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Organogenesis in leafy spurge

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The process of organogenesis in leafy spurge (*Euphorbia esula* L.) continues to be the major research project in this laboratory, since it is believed that the long range control of leafy spurge will require a combination of biological and chemical methods. The main target sites for chemical control are likely to be the tissues and cells that regenerate into shoot or root buds. The most effective herbicides (picloram, 2,4-D and dicamba) are thought to have physiological effects similar to auxin under certain conditions. The action of 2,4-D, picloram, and the natural auxin, indoleacetic acid (IAA) and its analog indole-butyric acid (IBA), have been determined in the model system that has been in use in this laboratory for several years. This model system consists of aseptic etiolated hypocotyl segments grown on agar media containing the nutrient formula of Gamborg *et al.* (1968), with 2% sucrose as the carbon source.

Both IAA and IBA increase root formation, but 2,4-D does not, unless the tissue is transferred to media free of 2,4-D after 2 to 5 days of treatment (Davis and Olson 1993). Picloram strongly inhibits organ formation, so that mostly callus tissues are formed. In contrast to IAA or 2,4-D, picloram at low concentrations did not increase root formation above the level of the control, even when the hypocotyl segments were transferred to basal medium without growth regulators. The inhibition of root formation by a specific inhibitor of ornithine decarboxylase, an enzyme involved in the biosynthesis of putrescine (the immediate precursor to the polyamines; Slocum and Flores 1991) was reversed by IAA and IBA, but not by 2,4-D. The possible reversal of the action of this inhibitor by picloram was not evaluated, since no stimulation of root formation by picloram at any concentration has been observed.

For the past two years the research emphasis has been on the action of the diamine putrescine, the polyamines spermidine and spermine, and the effects of inhibitors of the biosynthesis of these compounds (Davis and Olson 1992). Canavanine and canaline (analogs of the putrescine precursors, arginine and omithine, respectively) were tested for their effects on the formation of roots and shoots on the hypocotyl segments. Inhibition of both root and shoot formation occurred with canavanine (10 μM or greater) and canaline (25 μM or greater). The inhibition by canavanine was not reversed by simultaneous treatment with arginine. Comparable experiments on the possible reversal of canaline inhibition by ornithine are underway.

The effects of a chemical (designated fraction F-117), extracted from Sunnhemp (*Crotalaria juncia* L.), that has shown activity against photosynthetic tissues of leafy spurge, causing a bleaching of the tissues (Leather 1993), has also been tested in the leafy spurge hypocotyl system. In darkness, this chemical inhibits root formation in etiolated leafy spurge hypocotyls only slightly at the highest concentration tested (0.5 mM).

Agar-solidified media is used in the model hypocotyl system. Efforts to improve the system are being tested continuously. A system whereby liquid nutrient medium replaces the agar containing medium is presently being developed to avoid the presence of low concentrations of additional nutrients or contaminants contained in the agar. Growth of both roots and shoots is better in the liquid cultures if the cultures are shaken continuously, with the result that the organs become entangled and are difficult to visualize unless they are physically separated.

Special tissue culture dishes are used in these experiments and they must be opened to facilitate organ counts, thereby exposing the tissues to possible contamination. One type of dish has a small opening to the atmosphere, so that some gas exchange with the external air is possible. Ethylene, ethane, ethanol, methyl jasmonate or other volatile components generated by the tissues and that may influence organ formation, can escape to the outside of the vessels. This reduces, but does not eliminate, the possible complication of their involvement in organogenesis. On the other hand, airborne contaminants may move into the tissue culture dish through the opening. Handling of these dishes takes considerably longer times for routine counts of organs than the petri dishes, so the simplicity and ease of counting organs in petri dishes is lost.

Agar is presently used to support the hypocotyls in the petri dishes, to allow oxygen to reach the tissues, and for efficient visualization of the roots and shoots without requiring opening of the dishes (which risks contamination and introducing inequalities of the environmental conditions between experiments). The possibility of replacing agar with Gelrite® (Scott Laboratories, Troy, MI) was investigated. Gelrite is transparent and gives a better appearing support than agar, but the possibility of the inadvertent release of micronutrients from the Gelrite may not be greatly different from that of agar. In one experiment, root production was more prolific on Gelrite, but these results were not substantiated in two other experiments. Therefore, the use of Gelrite as an agar replacement did not appear to be advantageous.

One cm lengths of hypocotyl segments for routine experiments were selected early in the development of the model system, based on the results of experiments comparing the regenerative capacities of hypocotyl segments of 2 to 15 mm in length. Hypocotyl segments 5 mm or less in length produced few or no organs in the absence of exogenous growth regulators. This limits the response that can be observed to only those chemicals that stimulate organ formation or growth, since inhibition of either organ will not be observed simply because almost no organs are formed in control tissues of that size. However, hypocotyl segments as short as 2 mm can be used to study the effects of exogenous chemicals on auxin-induced root formation, and/or cytokinin-induced shoot formation, nearly independent of the formation of the other organ.

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Progress on an integrated leafy spurge (*Euphorbia esula*) management system combining sheep grazing with fall-applied herbicides

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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 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 objective of our research was to determine if fall-applications of herbicides at reduced rates, preceded by sheep grazing, provided equivalent control to higher rates of those herbicides applied alone in spring or fall.

Materials and methods

The experiment was initiated in 1991 at Cherry Creek State Park in Aurora, CO. The design was an 8 (herbicides) by 3 (management approaches) factorial arranged in a strip-plot with four replications. The eight herbicide treatments (Table) comprised the 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 re-growth) were subplots. 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.

Table 1. Leafy spurge cover and density in fall, 1992, as impacted by three management approaches.

		Management approaches					
		Spring-applied herbicides		Fall-applied herbicides		Graze + fall-applied herbicides	
Herbicides	Rate	% Cover[1]	Density[2]	% Cover[1]	Density[2]	% Cover[1]	Density[2]
	(lb ai/A)						
Picloram	0.13	34 a-d	7 b-f	11 cd	6 b-f	10 cd	4 def
	0.25	2 d	1 f	19 bcd	5 b-f	36 abc	9 a-e
	0.5	23 bcd	5 b-f	34 abc	11 a-d	13 cd	3 ef
Picloram + 2,4-D		27 a-d	8 a-e	30 a-d	10 a-e	61 a	16 a
	0.25 + 1.0	8 cd	2 def	52 ab	13 abc	37 abc	11 abc
	0.5 + 1.0	15 cd	4 b-f	23 a-d	7 a-f	4 cd	2 def
Dicamba + 2,4-D	1.0 + 2.0	13 cd	5 c-f	15 cd	3 def	24 bcd	6 b-f
Non-spray control	0	17 bcd	6 b-f	10 cd	4 c-f	23 bcd	12 abc

[1]Means followed by the same letter do not differ, LSD (0.05). Compare cover means for all management approaches.
[2]Means followed by the same letter do not differ, LSD (0.05). Compare density means for all management approaches.

The impact from each management approach was assessed on the entire plant community. Leafy spurge, downy brome (*Bromus tectorum*), smooth brome (*Bromus inermis*), and western wheatgrass (*Agropyron smithii*) cover (Daubenmire) and leafy spurge density were estimated three times per season; before sheep were introduced into the study area in spring, approximately one month after they were removed in summer, and in fall before herbicides were applied. Repeat cover and density determinations were taken from the same locations within plots. Leafy spurge soil seed reserve, plant community biomass (weeds separated from desirable forage), and % control also were taken however, only cover and density from fall 1992 are presented.

Results

Cover and density data were subjected to analysis of variance as arc sine transformations. Means were separated by LSD (0.05) and are presented in their original scale. No differences occurred among herbicide treatments when averaged over all management approaches and no differences occurred among management approaches when averaged over all herbicide treatments. However, a herbicide by management approach interaction was observed.

