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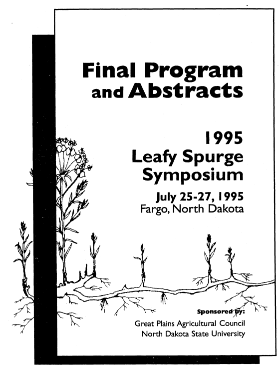

Leafy Spurge Task Force meeting and symposium

25 - 27 July, 1995
Radisson Hotel, Fargo, North Dakota

Program

Tuesday, July 25

10:00 Registration - Coffee

1:10 Welcome - Opening Remarks
Don Galitz
Dr. Robert Todd - Director North Dakota Agricultural Experiment Station

Cal Messersmith - Moderator

1:20 Insects & informationage - Neal Spencer, USDA/ARS, Range Weeds and Cereals Research Unit, Sidney, MT.

1:40 USDA APHIS field insectory establishment and redistribution of leafy spurge biological control agents - How it's working on a national scale - Bob Richard, USDA/APHI/PPQ, Forestry Sciences Lab, Montana State University, Bozeman, MT.

2:00 PPQ insectary sites in North Dakota - Keith Winks, USDA/APHIS/PPQ, Bismarck, North Dakota.

2:20 *Chamaesphecia hungarica* - current status of quarantine clearance and field establishment - Paul Parker, USDA/APHIS, Mission Biological Control Center, Mission, Texas

2:40 Collection and redistribution of leafy spurge biological control agents from North Dakota insectary and research sites - R.B. Carlson, Entomology Department, North Dakota State University, Fargo, North Dakota.

3:00 Break

Bob Carlson - Moderator

3:20 Dispersion of *Aphthona Species* within release sites - Donald Mundal* and R.B. Carlson, Entomology, North Dakota State University, Fargo, North Dakota.

3:40 DNA characterization of *Aphthona* spps. - Lloyd Wendel, USDA/APHIS, Mission, Texas.

4:00 Simple techniques for distributing *Aphthona* flea beetles from established field insectary sites - Rich Hansen, USDA/APHIS, Forestry Sciences Lab, Montana State University, Bozeman, MT.

4:20 Systems approach with biological agents for leafy spurge control - Jeff Nelson, Department of Plant Sciences, North Dakota State University, Fargo, North Dakota.

4:40 Biocontrol of leafy spurge in Manitoba - Progress to date and prospects for the future - Carla Allen, Manitoba Agriculture, Carman, Manitoba.

Wednesday, July 26

Rod Lym - Moderator

8:00 The economic impact of leafy spurge on conservation reserve program land in North Dakota - Steven Hirsch, USDA/APHIS/PPQ, Bismarck, North Dakota.

8:20 Leafy spurge management by combining sheep grazing with fall-applied herbicides - George Beck, Colorado State University, Ft. Collins, CO.

8:40 Defoliation effects of leafy spurge on sheep rumen microorganisms - JoAnna Roberts, Department of Animal & Range Sciences, Montana State University, Bozeman, MT.

9:00 Multi-species grazing of leafy spurge infested rangeland in North Dakota. Chad Prosser*, Kevin Sedivec and William T. Barker.

9:20 Intensive grazing of angora goats on leafy spurge infested rangeland. Kevin Sedivec*, William T. Barker and Chad W. Prosser, Animal and Range Sciences, North Dakota State University, Fargo, North Dakota.

9:40 Break - Coffee

Dave Davis - Moderator

10:00 On the Biology of Leafy Spurge - Don Galitz, Department of Botany, North Dakota State University, Fargo, North Dakota.

10:20 Cloning of GRP (growth response) genes which are induced during breaking of quiescence in root buds of leafy spurge - David Horvath, USDA/ARS, Biosciences Research Lab, Fargo, North Dakota.

10:40 Relatedness of North American and European leafy spurge based on DNA markers - Martha Rowe, Department of Agronomy, University of Nebraska-Lincoln, Lincoln, Nebraska.

11:00 Esters of quinclorac as possible leafy spurge herbicides: absorption, translocation, metabolism and toxicity. - G.L. Lamourex* and D.G. Rusness, USDA/ARS, Biosciences Research Lab, Fargo, North Dakota.

11:20 Effect of leafy spurge biotype and herbicide application on *Aphthona* spp. establishment. - R.G. Lym*, R.B. Carlson and D.A. Mundal, Department of Plant Sciences, North Dakota State University, Fargo, North Dakota.

12:00 Luncheon provided

Don Galitz - Moderator

1:00 Preliminary assessment of effect of prescribed fire upon establishment of *Aphthona nigriscutis* in leafy spurge - David Fellows, National Biological Service, Northern Prairie Science Center, Jamestown, North Dakota.

1:20 Herbicides and grass competition for leafy spurge control in North Dakota - 1995. K.M. Christianson*, R.G. Lym and C.G. Messersmith, Department Plant Sciences, North Dakota State University, Fargo, North Dakota.

1:40 Methods to enhance competitiveness of other species with leafy spurge. - R.T. Wallander* and B.E. Olson, Department of Animal and Range Sciences, Montana State University -Bozeman, Bozeman, MT.

2:00 Leafy spurge control with imazameth. - R.A. Masters* and F. Rivas-Pantoja, Agricultural Research Service, University of Nebraska-Lincoln, Lincoln, Nebraska.

2:45 Leave for Ekre Ranch - transportation provided
History of ranch

Plots
Spurge control program

6:00 BBQ at Runck Ranch

Thursday, July 27

8:00 Discussion Groups

9:30 Business Meeting: There will be a discussion of the future of this group's meetings, as the Great Plains Agricultural Council (GPAC) will terminate its existence on September 30, 1995. The Leafy Spurge Task Force has existed under the auspices of the GPAC.

Leafy Spurge Symposium Posters

Jennifer Birdsall, USDA-ARS Rangeland Weeds Lab

Title: *Image Analysis to Determine Vegetative Cover of Leafy Spurge*

By: J.L. Birdsall, P.C. Quimby, Jr., T. Svejror & B. Sowell.

David G. Davis, USDA-ARS

Title: *IAA Partially Counters the Effect of a Wide Range of Root Formation*

Inhibitors in Leafy Spurge.

By: David G. Davis

D. Stuart Frear, USDA-ARS

Title: *Induced Frost Tolerance in Leafy Spurge Roots: Changes in Carbohydrate Metabolism*

By: D. Stuart Frear and Harley R. Swanson

John Lankow, Dept of Botany, NDSU

Title: *Ultrastructure of Euphorbia esula Root and Crown Buds*

By: John Lankow and Don Galitz

Robert A. Masters, University of Nebraska

Title: *Imidazolinones Improve Ecological Restoration of Great Plains Grasslands*

By: R.A. Masters, D.D. Beran, and R.E. Gaussoin

Julie Schroer, Dept of Botany, NDSU

Title: *G.I.S. Analysis of Leafy Spurge Distribution*

By: Julie Schroer, C.S. Balachandran, Don Galitz

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Biological control of leafy spurge in Manitoba – Progress to date and prospects for the future

CARLA L. ALLEN

Weed Specialist, Manitoba Agriculture

Manitoba Agriculture has been involved in biological weed control since 1974. To date more than 25 different insect species have been released on nine different weeds. Most efforts have concentrated on leafy spurge as a number of different agents have been released on this noxious weed. The *Aphthona nigriscutis* and *Aphthona cyparissiae* have provided the greatest suppression of leafy spurge since the first release in Manitoba in 1983. The Manitoba Weed Supervisors Association has been an excellent vehicle for re-distribution of insects. As of 1994, there were more than 850 *Aphthona nigriscutis* and more than 250 *Aphthona cyparissiae* release sites in the province. Many of these sites are harvestable and show significant weed suppression. *Lobesia euphorbiana* has successfully established in Manitoba and reduces seed set considerably. Two other *Aphthona* spp. have established in Manitoba: *A. lacertosa* and *A. czwalinae.* Both insects thrive on heavier soil types and *A. czwalinae* has survived spring flooding.

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Leafy spurge (*Euphorbia esula*) management by combining sheep grazing with fall-applied herbicides

K. G. BECK and J. R. SEBASTIAN

Dept. of Plant Pathology and Weed Science, Colorado State University

Introduction

Leafy spurge (*Euphorbia esula*) is an aggressive, perennial rangeland weed infesting millions of acres in the northern tier of the Great Plains states. It reduces cattle carrying capacity of rangeland and causes extreme economic losses to cattle producers and wildland areas (2).

