1989, the root dry weight and root buds decreased 5% and the stem counts decreased by almost 60%. The root segments increased 3% in 1989. In 1990, the stems increased 8%, but the root dry weight decreased 38%, root segments decreased 18%, and the root buds decreased by 80%. The sampling for root damage ended in 1991 because of the reduced number of adult flea beetles in the springs of 1990 and 1991 (Table 2).

The results at site FL11-26BA show that all of the plant data variables increased after the first year of feeding by *A. flava* larvae (Fig. 1G). In 1991, there was an increase in the root dry weight (53%), number of root segments (27%), root buds (84%), and leafy spurge stem density (78%). From 1992 to 1994, there was a steady decrease in the leafy spurge stem density and root mass. By 1995, the feeding of *A. flava* larvae on the root system reduced the root dry weight by 46%, root segments (36%), root buds (57%), and the spurge stem density by 60%.

Open release sites, from shipments 10, 11 and 12, of larger numbers of *A. flava* adults did not establish at fourteen of the additional sixteen release sites selected. At the two sites, where adults could be collected, the average sweep counts were less than ten adults per five sweeps. The impact on leafy spurge was minimal at release sites with less than 100 adults per five sweeps. Therefore, these sixteen insect release sites were not used in this study.

Discussion

The sampling for leafy spurge root structure damage by *A. flava* larvae ended in 1991 at sites FL1-09BA, FL2-22BA, and FL3-23BA, because the populations levels steadily decreased to 0 (Table 2). The decline in population levels during 1988 and 1989 may have been due to primarily spring and summer climatic conditions for ND. The average 1988 ambient temperature departure from normal was +1.83^{o} C in May, +3.6^{o} C in June, and +6.1^{o} C during July. The average 1988 rain fall during the same months were below normal; -3.6 cm in May, -1.22 cm in June, and -4.1 cm during July. The average 1989 temperatures were slightly lower; May, -0.55^{o} C; June, -1.27^{o} C; and July, -0.4^{o} C. The average rain fall amounts were +0.41 cm for May, -1.06 cm in June, and -3.7 cm for July (Climatological Data North Dakota 1988, 1989). Other factors may have contributed to reduction in population levels of the flea beetles at the three study sites such as egg dessication, reduced nutrients in the leafy spurge roots, and dry hard soil; all of which can occur during periods of higher temperatures and lower soil moisture.

The population levels of *A. flava* and the increase or decrease of percent change in the leafy spurge plant data variables appear to have some correlation. A population level of 35 adults per m^2 had a minimum impact on the leafy spurge, but a sustained population level of 50 to 89 adult *A. flava*, evenly distributed over a leafy spurge infestation per m^2, reduced the root system and stem density by 40 to 60% within five years.

The size of the areas at any of the release sites, where the leafy spurge was reduced by the flea beetles, were small. The number of hectares at site FL4-25BA was 0.01 and 0.04 at site FL11-26BA. At the other five release sites, when *A. flava* adults could be collected, the areas producing adults were smaller than 0.04 ha.

Table 1. Shipments of *Aphthona flava* Received by The Department of Entomology at NDSU from USDA-ARS-WR & USDA-APHIS-PPQ between 1986 & 1994

#	Year	Quarantine/ Release File No.	Date Collected	Date Shipped	Date Received or Released	Number of Specimens Received	[d] Release Site Legal Description	Days in Captivity
1	1986	[a] BCWRL-86-22	12 June 1986	7 July 1986	8 July 1986	100 male 100 female	Twp 142N R 58W-S16	26 days
2	1986	BCWRL-86-28	3 July 1986	22 July 1986	23 July 1986	110 male 110 female	Twp 142N R 58W-S16	19 days
3	1988	[b] BCWLA-19-88-32	25 June 1988	19 July 1988	20 July 1988	250 females	Twp 133N R 59W-S32	25 days
4	1988	BCWLA-7-88-17	3 June 1988	26 June 1988	27 June 1988	20 females	Twp 140N R 48W-S29	24 days
5	1988	BCWLA-20-88-33	29 June 1988	19 July 1988	20 July 1988	82 females	Twp 133N R 59W-S32	21 days
6	1990	BCWLA-12-90-13	19 June 1990	17 July 1990	18 July 1990	250 females	Twp 149N R 63W-S24	29 days
7	1990	BCWLA- 11-90-4	9 July 1990	30 July 1990	31 July 1990	186 females	Twp 140N R 48W-S29	22 days
8	1990	BCWLA-07-90-21	19 June 1990	29 July 1990	30 July 1990	353 adults	Twp 138N R 58W-S34	40 days
9	1990	BCWLA-13-20-22	24 June 1990	30 July 1990	31 July 1990	86 females	Twp 140N R 48W-S29	37 days
10	1993	[c] BBCF-APFL-93-32	11 Aug 1993	11 Aug 1993	12 Aug 1993	4200 adults	Twp 136N R 51W-S22	3 days
11	1993	BBCF-APFL-93-ND	3 Aug 1993	3 Aug 1993	5 Aug 1993	3000 adults	Twp 142 R 58W-S6	3 days
12	1993	BBCF-APFL-93-33	17 Aug 1993	18 Aug 1993	19 Aug 1993	6000 adults	Twp 141N R 58W-S7	3 days
13	1994	BBCF-APFL-94-10	4 Aug 1994	8 Aug 1994	9 Aug 1994	4000 adults	Twp 149N R 63W-S24	5 days
14	1994	BBCF-APFL-94-11	21 July 1994	28 July 1994	29 July 1994	2000 adults	Twp 141N R 58W-S8	8 days

[a] Biological Control of Weeds Rome Laboratory

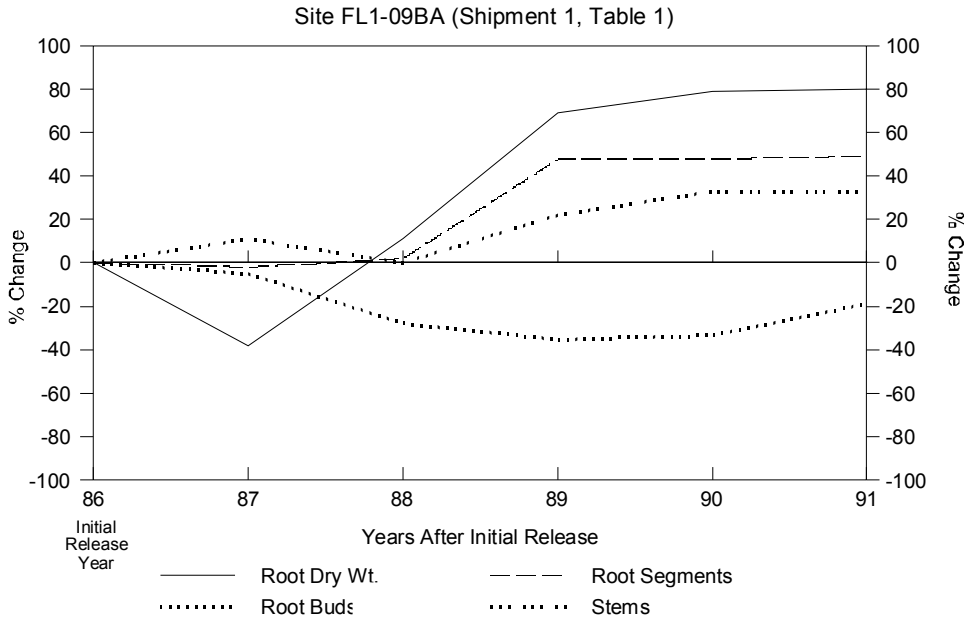
[b] Biological Control Western Laboratory Albany

[c] Bozeman Biological Control Facility

[d] Twp = Township, R = Range, and S = Section for a county

Figure 1. The Impact of *Aphthona flava* on Leafy Spurge Root System and Stem Density

A



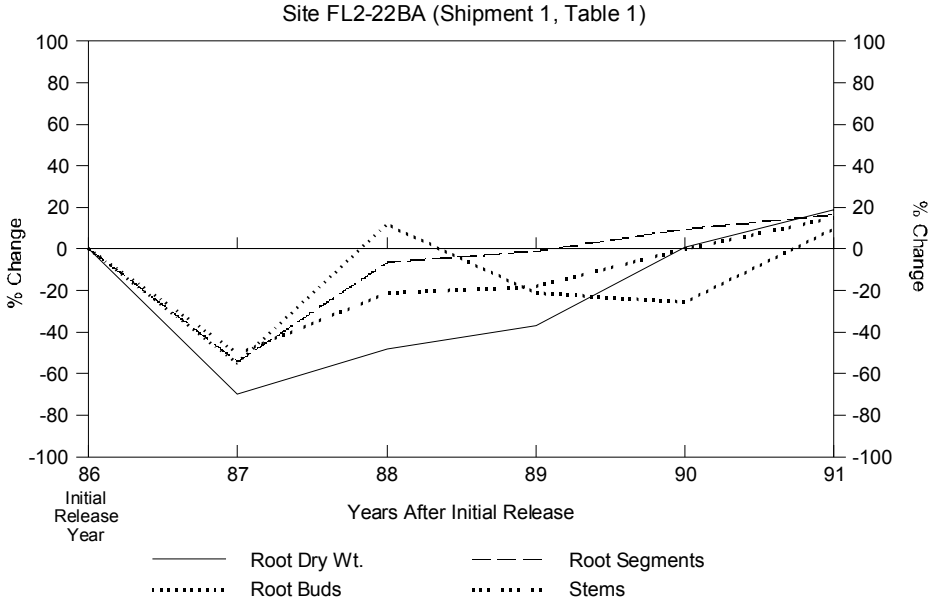
2.68 grams (Initial release year) mean root dry wt.

9.6 (Initial release year) mean root segments

47.4 (Initial release year) mean root buds

1.8 (Initial release year) mean stems

B



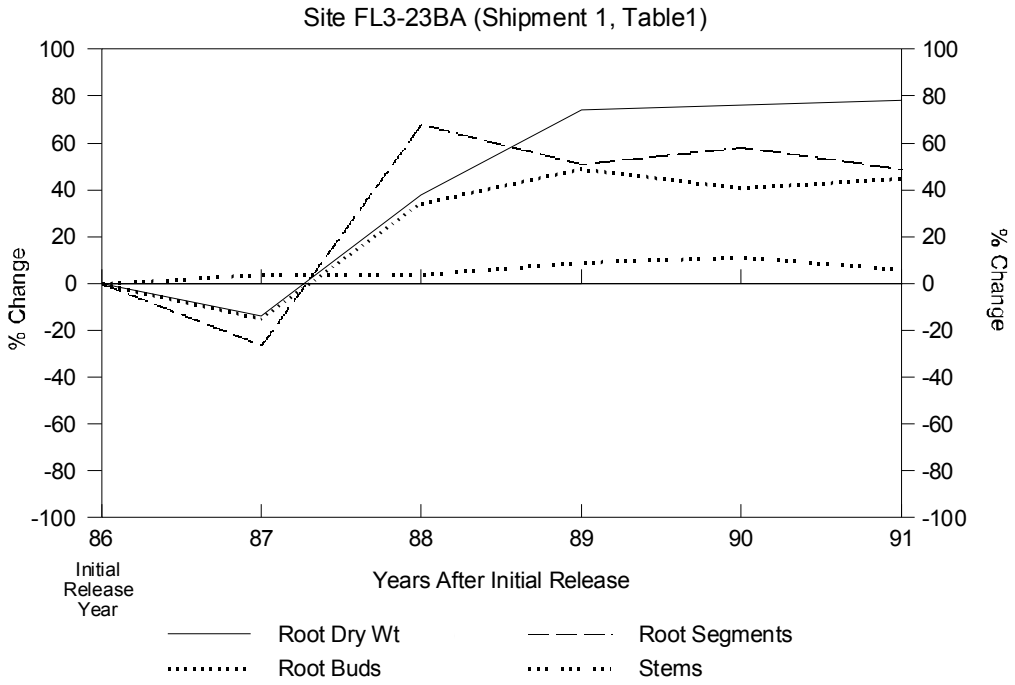
4.88 grams (Initial release year) mean root dry wt.

14.2 (Initial release year) mean root segments

58.6 (Initial release year) mean root buds

4.8 (Initial release year) mean stems

C



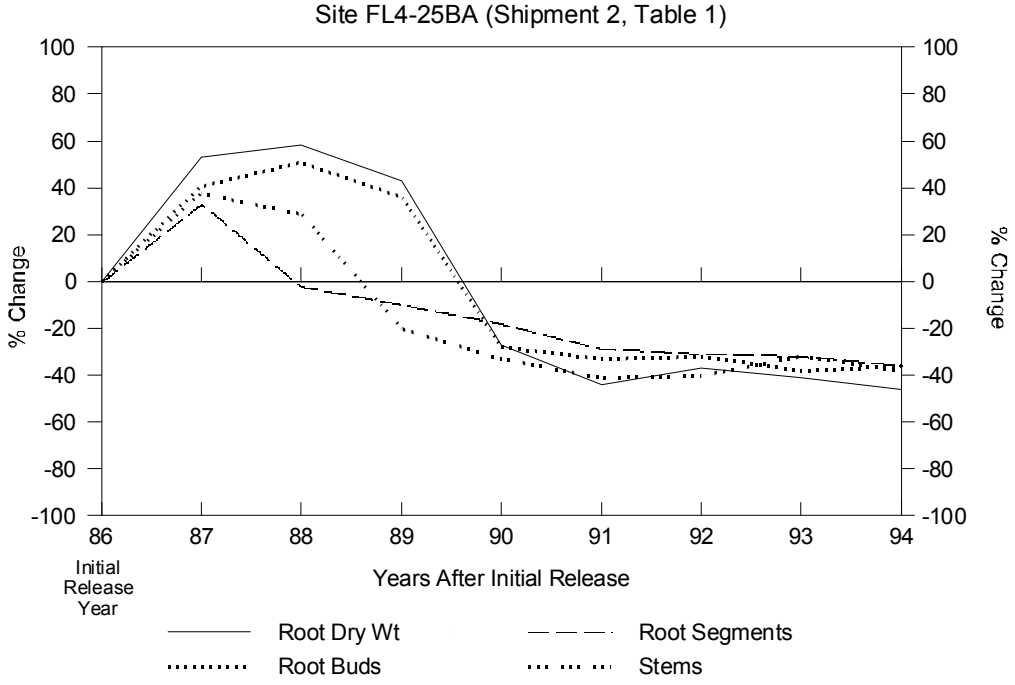
4.62 grams (Initial release year) mean root dry wt.