Treatments did not differ for downy brome or smooth brome cover. Leafy spurge density and cover differed among treatments. No differences occurred among management approaches with picloram at 0.13 lb/A but there was a trend for decreased leafy spurge density with the graze plus fall-applied herbicide approach (Table). There were fewer leafy spurge shoots with spring-applied picloram at 0.25 lb/A compared to the graze plus fall-applied picloram at this rate. Leafy spurge density was greater with picloram plus 2,4-D at 0.25 + 1.0 lb/A applied alone in fall or preceded by grazing compared to this herbicide treatment spring-applied. No differences occurred among management approaches within the picloram plus 2,4-D treatment at 0.5 + 1.0 lb/A although, there was a trend for decreased leafy spurge density with the graze plus fall-applied herbicide approach. Leafy spurge density did not vary among management approaches within the dicamba plus 2,4-D herbicide treatment. Leafy spurge density did not vary among the non-sprayed control plots but there was a tendency for increased shoots in the grazed plots.

Leafy spurge cover did not differ among management approaches within picloram alone at 0.13 or 0.5 lb/A, although, there was a tendency for reduced cover with the grazing plus fall-applied picloram at these rates. However, picloram at 0.25 lb/A spring-applied reduced leafy spurge cover compared to the other management approaches. No differences among management approaches for leafy spurge cover occurred within picloram plus 2,4-D at 0.13 + 1.0 lb/A and grazing plus this herbicide treatment fall-applied tended to increase leafy spurge cover, which is the opposite trend as that observed for picloram alone at 0.13 lb/A. Leafy spurge cover was greater with picloram plus 2,4-D at 0.25 + 1.0 lb/A applied alone in fall compared to this rate spring-applied. Leafy spurge cover did not differ among management approaches within picloram plus 2,4-D at 0.5 + 1.0 lb/A and there was a trend for reduced leafy spurge cover when this treatment was fall-applied and preceded by grazing. Leafy spurge cover did not differ among the non-sprayed control plots.

Differences for western wheatgrass cover among management approaches occurred however, the stand was not uniform and this may have impacted results. Western wheatgrass cover ranged from 0 to 29%. Western wheatgrass cover was 29% in the fall-applied herbicide management approach and this was different only from those treatments that had less than 1% western wheatgrass cover (data not shown). No western wheatgrass was found in any spring-applied picloram treatment or in any of the 0.5 lb picloram treatments regardless of management approach. However, western wheatgrass cover was 16, 18, and 25% within the picloram plus 2,4-D at 0.5 + 1.0 lb/A in the spring-applied, fall-applied, and graze plus fall-applied management approaches, respectively. Western

wheatgrass is increasing in the study area and with time, differences among management approaches and/or herbicide treatments may become evident.

The management aspects of this experiment will continue through 1994. Plant community measurements will be taken for 2 years following cessation of management input.

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Imazethapyr tankmixes for control of leafy spurge

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This research was conducted near Devil's Tower, Wyoming to evaluate leafy spurge control with imazethapyr alone or in combination with dicamba, glyphosate, 2,4-D LVE, or picloram. Plots were 10 by 13.5-ft. with four replications arranged in a randomized complete block. Spring treatments were applied broadcast with a CO_2 pressurized six-nozzle knapsack sprayer delivering 40 gpa at 40 psi June 10, 1991 (air temp. 74º F, soil temp. 0 inch 80º F, 1 inch 75º F, 2 inch 70º F, 4 inch 70º F, relative humidity 58%, wind south at 3 mph, sky clear). Late summer treatments were applied September 11, 1991 (air temp. 70º F, soil temp. 0 inch 85º F, 1 inch 80º F, 2 inch 80º F, 4 inch 75º F, relative humidity 55%, wind west at 3 mph, sky 50% cloudy). The soil was classified as a silt loam (22% sand, 58% silt, and 20% clay) with 1.8% organic matter and a 6.3 pH. Leafy spurge was in the full bloom stage and 14 to 18 inches in height, for the spring treatments and past seed production and 14 to 20 inches in height, for the late summer treatments. Infestations were heavy throughout the experimental area. Visual evaluations were made June 11, 1992.

No spring or fall applied treatment provided adequate control of leafy spurge in 1992. The treatment which provided the most control was imazethapyr + picloram at 0.125 + 0.25 lb/A. This combination provided better control than either imazethapyr or picloram applied alone. Fall applied treatments provided better leafy spurge control than spring applied treatments.

Leafy spurge control

Treatment	Rate	1991 application date/evaluation date	
		June 10/ June 11, 1992	Sept 11/ June 11, 1992
	(lb ai/a)	----------(percent control[1]) ----------	
imazethapyr[2]	0.063	0	20
imazethapyr[2]	0.125	0	28
imazethapyr + 2,4-D LVE[2]	0.063 + 1.0	8	30
imazethapyr + dicamba[2]	0.063 + 1.0	3	23
imazethapyr + picloram[2]	0.063 + 0.25	8	45
imazethapyr + glyphosate[2]	0.063 + 0.38	3	38
imazethapyr + 2,4-D LVE[2]	0.125 + 1.0	20	68
imazethapyr + dicamba[2]	0.125 + 1.0	13	54
imazethapyr + picloram[2]	0.125 + 0.25	18	78
imazethapyr + glyphosate[2]	0.125 + 0.38	0	54
2,4-D LVE[2]	1.0	5	15
dicamba[2]	1.0	5	23
picloram[2]	0.25	3	35
glyphosate[2]	0.38	0	20
(LSD 0.05)		10	18
(CV)		132	35

[1]Percent control by visual estimation.
[2]Surfactant (X-77) added at 0.25% v/v. 32-0-0 liquid fertilizer added at 1.0 quart N/acre.

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Picloram with or without surfactant (Sylgard®) for control of leafy spurge

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This research was conducted near Devil's Tower, Wyoming to evaluate control of leafy spurge with picloram, with or without surfactant, for control of leafy spurge. Plots were 10 by 27 ft. with four replications arranged in a randomized complete block. Treatments were applied June 09, 1992 (air temp. 82º F, soil temp. 0 inch 125º F, 1 inch 110º F, 2 inch 95º F, 4 inch 85º F, relative humidity 27%, wind south at 5 mph, sky 20% cloudy). The soil was classified as a silt loam (22% sand, 58% silt, and 20% clay) with 1.8% organic matter and a 6.3 pH. Leafy spurge was in full bloom and 14 to 20 inches in height. Infestations were heavy throughout the experimental area. Visual evaluations were made September 23, 1992 and June 21, 1993.

Evaluations four and eight months after application show the surfactant Sylgard® to have no effect on leafy spurge control with picloram at any rate.

Leafy spurge control

Treatment[1]	Rate	Control[2]	
		9-23-92	6-21-93
	(lb ai/a)	%	
picloram + Sylgard[1]	0.25	10	0
picloram + Sylgard[1]	0.5	40	5
picloram + Sylgard[1]	1.0	90	25
picloram	0.25	10	0
picloram	0.5	40	0
picloram	1.0	91	38
(LSD 0.05)		11	11
(CV)		19	79

[1]Surfactant (Sylgard®) added at 0.25% v/v.
[2]Visual evaluations September 23, 1992 and June 21, 1993.

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Dicamba, picloram, 2,4-D tankmixes for control of leafy spurge

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This research was conducted near Devil's Tower, Wyoming to evaluate leafy spurge control with tankmixes of dicamba, picloram, and 2,4-D amine. Plots were 10 by 13.5 ft. with four replications arranged in a randomized complete block. Spring treatments were applied broadcast with a CO_2 pressurized six-nozzle knapsack sprayer delivering 40 gpa at 40 psi June 11, 1991 (air temp. 86º F, soil temp. 0 inch 95º F, 1 inch 85º F, 2 inch 80º F, 4 inch 80º F, relative humidity 30%, wind south at 5 mph, sky clear). Late summer treatments were applied September 11, 1991 (air temp. 70º F, soil temp. 0 inch 85º F, 1 inch 80º F, 2 inch 80º F, 4 inch 75º F, relative humidity 55%, wind west at 3 mph, sky 30% cloudy). The soil was classified as a silt loam (22% sand, 58% silt, and 20% clay) with 1.8% organic matter and a 6.3 pH. Leafy spurge was in the full bloom stage and 14 to 18 inches in height, for the spring treatments and past seed production and 14 to 20 inches in height, for the late summer treatments. Infestations were heavy throughout the experimental area. Visual evaluations were made September 25, 1992.

Late summer applications of picloram+dicamba+2,4-D provided significantly better leafy spurge control than spring applications of picloram+dicamba+2,4-D. Herbicide combinations provide better control than individual herbicides at both dates. The addition of surfactant to combination treatments had no effect on leafy spurge control.