Leafy spurge is very difficult to control and a combination of treatments, i.e., integrated weed management, may provide long-term leafy spurge population reductions. University of Wyoming research indicates that sequential applications of glyphosate followed by seeding perennial grasses controlled leafy spurge 83% on the average across all tilled plots three years after treatments were invoked (3).

Sheep will graze leafy spurge. Sheep readily consumed leafy spurge up to 50% of their diet free choice and showed no deleterious signs (1). While sheep grazing may not reduce leafy spurge populations, they may consume enough leafy spurge to release grasses from weed competition and thus, allow the area to be grazed by cattle. Additionally, sheep grazing in spring and summer may stress leafy spurge sufficiently to make it more susceptible to fall-applied herbicides.

The objectives of our research were to determine if sheep grazing of leafy spurge followed by fall applied herbicides would enhance control compared to herbicides applied alone in spring; and whether grazing would enhance the susceptibility to fall applied herbicides such that reduced herbicide rates would control leafy spurge similarly compared to higher rates.

Materials and methods

The experiment was initiated in 1991 at Cherry Creek State Park in Aurora, CO. The design was an eight (herbicides) by three (management approaches) factorial arranged in a strip-plot with four replications. The eight herbicide treatments (Table 1) comprised the horizontal factors (main plots) and the three management approaches (spring-applied herbicides at flowering, fall-applied herbicides to regrowth, or grazing followed by fall-applied herbicides to regrowth) were the vertical factors (sub-plots). Two sheep grazed their assigned plots (0.33 A) for 75 days per year. All herbicides were applied with a CO_2 backpack sprayer at 24 GPA. Spring applied herbicides, fall-applied herbicides, and graze plus fall-applied herbicides treatments were invoked for 4 consecutive years.

The impact from each management approach and herbicide treatment was assessed on the entire plant community. Leafy spurge, downy brome (*Bromus tectorum*), smooth brome (*Bromus inermis*), western wheatgrass (*Agropyron smithii*), Kentucky bluegrass (*Poa pratensis*), litter, and bareground cover (Daubenmire) and leafy spurge density were estimated twice per season; before sheep were introduced into the study area in spring (April) and in fall before herbicides were applied (October). Repeat cover and density determinations were taken from the same locations within plots. Percent control of leafy spurge was estimated visually each year in April, before sheep grazing began and in October before herbicides were applied. Cover, density, and control data from spring, 1995 are presented.

Results

Perennial grasses: Western wheatgrass and Kentucky bluegrass cover was not influenced by herbicide treatment or management approach. Smooth brome cover was affected by herbicide treatment (Table 1), but not by management approach. Smooth brome cover was 3 to 5 times less from picloram plus 2,4-D at 0.25 + 1.0 lb and 0.5 + 1.0 lb compared to picloram at 0.25 lb, picloram plus 2,4-D at 0.13 + 1.0, and dicamba plus 2,4-D. The highest rate of picloram alone and the two highest rates of picloram plus 2,4-D may have injured the smooth brome. Total perennial grass cover (sum of all perennial grasses) was influenced by herbicide treatment (Table 1). Total perennial grass cover was 1.5 to 2.5 times greater in plots treated with dicamba plus 2,4-D than in plots treated with picloram at 0.13 and 0.5 lb, and picloram plus 2,4-D at 0.25 + 1.0 lb. Total perennial grass cover was 1.6 to 1.9 times less in plots treated with picloram plus 2,4-D at 0.25 + 1.0 lb than in plots treated with picloram at 0.25 lb, picloram plus 2,4-D at 0.13 + 1.0 and 0.5 + 1.0, and the non-sprayed control.

Litter and bareground: Litter and bareground cover was influenced by management approach (Table 2), but not by herbicide treatments. Sheep grazing increased the amount of bareground and decreased the amount of litter. There was 8 to 16 times more bareground in plots where the graze plus fall herbicide management approach was used than in plots where herbicides were applied alone in spring or fall, respectively. There was 1.2 times more litter in plots where herbicides were applied alone in spring or fall than in plots where the graze plus fall-applied herbicides treatments were invoked.

Leafy spurge cover, density, and control: Leafy spurge cover and density were influenced by herbicide treatments and the different management approaches, but the herbicide by management interaction was insignificant. Leafy spurge cover (36%) and density

(19 shoots/0.1 m^2) were greater in the non-sprayed control plots than in all herbicide treated plots (Table 3). Leafy spurge cover was 4 to 8 times less and density was 2 to 5 times less in plots treated with picloram at 0.5 lb and picloram plus 2,4-D at 0.5 + 1.0 lb than in plots treated with picloram at 0.13, 0.25 lb, and picloram plus 2,4-D at 0.13 + 1. 0 lb. - Leafy spurge cover was 2 times less and density was 2.5 times less in plots where the graze plus fall-applied herbicide treatments were made than in plots that received only spring applied herbicides, whereas plots that received only fall applied herbicides were not different from the other management approaches (Table 4). It appears that the management system of grazing plus fall applied herbicides may have decreased leafy spurge more effectively than the traditional approach of only applying herbicides in spring, but these data were collected 6 and 11 MAT, respectively, and this artifact may have influenced our spring 1995 results.

A significant herbicide by management approach interaction was observed for leafy spurge control. Leafy spurge control did not vary within a herbicide treatment when compared among management approaches (Table 5). However, 38% of leafy spurge was controlled in plots that were grazed and where no herbicides were applied compared to no control in the non-grazed, non-sprayed plots. When comparing leafy spurge control from herbicide treatments within a management approach, picloram plus 2,4-D at 0.5 + 1.0 lb applied in spring controlled 34 and 28% more leafy spurge than the lowest rates of picloram and picloram plus 2,4-D, respectively. Picloram at 0.5 lb applied in fall controlled 45% more leafy spurge than picloram at 0.13 lb and 39 % more than picloram at 0.25 lb. After 4 consecutive years of applying the integrated management approach, leafy spurge control from grazing plus fall applied picloram at 0.13 lb was not different from grazing plus fall applied picloram at 0.5 lb.

It is apparent that grazing of leafy spurge by sheep did not enhance the susceptibility of leafy spurge to herbicide treatments when comparing a rate of a herbicide among the three management approaches. However, grazing enhanced the susceptibility of leafy spurge to fall applied herbicides sufficiently enough that the weed was controlled equivalently from picloram at 0.13 and 0.5 lb as long as these treatments were preceded by grazing.

Literature cited

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3. Whitson, T.D., D.W. Koch, A.E. Gade, and M.E. Ferrell. 1990. The control of leafy spurge (*Euphorbia esula* L.) by the integration of herbicides and perennial grasses. Proc. Leafy Spurge Symp., July 10-12, Gillette, WY. p.26-27.

Table 1. Smooth brome and total perennial grasses cover in spring 1995, as influenced by 4 consecutive years of herbicide treatments to control leafy spurge, when treatments were averaged over management approaches.

Herbicides	Rate	Cover[1]	
		Smooth brome	Total perennial grasses
(lb ai/A)	%		
Picloram	0.13	12 bcd	36 bc
	0.25	23 abc	41 ab
	0.5	10 cd	27 bc
Picloram + 2,4-D	0.13 + 1.0	21 ab	36 ab
	0.25 + 1.0	7 d	22 c
	0.5 + 1.0	5 d	39 ab
Dicamba + 2,4-D	1.0 + 2.0	29 a	56 a
Control	0	10 bcd	39 ab

[1]Data were analyzed as square root transformations, but are presented as their original values. Means within a column followed by the same letter do not differ, LSD (0.05).

Table 2. Litter and bareground cover in spring 1995, as influenced by management approach invoked for 4 consecutive years, when management approaches were averaged over all herbicide treatments.

Management approach	Cover[1]	
	Litter	Bareground
Spring-applied herbicides	93 a	1 b
Fall-applied herbicides	95 a	2 b
Graze + fall-applied	78 b	16 a

[1]Data were analyzed as square root transformations, but are presented as their original values. Means within a column followed by the same letter do not differ, LSD (0.05).