10.6 (Initial release year) mean root segments

35 (Initial release year) mean root buds

4.6 (Initial release year) mean stems

D



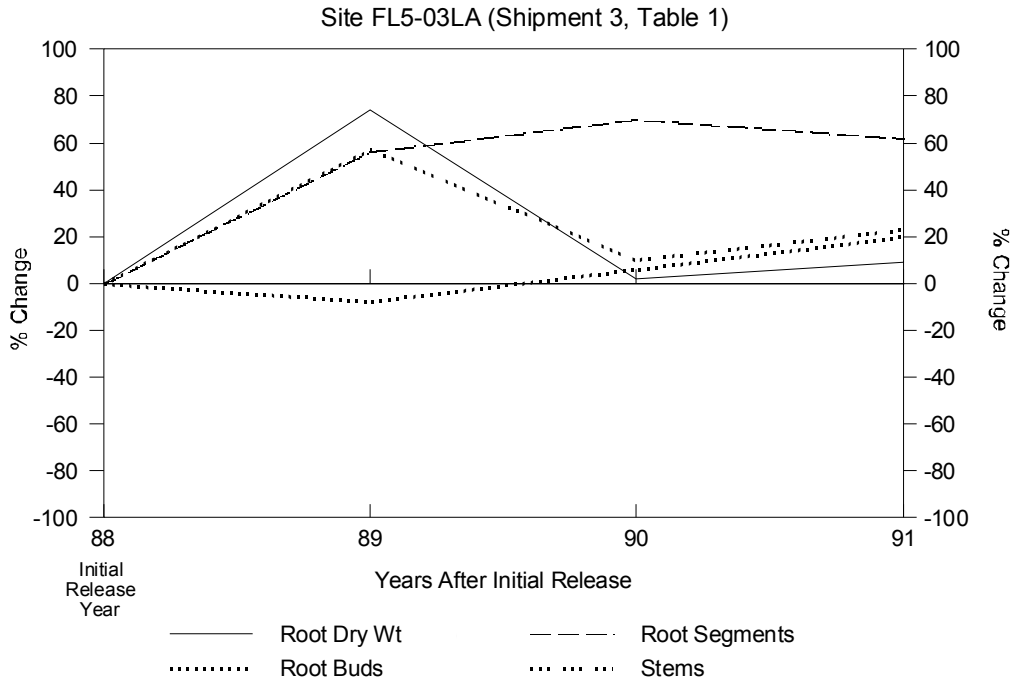
2.07 grams (Initial release year) mean root dry wt.

16 (Initial release year) mean root segments

55 (Initial release year) mean root buds

2 (Initial release year) mean stems

E



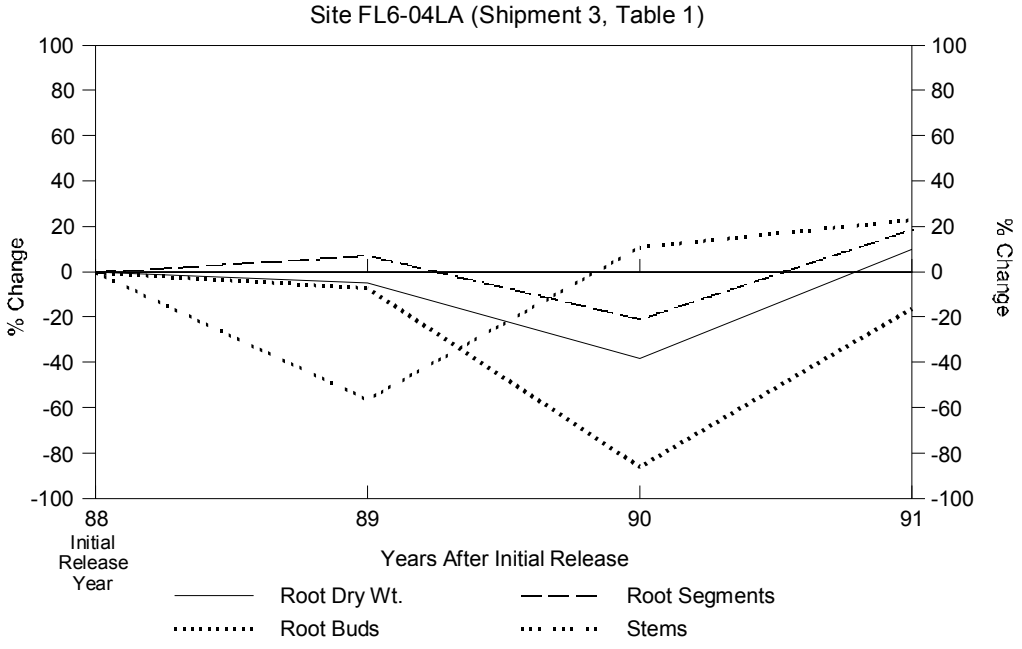
2.48 grams (Initial release year) mean root dry wt.

10.2 (Initial release year) mean root segments

56.8 (Initial release year) mean root buds

4 (Initial release year) mean stems

F



3.13 grams (Initial release year) mean root dry wt.

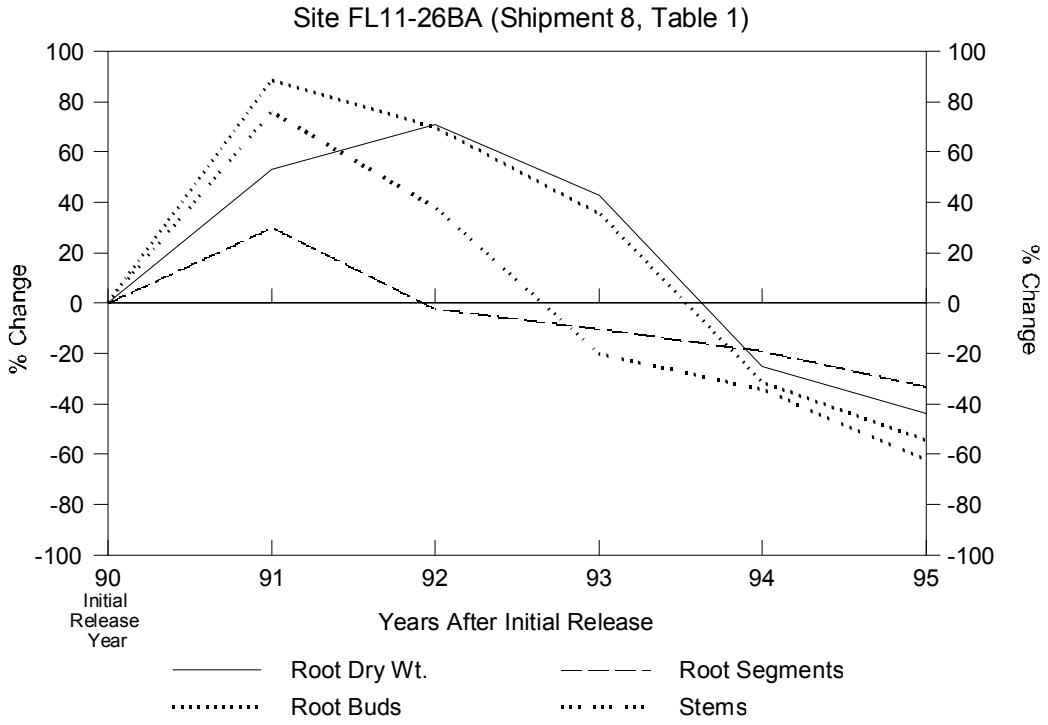
12.4 (Initial release year) mean root segments

62.4 (Initial release year) mean root buds

5.2 (Initial release year) mean stems

Figure 1. continued

G



3.56 grams (Initial release year) mean root dry wt.

20 (Initial release year) mean root segments

97 (Initial release year) mean root buds

6 (Initial release year) mean stems

Table 2. Mean (n=5) Yearly Sweep Counts of Adult *Aphthona flava* at Peak Emergence Per m^2

Release Site	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995
FL1 - 09BA	0	56	48	10	0	0				
FL2 - 22BA	0	35	31	6	0	0				
FL3 - 23BA	0	22	22	4	0	0				
FL4 - 25BA	0	78	62	58	50	89	83	77	81	
FL5 - 03LA			0	3	0	0				
FL6 - 04LA			0	55	12	58	2			
FL11 - 26BA					0	48	59	82	84	72

Site Descriptions A

FL1-09BA and FL2-22BA. (Twp-142N R-58W S-16) These two sites were inside a planted tree claim of Chinese elm, juniper, green ash, box elder trees and other unidentified woody plants, and native grasses. This site has a flat topography, the soil is loam, the average annual rain fall is 48.26 cm, 65-75% direct sunlight and is exposed to south and north winds. The leafy spurge density was 185 stems per m^2 and the average stem height, at flowering, was 60.96 cm.

FL3-23BA. (Twp-142N R-58W S-16) This site consists of an open pasture that has some grazing 73 m lower in elevation than sites 1 and 2. It is also at the bottom of a natural east facing slope and adjacent to the natural water drainage for the south section of Katie Olson Wildlife Management study area. The only vegetation was natural grasses and leafy spurge. The topography is flat, the soil is clay loam and the average annual rain fall is 48.25 cm per year. It has approximately 95% direct sunlight, with east, south, and north wind exposure. The leafy spurge density was 145 stems per m^2 and the average stem height, at flowering, was 48.26 cm.

FL4-25BA. (Twp-142N R-58W S-16) The vegetation is only leafy spurge, the topography is a rolling slope terrain which faces east, the soil is a clay loam and the average annual rain fall is 48.25 cm per year. The amount of direct sunlight is approximately 95%, with east, south, and north wind exposure. The stem density was 329 stems per m^2, height, at flowering, was 45.72 cm.

FL5-03LA and FL6-04LA. (Twp-133N R-59W S-32) The vegetative cover, other than leafy spurge at this location, was native grasses and a three row wind break planted east to west, consisting of honey suckle, cedar, and apple trees. The topography is flat and the soil is a fine sand loam. The climatic environment consist of an average rain fall of 49.53 cm per year, 100% direct sunlight and wind exposure from all directions except north. The leafy spurge density was 371 stems per m^2 and the average plant height, at flowering, was 53.34 cm.

FL11-26BA. (Twp-138N R-58W S16) The vegetation consists of warm and cool crested wheat grasses mixed with leafy spurge. This site lies in a pasture situation near the Sheyenne river basin and has a flat topography. The soil is silt clay loam, an average annual rain fall of 45.72 cm with 90% direct sunlight, and exposed to the wind from all directions. The spurge stem density was 127 per m^2 with a average height, at flowering, of 35.56 cm.

Aphthona cyparissiae

Materials and Methods

The Entomology Department, North Dakota State University, received six shipments of *A. cyparissiae* between 1986 and 1991 from USDA-ARS or APHIS-PPQ (Table 3). The first four shipments, of 160, 240,131 females and 206 adults, respectively, were released at four caged sites in the Katie Olson Wildlife Management area. The fifth shipment, 400 females, was divided into lots of 200 and released at two caged sites at Kulm waterfowl production area, Lamoure Co. ND (Site Description B). A sixth shipment, 1000 adults, was released at one caged site in the Sheyenne National Grasslands, Ransom Co. ND.

Additional collections were made at Maxim, Saskatchewan Canada by the NDSU Entomology Department. These insects were released, in 250 adult lots, at nine leafy spurge impact evaluation release sites: two each in Grant Co., Williams Co. and Theodore Roosevelt National Park Billings Co, and one each in Wells, Richland, and Cass Co.

Only five of the fifteen *Aphthona cyparissiae* release sites were evaluated for larval feeding impact on leafy spurge because *A. cyparissiae* at the remaining ten release sites did not establish or the population levels were to low to have an impact on the leafy spurge infestation (Table 4). These five sites include two from shipment 5 (CY5-01LA and CY6-02LA) and 3 (CY10-01WE, CY11-02RO, and CY15-02WA) from collections made in Maxim, Saskatchewan Canada (Site Description B).

Results

During the three evaluation years following the initial release year, each site (CY5-01LA, CY6-02LA, CY10-01WE and CY11-02RO), root dry weights, segments, buds and stem densities showed fluctuating increases (Fig. 2A, B, C and D).

One release location, site CY15-02WA, located in the northwest region of North Dakota in Ward Co., showed a reduction of leafy spurge root mass as a result of *A. cyparissiae* larval feeding (Fig. 2E). Between 1989 and 1992, there was an 18% reduction in root dry weight, a

36% reduction in root buds, and the stem density was reduced 18% by 1992. In 1992, the average number of root segments was nearly the same as that recorded for the initial release year. The sweep counts in 1992 were 31 adults per five sweeps.

The evaluation of the leafy spurge root system at site CY15-02WA ended in 1992 because the release location was contaminated by *A. nigriscutis* from another release study within the same area. The leafy spurge impact evaluation ended at sites CY5-01LA and CY6-02LA in 1991, CY10-01WE in 1992 and CY11-02RO in 1994 because of contamination by *A. lacertosa* from another release study within the same area.

At the remaining release sites *A. cyparissiae* either failed to overwinter, or the population level the next year consisted of less than ten adults per m^2.

Discussion

Shipments 1- 5, of 160 to 400 adults, were released during a dry period that began in 1986 and continued through 1989. In addition, shipments 3 and 5 contained only females. These three conditions (drought, low release numbers or one gender) may have had an effect on flea beetle survival at some sites. But the *A. cyparissiae* release sites from the 6^{th} shipment and collections from Canada were released during more moist conditions, the release numbers were higher and both genders were present, yet the results were poor.

The results from our studies indicate that *A. cyparissiae* may not be a suitable biocontrol agent for leafy spurge in North Dakota. Other individuals involved in the control of leafy spurge have shown that there are areas in North Dakota producing adult *A. cyparissiae* in collectable numbers. This suggests that more habitat studies are needed to determine the optimal environment(s) where *A. cyparissiae* might be a suitable biocontrol agent for leafy spurge in North Dakota.

Site Description D

NI1-06LA. (Twp-133N R-59W S-32) This location has native grasses, sweet clover, and the topography is flat. A three row wind break of honey suckle, cider, and apple trees planted east to west in addition to leafy spurge. The climatic environment consist of 100% direct sun light, wind exposure from all directions except north, and an average rain fall of 45.72 to 53.34 cm per year. The soil class in this location is a fine sand loam. The leafy spurge density was 171 stems per m^2 and the average plant height was 45.72 cm.

NI2-01RI. (Twp-135N R-52W S-11) The soil is classified as fine sand and the topography is flat. Vegetation at this release site consists of native grasses and leafy spurge 30.48 to 35.56 cm tall with a density of 87 stems per m^2. The average rain fall is 40.64 to 50.80 cm annually. There is approximately 95% direct sun light and exposed to winds from all directions.