Leafy spurge control

Treatment	Rate	Application date/evaluation date: June 11, 1991/ Sept. 25, 1992	Application date/evaluation date: Sept. 11, 1991/ Sept. 25, 1992
	(lb ai/a)	----------(percent control[1])----------	
picloram + dicamba + 2,4-D amine[2]	0.25 + 1.0 + 1.0	18	63
picloram + dicamba + 2,4-D amine	0.25 + 1.0 + 1.0	13	S3
picloram + dicamba + 2,4-D amine[2]	0.25 + 2.0 + 1.0	23	71
picloram + dicamba + 2,4-D amine	0.25 + 2.0 + 1.0	55	78
picloram + dicamba + 2,4-D amine[2]	0.5 + 1.0 + 1.0	28	89
picloram + dicamba + 2,4-D amine	0.5 + 1.0 + 1.0	64	86
picloram + dicamba + 2,4-D amine[2]	0.5 + 2.0 + 1.0	39	78
picloram + dicamba + 2,4-D amine	0.5 + 2.0 + 1.0	61	83
picloram	0.25	0	18
picloram	0.5	23	68
dicamba[2]	1.0	0	15
dicamba[2]	2.0	0	8
2,4-D amine	1.0	5	5
(LSD 0.05)		26	22
(CV)		78	30

[1]Percent control by visual estimation. An LSD (0.05) of 24 is valid for comparison of treatment means between application dates (CV = 45%).

[2]Surfactant (X-77) added at 0.5% v/v.

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Quinclorac tankmixes for control of leafy spurge

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This research was conducted near Devil's Tower, Wyoming to evaluate leafy spurge control with early or late summer applications of quinclorac, alone or in combination with other herbicides. Plots were 10 by 27-ft. with four replications arranged in a randomized complete block. Spring treatments were applied June 10, 1991 (air temp. 70º F, soil temp. 0 inch 115º F, 1 inch 80º F, 2 inch 75º F, 4 inch 70º F, relative humidity 65%, wind south at 5 mph, sky 40 % cloudy). Fall treatments were applied September 25, 1990 (air temp. 65º F, soil temp. 0 inch 70º F, 1 inch 65º F, 2 inch 60º F, 4 inch 60º F, relative humidity 34%, wind south at 3 mph, sky clear). The soil was classified as a silt loam (22% sand, 58% silt, and 20% clay) with 1.8% organic matter and a 6.3 pH. Leafy spurge was in full bloom and 14 to 20 inches in height for the spring treatments or past seed production and 14 to 20 inches in height for the fall treatments. Infestations were heavy throughout the experimental area. Visual evaluations were made June 18, 1991 and June 10, 1992 or September 25, 1992.

Fall applications of quinclorac + picloram (1.0 + 0.5 lb/A), provided 80% control of leafy spurge nine months after treatment. However, control had dropped to 51% by June 1992. No other treatments provided effective leafy spurge control.

Leafy spurge control

Treatment	Rate	Application date/evaluation date Sept. 25, 1990/ June 18, 1991	 Sept. 25, 1990/ June 10, 1992	 June 10, 199 1 Sept. 25, 1992
	(lb ai/a)	----------(control[1])----------		
quinclorac[2]	0.5	25	10	30
quinclorac + 2,4-D LVE[2]	0.5+1.0	35	18	51
quinclorac + dicamba	0.5+1.0	36	15	48
quinclorac + picloram[2]	0.5+0.5	46	20	60
quinclorac[2]	1.0	64	33	55
quinclorac + 2,4-D LVE[2]	1.0+1.0	71	33	65
quinclorac + dicamba	1.0+1.0	75	36	60
quinclorac + picloram[2]	1.0+0.5	80	51	65
(LSD 0.05)		11	20	19
(CV)		16	57	27

[1]Percent control by visual evaluation.
[2]Crop oil concentrate (Sunit) added at 1 quart/acre.

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Late summer applications of quinclorac or imazethapyr for control of leafy spurge

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This research was conducted near Devil's Tower, Wyoming to evaluate leafy spurge control with late summer applications of quinclorac and imazethapyr, alone or in combination. Plots were 10 by 13.5-ft. with four replications arranged in a randomized complete block. Late summer treatments were applied September 11, 1991 (air temp. 76º F, soil temp. 0 inch 85º F, 1 inch 90º F, 2 inch 90º F, 4 inch 85º F, relative humidity 40%, wind west at 5 mph, sky 30% cloudy). The soil was classified as a silt loam (22% sand, 58% silt, and 20% clay) with 1.8% organic matter and a 6.3 pH. Leafy spurge was past seed production and 14 to 20 inches in height. Infestations were heavy throughout the experimental area. Visual evaluations were made June 11, 1992.

Late summer applications of quinclorac and imazethapyr, alone or in combination, did not provide adequate control of leafy spurge nine months after treatment.

Leafy spurge control

Treatment[1]	Rate	Control[3]
	(lb ai/a)	(%)
quinclorac[2]	0.25	0
imazethapyr[2]	0.06	0
imazethapyr[2]	0.13	5
quinclorac + imazethapyr[2]	0.25+0.06	40
quinclorac + imazethapyr[2]	0.25+0.13	50
picloram	1.0	91
(LSD 0.05)		15
(CV)		26

[1]Treatments applied September 11, 1991.

[2]Crop oil concentrate (Sunit) added at 1 quart/acre.

[3]Visual evaluations June 11, 1992.

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Leafy spurge control with picloram dry acid and picloram liquid

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This research was conducted near Devil's Tower, Wyoming to evaluate leafy spurge control comparing picloram 70% dry acid with picloram 2.0 lb/gal liquid. Plots were 10 by 27-ft. with four replications arranged in a randomized complete block. Spring treatments were applied June 9, 1992 (air temp. 82º F, soil temp. 0 inch 120º F, 1 inch 110º F, 2 inch 95º F, 4 inch 85º F, relative humidity 27%, wind south at 5 mph, sky 20% cloudy). Fall treatments were applied September 23, 1992 (air temp. 82º F, soil temp. 0 inch 115º F, 1 inch 105º F, 2 inch 95º F, 4 inch 75º F, relative humidity 23%, wind south at 3 mph, sky clear). The soil was classified as a silt loam (22% sand, 58% silt, and 20% clay) with 1.8% organic matter and a 6.3 pH. Leafy spurge was in full bloom and 8 to 16 inches in height for spring applications. Leafy spurge was past seed production and 14 to 18 inches in height for fall applications. Infestations were heavy throughout the experimental area. Visual evaluations were made September 25, 1992 and June 21, 1993 for spring applications and June 21, 1993 for fall applications.

Evaluations show there to be no significant differences between picloram 70% dry acid and picloram 2.0 lb/gal liquid formulations for leafy spurge control.

Leafy spurge control

		Application date/evaluation date		
Treatment	Rate	6-9-92/9-25-92	6-9-92/6-21-93	9-23-92/6-21-93
	(lb ai/a)	(% control[1])		
picloram 2.0 lb. liquid	0.25	0	0	8
picloram 2.0 lb. liquid	0.5	15	0	46
picloram 2.0 lb. liquid	1.0	94	43	86
picloram 70% dry acid	0.25	0	0	0
picloram 70% dry acid	0.5	13	0	50
picloram 70% dry acid	1.0	91	48	91
(LSD 0.05)		13	8	15
(CV)		29	44	24

[1]Percent control by visual estimation.

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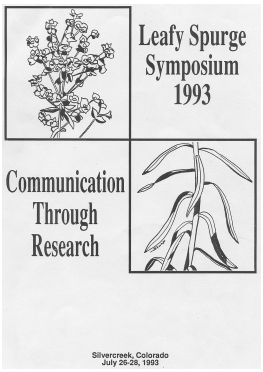

1993 Leafy Spurge Symposium "Communication through research"

Foreword / Business Meeting Minutes

The 1993 Great Plains Agricultural Council Leafy Spurge Task Force Symposium was held July 26-28 at Silvercreek, Colorado. The Symposium was attended by scientists, weed district supervisors, and land managers from the private and public sectors. The Leafy Spurge Symposium is an opportunity for interested people to assemble, listen, and discuss the most current scientific information concerning leafy spurge – i.e., an opportunity to communicate through research.