Table 3. Leafy spurge cover and density in spring 1995, as influenced by 4 consecutive years of herbicide treatments when treatments were averaged over all management approaches.

		Leafy spurge[1]	
Herbicides	Rate	Cover	Density
	(lb ai/A)	%	(shoots/0.1 m^2)
Picloram	0.13	8 b	5 b
	0.25	4 b	2 bc
	0.5	<1 c	< 1 d
Picloram + 2,4-D	0.13 + 1.0	7 b	3 bc
	0.25 + 1.0	2 bc	<1 cd
	0.5 + 1.0	<1 c	<1 d
Dicamba + 2,4-D	1.0 +2.0	2 bc	<1 cd
Control	0	36 a	19 a

[1]Data were analyzed as square root transformations, but means are presented as their original values. Means within a column followed by the same letter do not differ, LSD (0.05).

Table 4. Leafy spurge cover and density in spring 1995, as influenced by management approach invoked for 4 consecutive years, when management approaches were averaged over herbicide treatments.

	Leafy spurge[1]	
Management approach	Cover	Density
	%	(shoots/0. 1m^2)
Spring-applied herbicides	10 a	5 a
Fall-applied herbicides	8 ab	4 ab
Graze + fall-applied	5 b	2 b

[1]Data were analyzed as square root transformations, but are presented as their original values. Means within a column followed by the same letter do not differ, LSD (0.05).

Table 5. Leafy spurge control in spring 1995, as influenced by herbicide treatments and management approaches invoked for 4 consecutive years.

		Management approaches[1]		
Herbicides	Rate	Spring-applied herbicides	Fall-applied herbicides	Graze + fall herbicides
	(lb ai/A)		% Control	
Picloram	0.13	65 a	55 a C	89 a AB
	0.25	79 a B	61 a C	83 a B
	0.5	83 a AB	100 a A	100 a A
Picloram + 2,4-D	0.13 + 1.0	71 a	78 a BC	84 a AB
	0.25 + 1.0	81 a AB	81 a ABC	100 a A
	0.5 + 1.0	99 a A	95 a AB	100 a A
Dicamba + 2,4-D	1.0 + 2.0	78 a AB	93 a AB	89 a AB
Control	0	0 b C	0 b D	38 a C

[1]Data were analyzed as arc sine square root transformations, but are presented as their original values. Use lower case letters to compare means within a row and upper case letters to compare means within a column. Means within a row or a column followed by the same letter do not differ, LSD (0.05).

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Image analysis to determine vegetative cover of leafy spurge

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USDA-ARS Rangeland Weeds Lab., MSU, Bozeman, MT 59717, USDA-ARS Eastern Oregon AG. Res. Ctr., Bums, OR 97720, Dept. Animal and Range Natl. Res., MSU, Bozeman, MT 59717

Introduction

Ocular plant canopy cover is useful to describe trends following certain treatments (Brown 1954). However, ocular estimates of cover can be highly biased and influenced by the subjectivity and judgement of the observer (Bonham 1989). Image analysis offers technology which could be useful to determine vegetative cover with less bias. In image analysis, a video image is digitized into pixels which are assigned numeric values. The pixels can then be quantified. Comprehensive personal computer based software packages for image analysis have recently been developed.

The objective of this study was to determine if an image analysis program could separate leafy spurge from other component species in a quadrant and measure leafy spurge cover, and to compare the image analysis method of estimating leafy spurge cover to the ocular cover method.

Materials and methods

Image generation

In 1993, leafy spurge canopy cover was measured ocularly in the field at forty $0.1m^2$ sampling loci in each of eighteen plots. Plots were located near Glasgow, MT, Grassrange, MT and Mackay, ID. At a later date, color print photos one meter above ground level were taken at the sampling loci using a 35mm camera. Two independent observers determined ocular cover for each photo. A cover value was also determined for each photo using Jandel Video Analysis Software.

Image processing

To obtain cover values using Jandel Video Analysis Software, each photo was converted to a digital image composed of pixels with grey values that could range from 0 to 255. Leafy spurge cover was estimated by selecting the grey value range that corresponded to leafy spurge in the image, producing a binary image where all pixels in the selected range were white and all pixels outside the range were black, and determining the percentage of white pixels.

Data analysis

To compare the precision of the ocular and image analysis cover estimates, 10 photos were selected which ranged in cover from 0 to 95 percent. Four independent observers ocularly estimated leafy spurge cover ten times for each photo on different days. Similarly, leafy spurge cover was estimated ten times for each photo using image analysis. Precision was defined as the relative measure of the reliability and repeatability of the estimates. The variable used to measure reliability was the mean cover value for each photo. The variable used to compare repeatability was the variance of the repeated measures for each photo. Analysis of variance procedures were used to compare the means and variances. The reliability of the image analysis method was also tested by comparing the image analysis cover estimates to the ocular cover estimates made in the field and by the two observers from the photos using analysis of variance procedures for all the sampling loci together and for the sampling loci at each of the eighteen plots. Tests for correlation were used to further compare the ocular and image analysis estimates.

Results and discussion

Image analysis was capable of separating leafy spurge from other component species in a quadrant. This is attributed to leafy spurge's significantly higher reflectance measurements which result in different digital values from those of associated vegetation and soil (Everitt *et al*. 1994).

Image analysis could estimate leafy spurge cover with the same level of precision as the ocular cover method. The means and variances of the repeated measures made on the ten photos by image analysis and ocularly by four observers did not differ ($P > .05$). The image analysis method had a lower variance than the three of the four observers. Nutter *et al*. (1993) found image analysis estimates of percent disease severity of bentgrass more precise than ocular estimates of disease severity.

Comparison of the means for the image analysis versus ocular cover methods for all the sampling loci together revealed that the ocular estimates of leafy spurge cover made in the field and from the photos by one of the two observers did not differ from the image analysis estimates ($P > .05$). The mean of the ocular estimates of the second observer differed ($P > .0001$) from image analysis by 4.2%. The correlations between image analysis and ocular estimates of cover produced r-values (P values >.0001) of 62%, 2) 77%, and 91%.Other researchers have calculated coefficients of determination (R ranging from 75 to 99% when image analysis was compared to other assessment methods (Meyer *et al*. 1988, Meyer and Davison 1987).

Comparison of the means for the image analysis versus ocular cover methods for the sampling loci at the eighteen plots revealed no differences between the image analysis estimates and any of the ocular estimates in at least 13 of 18 or 72% of the tries (P>.05). When image analysis did differ from the ocular estimates it was not consistently higher or lower than the ocular estimates for any given plot.

Conclusions

Image analysis can separate leafy spurge from surrounding vegetation and soil and quantify leafy spurge cover. The image analysis method of determining leafy spurge cover is as precise as the ocular method and in this study agreed with the ocular method at least 72% of the time. An advantage of the image analysis method is that the photographic images provide a permanent record. Disadvantages are the cost of the system and the additional time taken to process the photos. Further research will compare the image analysis method to biomass estimates of leafy spurge.

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Herbicides and grass competition for leafy spurge control in North Dakota – 1995

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Picloram is one of the most effective herbicides for leafy spurge control. Previous research at North Dakota State University has shown that picloram plus 2,4-D at 0.25 plus 1 lb/A will provide approximately 85% control or better after 3 to 5 years of annual treatment. Glyphosate plus 2,4-D at 0.4 plus 0.6 lb/A will provide 70 to 90% leafy spurge control after one treatment but can cause severe grass injury with repeated applications. A series of experiments was established to compare cost and efficacy of glyphosate plus 2,4-D as part of a long-term management program for leafy spurge control.

The initial treatments of glyphosate plus 2,4-D or picloram plus 2,4-D were applied in late June of 1993 and were retreated with the same or an alternate treatment in 1994. Visual evaluations were taken in 1993, 1994, and 1995. Glyphosate plus 2,4-D provided 75% leafy spurge control 12 months after treatment (MAT) compared to 30% for picloram plus 2,4-D. Glyphosate plus 2,4-D after two consecutive treatments provided 60% control at a cost of $16. Glyphosate plus 2,4-D followed by an auxin herbicide averaged 68% control at cost of $21 while two consecutive applications of picloram plus 2,4-D provided only 48% control at a cost of $26. There was no significant grass injury for any treatment. Application costs were not included in the treatment cost, because one application occurred each year.