NI4-01WA. (Twp-161N R-88W S18) The topography is a steep south facing slope. The vegetation is native grasses and leafy spurge with a stem height of 27.94 cm and a density of 146 stems per m^2. The soil class is loam. It is exposed to direct sun light 70% of the time and south winds. The average precipitation is 50.8 cm per year.

NI5-12BA. (Twp-138N R-58W S-34) The topography is a rolling slope terrain which faces east. The vegetation is only leafy spurge (*Euphorbia esula*) with a stem height of 45.72 cm, a density of 229 stems per m^2, and a silt clay loam soil. The average precipitation is 48.25 cm per year, approximately 95% direct sun light, and east, south, north wind exposure.

NI6-01ED. (Twp-149N R-63W S-23) The topography is flat and in a pasture situation near the Sheyenne River basin. The vegetation consists of warm and cool season crested wheat grasses mixed with leafy spurge. The spurge height was 45.72 cm with a density of 127 stems per m^2. The climatic conditions are 53.34 cm annual rain fall, 90% direct sunlight, and exposed to the wind from any direction. The soil is a fine sand loam.

NI3-01RO. (Twp-135N R-52W S-19) Site 26 is situated in a rolling terrain topography with high prairie to tree type habitats. There is little wind exposure and the annual rain fall is

Table 3. Shipments of *Aphthona cyparissiae* Received by The Department of Entomology at NDSU from USDA-ARS-WR & USDA-APHIS-PPQ Between 1986 & 1991

#	Year	Quarantine/ Release File No.	Date Collected	Date Shipped	Date Received or Release	Number of Specimens Received	[d] Release Site Legal Description	Days in Captivity
1	1986	[a] BCWRL-86-36	12 July 1986	31 July 1986	1 Aug1986	160 adults	[d] Twp 142N R 58W-S16	20 days
2	1987	BCWRL-87-29	6 July 1987	18 July 1987	19 July 1987	240 adults	Twp 142N R 58W-S16	13 days
3	1988	[b] BCWLA-7-88-16	9 June1988	29 June1988	30 June 1988	131 female	Twp 142N R 58W-S16	21 days
4	1988	BCWLA-8-88-14	10 June 1988	29 June1988	30 June 1988	70 male 136 female	Twp 142N R58W-S16	20 days
5	1988	BCWLA-9-88-22	23 June 1988	6 July 1988	7 July 1988	400 female	Twp 133N R 59W-S32	14 days
6	1991	[c] BBCF-APCY 91-15	12 July 1991	15 July 1991	16 July 1991	1000 adults	Twp 135N R 53W-S32	4 days

[a] Biological Control of Weeds Rome Laboratory

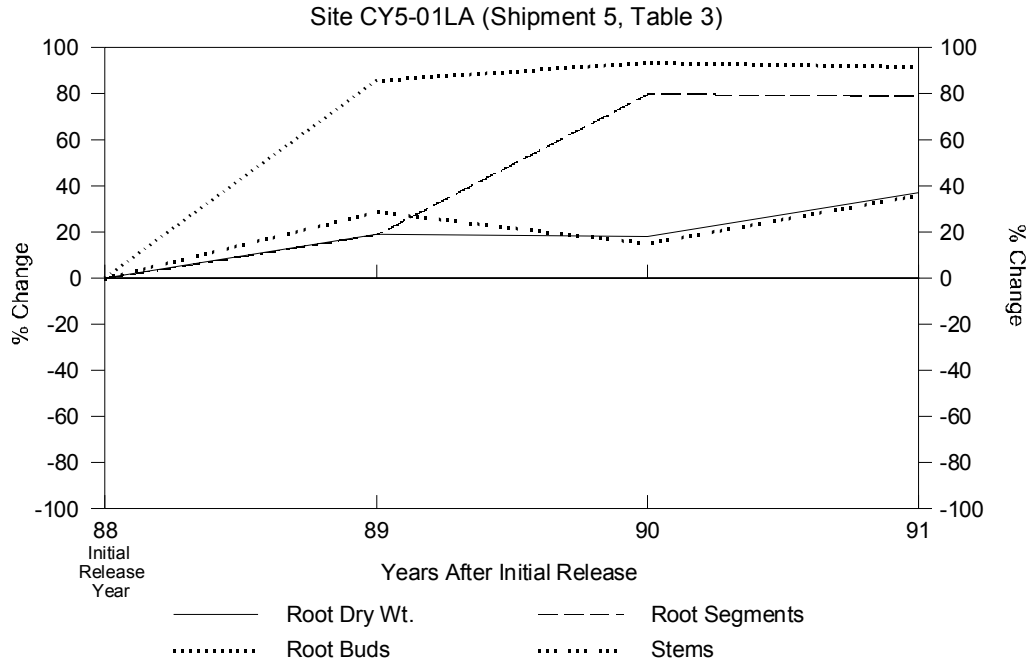
[b] Biological Control Western Laboratory Albany

[c] Bozeman Biological Control Facility *Aphthona cyparissiae*

[d] Twp = Township, R = Range, S = Section for a county

Fig. 2. The Impact of *Aphthona cyparissiae* on Leafy Spurge Root System and Stem Density

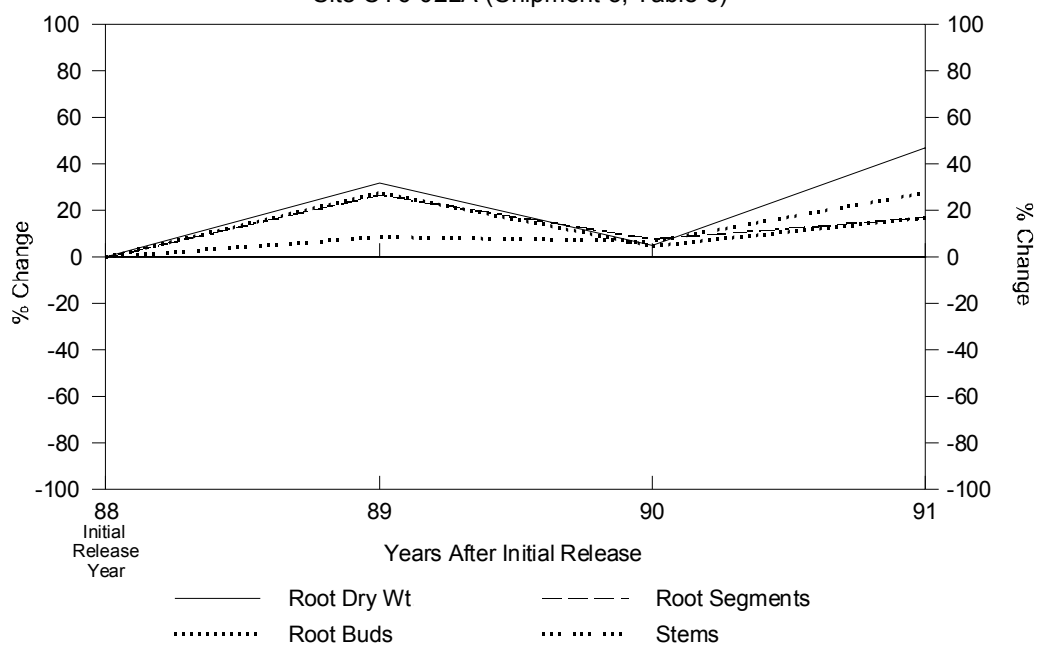
A



1.81 grams (Initial release year) mean root dry wt. 5.2 (Initial release year) mean root segments

25.6 (Initial release year) mean root buds 1.4 (Initial release year) mean stems

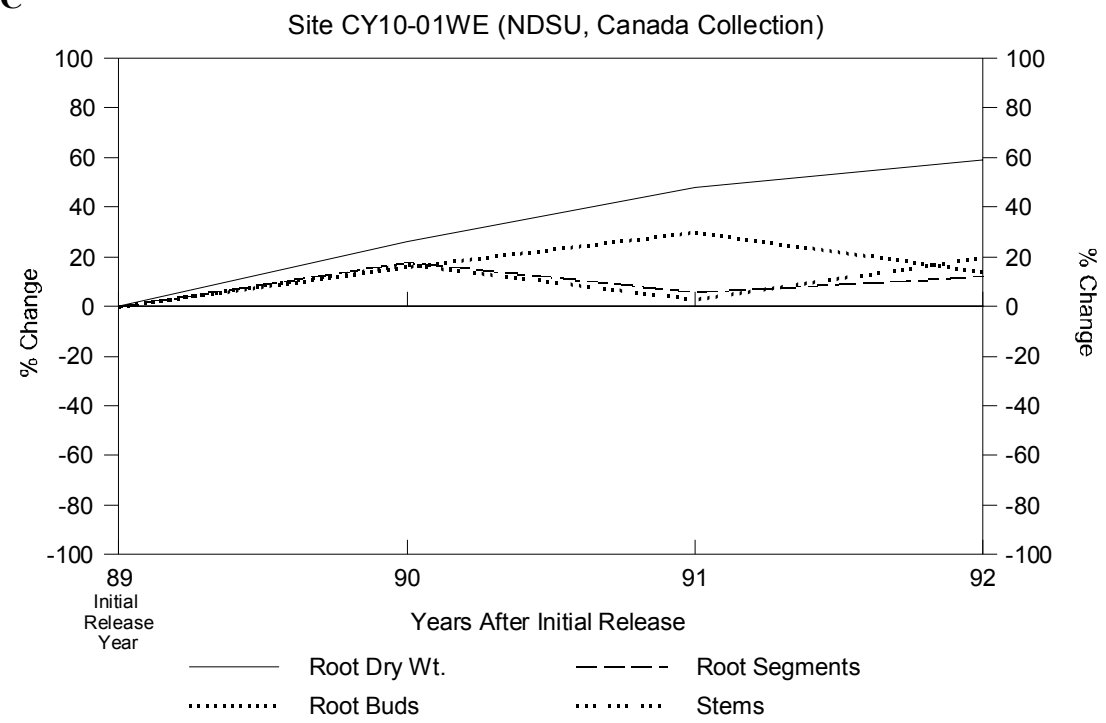
B



3.99 grams (Initial release year) mean root dry wt. 12.4 (Initial release year) mean root segments

73.4 (Initial release year) mean root buds 6.4 (Initial release year) mean stems

C



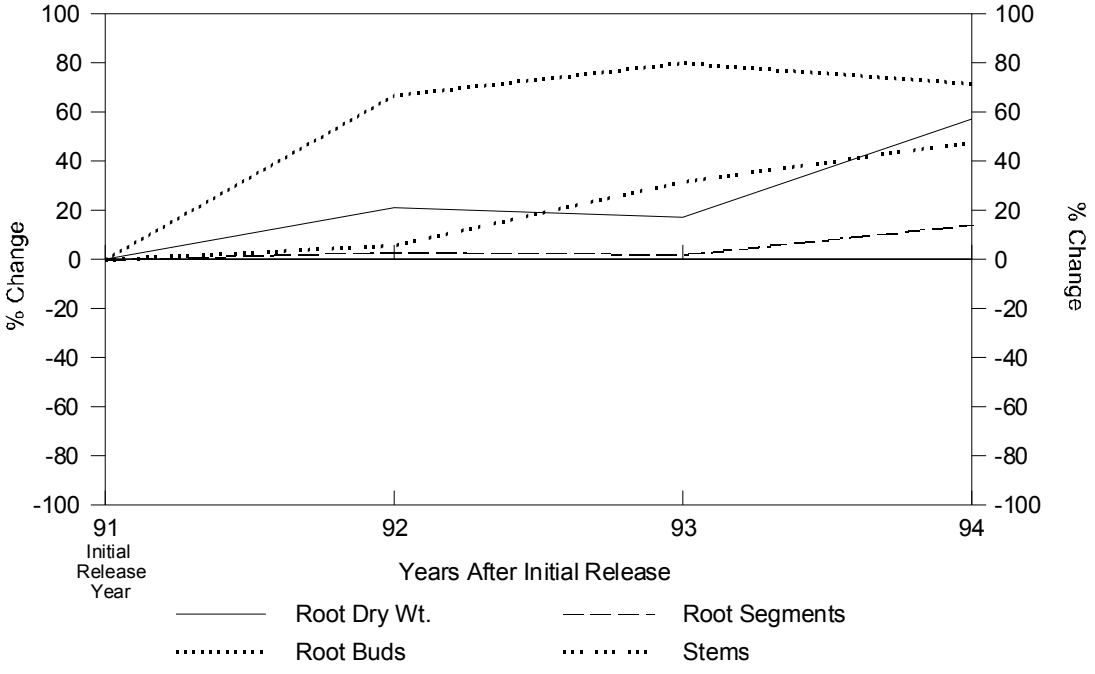
2.2 grams (Initial release year) mean root dry wt.

11 (Initial release year) mean root segments

34 (Initial release year) mean root buds

3 (Initial release year) mean stems

D



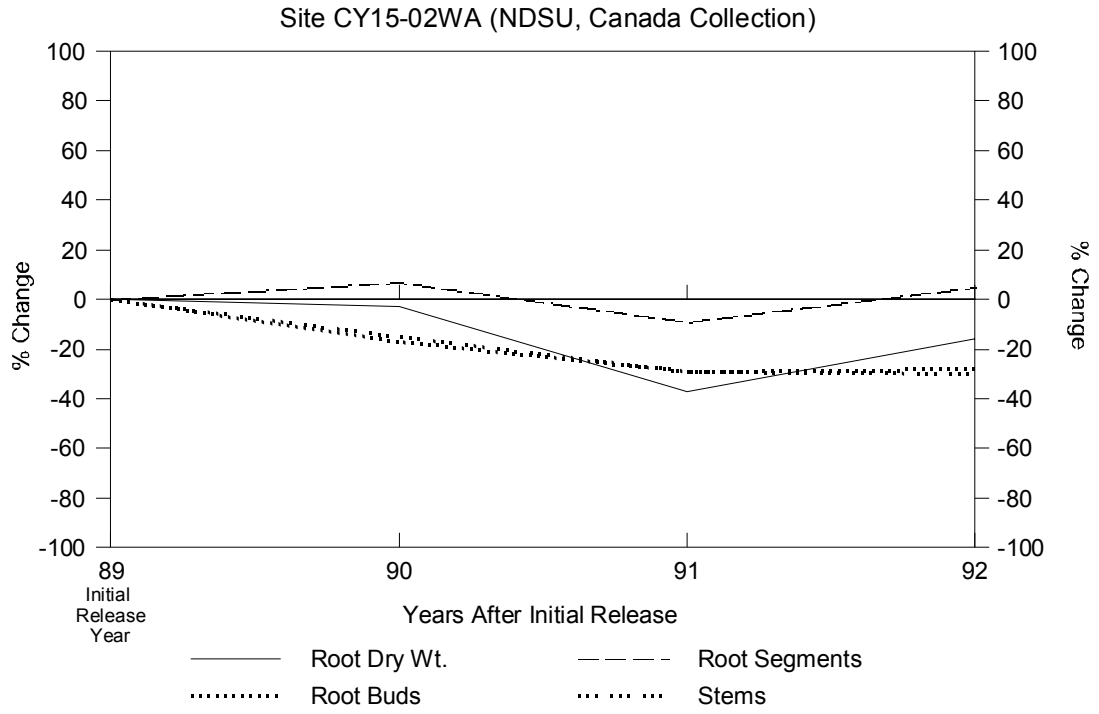
2.25 grams (Initial release year) mean root dry wt.