The research papers presented at this symposium were balanced among biological control, chemical control, integrated leafy spurge management systems, and physiological/biological aspects of this important noxious weed. The primary purpose of the Leafy Spurge Symposium is for scientists who are conducting research on leafy spurge to discuss and critique their latest findings such that continual progress is made toward enhancing our knowledge base. It is anticipated that weed supervisors and land managers will take the research information presented and incorporate it into their weed management practices and systems.

This was the first time, hopefully not the last, that the Symposium was held in Colorado. A good exchange occurred and this will continue at future Symposia. The 1994 GPAC Leafy Spurge Task Force Symposium will be held in Bozeman, Montana, July 26-29. For information concerning the 1994 meeting, contact Dr. Neal Spencer, USDA-ARS Sidney, Montana, at (406) 482-2020.

K. George Beck
President, GPAC Leafy Spurge Task Force, 1993
Colorado State University
Ft. Collins, Colorado 80523

Minutes of the business meeting 1993 Leafy Spurge Symposium

Granby, Colorado
July 27, 1993

The meeting was opened by Dr. George Beck, President of the 1993 Symposium and Weed Scientist, Colorado State University, Ft. Collins, Colorado.

I. A motion was made by Dr. Rod Lym (weed scientist, North Dakota State University (NDSU)) to hold the leafy spurge meeting every other year.

A. The motion was seconded by Dr. David Davis (USDA/ARS, Fargo, ND)

B. Discussion:

1. Dr. Lym - lack of new data at yearly increments.
2. Cost of attendance of a yearly meeting is a decision factor.
3. Dr. Beck agreed with the discussion for holding meeting every other year.
4. Dr. Davis agreed with the concept of biennial meetings, but thought Dr. Don Anderson,
   Assist. Dean of Agriculture at NDSU preferred the meeting to be held on a yearly basis.
5. Dr. Claude Schmidt (USDA/ARS - retired) stated GPAC - 14 may need to be consulted as changes in the meeting schedule may be in opposition to the charter.
6. Dr. Calvin Messersmith (weed scientist, NDSU) suggested we confer with Dr. Anderson and GPAC-14. A revision could be made at next year's meeting.

The Motion carried.

II. The next meeting is scheduled for July 26-29, 1994 in Bozeman, Montana.

III. The meeting following the Bozeman symposium would be scheduled for 1996 in Fargo, ND (if the above motion does not go against the GPAC - 14 charter).

IV. Dr. David Davis made a motion for the meeting ensuing the Fargo, ND meeting to be held in province of Manitoba, Canada.

A. Dr. Rod Lym seconded the motion.

B. A discussion was held on whether officers of the Leafy Spurge Symposium could be from Canada.

C. Dr. Calvin Messersmith suggested the issues such as officers for the Manitoba meeting could be resolved at the Bozeman meeting in 1994.

The motion carried.

V. Clay Vint was nominated for the position of secretary for the 1998 meeting in Manitoba.

The motion carried.

VI. Dr. Beck led a discussion on the aims and goals of the Leafy Spurge Symposiums i.e. research, extension, or some combination of the two.

A. Dr. Lym felt the objective for the meetings was for researchers to communicate with one another. Too many people in attendance tend to restrict discussion between researchers.

B. Attendees of the business meeting joined into the discussion of the objectives for the symposium. The general consensus was directing the goal of the Leafy Spurge Symposium toward the sharing of research data.

VII. Neal Spencer, USDA/ARS Sidney, Montana is chairing the 1994 Leafy Spurge Symposium to be held at the Holiday Inn in Bozeman, Montana on July 26-29, 1994. On Tuesday evening, July 26, a series of group discussions will be convened. These groups will cover the principal areas of interest to the symposium attendees. Each will be led by one or more "facilitators". Thursday afternoon and Friday will be a field trip to several areas in Montana where leafy spurge controls are being applied. It promises to be a good meeting.

VIII. Dr. Beck discussed the format for the symposium proceedings.

A. Dr. Beck stated, and Dr. Robert Masters (USDA/ARS Lincoln, NE) agreed, it is difficult to obtain written material from the speakers.

B. The group consensus was to encourage researchers to write a paper and have a proceedings in the hands of the attendees.

C. Dr. Lym stated early publication would facilitate having the information to the user when it was needed.

D. Dr. Masters reiterated difficulty in obtaining papers from the speakers and a resultant delay in publishing the symposium proceedings.

E. Dr. Beck stated he was establishing a deadline of August 31, 1993 for submission of manuscripts for the 1993 Proceedings. Those papers received by that time would be published; the remaining ----.

F. Neal Spencer plans to ask for long abstracts prior to the 1994 meeting and distribute the publications with the meeting packet of information.

IX. Dr. Schmidt told the group that Dr. Don Anderson had been unable to attend the meeting this year. Dr. Russ Lorenz was also unable to attend. Dr. Schmidt would be doing more of the work on putting together the Leafy Spurge News.

A. The Leafy Spurge News is scheduled for publication 3 to 4 times a year.

B. Please continue to submit information to the Editors that would be of interest to the readers. Communicate!

C. Abstracts from the meeting will be featured in the newsletter soon.

Submitted by Neal R. Spencer

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Multi-state sampling protocols for *Aphthona* flea beetles, leafy spurge biocontrol agents

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Summary

We employ sweep-net sampling to document establishment and estimate population size at *Aphthona* spp. field insectary sites in 15 states. In order to calibrate sweep sample results (a relative sampling method), sweep-net catches were compared to mark/recapture and vacuum sampling data (absolute sampling methods). However, preliminary experiments showed that paint marks or powders either did not persist or were toxic to marked insects, so further work with mark/recapture data was abandoned.

Sweep-net and vacuum sampling data were compared at *Aphthona cyparissiae*, *A. flava*, and *A. nigriscutis* field insectary sites in Montana. Vacuum sampling is an absolute sampling method, wherein a portable suction device collects all adult flea beetles in a fixed area. Paired sweep-net and vacuum samples, separated by 2 m, were collected at 2-m intervals, along each cardinal direction from the initial release point.

By regressing sweep-net catches (beetles/sweep) on vacuum catches (beetles/m^2), a significant, positive linear relationship was discovered. A first attempt at calibrating *Aphthona* sweep-net data was developed, using the "inverse" of this relationship (i.e. no. beetles/sweep becomes the independent or "x" variable):

$Y = (X-0.276)/0.054$, where

Y = no. beetles/m^2

X = no. beetles/sweep

With further refinement, we believe this strategy will enable us to estimate the number of beetles at field insectary sites throughout the U.S., based on sweep-net catches.

Aphthona spp. sampling programs are directed at adult beetles, and predictions of adult activity periods would enable "optimal" scheduling of sweep-net samples. This effort has two components: a general phenological model for adult *Aphthona* emergence, and a "temperature network" of weather stations that supplies temperature data for broad application of the general phenological model. Adult phenology data were collected from

populations of *Aphthona cyparissiae*, *A. flava*, and *A. nigriscutis* near Bozeman. Emergence cages were placed at 2 and 6 m from the initial release point, along each cardinal direction (eight cages total for each species). Cages enclosed an area of 0.25 m^2 from which all aboveground spurge vegetation was periodically removed. Cages were checked at two- or three-day intervals and all "trapped" beetles removed and counted. An electronic weather station at the site (within 0.5 km of all releases) recorded air temperatures at 15-minute intervals; daily minimum and maximum temperatures were used to calculate daily and accumulated degree-days with a lower developmental threshold of 10º C.

The total number of beetles caught, by species, was divided by the cumulative number of beetles caught at a given date, to generate a "cumulative percent emergence curve"; *A. nigriscutis* data were discarded because so few adult beetles were caught (<25 total). Julian date was then replaced by accumulated degree-days as the independent (x) variable for the emergence curves. Curves for *A. cyparissiae* and *A. flava* were nearly identical; both described 50%, or "median", cumulative emergence occurring at 425 degree-days (> 10º C).