A regional research experiment was established by scientists in Colorado, Minnesota, Montana, Nebraska, Wyoming, and North Dakota to evaluate leafy spurge control with quinclorac. Leafy spurge control was 99% regardless of the quinclorac rate at all locations except Wyoming where control was 75%. Quinclorac plus picloram at 12 plus 8 oz/A was >90% at all locations. Picloram plus 2,4-D at 0.5 plus 1 lb/A and picloram at 0.5 lb/A averaged >95% control for all states, except Wyoming where the control was 45% and 63%, respectively.

Grass competition is one means of controlling the rate of spread of leafy spurge based on data from an experiment established in 1990 at Fargo. A second experiment was established near Jamestown to evaluate competitive grass species on a soil type more typical of North Dakota than found at Fargo. Glyphosate plus 2,4-D at 0.4 plus 0.6 lb/A was applied to all plots except the untreated control when leafy spurge was in the flowering growth stage in June and again in July. The seedbed was prepared and grass species were planted August 24, 1993 in all plots except the untreated control and planted August 24, 1993 in all plots except the untreated control and glyphosate plus 2,4-D plots.

All the grass species selected were competitive and reduced leafy spurge production compared to the control. The four grass species that provided > 70% control were ‘Rebound’ smooth brome, ‘Arthur’ Dahurian wildrye, ‘Reliant’ intermediate wheatgrass, and ‘Pryor’ slender wheatgrass. These grass species also had greater than 1,440 lb/A total forage production in 1994.

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IAA partially counters the effects of root formation inhibitors in leafy spurge

DAVID G. DAVIS[1] and KRISTI A. BREILAND[2]

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In aseptic, isolated hypocotyl segments of the perennial weed leafy spurge (*Euphorbia esula* L.), root formation is inhibited, whereas shoot formation is stimulated by cool white fluorescent light. Exogenous IAA stimulates root formation in light or darkness. In most cases, shoot formation is inhibited by IAA, irrespective of the presence or absence of light. Canaline and canavanine, inhibitors of polyamine biosynthesis, inhibit root formation. The inhibition by canaline is partially overcome by IAA, but the results of canavanine treatment varied. Arcaine and pentamidine, two presumed competitors of polyamine interactions with N-methyl-D-aspartate in animals, also inhibit root formation in leafy spurge. These results support the hypothesis that IAA (or a major metabolite of IAA) is involved in a regulatory mechanism that controls root formation in leafy spurge hypocotyl segments, but is not the sole regulator of root formation.

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Preliminary assessment of effect of prescribed fire upon establishment of *Aphthona nigriscutis* in leafy spurge

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Preliminary data from *A. nigriscutis* release sites on 5 Waterfowl Production Areas (WPA's) in South Central ND suggest that prescribed burning in the fall or spring preceding release of the beetles may increase the chances of successful colonization. One hundred and fifty beetles were released on each of 10 sites/WPA in June 1994. Of the 10 sites on each WPA, 2 had been burned in October 1993 and 2 had been burned in early May, 1994. In June, 1995, beetles were recovered in sweep net samples on 90% of the fall-burn sites and 80% of the spring-burn sites, compared to only 34% of the unburned sites; the proportion of sites where beetles were recovered was significantly greater among burned than unburned sites ($P < 0.01$). Taking all release sites into consideration, significantly more beetles were captured on the burned ($\bar{x}$ = 12.4/fall burned site and $\bar{x}$ = 13.3/spring burned site) sites than on the unburned sites $\bar{x}$ = 2.6 beetles/site) ($P < 0.01$). Considering only those sites where beetles were captured, the mean number of captures tended to be higher on the burned ($\bar{x}$ = 13.8 fall, $\bar{x}$ = 16.6 spring) than on the unburned ($\bar{x}$ =6.7) sites, but the difference was not significant (P=0.53).

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Induced frost tolerance in leafy spurge roots: Changes in carbohydrate metabolism

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The resistance of leafy spurge (*Euphorbia esula* L.) to conventional weed control practices is due primarily to an extensive perennial root system that is well adapted to survive many different chemical, biological and environmental stress situations, including the harsh winters of the Northern Great Plains.

If the biochemical and physiological mechanisms that enable leafy spurge roots to acquire and maintain tolerance to freezing were understood, alternative control strategies, in addition to herbicide treatments and/or the use of biological control agents, might be developed that would reduce the ability of the root system to overwinter and effectively limit the plant's aggressive pattern of vegetative regrowth and infestation. Laboratory studies with 2-month-old greenhouse-grown leafy spurge plants showed that the tolerance of the roots to freezing at -7°C for 1 week was induced over a period of 3 or 4 weeks of acclimation at 4°C. Cold-induced frost tolerance in roots was marked by a rapid reduction in reserve starch levels and a corresponding increase in soluble carbohydrates. After a cold treatment for 4 weeks, starch levels were reduced over 50% and soluble carbohydrate levels increased over 4-fold.

Analysis of the soluble carbohydrates showed that sucrose levels increased more than 6-fold after 4 weeks of cold acclimation and represented over 84% of the total soluble carbohydrates in the roots. In contrast to sucrose, glucose levels were less than 7% of the total soluble carbohydrates and increased to only a limited extent after 4 weeks of cold acclimation. Even though endogenous sucrose concentrations increased dramatically during the induction of frost tolerance, concentrations were not high enough by themselves to provide effective cryogenic protection against freezing injury at -7°C.

The rapid loss of starch reserves in roots during the induction of frost tolerance was associated with a 3-fold increase in α-amylase activity and a 2-fold increase in phosphorylase activity. Important enzymes responsible for sucrose synthesis were UDPglucose pyrophosphorylase and sucrose synthase. The activity of these two enzymes together with differential rates of starch hydrolysis and the absence of measurable invertase activity in both control and cold-induced roots may account for the observed accumulation of sucrose during the development of frost tolerance.

Even though a direct relationship between frost tolerance and carbohydrate metabolism was not established in these studies, key enzymes responsible for starch hydrolysis and sucrose synthesis may indirectly affect the establishment of frost tolerance and serve as possible early targets for biochemical regulation and reduced winter survival of the plant.

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Simple techniques for distributing *Aphthona* flea beetles from established field insectary sites

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Once the population of *Aphthona* sp. adults has exceeded a predetermined threshold, beetles may be collected and distributed to other leafy spurge-infested areas. Adult beetles are simply collected from the field insectary site using a standard sweep net. Four methods for "processing" the collected adults are described, in increasing order of relative sophistication: 1) the "sweep and dump" method; 2) the "sweep, scoop, and dump" method; 3) use of the "Wyoming sorter" and 4) the use of vacuum sorting. The first three methods are quick and require simple equipment, but may incorporate large numbers of nontarget insects in the collected material. Generally, they provide only crude estimates of the number of *Aphthona* sp. adults harvested. Vacuum sorting provides generally contaminant-free material and precise quantification, but is more equipment- and time-intensive. The first three processing techniques are appropriate for within-site, within-county, and generally, within-state distribution. Vacuum sampling should be used for inter-state movement of flea beetle adults, or for distribution to "environmentally-sensitive" areas.

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Economic impact of leafy spurge on alternative post-Conservation Reserve Program land uses

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Leafy spurge infests some of the 2.9 million acres of Conservation Reserve Program (CRP) land in North Dakota. The CRP provides water quality and wildlife habitat benefits in addition to lowering crop surpluses, raising commodity prices, and providing income support for farmers. However, the CRP may have the unforeseen and unintended consequence of facilitating the spread of leafy spurge, a noxious weed. Once established, leafy spurge will reduce the expected benefits of the CRP and impact the returns to some post-CRP land uses.

Alternative land uses are (1) enrolling post-CRP land in a similar permanent vegetative cover program, (2) converting post-CRP land to grazing land, and (3) returning post-CRP to cropland. The study assumes 1/3 of total CRP acres in the state, about 967,000 acres, will be converted to each alternative land use and 4 percent, about 41,000 acres, of each land use is infested with leafy spurge. The estimated annual direct economic impacts are $327,000 on permanent cover post-CRP land, $1,331 million on post-CRP grazing land, and negligible (assumed $0) on post-CRP cropland, for a total of $1,658 million. Total annual direct and secondary economic impacts to North Dakota's economy are $5,263 million, which would support about 66 jobs.