10 (Initial release year) mean root segments

28 (Initial release year) mean root buds

4 (Initial release year) mean root stems

E



5.73 grams (Initial release year) mean root dry wt.

10 (Initial release year) mean root segments

43 (Initial release year) mean root buds

4 (Initial release year) mean stems

Table 4. Mean (n=5) Yearly Spring Sweep Counts of Adult *Aphthona cyparissiae* at Peak Emergence Per m^2

Release Site	1988	1989	1990	1991	1992	1993	1994
CY5- 01LA	0	6	4	0			
CY6 - 02LA	0	5	10	0			
CY10 - 01WE		0	22	4	7		
CY11 - 02RO				0	10	9	10
CY15 - 02WA		0	29	43	45		

Site Descriptions B

CY5-01LA and CY6-02LA. (Twp-133N R-59W S-32) The following is a combined description of the release locations CY5-01LA and CY6-02LA. The vegetation other than leafy spurge was native grasses. The topography is flat and the soil classes are fine sand loam (CY5-01LA) and loam fine sand (CY6-02LA). The climatic conditions are an average annual precipitation of 53.34 cm, 95% sunlight and a east, south and west wind exposure. Leafy spurge stem densities averaged 204 stems per m^2 and the stem heights, at flowering, averaged 50.8 cm at both sites.

CY10-01WE. (Twp-146N R-68W S-13) The vegetation other than leafy spurge consisted of native grasses and sweet clover. The topography has east to southwest facing slopes of loam soil with leafy spurge stem densities averaging 311 stems per m^2 and the stem height, at flowering, was 68.58 cm. The climatic conditions are 53.34 cm average annual precipitation, 90% sun light and a east, south and northwest wind exposure.

CY11-02RO. (Twp-135N R-52W S-11) This site is in an area used for livestock grazing that the only vegetation available is leafy spurge. The topography is flat and the soil is fine sand. The climatic conditions are 51.32 cm average annual precipitation, 100% sun light and has wind exposure from all directions. The leafy spurge stem density was 110 stems per m^2 and the stem height, at flowering, was 46.58 cm.

CY15-02WA. (Twp-161N R-88W S-11) The vegetation consists of native grasses and leafy spurge. The topography is a steep south facing slope with a loam soil. It is exposed to direct sun light 70% of the time and south winds. The average rain fall is 50.80 cm per year. The stem height, at flowering, was 27.94 cm with a density of 146 stems per m^2.

Aphthona czwalinae / A. lacertosa

Materials and Methods

North Dakota State University received one shipment containing both *A. czwalinae* and *A. lacertosa* on June 30, 1988 (Table 5). The combined number of adults, consisting of 64% *czwalinae* and 36% *lacertosa*, was 105 (40 males and 65 females). The collections originated in Austria, near St. Polten, on June 10, 1988 and the leafy spurge host was *Euphorbia esula*. Eighty adult flea beetles were released in a 1.8 m^3, 0.16 cm nylon mesh cage the same day they were received. The remaining adults were kept as voucher specimens. This release site, CZLA1-01BA (Site Description C), was located at the Katie Olson Wildlife Management area in Barnes county, North Dakota. Seven additional insect release locations, (CZLA2-01GO, CZLA3-01ED, CZLA4-02RI, CZLA5-01BI, CZLA6-03WA, CZLA7-03RO, and CZLA8-01BE) of 250 adults each, were supplied with adult *A. czwalinae* and *A. lacertosa* collected from site CZLA1-01BA in 1991 and 1992.

Results

The root system showed no reduction in 1989, the first year after releasing *Aphthona* adults at site CZLA1-01BA (Fig. 3A). The root dry weight and buds increased by 60% and the root segments by 20%. In 1990, the leafy spurge data variables were reduced substantially. Root segments and root buds were reduced by 20% from the original base-line data. The root dry weight was reduced by 60% and stem densities were reduced by 80% per m^2. By 1992, the number of leafy spurge stems per m^2 had been reduced 98%. This percent reduction in stem densities was maintained by *A. czwalinae* and *A. lacertosa* flea beetles through 1996. The root system dry weight, buds, and segments were reduced 89%, 82%, and 80% respectively by 1996. There was a substantial increase each year in the adult spring sweep counts through 1994 (Table 6).

Larval feeding activity had a substantial impact on the leafy spurge at site CZLA2-01GO (Fig. 3B). By 1994, the leafy spurge plant's root dry weight was reduced 79%. The number of root buds and spurge stems were reduced 88%, and root segments decreased 60%. The adult

sweep counts increased each year through 1994 (Table 6). Yearly soil sampling for root evaluation ended in 1994 because *A. nigriscutis* moved into this site.

The leafy spurge at site CZLA3-01ED increased the first year (1991) after releasing *A. czwalinae /A. lacertosa* adults. From 1994 through 1996, the spurge data variables were reduced by *Aphthona* larval feeding , but the percent reduction in the root dry weight, segments, buds, and stem density was only 19, 2, 19, and 10%, respectively (Fig. 3C). The spring sweep counts of adults population levels were also low (Table 6).

Population levels at site CZLA4-02RI failed to increase more than 8 to 15 adults per m^2 through 1996 (Table 6). Leafy spurge root system variables and stem densities steadily increased to amounts between 22 and 70% more than the base-line data (Fig. 3D).

Reductions in both the above and below ground portions of the leafy spurge plant systems were substantially reduced by 1996 at site CZLA5-01BI. Root dry weight was reduced 70%, root segments 72%, root buds 71%, and stem density 70% (Fig. 3E). Also, the adult population levels increased 75% during the same period of time (Table 6).

The over all impact at site CZLA6-03WA by the larvae on the leafy spurge was substantial at the end of 1996 (Fig. 3F). The leafy spurge stem density was reduced 89%, root dry weight by 76%, root buds 50%, and the number of root segments by 46%. There was also an increase in the number of adults collected each spring from 1993 to 1996 (Table 6).

Between 1993 and 1996 larval feeding reduced the leafy spurge root dry weight 78%, root segments 47%, root buds 52%, and stems per m^2 90% at site CZLA7-03RO (Fig. 3G). Adult population levels increased each year after the initial insect release year (Table 6).

The ability of *Aphthona* flea beetles to survive the winters was poor at site CZLA8-01BE (Fig. 3H). Sweep samples, taken in the spring of each year after initial release, produced an average of 10 adults per m^2 for the years 1993 through 1996 (Table 6). These low numbers of adults were also reflected in the reduced impact by the larvae on the leafy spurge root system and stem densities. By 1996, the root dry weight increased 37%, root segments 36%, root buds 49%, and stem densities 51% (Fig. 3H).

Discussion

The flea beetle shipments consisted of a mix of 36% *A. lacertosa* and 64% *A. czwalinae*. By 1990, the ratio at the original release point was 1:1. But the ratios, in similar habitats fifty meters away, were shifting towards *A. lacertosa*. In 1993, adults collected for redistribution were 75% *lacertosa*. The population of flea beetles consisted of 98% *A. lacertosa* after 1993. This indicates that the percent reduction of the leafy spurge root system and stem density by the larval feeding activity at all of the *A. lacertosa /A. czwalinae* release sites were *A. lacertosa* influenced. Both species are credited with having a positive impact on the leafy spurge at all the release sites evaluated, because *A. czwalinae* adults were still collectable in spring sweep net samples.

There was a reduction in the leafy spurge plants regeneration capabilities in six of the eight release sites evaluated. The most significant impacts (60 to 98% reduction in all data variables) were from those sites (CZLA1-01BA, CZLA2-01GO, CZLA5-01BI, CZLA7-03RO) where the soil classification is a silt loam, loam, clay loam, or silt clay loam. The least impact (8 to 20% reduction in all data variables) was from the location (CZLA3-01ED) where the soil classification is a loam fine sand. There were two different study locations (CZLA4-02RI and CZLA8-01BE) where *A. czwalinae* and *A. lacertosa* larvae had no impact on the leafy spurge infestation. The soil characteristic associated with these locations was a fine sand classification.

The adult population levels continued to increase at sites CZLA1-01BA, CZLA2-01GO, CZLA5-01BI, CZLA6-03WA, and CZLA7-03RO (Table 6) for four years after the initial release year. After the fourth year the adult population levels appeared to be related to the leafy spurge stem density at that particular location. At site CZLA3-01ED the adult populations never reached a level that would appear to have a noticeable impact on the leafy spurge infestation. At sites CZLA4-02RI and CZLA8-01BE, adult population levels were almost nonexistent.

Field observations of spurge infestation at the release locations indicated that the impact of adults moving away from the initial release points was substantial. This impact was consistent with the results at the initial release sites. Each year, adult *A. czwalinae /A. lacertosa* were

collected at increasing distances from the point of release.

The amount of damage caused by *A. czwalinae* and *A. lacertosa* larval feeding on the root system of leafy spurge would indicate these two species are excellent candidates for biological control of leafy spurge in North Dakota.

Table 5. Shipments of *Aphthona czwalinae* & *A. lacertosa* Received by The Department of Entomology at NDSU from USDA-ARS-WR & USDA-APHIS-PPQ in 1988 and NDSU Collections from Site CZLA1-01BA between 1990 & 1993

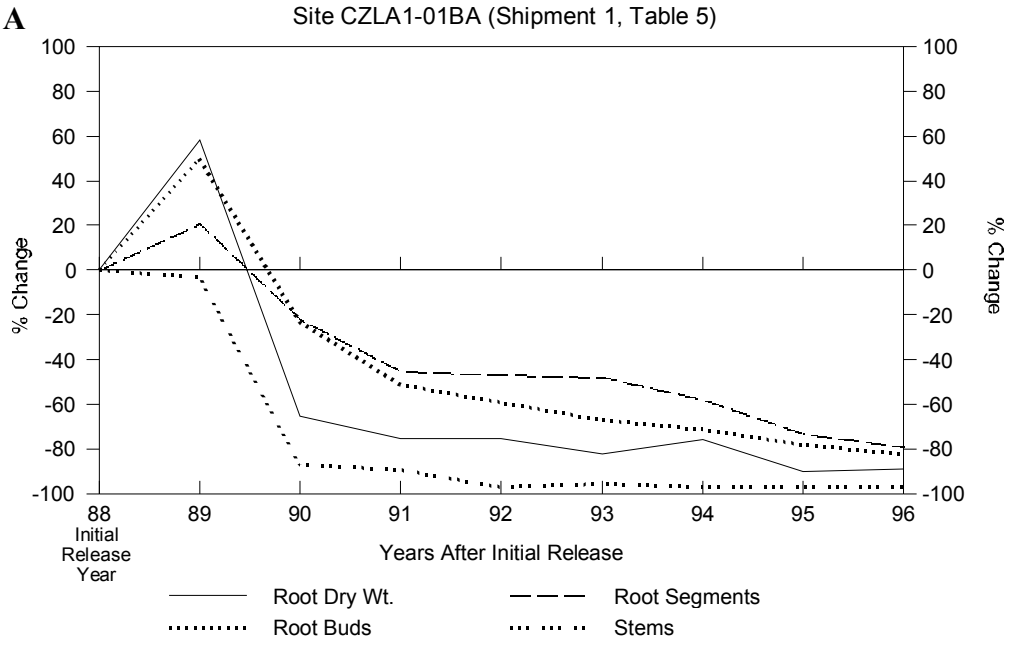
#	Year	Quarantine/ Release File No.	Date Collected	Date Shipped	Date Received or Released	Number of Specimens Received	[c] Release Site Legal Description	Days in Captivity
1	1988	[a] BCWLA-8 - 88-15	10 June 1988	29 June 1988	30 June 88	105 adults	Twp 142N R 58W-S16	20 days
2	1990	[b] Site CZLA1-01BA	25 June 1990		25 June 1990	250 adults	Twp 138N R 58W-S34	14 days
3	1992	Site CZLA1-01BA	12 June 1992		12 June 1992	250 adults	Twp 149N R63W-S24	12 days
4	1992	Site CZLA1-01BA	15 June 1992		15 June 1992	250 adults	Twp 136N R 51W-S13	15 days
5	1992	Site CZLA1-01BA	19 June 1992		19 June 1992	250 adults	Twp 140N R101W-S2	19 days
6	1993	Site CZLA1-01BA	5 June 1993		5 June 1993	250 adults	Twp 140N R86W-S29	12 days
7	1993	Site CZLA1-01BA	5 June 1993		7June 1993	250 adults	Twp 172N R70W-S30	14 days
8	1993	Site CZLA1-01BA	5 June 1993		7 June 1993	250 adults	Twp 172N R 66W-S29	14 days

[a] Biological Control Western Laboratory Albany

[b] 1988 Release site CZLA1-01BA

[c] Twp = Township R = Range S = Section for a County

Figure 3. The Impact of *Aphthona czwalinae* / *A. lacertosa* on Leafy Spurge Root System and stem Density

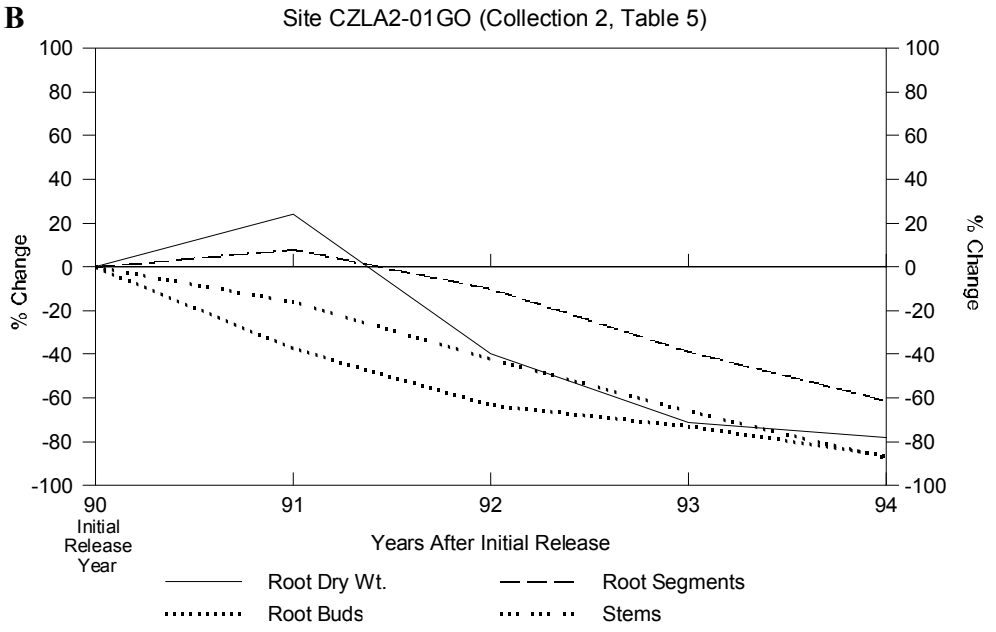


3.65 grams (Initial release year) mean root dry wt.