A network of 97 weather stations in 15 states was established that provides daily minimum and maximum air temperatures on a real-time basis. Using temperature data from 1980 through 1992 (where available), degree-days were calculated and the dates at which 425 degree-days were accumulated were determined. These dates were averaged for each station and then for each state to derive a "projected" date for median emergence in 1993. For each state, the three *Aphthona* sampling dates were set at this "median" date and two weeks before and two weeks after, in an attempt to bracket flea beetle emergence. Thus, initial 1993 sampling dates range from May 31 in New Mexico and Iowa to July 5 in Nevada.

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Effects of *Aphthona* flea beetles and sheep grazing in leafy spurge stands

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Summary

The objectives of this experiment include: 1) documenting individual effects of *Aphthona* spp. populations in leafy spurge stands; 2) documenting effects of both agents acting together in leafy spurge stands; and 3) determining if sheep grazing has a detrimental impact on establishment of *Aphthona* spp. populations. Two Montana sites have been utilized: the "Raynesford" site in Judith Basin Co., and the "Story Hills" site in Gallatin Co. At both sites, a randomized complete block design has been employed, using square plots ("blocks") roughly 15.3 m (50 feet) on a side. Twelve plots were established at each site, representing three replications of four experimental treatments. The four treatments are: 1) *Aphthona* spp. flea beetles alone; 2) sheep grazing alone; 3) *Aphthona* flea beetles and sheep grazing; and 4) no beetles or grazing (control). All plots experiencing grazing were fenced to confine sheep.

Adult beetles were released in the center of each ungrazed or grazed *Aphthona* plot; Raynesford plots received 500 *Aphthona nigriscutis* in July 1990, while Story Hills plots received 1,000 *Aphthona flava* in July 1991. Grazed plots received two grazing episodes per year, beginning in 1991. These plots were first grazed in the spring, up to approximately the second week in June, and grazed again in late August to early September. The number of sheep used per plot varied by site and year, but grazing was allowed to proceed until all palatable vegetation had been cropped.

Forty permanent 0.1-m^2 subplots were established along three parallel transects in each plot. Data collected within each subplot included leafy spurge density and stem height, and "canopy cover" attributable to spurge, grasses, forbs, plant litter, and bare ground. Five "biomass" samples were randomly-collected from each plot; all aboveground vegetation within a 0.1-m^2 area was removed, separated into spurge, grass, and forb components, oven-dried, and weighed. In 1991, sweep-net samples were conducted to note presence or absence of released flea beetles. In 1992, five net sweeps were made at 1, 3, 5, and 7 m from the initial release point along each cardinal direction (80 sweeps per plot), and all beetles collected were counted.

1993 data are not yet available, but two years of sheep grazing have had no apparent effect on the establishment and subsequent size of *Aphthona nigriscutis* and *Aphthona flava* populations, given the grazing schedule employed in this study.

Through 1992, sheep grazing has reduced leafy spurge and grass aboveground biomass. By 1993, grazing has reduced spurge density at Raynesford but not at Story Hills; grazing has reduced spurge stem height at both sites. Sheep grazing has caused reductions in spurge "cover" (Raynesford only), grass cover, and forb cover while increasing "cover" attributable to plant litter or bare ground. These results are not unexpected, since data were collected in leafy spurge stands following an early-season grazing episode, and thus represent measurements of "regrowth" spurge. Reduced grass and forb cover and grass biomass simply confirm that sheep ate these plants as well as leafy spurge.

Within two years after initial release (1992), *Aphthona* flea populations have had no significant impact on aboveground vegetative biomass. Through 1993, *A. nigriscutis* (Raynesford) has significantly reduced leafy spurge density, "canopy" cover, and stem heights; reductions in leafy spurge may be accompanied by increases in grasses and non-spurge forbs. At Story Hills, *A. flava* populations have had no consistent impact on various vegetation indices.

By 1993, sheep grazing and *A. nigriscutis* populations (Raynesford) appear to have reduced leafy spurge stem densities more than either agent acting alone.

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Dietary preference of angora goats grazing leafy spurge-infested rangeland

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Introduction

Leafy spurge infests approximately 1.2 million acres in North Dakota accounting for over $137 million per year depreciation losses and $75 million in forgone business activity (Thompson *et al*. 1990). Due to its aggressive spread and tremendous survival, integrated methods of control are being used to complement the traditional chemical means. One of those methods is grazing by goats. Goats consume leafy spurge without the apparent negative effects experienced by cattle and horses. Cattlemen, however, are concerned with the dietary overlap of goats with cattle and the competition for forage selected by cattle.

Materials and methods

The study was conducted on the Sheyenne National Grasslands. The Grasslands are managed by the U.S. Forest Service for multiple purposes in southeastern North Dakota. Angora goats were introduced into areas heavily infested with leafy spurge which are co-grazed by cattle. Two herds of 1300 Angora goats were rotated through allotments in 1992. Diets were collected following a video tape evaluation and analyzed for nutrient composition. Fecal samples were collected at the same time and sent to the Composition Analysis Laboratory in Fort Collins, Colo. for microhistological analysis. Available forage in these allotments was measured prior to the Angora goats introduction. Fifteen 0.1 m^2 quadrats were randomly sampled in each of the general cover types. A Preference Index was developed using the formula (Durham and Kothmann 1977):

$$\text{Relative Preference} = \frac{\text{\% in diet - \% available}}{\text{\% in diet + \% available}} \text{ X10}$$

A relative preference of 0 indicates selection in accordance with plant availability. A positive value indicates preference for a plant while a negative number indicates nonpreference or avoidance.

Results and discussion

Leafy spurge and Kentucky bluegrass were the most available species in all allotments at all times of the grazing season. The fecal analysis determined that leafy spurge was a predominate part of the diet. Warm season grasses made up a significant portion of the diet in June (20%) and early August (30%). The goats' fecal analysis showed this to be primarily sand dropseed which was found only when the Angora goats grazed in one allotment at these times. The Angora goats' feces also contained large amounts of shrub and tree material at all times of the year. The relative preference index (Table 1) showed a positive preference for leafy spurge and shrubs-trees at all times of the growing season except during late June sampling. The allotment grazed at this time had such a heavy spurge infestation that selection was equal to availability.

Table 1. Relative preference index[1] for forage selected by Angora goats.

			Date		
Forage class	6-8	6-13	6-27	1-28	8-2
Leafy spurge	2	4	0	4	3
Forbs	-8	-4	7	-5	-5
Shrubs	8	7	8	7	5
Kentucky bluegrass	-10	-10	-10	-10	-10
OCS[2]	-6	3	-9	-8	-8
WS[2]	7	0	-2	4	-3
OGL[2]	-4	-1	-6	-5	-9

[1] 0 = selection in accordance with availability
> 0 = preference for plant class
< 0 = nonpreference or avoidance of plant class
[2] OCS = other cool season grasses, WS =warm season grasses, OGL=other grass-like plants.

With few exceptions, the vegetation classes most preferred by cattle were not preferred by the Angora goats. Certain species of warm and cool season grasses were preferred by the goats at different times of the grazing season relating to their occurrence in the different grazing allotments.

The nutritional composition of the diet selected by the goats is shown in Table 2. Although the quality of the diets declined with advancing plant maturities, the nutritional requirements of nursing does was met throughout the growing season.

Table 2. Nutritional composition of leafy spurge based diets selected by Angora goats[1].

	Stage of Growth[2]					
Nutrient	V	F	EM	2	LMM	R
DM[3]	92	92	93	95	95	87
Ash	7.5	8.0	8.3	9.4	8.7	7.2
CP	19.2	17.6	17.9	18.8	17.8	13.2
IVDMD	76	73	70	63	68	73
Phos	0.38	0.37	0.36	0.35	0.33	0.27
ADIN	0.20	0.21	0.25	0.44	0.31	0.21
ADF	22.1	24.4	22.8	30.2	23.6	19.2
NDF	28.8	32.1	32.6	43.5	33.6	26.6
ADL	5.3	5.9	6.3	8.4	7.4	5.4

[1] Mean-1991, 1992
[2] V=vegetative, F=flowering, EM=early mature, 2=second grazing at flowering, R=late season regrowth
[3] DM=dry matter, CP=crude protein, ADIN=acid detergent insoluble nitrogen, IVDMD=in vitro dry matter digestibility, ADF=acid detergent fiber, NDF=neutral detergent fiber, ADL=acid detergent lignin, Phos=phosphorus.