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Cloning of *gro* (Growth Response) genes which are induced, during breaking of quiescence in root buds of leafy spurge

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Under normal growing conditions, root buds of leafy spurge are maintained in a quiescent state by an unknown molecular mechanism. However, if the leafy portion of the plant is destroyed or removed, the root bud will break quiescence, and grow as shoots. This is a major problem in controlling this noxious weed since the root buds are much more resistant to herbicides than the leafy portion of the plant. In order to learn more about the mechanisms and signal transduction pathways involved in maintaining and breaking of quiescence in root buds of leafy spurge, we utilized differential display technology to identify and then clone two genes that are specifically induced in root buds after mechanical removal of the stem. Work is currently underway to identify the proteins that are encoded by these genes, and to identify and isolate the *cis* acting elements that are responsible for the induction of these genes during the breaking of quiescence. Work is also underway to determine the tissue specificity and other environmental factors that play a role in the regulation of these genes.

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Esters of quinclorac as possible leafy spurge herbicides: Absorption, translocation, metabolism, and toxicity

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Quinclorac is one of the more effective herbicides that has been tested for the control of leafy spurge, but it is not registered for this use. Its efficacy is limited by metabolic detoxification, rapid efflux from the roots and sequestration. In an effort to overcome these deficiencies, the following quinclorac esters were synthesized and are being evaluated in the greenhouse and the laboratory: the methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *sec*-butyl, *n*-pentyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *iso*-octyl, hexadecyl, butoxy-ethanol, and the 1,3-propanediol esters.

The [^{14}C]quinclorac esters were readily absorbed by the leaves of leafy spurge where they were metabolized at varying rates. The *n*-pentyl, *n*-hexyl and *n*-octyl esters were metabolized more rapidly than the short-chain or branched-chain esters. Metabolism did not proceed by simple hydrolysis and only low levels of free quinclorac were produced. Limited translocation was observed with some of the esters. The esters were generally less toxic than quinclorac when applied to the foliage, but additional dose response studies must be conducted.

The [^{14}C]quinclorac esters were absorbed by the roots when leafy spurge was grown and treated in hydroponic culture. Following treatment, much of the radioactivity was effluxed from the roots back into the nutrient solution. The 4-hydroxybutyric acid ester of quinclorac was the major product detected in the nutrient solution following treatment with the *n*-hexyl ester. The 4-hydroxybutyric acid ester was produced by microorganisms in the nutrient solution, the potting material, and the soil, but it was not produced by leafy spurge. It was produced by elimination of a 2-carbon fragment following omega- and beta-oxidation. The short chain esters were not metabolized by the nutrient solution, the n-heptyl and n-octyl esters were metabolized at the highest rate and a long chain ester (hexadecyl) was metabolized at an intermediate rate. No metabolism of the esters was observed in filter-sterilized nutrient solution. Free quinclorac was liberated from the odd chain-length esters (*n*-pentyl, *n*-heptyl and 1,3-propanediol) while the 4-hydroxybutyric acid ester and another metabolite, probably the 2-hydroxyacetic acid ester, were liberated from the even chain-length esters (*n*-hexyl, *n*-octyl, and hexadecyl). Non-sterile soil from the Red River Valley metabolized the *n*-pentyl and 1,3-propanediol esters to quinclorac and the, *n*-hexyl ester to the 4-hydroxybutyric acid ester. Soil-applied *n*-pentyl and 1,3-propanediol esters of quinclorac appeared to be more toxic to leafy spurge than quinclorac; however, the *n*-heptyl ester would be predicted to be the most effective of these esters when-soil applied.

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Effect of leafy spurge biotype and herbicide application on *Aphthona* spp. establishment

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The timing of herbicide treatments on *A. nigriscutis* and *A. cwalinae* survival and establishment was evaluated. Two locations of *A. nigiiscutis* and one location of *A. czwalinae* were established. The treatments included picloram plus 2,4-D at 4 plus 16 oz/A spring applied picloram plus 2,4-D at 8 plus 16 oz/A fall applied, and *Aphthona* spp. alone. Stem density was evaluated in the spring, and adult sweep counts were conducted through the summer. For the first experiment, *A. nigriscutis* were released in 1989 and herbicide treatments were initiated in spring (June) of 1992 at Cuba. Stem density in the insect-only treatment declined by 97% from 1992 to 1995. The most rapid control was from the insect plus fall-applied herbicide treatment which resulted in a 97% decline in leafy spurge stem density in only 2 years. The spring-applied herbicide plus insect treatment reduced leafy spurge less than the insects alone. The *A. nigriscutis* population in the non-herbicide treatments increased from 11 beetles/m^2 in 1992 to 125 beetles/m^2 in 1994 but then declined to an average of 70 beetles/m^2 in 1995 as leafy spurge stem density declined. The *A. nigriscutis* population averaged 76 beetles/m^2, when herbicides were spring applied but only 30 beetles/m^2 when herbicides were fall-applied as very little leafy spurge remained in those plots.

Similar experiments were started in 1993 with 3,000 *A. nigriscutis* released at the Ekre Research station near Walcott and 30,000 *A. czwalinae* released at Camp Grafton South, near McHenry. As in the previous experiment, *Aphthona* spp. combined with a fall-applied herbicide treatment resulted both in better leafy spurge control than either control method used alone and a more rapid increase in the flea beetle population.

The establishment and movement of *A. nigriscutis* on leafy spurge patches is currently being evaluated. *A. nigriscutis* was released as 100, 200, 300, 400, or 500 adults per site along a 2.5 mile stretch of the Burlington Northern railroad right-of-way near Buffalo. The insects were released in dense stands of leafy spurge on June 28, 1993. Stem density and adult flea beetle population and spread have been determined annually in June. *A. nigriscutis* flea beetles were found at all release sites 1 year after release, and leafy spurge stem density began to decline in 1995, which was 2 years after release. In general, the greater the original number released, the more rapid the decline in stem density. The greatest leafy spurge decrease was from 17 stems/0.25 m^2 in 1993 to 10 stems/0.25 m^2 in 1995 when 500 insects/site were released. The insects had spread an average of 55 feet from the release site when 500 insects/site were released but only an average of 25 feet when less than 500 insects/site were released.

The survival of *A. czwalinae, A. flava,* and *A. nigriscutis* was evaluated on leafy spurge biotypes from Austria, Manitoba, Montana, Nebraska, North Dakota, South Dakota, and Wyoming. The seven biotypes were grown in a greenhouse for 4 to 5 months in 2.5-by 8-inch pots. These pots were planted directly outside in April. The pots were arranged in a RCB design with 12 replications in a 36 ft^2 area. Cages were placed over the experiments and 200 *Aphthona* spp. were released. The pots were dug in November, placed in a cooler at 3º C for 8 weeks, and then placed under greenhouse lights (16 hours) at 24º C.

The greatest number of *Aphthona* adults emerged from a Nebraska leafy spurge biotype followed by biotypes from Austria and South Dakota. The least number of adults emerged from biotypes from North Dakota and Montana. These are the same biotypes that, in previous research, had the greatest (NE, AU, and SD) and least (ND and MT) number of galls and larvae per gall when exposed to the leafy spurge gall midge (*Spurgia esulae*).

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Systems approach with biological agents for leafy spurge control

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The flea beetle, *Aphthona nigriscutis* Foudras, can be successfully integrated with herbicide treatment for control of leafy spurge. The compatibility of these methodologies (biological, chemical) is exemplified by an accidental overspray of established *A. nigriscutis*. The beetle population increased and leafy spurge density decreased the following season. The biological basis for this potential synergism will be investigated.

The effect of date of picloram and 2,4-D application on *A. nigriscutis* population in leafy spurge will be determined. This experiment will be conducted at two locations, Chaffee and Fort Ransom, North Dakota. Leafy spurge at Chaffee and Fort Ransom averaged 90 and 63 stems/m^2, respectively. Approximately 350 *A. nigriscutis* adults were released into 1.8- by 1.8- by 1.8-m screened cages on June 22, 1995. An additional 100 *A. nigriscutis* adults were released on July 14. Leafy spurge and *A. nigriscutis* will be oversprayed with picloram plus 2,4-D at 0.56 plus 1.1 kg ha-respectively, beginning August 15 and continuing every two weeks until October 1.