15.4 (Initial release year) mean root segments

47.8 (Initial release year) mean root buds

7.6 (Initial release year) mean stems



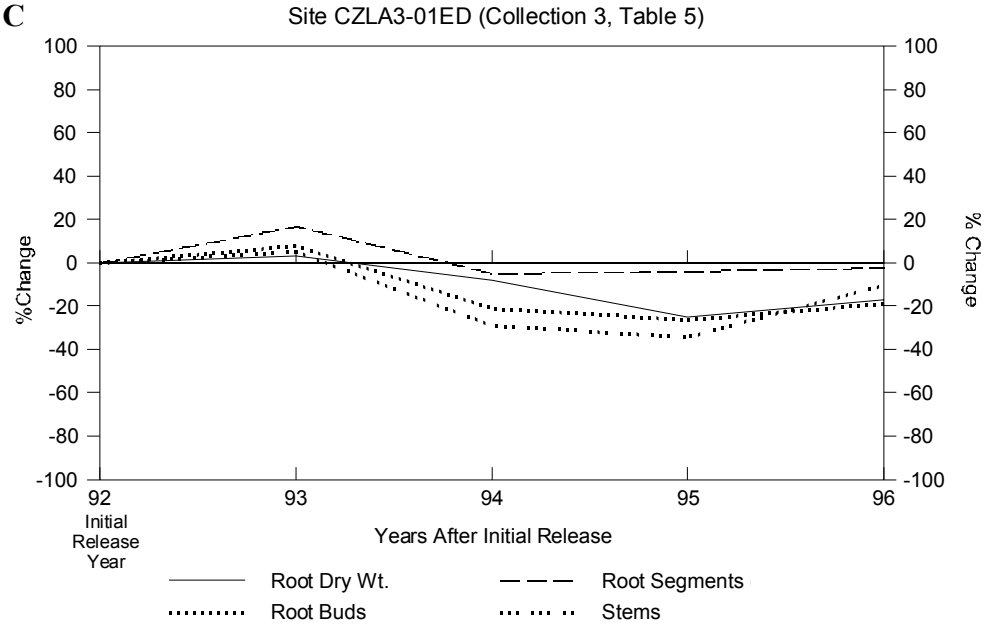
5.86 grams (Initial release year) mean root dry wt.

18 (Initial release year) mean root segments

51 (Initial release year) mean root buds

5 (Initial release year) mean stems

C



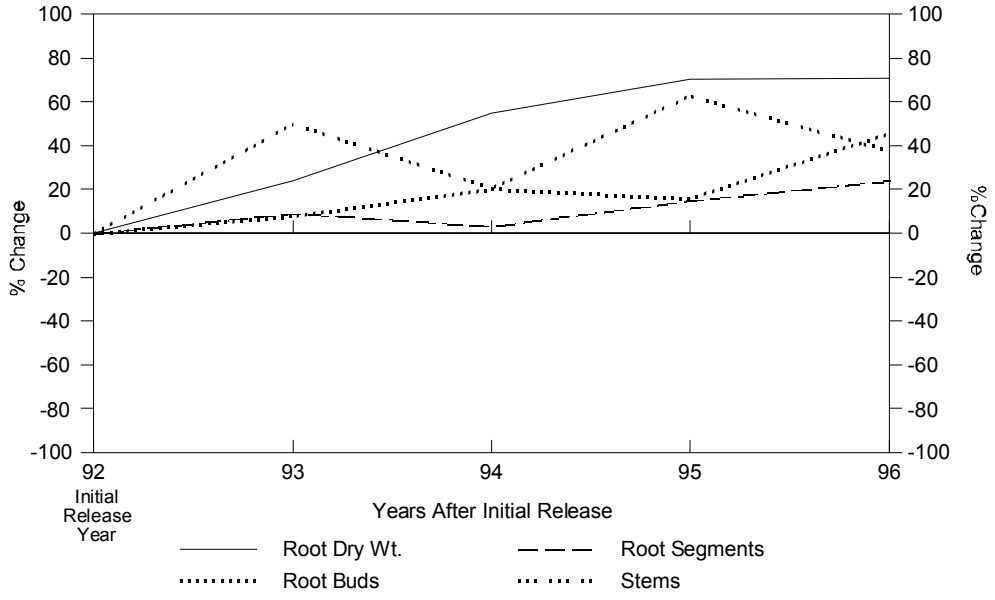
3.4 grams (Initial release year) mean root dry wt.

9 (Initial release year) mean root segments

44 (Initial release year) mean root buds

4 (Initial release year) mean stems

D



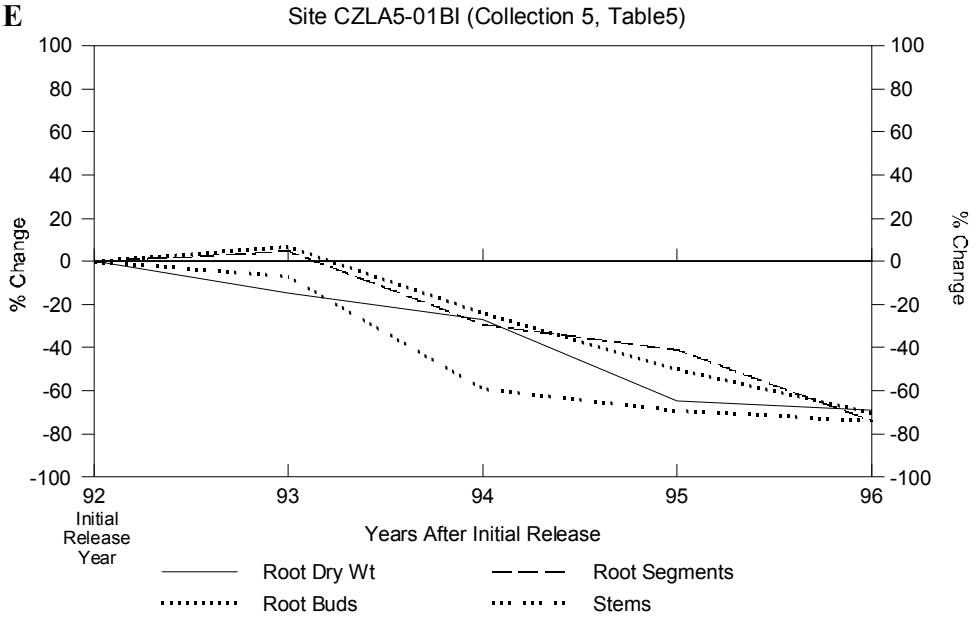
2.13 grams (Initial release year) mean root dry wt.

16 (Initial release year) mean root segments

61 (Initial release year) mean root buds

6 (Initial release year) mean stems

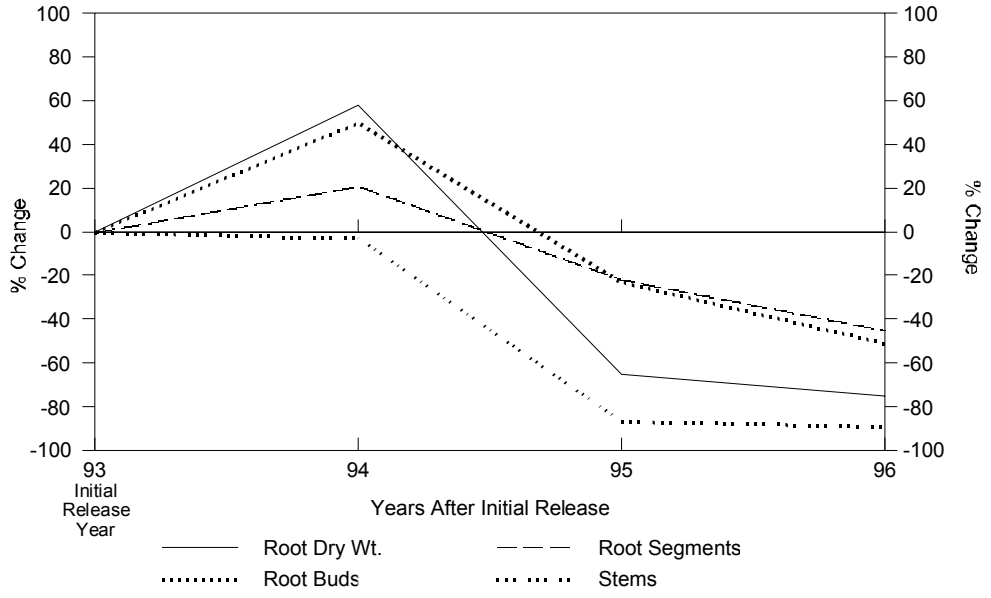
E



2.55 grams (Initial release year) mean root dry wt. 11 (Initial release year) mean root segments

39 (Initial release year) mean root buds 3.8 (Initial release year) mean stems

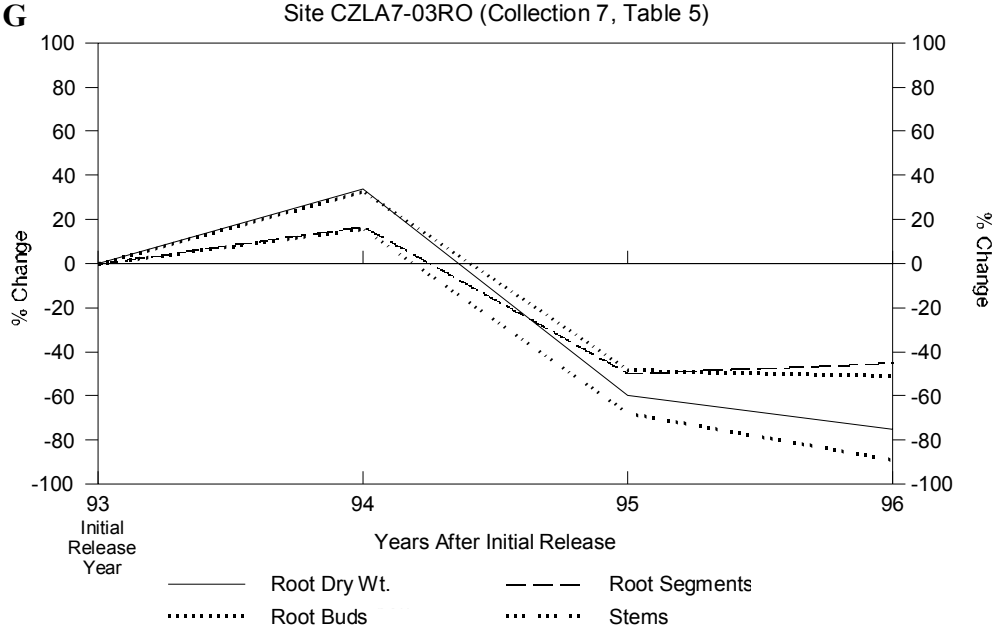
F



3.23 grams (Initial release year) mean root dry wt. 12 (Initial release year) mean root segments

51 (Initial release year) mean root buds 4.1 (Initial release year) mean stems

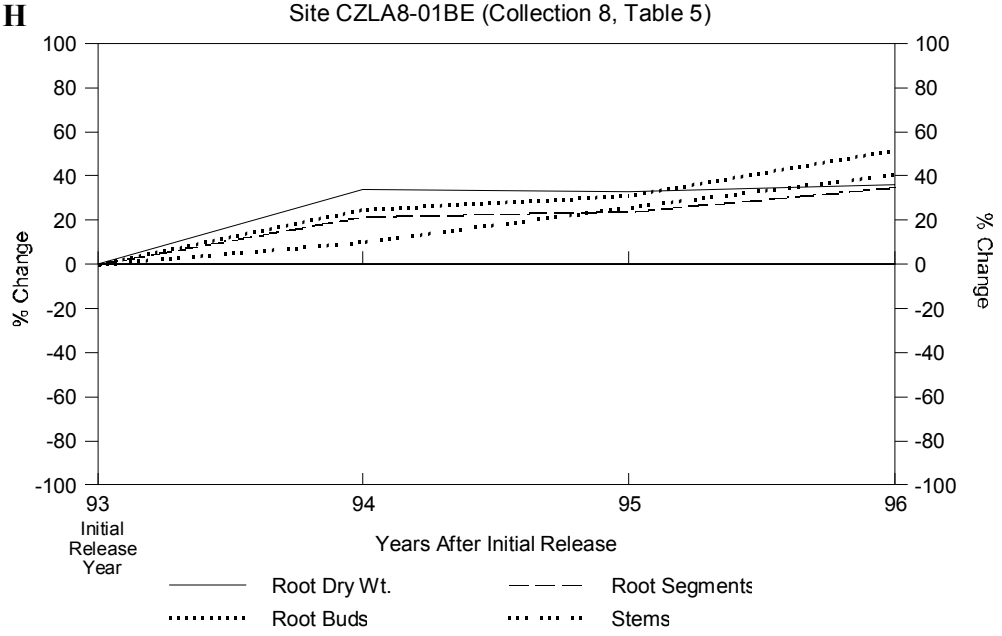
G



5.21 grams (Initial release year) mean root dry wt. 11.4 (Initial release year) mean root segments

57 (Initial release year) mean root buds 5.3 (Initial release year) mean stems

H



3.89 grams (Initial release year) mean root dry wt. 9.65 (Initial release year) mean root segments

49 (Initial release year) mean root buds 3.25 (Initial release year) mean stems

Table 6. Means (n=5) Yearly Spring Sweep Counts of Adult *Aphthona czwalinae / A. lacertosa* at Peak Emergence Per m^2

Release Site	1988	1989	1990	1991	1992	1993	1994	1995	1996
CZLA1 - 01BA	80	270	550	625	3000	3000	1000	800	350
CZLA2 - 01GO			250	325	400	525	500	600	300
CZLA3 - 01ED					250	200	260	250	280
CZLA4 - 02RI					250	10	7	12	13
CZLA5 - 01BI					250	325	400	375	425
CZLA6 - 03WA						250	375	575	725
CZLA7 - 03RO						250	375	500	650
CZLA8 - 01BE						250	5	6	4

Site Descriptions C

CZLA1-01BA. (Twp-142N R-58W S-16) The plant communities other than leafy spurge were minimal and the point of release was at the bottom of a south facing slope adjacent to a natural water shed swale. The soil profile is classed a silt loam. The climatic conditions are 45.72 cm annual rain fall, 100% direct sunlight, and exposed to south, east and west winds. The Leafy Spurge average plant height, at flowering, was 78.74 cm with an average stem density of 367 stems per m^2.