Summary

Angora goats prefer leafy spurge along with shrubs and trees in southeastern North Dakota rangelands infested with leafy spurge. Their dietary overlap with cattle appears to be minimal making them a good biological control medium in rangelands grazed by cattle.

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Effect of leafy spurge biotypes and herbicides on *Aphthona* spp. establishment

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The effect of herbicides applied to leafy spurge on *Aphthona* spp., survival and establishment was evaluated. The experiment was established at seven locations, four *A. nigriscutis*, two *A. cyparissae*, and one *A. flava* location. *Aphthona* spp. were released in the center of a 100 feet by 100 feet plot divided into four equal 50 feet by 50 feet quadrants. The herbicide treatment of picloram plus 2,4-D at 8 plus 16 oz/A was applied to quadrants one and two the first year, to quadrants two and three the second year, and so on. In general, most adults were found in quadrants that were sprayed the previous or current year, or quadrants that have never been sprayed, but not in quadrants treated 2 years consecutively.

The timing of herbicide treatment on *A. nigriscutis* survival and establishment was evaluated. *A. nigriscutis* was released in 1989 and herbicide treatments were initiated the spring of 1992 in a dense stand of leafy spurge near Cuba, ND. The treatments included spring-applied picloram plus 2,4-D at 4 plus 16oz/A fall-applied picloram plus 2,4-D at 8 plus 16 oz/A, and a control of no herbicide. Stem density was evaluated in June of 1992 before herbicide treatment and May of 1993. Stem density increased in the spring-treated plots from 43 stems/ml in 1992 to 52/m^2 in 1993. A large decrease in the fall-treated plots from 43 stems/m^2 in 1992 to 3/m^2 in 1993 was observed due to the herbicide treatment. A decrease from 57 stem/m^2 to 36/m^2 was observed in the control. The *A. nigriscutis* population declined when the herbicide was applied in the spring from an average of 2 beetles/m^2 to 0.3/m^2 12 months after treatment (MAT). The fall-applied herbicide treatment was less disruptive to the *A. nigriscutis* population which was averaged 19 beetles/m^2 compared to 32/m^2 in the control 12 MAT.

Survival and growth of *A. cyperissae, 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 in 6-inch pots were placed in a cage with approximately 50 adults. The plant position in the cage was rotated every 3 days. Plants were replaced after 9 days for a total of three replications per year, and the experiment was repeated 2 years. Adult feeding was monitored, by counting the number of insects on each biotype at the same time each day. No feeding preference was found except slightly less by *A. flava* on the Nebraska biotype. Eggs were found in all pots but there was poor adult emergence in 1991 and no adult emergence in 1992.

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Quinclorac applied with additives and other herbicides for leafy spurge control

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Preliminary research has shown that quinclorac (3,7-dichloro-8-quinolinecarboxylic acid) will control leafy spurge. Quinclorac (trade name Facet) is a systemic herbicide registered for use in rice to control annual grass and broadleaf weeds. Quinclorac moves both upward and downward in plants, and weed control is thought to come from a combination of both traits. Injury to grass plants in turfgrass, rangeland, and small grain experiments has been minimal.

The objective of this research was to determine the most cost-effective quinclorac treatment to control leafy spurge. Field and greenhouse research was begun to evaluate the most effective application time and whether quinclorac plus surfactant, oil, or nitrogen adjuvants, or other herbicides will improve leafy spurge control compared to quinclorac applied alone.

Adjuvants sometimes have enhanced leafy spurge control with quinclorac. Quinclorac at 0.56 to 1.7 kg ae/ha applied with Scoil (methylated seed oil) or Dash adjuvants, evaluated 9 months after treatment (MAT), provided 91% leafy spurge control compared to 86% with quinclorac applied alone. The adjuvants Scoil and Dash increased leafy spurge control with quinclorac more than X-77 (nonionic surfactant), L-77 (copolymer wetting agent), or Triton CS7 (blend of alkyl aryl polyethoxylate and sodium salt of alkylsulfonatedalkylate).

Quinclorac provided better leafy spurge control when applied in the fall than spring. Quinclorac at 1.14 and 1.7 kg/ha plus Scoil applied on September 1 or 15, 1992 averaged 97% control 9 MAT, but only averaged 71% control when applied on October 1 or 15, 1992.

Nitrogen applied to leafy spurge stimulates growth of dormant buds. Activating dormant buds in leafy spurge has the effect of creating metabolic sinks which can increase translocation of herbicides throughout the plant. A nitrogen adjuvant added to quinclorac has increased leafy spurge control in greenhouse experiments, but the increase was less than that with seed oil adjuvants.

Herbicide combination studies have been conducted in both the field and greenhouse. Quinclorac plus another herbicide increased both leafy spurge control and reduced the

amount of quinclorac required for 80% or higher control. Quinclorac at 1.14 kg/ha plus picloram at 0.56 kg/ha provided 85% control 9 MAT compared to 49% control when quinclorac was applied alone.

Quinclorac has potential to become a widely used leafy spurge control herbicide. However, BASF has been reluctant to pursue a label for this product in rangeland.

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Effect of herbicide treatments and leafy spurge biotype on *Spurgia esulae* population

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The leafy spurge gall midge (*Spurgia esulae* Gagné) has established in several areas of North Dakota especially when released in wooded areas or near shelterbelts. Around four generations of gall midges are produced each growing season in North Dakota. Only a portion of the leafy spurge stem tips generally are infested by the gall midge, so a second control method is needed to reduce the infestation. 2,4-D applied alone or with low rates of picloram may be useful in conjunction with the gall midge for leafy spurge control near trees. The purpose of the first series of experiments was to determine the compatibility of the gall midge biocontrol agent with several herbicides in a leafy spurge management program.

The experiment was established in a 3-acre area between two shelterbelts, which had a well established gall midge population. Herbicides were applied during the first or second gall midge generation in 1991 and 1992 for a total of four trials. The treatments were 2,4-D at 16 oz/A, picloram plus 2,4-D at 4 plus 16 oz/A, and imazethapyr at 2 oz/A. The effect of herbicide treatment on subsequent gall midge population was determined by monitoring both gall density and the number of larvae per gall.

The leafy spurge top-growth died in herbicide treated plots and gall density declined from an average of 30/m^2 to 1.5/m^2 1 month after treatment (MAT) compared to 26/m^2 in the untreated control. Galls were common on regrowth of treated leafy spurge 3 MAT and averaged 9 galls/m^2 compared to 15/m^2 in the control. Gall density was similar regardless of treatment 12 MAT. No treatment affected the number of larvae per gall which declined from 11/gall prior to herbicide treatment to >1/gall by the end of the growing season. There was an average of 10 larvae/gall regardless of treatment 12 MAT.

The effect of leafy spurge biotypes on gall midge survival was evaluated. The leafy spurge biotypes included plants from Austria, Manitoba, Montana, Nebraska, North Dakota, South Dakota, and Wyoming. Galls were collected from the field and placed in a cage with six plants (repetitions) of each biotype in a randomized complete block design. The adults emerged, laid eggs, and galls formed. The percentage of tips galled per biotype and the number of larvae per gall were averaged over eight trials.

An average of 58% of tips were galled on biotypes from South Dakota, Nebraska, and Wyoming compared to 25 and 11% for plants from North Dakota and Montana, respec-

tively. The most larvae per gall, averaging 32, occurred on the biotypes from South Dakota and Nebraska. No larvae survived on the Montana biotype and only one larvae per two galls survived on the Manitoba biotype.

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Report on exploration for leafy spurge and associated plant pathogens and insects in Russia

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The purpose of the foreign exploration was to collect leafy spurge genotypes and associated insects and plant pathogens from plant communities in Russia. I was responsible for collecting leafy spurge specimens, Anthony Caesar, Plant Pathologist, USDA-ARS, Bozeman, MT was responsible for collecting pathogens, and Luca Fornasari, Entomologist, USDA-ARS, Montpellier, France surveyed and collected insects.