The *A. nigriscutis* population will be monitored in three ways. First, adults will be collected from soil cores harvested in the fall. Second, *A. nigriscutis* larvae will be counted from soil cores harvested in the spring the following year. Third, adults will be collected in the spring from emergent trap chambers in the field.

Emergence of *A. nigriscutis* adults in the laboratory will be quantified with four soil cores taken from each *A. nigriscutis*-infested subplot. Soil samples will be harvested in late October with a golf-cup cutter. The soil cores will be 10.8-cm diameter to a depth of 15 cm and held at 3º C for 75 days. Each sample then will be placed into a 0.9-L plastic cup and covered by a clear plastic cylinder with a mesh top. Trap chambers with soil cores will be maintained in the laboratory at 21º C with a 16-hour photoperiod. Adult *A. nigriscutis* will be collected and quantified for each soil core. The second estimate of population will be with soil cores harvested from *A. nigriscutis* infested subplots in the middle of May of the following year. Two soil cores will be harvested from each subplot and dissected to quantify *A. nigriscutis* larvae. Adult emergence in the field will be evaluated using trap chambers. Trap chambers consist of 20-cm diameter and 20 cm-long PVC pipe recessed into the soil even with the soil surface. A mesh screen will cover the

PVC pipe to capture adults. Two trap chambers will be placed in each *A. nigriscutis*-infested subplot.

Leafy spurge root material will be harvested in subplots not infested with *A. nigriscutis* to quantify carbohydrate and protein content. Root material will be collected beginning August 15 and continuing until soil freeze-up. Root samples also will be collected in April and May the following spring. Leafy spurge roots cannot be sampled in the presence of *A. nigriscutis* because larvae inhabiting root tissue will alter protein and carbohydrate quantitation. Quantitation of leafy spurge root material may explain potential differences in counts of *A. nigriscutis* adults and larvae across herbicide application dates. Root nutrients will be compared between caged and uncaged leafy spurge to determine the effect of caging on the chemical composition of roots.

The effect of *A. nigriscutis* larval feeding on picloram, 2,4-D, and photosynthate translocation in leafy spurge will be determined through a series of greenhouse studies. Leafy spurge plants will be subjected to *A. nigriscutis* larval feeding for 60 days prior to application of ^{14}C-2,4-D, ^{14}C-picloram, or ^{14}C-sucrose. Plants will be sectioned and combusted, with ^{14}C tissue concentration determined by liquid scintillation techniques.

Data collected from the field experiment will illustrate the most beneficial herbicide application date over *A. nigriscutis* populations. Combined biological and chemical control will likely shorten the time needed to reduce leafy spurge populations to acceptable densities. In addition, integration will establish long term control, reduce chemical inputs, and reduce the economic losses on both private and public lands. The greenhouse experiments will clarify the biological basis for synergism between biological and chemical control observed in the field. Understanding the basis for this synergism may lead to the integration of additional biological and chemical control methodologies on noxious weeds.

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Chamaesphecia hungarica - Current status of quarantine clearance and field establishment

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Chamaesphecia hungarica, a member of the Aegeriidae family, is a clear-winged moth native to southeastern Czechoslovakia, Austria, Hungary, Serbia and Croatia. In Europe it is usually found in moist loamy soils and partial shade near riverbanks, swampy areas and ditches. Adults emerge from mid May to late July depending upon climatic conditions at the site. Mating usually occurs within 24 hours. Larvae mine the stem and move into the pith area. Larvae overwinter in the roots and migrate to the stem base in spring where pupation occurs. The International Institute of Biological Control, Delémont, Switzerland collected larvae infested roots in Serbia and shipped them to the United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine Laboratory (USDA-APHIS-PPQ) quarantine laboratory at Mission, Texas. The material was placed in moistened soil and maintained at a temperature range of from 20° C to 25° C. Upon eclosion of adults, sampling was performed for pathology examination and identification verification. Some adults were directly transported and released at field sites, others were mated in quarantine and eggs deposited onto plants or paper. Infested plants and eggs were transported to the field site in Montana where releases were made by the USDA-APHIS-PPQ, Bozeman Biological Control Facility. The first recovery of this species was made at the Montana release site in September of 1994. Identification of the recovery was verified by the Systematic Entomology Laboratory, Beltsville, MD. Another recovery was made in late June 1995.

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Multi-species grazing using goats and cattle to control leafy spurge

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Leafy spurge (*Euphorbia esula* L.), a herbaceous, deep-rooted, dicotyledonous, perennial, is a noxious weed which infests at least 458 counties in 26 states and six Canadian provinces (1). Leafy spurge is distributed on several habitats ranging from xeric to subhumid, and from subtropical to subarctic. This plant, since introduced from Eurasia, has become a troublesome weed in the Great Plains region of North America where it grows largely devoid of insect and disease pests which keep leafy spurge controlled in its native habitats (2). The weed, extremely persistent and competitive, contributes to significant economic losses to livestock producers.

Angora goats were introduced to Camp Grafton South in southeastern Eddy County as a biological control for leafy spurge. Project objectives were to determine: 1) the effect of angora goat grazing on leafy spurge stem density and associated herbaceous production, 2) determine the differences between multi-species grazing and single species grazing on leafy spurge infested rangeland in regard to herbaceous species utilization patterns.

The study area consisted of 85.5 hectares located in Sections 12 and 13, T. 149 N., R. 63 W. on Camp Grafton South in southeastern Eddy County. The 85.5 hectares was divided into a 37.3 ha cattle only treatment (CO) and a 41.4 ha cattle/goats (multi-species) treatment (CG). Goats only treatment bordered the CG and CO treatments, consisting of two replications (GO1, G02) of 3.5 and 3.2 hectares.

Leafy spurge stem counts were conducted prior to the introduction of angora goat grazing in late May, 1993-1995 to achieve initial stand counts and differences after one and two years of grazing. Stems were counted using $0.1m^2$ frame on ten line transacts. Paired-plot clipping technique was used to determine forage production and degree of use for leafy spurge, graminoids, shrubs and other forbs. Leafy spurge stem counts were tested for significant ($P<0.05$) main effects using multi-response permutation procedure (MRPP) (3).

The cattle only pasture was grazed by 21 cow/calf pairs from 6/15 to 11/1, with a stocking rate of 0.39 ha/AUM for both years. The goats only treatments were grazed by 15 and 16 angora goat nannies per cell in 1993 and 1994, respectively. The stocking rates for the goats only trials were 0.42 ha/AUM and 0.38 ha/AUM, respectively, in 1993 and 1994. The grazing dates were May 27 through September 11 in 1993 and June 1 through

September 1 in 1994. The CG treatment consisted of 41.4 hectares grazed by 21 cow/calf pairs from 7/15-11/1 (0.55 ha/AUM) and 6/1-11/1 (0.39 ha/AUM) in 1993 and 1994, respectively. A total 191 (0.36 ha/AUM) and 156 (0.43 ha/AUM) angora goats grazed from 5/27-9/11 and 6/1-9/24 in 1993 and 1994, respectively.

Leafy spurge stem densities in the CO treatment had a slight increase of 3.1 percent increase after two years of grazing, however, no significant ($P > 0.05$) differences were noted (Table 1). The leafy spurge stem densities were reduced ($P>0.05$) from 11.6 stems to 3.3 stems or a reduction of 71.5 percent on the GO treatment. Stems densities were reduced ($P > 0.05$) in the CG treatment from 12.4 stems to 7.1 stems with a reduction of 42.7 percent.

Table 1. Initial leafy spurge stem density counts (stems/0. Lm²) prior to goat turnout at Camp Grafton South, 1993-1995.

	Stem Density[1]				
Treatment	1993	1994	1995	Percent Change	P-value
Cattle Only	12.8[a]	11.6[a]	13.2[a]	+ 3.1	.4
Goats Only	11.6[a]	8.9[a]	3.3[b]	-71.5	$.2^{-10}$
Cattle/Goats Together	12.4[a]	9.4[a]	7.1[b]	-42.7	$.1^{-3}$

[1]Percentages by year with the same letter are not significantly ($P > 0.05$) different.