CZLA2-01GO. (Twp-138N R58W S-34) The vegetation consists of warm and cool crested wheat grasses mixed with leafy spurge. The topography is flat and in a pasture situation near the Sheyenne river basin and the soil is a silt clay loam. The climatic conditions are 45.72 cm average annual rain fall, 90% direct sunlight, and exposed to the wind from all directions. The spurge height, at flowering, was 34.56 cm at a density of 127 stems per m^2.

CZLA3-01ED. (Twp-149N R-62W S-18) The point of flea beetle release was in a wooded habitat with a flat topography. Other than leafy spurge, the vegetation was native grasses. The soil classification is a loam fine sand. The average precipitation per year is 50.30 cm and 75% direct sunlight with minimal wind exposure. The leafy spurge stem density was 119 stems per m^2 and the spurge plant height, at flowering averaged

30.48 cm.

CZLA4-02RI. (Twp-135N R-52W S-11) Site 4 is located in the Sheyenne National Grasslands. Vegetation at this release site consists of native grasses and leafy spurge. The soil is classified as fine sand and the topography is flat. The leafy spurge averaged 32.48 cm tall with a density of 87 stems per m^2 at flowering. The average rain fall is 45.64 cm annually. There is approximately 95% direct sun light and exposed to winds from all directions.

CZLA5-01BI. (Twp-140N R-102W S-4) This release site is located along Knutson Creek in Theodore Roosevelt National Park. The topography is a rolling slope terrain which faces east and a clay loam soil. The vegetation is native grasses and leafy spurge with a stem height, at flowering, of 38.10 cm with a density of 129 stems per m^2.

CZLA6-03WA. (Twp-155N R-84W S-2) This site is located in a prairie habitat on a south facing steep slope. Other than leafy spurge, with a average density of 98 stems per m^2, average height, at flowering, is 33.48 cm , the vegetation was native prairie grasses. The soil is classified as a loam to loam fine sand and the annual precipitation is 49.72 cm. The site is exposed to direct sun light 80% of the time and is exposed to winds from an east, west or south direction.

CZLA7-03RO. (Twp-162N R-70W S-19) This site is located 48 kilometers south of the Canadian border in Rolette county. The vegetation is leafy spurge and native grasses situated in a rolling terrain topography with prairie to wooded habitats. The soil is a loam clay or clay loam. The leafy spurge averaged 73.96 cm tall with a density of 187 stems per m^2 at the flowering stage. There is little wind exposure, 75% direct sunlight and the annual rain fall averages 50.55 cm.

CZLA8-01BE. (Twp-156N R-71W S-9) Most of the vegetation was leafy spurge at this location. The topography is flat with a prairie habitat. The soil is a loam/fine sand loam. Precipitation in this region averages 51.26 cm per year, 98% direct sunlight with a average stem density of 127 per m^2 and a height, at flowering, of 38.1 cm. This location is exposed to the wind from all directions.

Aphthona nigriscutis

Materials and Methods

Eight locations were selected for the evaluation of *A. nigriscutis* adults populations on leafy spurge to determine the effect *A. nigriscutis* has on the root system and stem density. The specimens used to supply site NI1-06LA (445)and NI2-01RI (230) were from the first shipment of 750 *A. nigriscutis* adult females collected in Baja, Hungary by USDA-APHIS from *E. cyparissiae.* The remaining 75 specimens from shipment 1 were kept as voucher specimens or used for greenhouse studies. The second shipment also collected in Baja, Hungary contained 250 adult females and was released in Rolette Co. at site NI3-01RO. Sites NI4-01WA and NI5-12BA were supplied from specimens collected at Glenboro, Canada by North Dakota State University Department of Entomology personnel; and collections in Ward county, North Dakota (Site NI4-01WA) in 1991, were released at sites NI6-01ED, NI7-03BE, and NI8-02GR (Site Descriptions D).

Results

The impact, at site NI1-06LA, by *A. nigriscutis* larval feeding reduced the leafy spurge between 1989 and 1996 (Fig. 4A). The percent change fluctuated, but by 1996 the percent reduction in root dry weight was 39%, root segments 50%, root buds 29%, and stem densities 68%. Spring adult sweep counts reached a peak in 1993 at 180 *A. nigriscutis* per m^2 (Table 6). The root evaluation ended in 1994 after this site was contaminated by an accidental release of *A. lacertosa*.

At site NI2-01RI *Aphthona nigriscutis* populations had not increased to more than 13 adults per m^2 by 1993 (Fig. 4B, Table 8). There was no distinguishable impact on the leafy spurge through 1993, and root evaluations were terminated in 1993 along with spring sweep counts.

By 1996, the leafy spurge root dry weight, buds, and segments, at site NI3-01RO, were reduced 60 to 82% from the release year (1989) base-line data (Fig. 4F). The stem density was reduced 90% during the same period. Adult populations peaked at 500 adults per m^2 in 1993 (Table 8).

There was a 40% increase in root dry weight and root segments at site NI4-01WA the first year (1989) after releasing the biocontrol agent (Fig. 4C). But the trend was a steady decrease in the spurge infestation. In 1996, the root system and vegetative portion of the leafy spurge infestation had been reduced 80 to 90%. The adult population levels peaked at 700 per m^2 in 1993 (Table 8).

At site NI5-12BA the leafy spurge root system was reduced 60 to 82% and the stem density was reduced 90% by 1996 (Fig. 4D). Adult populations peaked at 500 per m^2 in 1993 (Table 8).

At site NI6-01BE, the first year (1989) after the initial release of the bio-control agent all of the data variables increased (Fig. 4E). There was a 20 to 57% reduction in the leafy spurge root and stem systems by 1996, but the area impacted was less than 100 m in diameter. The adult populations reached its highest point in 1996, 221 adults per m^2 (Table 8).

Aphthona nigriscutis ability to survive the winters was poor at site NI7-03BE. Sweep samples taken in the spring of each year, after the initial release year, produced an average of 3 adults per m^2 for years 1989 through 1996 (Table 8). These low numbers of adults were also reflected in the reduced impact by the larvae on the leafy spurge root system (Fig. 4G).

At site NI8-02GR, the leafy spurge root system increased for two years after the initial release of *A. nigriscutis* (Fig. 4H). From 1992 through 1996, the impact of *A. nigriscutis* larval feeding reduced the root system, but the reduction in the root dry weight, root segments and root buds was only 15 to 25%. The stem density was reduced 39% by 1996. The spring sweep counts for adults ranged between 45 and 52 per m^2 from 1989 through 1996 (Table 8).

Discussion

The amount of control or percent reduction of a leafy spurge infestation treated with a biocontrol agent appears to be related to how many of the habitat characteristics are suitable for *A. nigriscutis* development and survival. Two of the locations, site NI4-01WA and site NI3-01RO, appeared to be suitable habitats for *A. nigriscutis*. At both locations the leafy spurge root system was reduced approximately 60% and stem densities 90%.

The overall impact on leafy spurge away from the initial release point differs between

site NI4–01WA and NI3-01RO. The effects of larval feeding damage on the spurge infestation could be measured in hectares at site NI4-01WA, but at site NI3-01RO the area of spurge effected was limited to meters. The habitat in site NI3-01RO changed to a wooded environment approximately 100 meters away form the initial release point. *Aphthona nigriscutis* did not appear to move into this wooded habitat and the control of leafy stopped at this point. There were also habitat restrictions at site NI4-01WA. The topography at this location was open rolling terrain with depressions or swales. Any *A. nigriscutis* impact on leafy spurge in the depressions was non existent or limited to the perimeter edges of the infestation.

The impact on the leafy spurge by larval feeding activity at sites NI1-06LA, NI5-12BA, NI6-01ED, and NI8-02GR was limited to an area of several meters in diameter. Though some of the habitat characteristics, suitable for *A. nigriscutis*, were available; it appears that the populations remained at a low level due to a lack of food resources. The reduction of the leafy spurge plant variables evaluated averaged 50% on stem densities, 35% in root dry weight, 32% in root segments, and 33% in root buds within the areas the adults were present.

Aphthona nigriscutis did not over winter at site NI7-03BE. This site is located in Benson county where late fall or early winter cold temperatures occur that have not been encountered at the other locations involved in this root evaluation study. The cold temperatures below the threshold level for the larvae development may have prevented overwinter survival.

At release site NI2-01RI, where all of the *Aphthona* species had been released at various times, the flea beetles did not establish.

Table 7. Shipments of *Aphthona nigriscutis* Received by The Department of Entomology at NDSU from USDA-ARS-WR & USDA-APHIS-PPQ between 1989 & 1991 and NDSU Collections in 1991

#	Year	Quarantine/ Release File No.	Date Collected	Date Shipped	Date Received or Released	Number of Specimens Received	[c] Release Site Legal Description	Days in Captivity
1	1989	[a] BCWLA-1-89-3	12 May 1989	13 June 1989	15 June 1989	750 female	Twp 136N R 51W-S22	34 days
2	1989	BCWLA-1-11	20 May 1989	21 June 1989	23 June 1989	250 female	Twp 172N R 70W-S30	33 days
3	1989	Canada	25 June 1989		25 June 1989	250 adults	Twp 156N R 84W-S30	0
4	1989	Canada	28 June 1989		29 June 1989	250 adults	Twp 140N R 48W-S29	1day
5	1991	[b] Site NI23-01WA	19 June 1991		20 June 1991	250 adults	Twp 133N R 59W-S32	1 day
6	1991	Site NI23-01WA	19 June 1991		20 June 1991	250 adults	Twp 142N R58W-S16	1 day
7	1991	Site NI23-01WA	19 June 1991		20 June 1991	250 adults	Twp 136N R 87W-S20	1 day
8	1991	Site NI23-01WA	19 June 1991		21 June 1991	250 adults	Twp 156N R 71W-S5	2 days

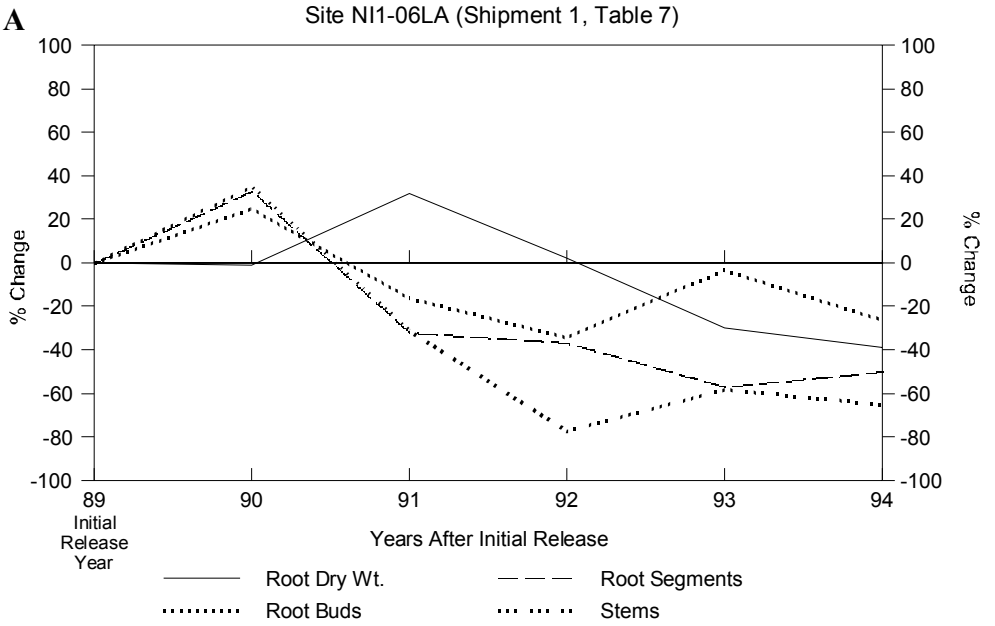
[a] Biological Control Western Laboratory Albany

[b] 1989 release site NI4-01WA Ward County ND

[c] Twp = Township R = Range S = Section for a County

Figure 4. The Impact of *Aphthona nigriscutis* on Leafy Spurge Root System and Stem Density

A



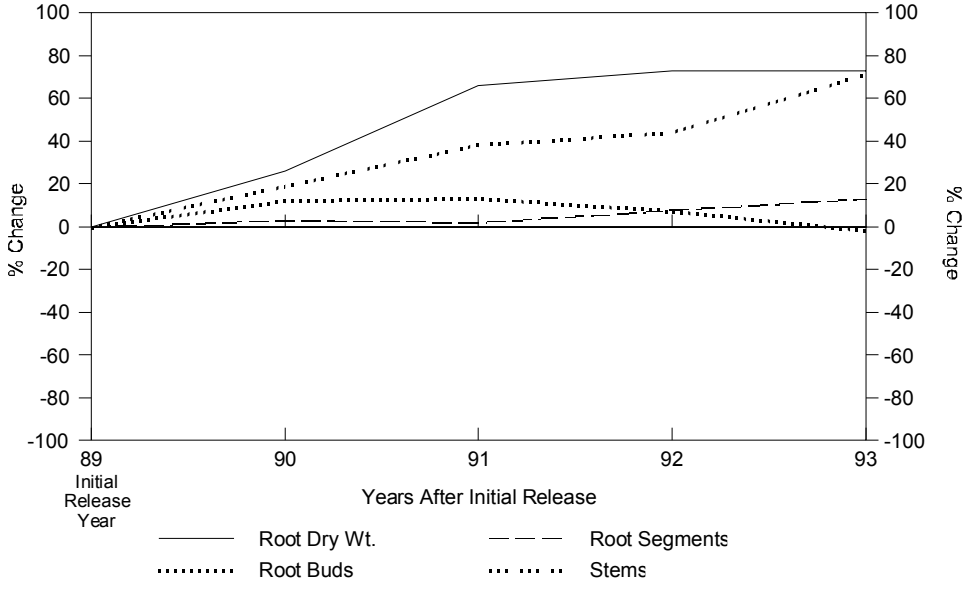
3.13 grams (Initial release year) mean root dry wt.