The highlights of the foreign exploration were the successful accomplishment of the exploration objectives and continued development of a very good working relationship with scientists affiliated with the Zoological Institute, Russian Academy of Sciences in St. Petersburg. The joint expedition occurred from June 15 through July 14, 1993 and specimens were evaluated along a 2,500-mile route through south central Russia [Table 1 (not available)]. Plants, pathogen, and insect specimens were collected and surveyed from June 17 through June 29, 1993 starting near Mineralnye Vody and then following a route through Kropotkin, Krasnodar, Tuapse, Maykop, Armavir, Stavropol, Elista, Volgograd, Kamyshin, and Saratov.

Tony Caesar departed from Saratov for St. Petersburg on June 30 and continued on to the European Biological Control Laboratory at Montpellier, France on July 3. This departure was necessary because of the extreme perishability of the pathogens that Tony had collected and the need to culture and isolate specimens. I also returned to St. Petersburg to resolve a problem with my visa. I returned to Saratov on July 3 to continue the joint exploration. From July 4 through July 9, 1993 plant and insect specimens were collected and surveyed along a route that originated from Saratov through Bozioglebsk, Tambov, Pensa, and back to Saratov.

Plant pathogens were collected or surveyed at 15 locations and plant and insect specimens were collected or surveyed from 32 locations during the expedition. The exploration route was traveled by truck and AEROFLOT was the airline used to fly from St. Petersburg to Mineralnye Vody on June 17, 1993 and from Saratov to St. Petersburg at the end of the expedition on July 9, 1993.

In the grasslands explored in the region south of Stavropol, *Euphorbia steposa* was the most common euphorb with *E. virgata, E. parillius, E. seguiriana*, and *E. iberica* encountered less frequently. Populations of *E. virgata* and *E. seguiriana* were found more frequently in the drier and more temperate grasslands north of Stavropol. *Euphorbia seguiriana* was the dominant euphorb found as the expedition continued into xeric grassland communities northeast of Stavropol to Elista. *Euphorbia virgata* was the dominant euphorb as the expedition continued north of Elista into more mesic grasslands north of Volgograd along the Volga River to Saratov. *Euphorbia virgata* and *E. esula* were the most common euphorbs found in pastures and roadsides along the route followed west of Saratov.

There was a great amount of variation in leaf shape and size and plant height of the *Euphorbia* spp. specimens collected. This high degree of variability in morphological traits underscores the need for basic research to determine information on the genetic variability among leafy spurge genotypes in North American and Eurasia. Root/crown fragments were collected from 510 individual plants during the expedition. These plant propagules have been planted and are currently being maintained along with several North American and Eurasian leafy spurge biotypes in a nursery at the University of Nebraska in Lincoln, Nebraska. These plants will be used in research to determine the Eurasian origins of North American leafy spurge and will be made available to other scientists interested in working with leafy spurge.

Specific identity of pathogens and insects collected from populations of *Euphorbia* is currently being determined by Tony Caesar and Luca Fornasari, respectively. Tony found *Rhizoctonia solani* on 90% of diseased *Euphorbia* spp. specimens collected. Flea beetle adults (possibly belonging to *Aphthona* genus) were the most common insects found in sweep net surveys conducted by Luca Fornasari. Insects that formed galls in the shoot apices and fruits of euphorbs were collected from a number of sites. Larvae feeding on the roots of *Euphorbia* species were found at 3 locations sampled near Stavropol. Dr. Nartshuck, Entomologist, Zoological Institute at St. Petersburg, determined that the larvae were dipterans and belonged to the family, Sciáridae. A large number of Hyles euphorbia larvae were found feeding on leafy spurge foliage at a site near Balashov. Larvae and pupae of an insect (Pegomyid) that formed a gall in the stems of *E. virgata* were collected near Bozioglebsk. Pathogens collected by Tony Caesar are being evaluated and maintained under quarantine at facilities in Bozeman, MT and Frederick, MD. Insects collected by Luca Fornasari are being evaluated and maintained at the USDA-ARS, European Biological Control Laboratory in Montpellier, France. These scientists can be contacted directly for additional information on specimens collected.

Recommendations:

1. Exploration activities by USDA-ARS scientists in Russia and other members of the Commonwealth of Independent States (CIS) should be expanded and intensified. Russia and other CIS countries contain the native environments for many plants that are exotic noxious weeds in the United States. The CIS countries are resources of great potential for insects and pathogens that can be used in USDA-ARS biological control research pro-

grams and ultimately in federal and state government efforts to control noxious weeds on North American rangelands.

2. Exploration for potential leafy spurge biological control organisms should be expanded in Russia to include the region north of Stavropol, west to Voronesh, north to Orel, and east to the Volga River. Exploration in the region east of a line that runs north from Elista to Samara and into the Kazakhstan should be expanded. This region contains large expanses of xeric grasslands that are similar in appearance and climate to plains grasslands commonly found in western Nebraska, North and South Dakota and in eastern Montana, Wyoming, and Colorado.

3. Decrease cost and increase efficiency of biocontrol research by strengthening ties with Russian institutions and increasing participation of Russian scientists in conduct of research. The Russian scientists that I worked with in 1992 and 1993 were very capable and fundamentally competent in their respective disciplines. I believe that scientists with the Zoological Institute could conduct preliminary investigations, such as literature reviews and identification of locations for survey and collection of potential biological control organisms. Russian scientists could also perform field surveys and collections of biocontrol agents and conduct experiments (i.e., life history, behavior, and host range studies) to determine suitability of collected agents as candidates for use in biological control programs.

4. Increase opportunity and funding for Russian scientists that are collaborating in joint biocontrol research projects to visit the United States so they can become familiar with American scientists, research institutions, agricultural production systems, and rangeland weed problems.

Conclusion

My experience in Russia this summer was extremely rewarding and enriching, both professionally and personally. I was able to successfully accomplish my defined mission. I found my Russian colleagues to be hardworking, capable, and honest. See Table 2 (not available) for information on how to contact Russian scientists I worked with during the summer of 1993. They were always attentive to my requests and those of Tony Caesar and Luca Fornasari. I feel that the USDA-ARS has an excellent opportunity to cultivate and establish a long-term collaborative relationship that is based on cooperation and mutual respect with the Zoological Institute. Such a relationship will greatly benefit biological control efforts in the United States and bolster the Zoological Institute through the difficult times that currently exist in Russia. I look forward to participating in future joint research efforts with the Zoological Institute.

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Controlling leafy spurge in Minnesota with competitive species and combined management practices

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The present study was performed to evaluate the interfering ability of several perennial grasses with an integration of additional weed management tools to decrease leafy spurge populations. Two field experiments (Plot A and Plot BC) were started in June of 1991 at the Minnesota Valley National Wildlife Refuge in Shakopee, MN. Set-up treatments were disking of Plot A and an application of glyphosate (4 lb/acre) followed 7 days later by disking of Plot BC. Plot A was used as one experiment consisting of three repetitions of eight grass treatments. The treatments were (1) 'Bozoisky' Russian wildrye (*Psathyrostachys juncea*); (2) 'Hakari' mountain brome (*Bromus sitchensis*); (3) a mixture of 'Luna' pubescent wheatgrass (*Agropyron intermedium* var. *trichophorum*), 'Ephraim' crested wheatgrass (*Agropyron cristatum*), and 'Arriba' western wheatgrass (*Agropyron smithii*); (4) a mixture of 'Hakari' mountain brome, 'Tiki' smooth brome (*Bromus inermis*), and 'Matua' brome; (5) a mixture of short native prairie grasses, side-oats grama (*Bouteloua curtipendula*), buffalograss (*Buchloe dactyloides*), and little bluestem (*Schizachvrium scoparium*); (6) a mixture of little bluestem and side-oats grama; (7) little bluestem; and (8) control - no species seeded. Plot BC was used as one experiment consisting of three repetitions of eleven grass treatments. The treatments included: (1) a mixture of big bluestem (*Andropogon gerardii*) and little bluestem; (2) a mixture of switchgrass (*Panicum virgatum*) and side-oats grama; (3) a mixture of short native prairie grasses - side-oats grama, buffalograss, and little bluestem; (4) a mixture of tall native prairie grasses - big bluestem, switchgrass, and indiangrass (*Sorghastrum nutans*); (5) 'Ramsey' alfalfa (*Medicago sativa*); (6) a mixture of 'Luna' pubescent wheatgrass, 'Ephraim' crested wheatgrass, and 'Arriba' western wheatgrass; (7) a mixture of 'Hakari' mountain brome, 'Tiki' smooth brome, and 'Matua' brome; (8) 'Bozoisky' Russian wildrye; (9) 'Luna' pubescent wheatgrass; (10) buffalograss; (11) control - no species seeded. The grasses were seeded at 100 PLS lb/acre into 1 m by 18 m rows.