Goat grazing within the goats only and multi-species treatments extensively grazed leafy spurge, providing an open canopy and competitive growth advantage for the graminoid species. The mean degree of use of leafy spurge was 68.7 and 71.6 percent in the goats only and multi-species treatments, respectively. The intensive use of the leafy spurge by goats allowed for a more suitable forage base for cattle, creating a higher degree of use of the graminoid species in the multi-species treatment. Graminoid degree of use in the multi-species treatment was 23.5 percent, significantly ($P<0.05$) higher than the 8.3 and 9.0 percent in the cattle only and goats only treatment, respectively (Table 2).

Table 2. Degree of use (percent) of all graminoid species in leafy spurge infested rangeland by treatment and year at Camp Grafton South, 1993 and 1994.

Treatment	1993[1]	1994	Mean[2]
Cattle Only	10.1	6.4	8.3[a]
Goats Only	7.5	10.4	9.0[a]
Cattle and Goats	27.8	19.3	23.5[b]

[1]No significant ($P > 0. 05$) differences occurred between years for either treatment.
[2]Means with the same letter are not significantly ($P > 0. 05$) different.

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USDA, APHIS field insectary establishment and redistribution of leafy spurge biological control agents – How it is working on a national scale

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APHIS has been involved in the implementation of biological control of leafy spurge since 1988 with initial efforts on a minor scale in four states. Since that time, it has been a focus of APHIS activity to establish Field Insectary Site (FIS) colonies of approved biological control agents (insects) targeted against leafy spurge in several states. Between 1988 and 1992, APHIS imported agents from populations in Canada and Europe. By 1993, collectable populations from U.S. domestic sources made collection in Canada unnecessary. Only recently approved biological control agents have been collected and imported from Europe through APHIS Quarantine in Mission, TX. In 1995 release of agents occurred in 19 states. Of the 9 species of USDA approved biological control agents for leafy spurge, only 3 were imported for initiating colonization in the U.S. They were *Aphthona abdominalis, Chamaesphecia hungarica,* and *Oberea erythrocephala.* The other 6 species have established well, and developing populations now are providing large numbers for collection and redistribution. Biological control agents for leafy spurge have been released in 180 counties in 19 states. Based on sampling of released insects during the 1994 field season, established populations exist in 131 counties in 15 states. Collectable populations exist in 35 counties (48 locations) in 11 states. Management of these thriving field insectaries are being turned over to cooperators for their management and continued efforts in redistribution to new locations. Cooperators with APHIS include state departments of agriculture, university, county, and federal land managers with weed control responsibilities. Technology transfer from APHIS to cooperators is being accomplished by development of field manuals and actual field training of cooperators by APHIS employees at field locations across the U.S. In 1995, cooperators utilizing the resources of APHIS field insectaries will collect and redistribute in excess of 5 million biological control agents of leafy spurge. APHIS will continue its effort to establish colonies of new biological control agents of leafy spurge. Biological control of weeds has been shown to be cost-effective, is self-sustaining, and environmentally sensitive. Use of biological control as an alternative, or to be used in conjunction with other management strategies, for the control of leafy spurge is now a reality in many weed infested areas in the U.S.

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Defoliation effects of leafy spurge on sheep rumen microorganisms

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Most herbivores avoid the noxious weed leafy spurge (*Euphorbia esula* L.) when grazing. However, ruminants, including sheep, will consume leafy spurge at levels up to 50% of their total diet. An herbivore may avoid a plant due to a nutritional deficiency in plant tissues, structural defenses, or the presence of adverse phytochemicals. Because leafy spurge is high in protein and has no structural defenses, we examined the presence of phytochemicals in the plant and the effects they may have on sheep rumen microorganisms. Plant secondary chemicals reduce an herbivore's ability to digest forages and assimilate nutrients, or are toxic at high levels. Plants may increase levels of secondary chemicals in regrowth as an evolutionary response to herbivory. Our objectives were: (1) to determine if defoliation increases the level of secondary plant chemicals in leafy spurge regrowth and (2) to determine if defoliation adversely affects sheep rumen microorganisms. We measured in vitro microbial gas production, purine accumulation and dry matter disappearance to evaluate sheep rumen microbial response to leafy spurge primary growth and regrowth. Primary growth and regrowth were collected on 9 June, 14 July, and 18 August, 1994. Forage quality of stems, leaves, and flowers was determined to establish baseline values to assess microbial response. All plant parts were analyzed using four ratios of leafy spurge and grass hay, and 100% grass hay was used as a control. We also analyzed primary growth and regrowth samples from each date for condensed tannins. The microbial assays indicate there is a positive associative effect with the addition of leafy spurge to grass hay. At very high levels of leafy spurge, the metabolic activity of rumen microorganisms is depressed, but at 75% leafy spurge, metabolic activity is higher than that of microbes consuming grass alone. However, purine concentrations indicate that microbial mass is the greatest at the highest levels of leafy spurge. Dry matter disappearance was the greatest in flowers and leaves, declined seasonally, and was lower for stem regrowth than for stem primary growth. Leafy spurge contains condensed tannins, they are highest in the stem regrowth, and they increase seasonally. We predict that at relatively high levels of leafy spurge early in the growing season, sheep rumen microorganisms are not adversely affected by secondary defense chemicals in the plant. However, as the growing season progresses microbial activity decreases. Defoliation increased the production of secondary defense chemicals in stems of leafy spurge.

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Relatedness of North American and European leafy spurge based on DNA markers

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Chloroplast DNA restriction fragment length polymorphism (cpDNA RFLP) and random amplified polymorphic DNA (RAPD) data were collected for leafy spurge populations from North America, Austria, Hungary, the Czech Republic, Italy, Ukraine, and Russia. CpDNAs of individuals from six other European species of *Euphorbia* were also examined. Three restriction enzymes and six mung bean cpDNA probes were used to assess polymorphism among chloroplast genomes; five 9-mer primers were used for PCR amplification of leafy spurge DNA. Data were scored as presence or absence of bands and a dendrogram based on genetic distance was constructed among genotypes by a cluster analysis program.

A preliminary analysis of the data indicated that cpDNA genotypes of *Euphorbia amygdaloides, E. seguierana, E. helioscopia,* and *E. palustris* were easily distinguishable from those of leafy spurge, but *E. salicifolia* and *E. cyparissias* cpDNA types were indistinguishable from leafy spurge cpDNA with the markers used. There was some evidence that the cypress spurge, which was collected in Austria and Hungary, had cpDNA more similar to cpDNA of leafy spurge from the same geographic area than to leafy spurge cpDNA from other countries.

Greater genetic variation was found among European leafy spurge cpDNAs than among cpDNAs from North America. Most North American leafy spurge cpDNA genotypes clustered closer to Russian, Ukraine, and some Czech cpDNAs, while one North American cpDNA genotype that was heavily represented in the sample showed greater similarity to types from Austria and Hungary. Italy had the most divergent cpDNA types. RAPD analysis demonstrated the greatest relatedness among individuals within a population of leafy spurge. The European population that showed greatest similarity to North American leafy spurge was the Russian population; Italy was the most divergent population.

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Intensive grazing of angora goats on leafy spurge infested rangeland

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Utilization and control of leafy spurge by angora goats were determined in 1991 through 1995. Project objectives were to determine 1) if intensive seasonlong grazing of angora goats will significantly reduce stem and herbage density of leafy spurge, and 2) determine if seasonlong grazing leafy spurge with angora goats will increase grass density in leafy spurge infested rangeland.

The study was conducted on a 6.1 hectare parcel located on Camp Grafton South in southeast Eddy County adjacent to the east shore line of North Twin Lake in Sec. 4, T 148 N, R 63 W. The study site was situated on a west-facing slope with the plant communities classified. These plant communities were classified as high, mid, and low prairie, and open woodland (Dix and Smeins 1967).

Angora goats were stocked at 0.14 ha/AUM in 1991, 0.34 ha/AUM in 1992 and 1993, and 0.49 ha/AUM in 1994. Leafy spurge and brush stem densities were collected in the third week of May in 1991, 1992, 1993, and 1994 and early June in 1995 to determine overall change in plant numbers using 0.1 m^2 plots on four replicated line transacts. Overall degree of use of leafy spurge, grass and brush were determined using the paired-plot clipping technique. Leafy spurge and shrub stem counts were tested for significant ($P < 0.05$) main effects using multi-response permutation procedure (MRPP) (Biondini *et al*. 1988). Each year was tested to determine number of years required to significantly ($P < 0.05$) reduce leafy spurge stem counts.