12.4 (Initial release year) mean root segments

62.4 (Initial release year) mean root buds

5.2 (Initial release year) mean stems

B



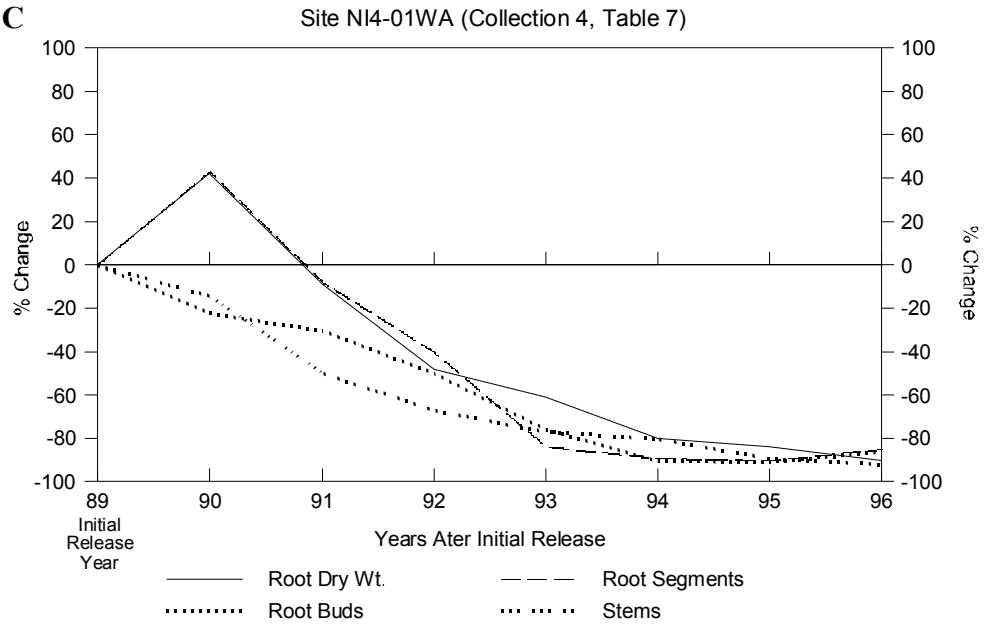
2.88 grams (Initial release year) mean root dry wt.

11.4 (Initial release year) mean root segments

51 (Initial release year) mean root buds

3.45 (Initial release year) mean stems

C



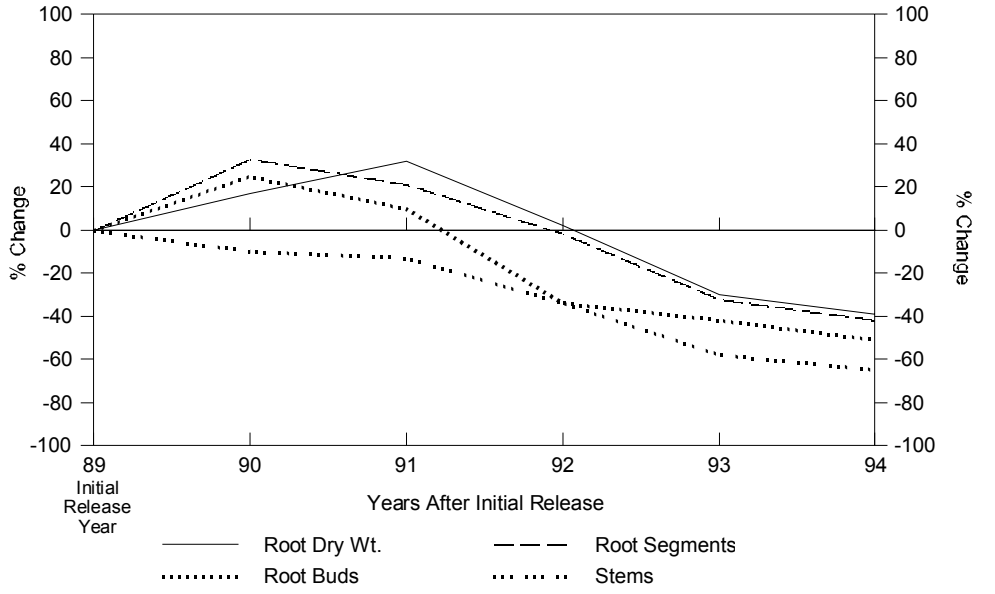
3.45 grams (Initial release year) mean root dry wt.

10.5 (Initial release year) mean root segments

52.5 (Initial release year) mean root buds

4.2 (Initial release year) mean stems

D



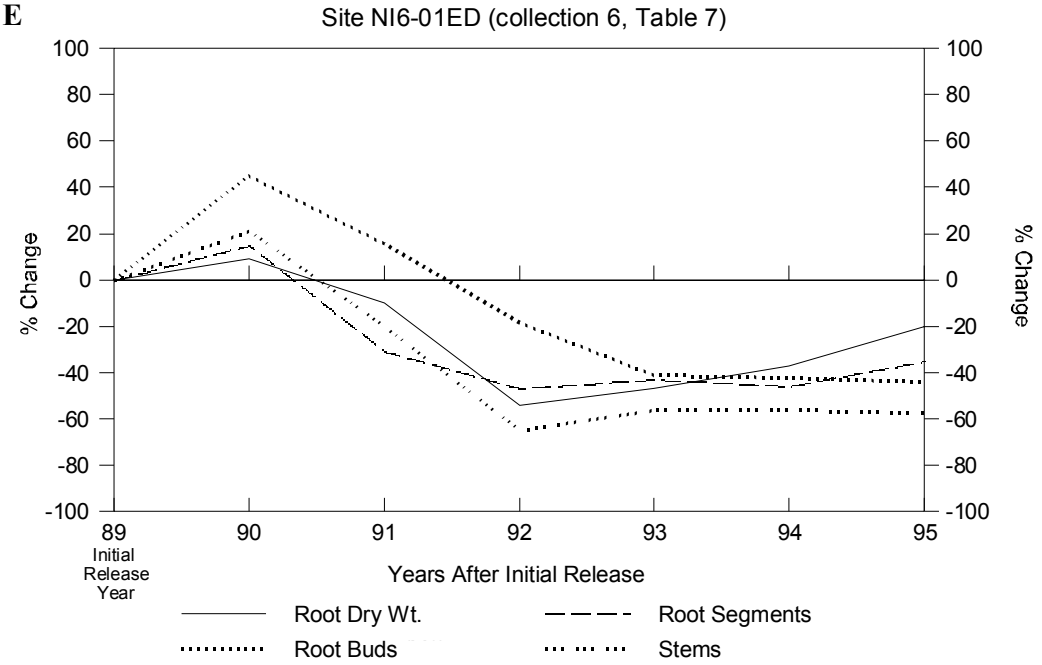
2.08 grams (Initial release year) mean root dry wt.

8.9 (Initial release year) mean root segments

32.5 (Initial release year) mean root buds

3.4 (Initial release year) mean stems

E



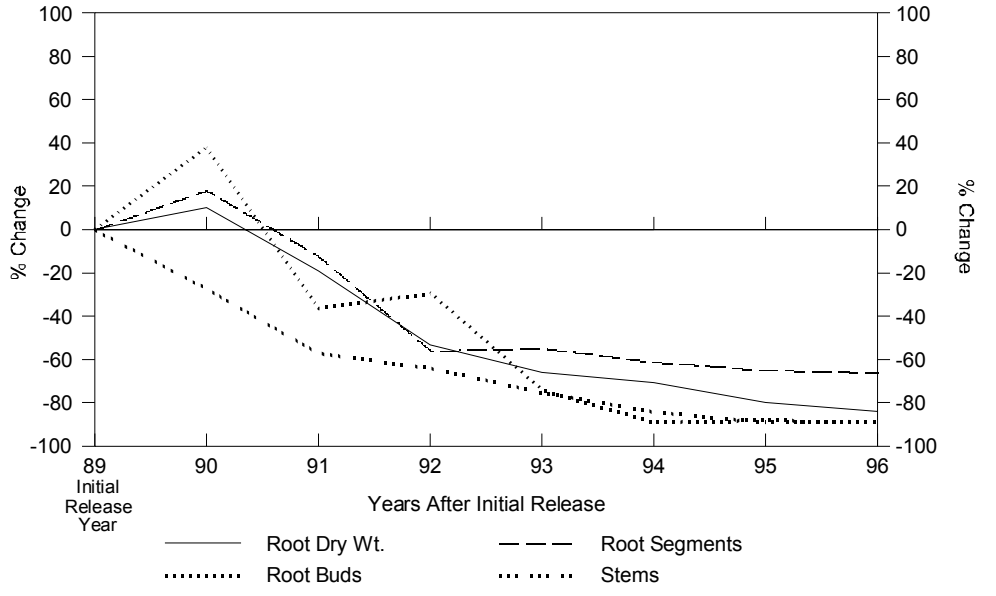
2.43 grams (Initial release year) mean root dry wt.

7.4 (Initial release year) mean root segments

49.44 (Initial release year) mean root buds

3.1 (Initial release year) mean stems

F



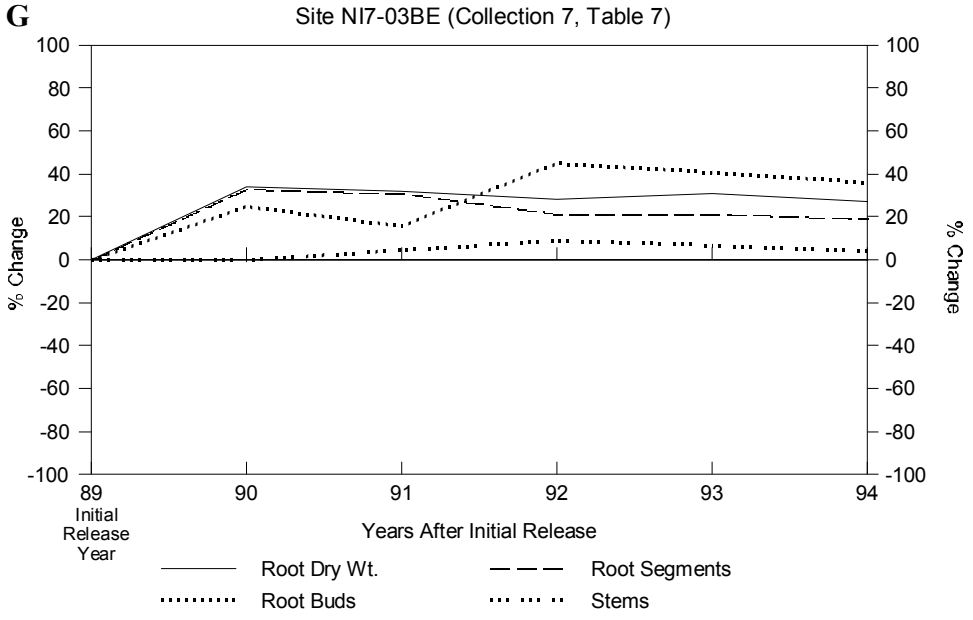
4.1 grams (Initial release year) mean root dry wt.

11.4 (Initial release year) mean root segments

58.35 (Initial release year) mean root buds

4.5 (Initial release year) mean stems

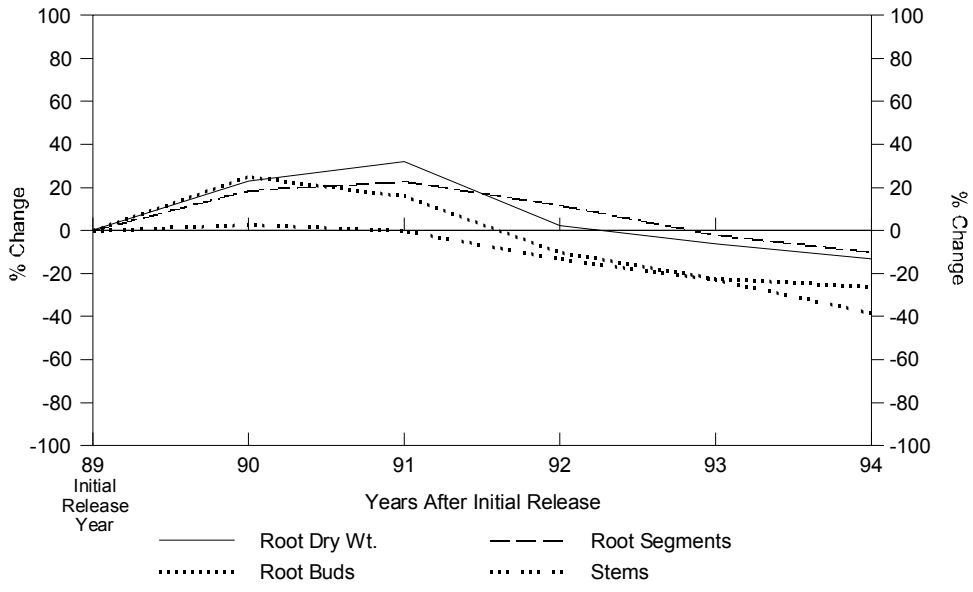
G



3.15 grams (Initial release year) mean root dry wt. 10.8 (Initial release year) mean root segments

43.5 (Initial release year) mean root buds 3.75 (Initial release year) mean stems

H



2.13 grams (Initial release year) mean root dry wt. 13 (Initial release year) mean root segments

53.5 (Initial release year) mean root buds 3.2 (Initial release year) mean stems

48.26 to 53.34 cm. The vegetation is 90% leafy spurge and native grasses. The leafy spurge was 35.56 to 45.72 cm tall with a density of 187 stems per m^2 , 70% direct sun light and the soil is loam.

NI7-03BE. (Twp-156N R-71W S-4) The soil type at this location is a loam/fine sand loam. Precipitation in this region is 48.26 to 55.88 cm per year. The topography is east sloping in a high prairie habitat. Most of the vegetation was leafy spurge with a stem density of 127 per m^2 and a height of 38.1 cm. This location is exposed to sun light 98% of the time and wind from all directions.

NI8-02GR. (Twp-136N R-88W S-14) This was in a high prairie habitat with a flat topography. Other than leafy spurge the vegetation was native grasses. The leafy spurge stem density was 119 stems per m^2 and the spurge plants height measured 30.48 cm. The average precipitation per year is 40.64 to 45.72 cm and 100% direct sun light. The soil is a loam.

Table 8. Mean (n=5)Yearly Sweep Counts of Adult *Aphthona nigriscutis* at Peak Emergence Per m^2

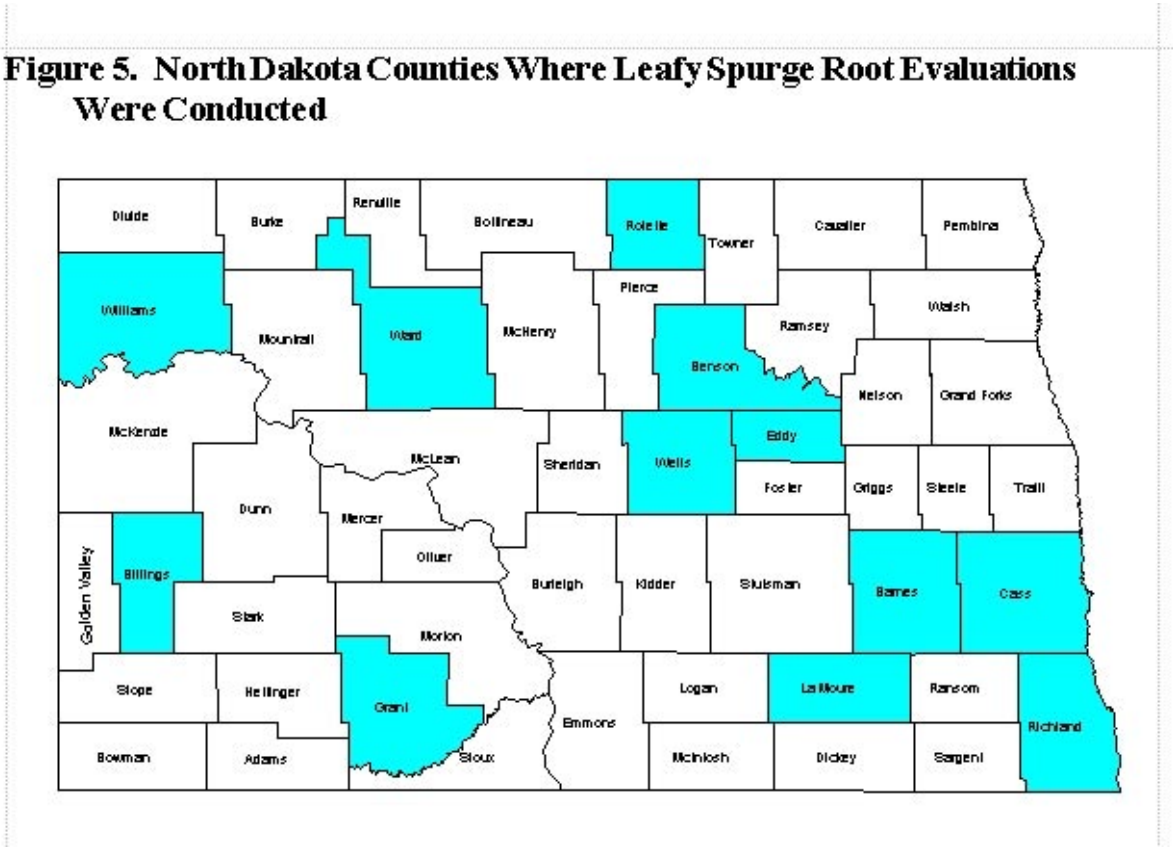
Release Site	1989	1990	1991	1992	1993	1994	1995	1996
NI21 - 06LA	250	56	48	62	180			
NI22 - 01RI	230	8	12	9	13			
NI25- 01ED	250	22	36	168	145	172	210	221
NI26- 01RO	120	178	260	400	500	437	175	90
NI24 - 12BA	250	135	240	350	400	370	125	95
NI23- 01WA	250	325	425	700	600	330	160	130
NI27 - 03BE	250	7	4	3	0	3	0	0
NI28 - 02GR	250	45	50	35	43	45	39	52

Table 9. Soil Analysis of Leafy Spurge Root System & Stem Density Evaluation Sites

SITE	Specie	CLASS	VCOS[a]	COSb	MSc	FSd	VFSe	SAND	SILT	CLAY	FSif	COSig	CO3-Ch	OMi	pH
FL1-09BA	flaj	L[n]	0.3	1.5	3.5	4.5	4.4	14.2	46.4	39.3	37.2	9.2	4.5	8.00	7.9
FL2-22BA	fla	L	3.5	2.8	6.2	26.3	19.0	57.7	26.8	15.5	13.6	13.1	0.9	6.70	7.7
FL3-23BA	fla	FSL[o]	0.1	1.2	13.7	42.2	13.9	71.0	20.0	9.0	11.4	8.6	0	3.10	6.6
FL4-25BA	fla	CL[p]	1.0	4.2	9.9	12.7	7.3	35.0	34.6	30.4	26.3	8.3	0	7.30	7.6
FL5-03LA	fla	FSL	0.0	1.2	12.8	38.8	14.3	66.9	19.9	13.2	8.0	11.9	0	2.80	7.1
FL6-04LA	fla	FSL	0.0	1.2	12.8	38.8	14.3	66.9	19.9	13.2	8.0	11.9	0	2.80	7.1
FL11-26BA	fla	SICL[q]	0.7	2.1	2.7	3.9	3.9	13.4	48.7	37.9	39.6	9.1	6.1	8.40	7.9
CY5-01LA	cyp[k]	FSL	2.7	3.1	2.5	6.8	8.9	24.1	42.4	33.5	28.1	14.3	0.9	3.80	7.1
CY6-02LA	cyp	LFS[r]	0.1	0.9	14.0	52.7	12.8	80.6	13.5	5.9	8.0	5.5	0	3.10	6.5
CY10-01WE	cyp	L	0.0	2.9	6.5	12.9	11.5	33.8	42.8	23.3	18.0	24.9	0	8.50	7.1
CY11-02RO	cyp	FS[s]	0.0	0.0	0.7	51.6	28.4	80.8	11.6	7.6	5.7	5.9	0	3.10	6.8
CY15-02WA	cyp	L	0.0	2.9	6.5	12.9	11.5	33.8	42.8	23.3	18.0	24.9	0	8.50	7.1
CZLA1-01BA	czla[l]	SIL[t]	0.7	3.1	6.1	10.9	12.3	33.1	42.1	24.8	24.5	17.6	1	9.28	6.8
CZLA2-01GO	czla	SICL	0.8	2.0	3.7	9.2	12.1	27.9	47.3	24.8	29.0	18.3	0.9	9.28	7.9
CZLA3-01ED	czla	LFS	0.0	1.0	18.3	51.6	13.4	84.4	9.8	5.8	3.7	6.1	0	2.40	6.5
CZLA4-02RI	czla	FS	0.0	0.0	0.7	51.6	28.4	80.8	11.6	7.6	5.7	5.9	0	3.10	6.8
CZLA5-01BI	czla	CL	0.5	3.3	6.8	8.4	6.3	25.2	46.4	28.3	25.8	20.7	0	10.10	7.2
CZLA6-03WA	czla	LFS[u]	0.0	3.3	18.4	43.3	19.4	84.4	8.5	7.2	2.7	5.8	0	2.80	7.0
CZLA7-03RO	czla	L	0.0	1.7	8.4	12.4	9.5	31.9	41.7	26.4	29.7	11.9	0	6.00	7.8
CZLA8-01BE	czla	FSL	0.1	3.2	18.8	25.3	11.1	58.4	27.3	14.2	16.3	11.0	0	6.40	7.4
NI1-06LA	nig[m]	FSL	0.1	1.2	15.3	46.2	13.8	76.7	13.3	10.0	7.5	5.9	0	2.50	7.5
NI2-01RI	nig	FS	0.0	0.0	0.3	80.9	13.8	95.1	2.5	2.4	1.1	1.5	0	0.90	7.2
NI4-01WA	nig	L	0.1	2.2	7.3	10.3	10.0	29.9	43.4	26.7	29.1	14.3	0	8.40	7.0
NI5-12BA	nig	L/FSL[v]	0.0	0.5	6.0	24.9	20.6	52.0	37.7	10.3	15.1	22.6	0	3.30	7.1
NI6-02ED	nig	FSL	0.0	1.3	9.3	41.7	22.6	74.9	15.6	9.5	5.8	9.9	0	4.30	7.4
NI3-01RO	nig	L	0.0	10.6	18.6	13.2	5.9	48.3	28.4	23.2	17.9	10.5	0	10.10	7.5
NI7-02ED	nig	FS/LFS[w]	0.0	3.5	25.0	48.5	11.8	88.8	6.9	4.3	3.7	3.1	0	2.00	6.9
NI8-02GR	nig	L	0.0	0.4	5.0	20.0	25.2	50.7	29.6	19.8	10.0	19.6	0	4.30	7.0

[a] Very coarse sand [b] Coarse sand [c] Medium sand [d] Fine sand
[e] Very fine sand [f] Fine silt [g] Coarse silt [h] Carbonate-Carbon
[i] Organic matter [j] flava [K] cyparissiae [l] czwalinae/lacertosa
[m] nigriscutis [n] Loam [o] Fine sand loam [p] Clay loam
[q] Silt clay loam [r] Loam fine sand [s] Fine sand [t] Silt loam
[u] Loam/fine sand loam [V] Loam/fine sand loam [w] Fine sand/loam find sand

Figure 5. North Dakota Counties Where Leafy Spurge Root Evaluations Were Conducted



Summary

The following life cycle data refers only to each species as they develop in North Dakota. *Aphthona czwalinae* and *A. lacertosa* usually emerge from late May to mid-June. They are followed by *A. nigriscutis* and *A. cyparissiae* approximately 4 to 6 days later in June. *Aphthona flava* is the last to emerge, usually in early July, but approximately 30 to 40 days after *A. czwalinae* and *A. lacertosa*. The longevity is also similar for all *Aphthona* spp. adults, lasting approximately 45 to 50 days from time of emergence. Egg laying begins 7 to 10 days after emergence and continues through the adult stage of the life cycle. The adult female will lay the eggs in the soil either at the base of the leafy spurge stem or in openings in the soil surface. The eggs hatch in 14 to 19 days. The first instar larvae begin feeding on the filamentous or hair roots. After the first molt, they move to secondary roots to feed. The third instar feed on primary roots. This instar has the longest feeding period, lasting approximately 4 to 6 months, with a dormant stage from the first freeze to the spring thaw, approximately November to April. Larval feeding continues in the spring until the larvae reach the pre pupa stage of their life cycle, approximately mid-May. At this point the larvae pupate and after 10 to 14 days the summer generation adults emerge.

There is approximately 850,000 acres infested with leafy spurge in North Dakota**.** The environments where this plant can survive ranges from metropolitan environments to sparsely inhabited rural areas. The magnitude of any one infestation can be as variable in size as there are places leafy spurge can grow. It is the ability of leafy spurge to thrive in diverse habitats that dictates the necessity for more than one control method.

The utilization of *Aphthona* spp. flea beetles as a biological control method on leafy spurge can be effective and economical. A single release of 80 adult *A. czwalinae* and *A. lacertosa* mix reduced a leafy spurge plant infestation by 98% over hundreds of acres, with *A. lacertosa* being the predominant species. An extremely high population developed from these 80 flea beetles that millions of adults were collected and redistributed to several other areas in North Dakota.

The soil environment conducive for *A. lacertosa* larval development are silt loam, silt clay loam, clay loam, loam or loam/fine sand loam, with a ph range of 6.8-7.9, and 6.0-9.28% organic matter (OM) (Table 9). Leafy spurge stem and root tissues are reduced an average of 70 to 90% under these conditions. A fine sand soil classification appears to restrict the development of filament roots within a few centimeters of the soil surface. Consequently, an accessible food source is not available for newly emerged *A. lacertosa* larvae, resulting in reduced impact and population development under these conditions. *Aphthona lacertosa* will establish and maintain leafy spurge, at very low plant stands, in habitats ranging from high and dry to wet dense wooded areas that have the above soil conditions.

Aphthona nigriscutis can have the same impact on leafy spurge infestations as *A. lacertosa*; however, its success is restricted to more specific habitats. The success of *A. nigriscutis* will vary among characteristically different habitats that often occur in very large infestations of leafy spurge. *Aphthona nigriscutis* is more successful when leafy spurge is growing under 90% direct sunlight in a well drained loam soil with a ph of 7.1 to 7.9 and 8 to 11% OM; and, the spurge stem height and density is less than 45.72 cm and 187 m^2. *Aphthona nigriscutis* can reduce a spurge infestation by 90% under these conditions. This flea beetle species will reduce the vegetative tissues of leafy spurge by 50 to 70% when the habitat consist of a loam or loam find sand soil with a ph of 6.5-7 and 2.4-4.3% OM; and, the stem density is 119 to 229 stems per m^2 with a plant height of 30.48 to 53.34 cm. *Aphthona nigriscutis* does not reach sufficient population levels to impact leafy spurge infestations in habitats where the soil is classified fine sand.

The habitat requirement for *A. flava* is more restricted than for *A. lacertosa* or *A. nigriscutis*. The feeding activity of *A. flava* was confined to a relatively small area at each evaluation release site. The only type of habitat where *A. flava* maintained a small population consisted of silt clay loam soil with a ph of 7.9 and 8.0-8.4% OM; and, a stem density and height ranges from 145-371 stems per m^2 and 45.72-35.56 cm. The habitats that are not conducive for *A. flava* survival and development consist of loam, find sand loam, clay loam soil, with a ph of 6.6-7.9 and 2.8-8.0% OM; and, stem density and height of 145-371 stems per m^2 and 48.26-

60.96 cm. The soil and root characteristics can vary, particularly, within a large spurge infestation. When using *A. flava* as a biological control agent for leafy spurge, it will be necessary to collect and specifically redistribute the flea beetle into its preferred habitat.

The habitat requirement of *A. cyparissiae* is still questionable. The only evaluation site that a population still exists has a loam soil. The ph is 7.1 and the percent OM is 8.5. The density of the leafy spurge is 146 stems per m^2 and the plants are 27.94 cm tall. The impact on the leafy spurge infestations was not significant.

Conclusion

Aphthona spp. flea beetles can be a successful biocontrol method for leafy spurge in North Dakota. One species *of Aphthona* flea beetle could be used in a leafy spurge infestation if the habitat characteristics are uniform and meet the requirements of the *Aphthona* spp. used. It is more likely that a leafy spurge infestation of more than an acre inhabits a wide range of habitats. When treating these types of infestations with *Aphthona* flea beetles, a multi species control method may be better.

The order of *Aphthona* spp. flea beetles productivity in controlling leafy spurge is *A. lacertosa/A. czwalinae* followed by *A. nigriscutis, A. flava*, and *A. cyparissiae*.

Acknowledgments

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