Leafy spurge stem densities were reduced 12.5% ($P>0.05$) after one year, 39.9% ($P < 0.05$) after two years, 53.7% ($P < 0.05$) after three years, and 84.2 percent ($P < 0.05$) after four years of grazing (Table 1). The shrub stem densities were significantly ($P < 0.05$) reduced by 91.6% after four years of grazing (Table 1). No significant ($P > 0.\ 05$) changes in relative frequency of leafy spurge and grass herbage production occurred after two years of grazing, however, after three years of grazing leafy spurge was significantly ($P < 0.05$) reduced and graminoids significantly ($P<0.05$) increased (Table 2).

The leafy spurge and shrub components were extensively grazed during all four years of the study. Degree of use by goats on leafy spurge was 64, 92, 97, and 91% in 1991, 1992, 1993, and 1994, respectively. Shrub degree of use was 94, 92, 89, and 81% in 1991, 1992, 1993, and 1994, respectively. Intensive seasonlong grazing of angora goat on leafy spurge infested rangeland significantly reduced leafy spurge stem densities after two, years of grazing. Leafy spurge herbage production was significantly reduced and graminoid species significantly increased after three years of seasonlong angora goat grazing.

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Table 1. Stem density reduction of leafy spurge and shrub species at Camp Grafton South near McHenry, ND from 1991 to 1995.

Number of stems per 0.1 m^2				
Date collected	Leafy spurge[1]	P-value[2]	Shrubs	P-value
May 25, 1991	3.9^{a}		---	
May 19, 1992	3.4^{ab}	0.517	1.6^{c}	
May 20, 1993	2.4^{bc}	0.039	0.9^{c}	0.437
May 26, 1994	1.9^{cd}	0.004	0.2d	0.001
June 5, 1995	0.7^{d}	0.49^{-6}	0.1^{d}	0.81^{-3}
Stem Density Reduction	84.7		91.6	

[1]Percentages with the same letter are not significantly (P>0.05) different.

[2]P-value represents comparison of 1991 and 1992, 1991 and 1993, 1991 and 1994, and 1991 and 1995.

Table 2. Percent change in herbage production by weight from 1991 to 1994 at Camp Grafton South near McHenry, ND.

Year	Leafy spurge	P-value	Grass species[1]	P-value[2]
1991	22.5^{a}		77.5^{c}	
1992	15.7^{a}	0.512	84.3^{c}	0.676
1993	22.1^{a}	0.988	77.9^{c}	0.967
1994	8.0^{b}	0.013	90.8^{d}	0.014
Percent change	-64.4		+17.2	

[1]Percentages with the same letter are not significantly (P > 0.05) different.

[2]P-value represents comparison of 1991 and 1992, 1991 and 1993, and 1991 and 1994 for both leafy spurge and grass.

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Enhancing the competitiveness of other species with leafy spurge

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The Eurasian noxious weed leafy spurge infests millions of hectares in North America. Herbicides are generally not cost-effective on rangelands, and biocontrol agents have limited effectiveness in cool, moist areas. We conducted a field trial and a greenhouse study to assess the effectiveness of interseeding alfalfa (field) and other species (field and greenhouse) into infestations of leafy spurge. We had three pretreatments: discing, mowing, and control at two field sites in northcentral Montana. We interseeded one of three alfalfa cultivars: Ladak, Spreador II, and Pioneer 5364 in late May 1993 across these three pretreatments. Standing crop in August 1994 ranged from 2700-3800 kg/ha^{-1}. More than half of the standing crop at both sites was alfalfa (54-79%). Discing enhanced leafy spurge at one site but not at the other, and was not necessary for alfalfa establishment. In the greenhouse, we grew leafy spurge with one of eight other species (cultivars): sheep fescue, Russian wildrye, crested wheatgrass, intermediate wheatgrass, western wheatgrass, and the same three alfalfa cultivars. Each combination was then treated with the following nutrients: none (control), 50 kg N ha^{-1}, 100 kg N ha^{-1}, 50 kg P ha^{-1}, 50 kg P ha^{-1}, 50 kg N ha^{-1}, 50 kg P ha^{-1} + 100 kg N ha^{-1}. Leafy spurge shoot biomass was lowest when grown with intermediate wheatgrass, western wheatgrass, and Ladak and Spreador II alfalfa, and highest when grown with sheep fescue. Root biomass of leafy spurge was lowest when grown with intermediate wheatgrass and highest when grown with sheep fescue. Leafy spurge shoot biomass was highest in pots with added P, although root biomass was unaffected by nutrient treatments. The other species had different responses to the nutrient treatments. Overall alfalfa, crested wheatgrass and intermediate wheatgrass appear to have the potential to compete effectively with leafy spurge.

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DNA Characterization of *Aphthona* spp.

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The recent increase in environmental awareness and bio-diversity issues by a more concerned and knowledgeable society has resulted in biological control based pest management practitioners pushing the envelope of classical taxonomy to the limit. State and federal regulations require that new introductions be identified. The classical morphological methods used in the past are based on lengthy studies. The time required to study and develop classical methods for identifying these exotic and often new species delays the introduction of possible new natural enemies. Today, the increased demand for use of non-chemical methods requires new approaches be developed for use by growers to manage agriculture pests. These new management approaches require more importation of the natural enemies from foreign countries. The time these natural enemies spend in quarantine facilities may compromise valuable genetic traits that provide control of a target weed. Now scientists have several new tools based on molecular techniques that provide definitive separation of closely related natural enemies. These new techniques provide rapid identification of natural enemies from foreign countries that facilitates release from quarantine. Personnel at the Mission Biological Control Center have successfully utilized randomly amplified polymorphic DNA to separate closely related species of *Aphthona.* This information is shared with all scientists committed to management of leafy spurge, systematists at the national museum and regulatory officials at the state and federal level.

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PPQ insectary sites in North Dakota

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PPQ insectary sites began in 1989. We started with two caged sites. The first releases made were with *nigriscutis* at one site and *cyparisiae* and *flava* at the other site. Neither one of these sites had done particularly well, but some insects were found at both sites before we terminated the sites. In 1990 we did releases at 15 sites. Several of these sites are producing where collections are now being made.

One of the 1990 sites is a *flava* site and is a producing insectary. This year the site was grazed to almost nothing by sheep. There was no spurge showing. We thought the site was lost. In July, the spurge started to grow back. We sampled the site and flava was found in large enough numbers so we were able to collect several thousand beetles.

We have six APHIS insectary sites that we could have made collections from this year. Due to the tremendous number of insects at the Valley City and Minot sites most of our resources were directed at these two sites. 99% of the insects were collected from these two sites. We helped collect and distribute approximately 2 1/2 million insects this year. This included *nigriscutis, czwalinae,* and *flava*. This work was accomplished at four field days from 6-29 through 7-12. The insects were given to county weed boards, state and federal agencies, and some private individuals.

Everyone wants beetles. However, everyone must realize this is an integrated approach and all the tools available to combat leafy spurge should be used.

APHIS has 41 insectaries. We will drop 17 sites. They are not producers. If our cooperators want to maintain these insectaries they can.

This is a great program to be involved with. Fifteen years ago many people were in despair. They thought there was no hope. Now they have smiles on their faces. With all the tools that are coming into place, leafy spurge will not be a major problem in the future.

Field day activities – 1995

Gladstone	6-29-95	376,000 *Nigriscutis*
		130,000 *Czwalinae*
		506,000 Total
Mandan	6-28-95	253,000 *Nigriscutis*
		158,000 *Czwalinae*
		411,000 Total
Minot	7-6-95	**759,600 *Nigriscutis* Total**
Valley City	7-12-95	64,000 *Nigriscutis*
		678,000 *Czwalinae*
		742,000 Total
		2,418,600 Total Redistribution

PPQ sites harvest 1995

	Nigriscutis	
Stark County - Gladstone	6-29-95	15,000
	7-10-95	5,000
Morton County - GMA	6-14-95	5,000
	7-10-95	65,000
Sioux County	7-18-95	20,000
	Flava	
Burleigh County	7-17-95	10,000
	7-10-95	4,000

1995 PPQ Distribution

48 Counties Total

Missing: Divide
Cavalier
Grand Forks
Pierce
Ramsey

10 Government Agencies