In the second year of the study, several management techniques were used to promote grass establishment and reduce leafy spurge. A burn was conducted on May 19, 1992 on Plot A and Plot BC. Herbicides were applied in 4.75 m wide blocks in Plot A and in 4.5 m wide blocks in Plot BC across all species treatments rows. The herbicide treatments

included: (1) picloram at 1 lb/acre applied on June 25, 1992; (2) picloram + 2,4-D at 0.25 + 1 lb/acre applied on June 25, 1992; (3) imazethapyr at 0.25 lb/acre applied on October 5, 1992; and (4) control - left untreated.

Herbicides were applied using a backpack sprayer. Both Plot A and Plot BC were mowed to a height of 6-8 inches in July 31, 1992 and fertilized with (10-20-16) on October 5, 1992. The percent cover of leafy spurge was monitored throughout each growing season. In Plot A, all three grass treatments containing little bluestem established well and were best at reducing leafy spurge cover.

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Effects of late season herbicide applications on the elongation and growth of leafy spurge vegetative buds

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The present study was performed to investigate the effects of applications in early October of picloram and glyphosate on the elongation of leafy spurge crown buds and on the recovery of treated plants in the field. Preliminary experiments were designed to collect information on crown bud behavior late in the season to serve as a baseline for comparisons to field observations. Field excavation experiments revealed that crown buds were still elongating late in October in Minnesota. Crowns were also harvested from the field in the fall and grown in pots in growth chambers in the laboratory to study bud elongation patterns. Although most buds began to elongate within 3 days, usually only a single bud per crown elongated beyond 20 mm. Crowns treated with picloram (1 lb/acre) and glyphosate (commercial rate) in the field on October 2, 1991 showed reduced bud elongation compared to untreated controls. Picloram was more effective than glyphosate in inhibiting bud elongation. In the field, the picloram treatment resulted in excellent control of leafy spurge into the second year. Although the glyphosate treatment reduced the percent cover of leafy spurge during the spring of 1992, by the summer of 1992, the percent cover was greater than the controls. Picloram and glyphosate solutions applied directly onto crown buds with a brush indicated that picloram could be absorbed directly by the crown buds at levels which could inhibit bud elongation. Apparently, only low levels of glyphosate could be absorbed by the crown buds since a stimulatory effect was observed. Persistence of picloram in soils combined with the ability to be absorbed directly by crown buds may explain why picloram applied late in the season is so effective at controlling leafy spurge.

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The gall midge, *Spurgia esulae* Gagne (Diptera: Cecidomyiidae): Notes on biology and impacts of hymenopteran predators

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Life history characteristics of *Spurgia esulae*, including gall counts, immature counts, adult emergence, fecundity, and occurrence of a hymenopteran predator are examined. Experiments in 1993 are in progress and data presented are initial evaluations and are not the final results.

Gall counts were taken on 0.6m^2 quadrats randomly located within study plots. Three plots, reflecting habitat differences, were sampled periodically. Counts of immature midges were made on harvested galls by removing each leaf individually and recording the number of immatures. Adult midge emergence was recorded in the lab from harvested galls placed in vials containing water and held in cylindrical emergence. Predator activity and counts were made during dissections of galls and during observations of midge emergence. Female midges, collected from emergence chambers, were dissected to determine fecundity.

Gall counts were grouped according to first and second generation with additional counts reported strictly by date. Mean, 1992, first generation gall counts were 0.4, 0.12, 6.5 per 0.6m^2 within plots I, II, and III respectively. Mean, second generation gall counts were 1.0, 0.51, 18.0 per 0.6m^2 within the three plots, respectively. After the second generation peak, gall counts declined over time. No galls were observed on or after September 28, 1992. Mean, 1993, first generation gall counts for plots I, II, III are .68, .30, and 14.4 per 0.6m^2 respectively.

Counts of immatures averaged 6.7 per gall for the first generation and 5.4 per gall for the second generation in 1992. Increases in immatures per gall for the first and second generation, 11.0 and 26.7 respectively, have occurred in 1993.

Adult emergence in 1992 for the first and second generations averaged 4.2 and 2.2 adults per gall. Adult emergence for 1993 averaged 8.8 adults per gall. Adult females, dissected to determine fecundity, averaged 107.8 and 104.3 eggs for the first and second generation in 1992. First generation females averaged 100.8 eggs in 1993. *Zatropis nigroaeneus* Ashmead (Hymenoptera: Pteromalidae) was present in 6.8% of the galls in the first generation and 57.8% of the galls in the second generation in 1992. The occurrence

of *Z. nigroaeneus* was greatly reduced in 1993, with 0% occurrence in the first generation and 12% in the second generation.

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Sheep grazing leafy spurge at the Greene Ranch

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Introduction

The epicenters of many leafy spurge infestations in North America have been abandoned crop- and haylands, and along streams and rivers. In these areas, the competing vegetation is usually dominated by *rhizomatous* grasses and forbs; many of which are introduced species themselves such as Kentucky bluegrass and smooth brome. Yet in the Northern Rocky Mountains leafy spurge is increasingly invading native range dominated by bunchgrasses such as bluebunch wheatgrass and Idaho fescue. Many bunchgrasses are not as competitive, or tolerant of grazing, as rhizomatous species.

Sheep producers have known for years that sheep will graze leafy spurge. We designed a grazing system to determine if timed, repeated grazing by sheep will reduce populations of leafy spurge without damaging associated, native perennial bunchgrasses. We are also assessing whether yearling sheep that were exposed to leafy spurge on rangeland as lambs graze leafy spurge more readily than yearlings that were not exposed to leafy spurge as lambs.

Materials and methods

This study began in May 1992 and will continue through May 1995. Our study site is 60 km west of Bozeman, MT on a 15-19 inch shallow range site. Within a non-uniform leafy spurge infestation, we enclosed an approximately 3 ha area with high tensile power fence. The 3 ha were divided into 3 blocks with polywire fence, each block was first divided in half lengthwise to compare the grazing behavior of “experienced” and “naïve” yearling sheep, and then further subdivided crosswise into three small paddocks to assess the affect of repeated grazing on leafy spurge and associated bunchgrasses. Sheep grazed each small pasture three times between early June and late September, thus all plants had an equal probability of being grazed at least three times during that four-month period.

In May 1992, we collected baseline information inside and outside exclosures in each small pasture. Prior to grazing in 1992, we set up permanent transects and determined the density of stems and seedlings of leafy spurge and of Idaho fescue, and noted the presence/absence of other plant species. Cover of bare ground and litter was estimated along each transect, and we took soil cores inside each pasture to determine the initial seedbank of leafy spurge before grazing. All of these variables, except the seedbank, are remeasured each year. The seedbanks will be resampled in May 1995.

In 1992, we randomly assigned five yearling Targhee ewes to each of the 6 treatment block combinations (3 blocks, 2 treatments – naive and experienced). The experienced yearlings had been raised as lambs on a summer pasture heavily infested with leafy spurge, whereas the naive yearlings had never been exposed to leafy spurge until they were placed on the pastures in this study. In 1992, we observed the grazing behavior of these yearlings in early June, early July, early August, and mid September on the small pastures. For the first two periods, leafy spurge was abundant, but for the latter two periods, the yearlings were grazing leafy spurge and grass regrowth on pastures that they had previously grazed.

Each block (naive and experienced) was observed by one individual. On day 1 of each period, sheep were turned into a new pasture. That evening and the following morning, their activities were observed for three rounds. A round consisted of 30 minutes of observation [15 minutes (3 minutes/sheep for 5 sheep) with naive yearlings, 15 minutes with experienced yearlings]. We noted whether they were grazing, ruminating, or traveling. While grazing, bites of leafy spurge, grass, or other forbs were recorded. These observations were repeated on Days 3, 5, and 7 during each period, except in September when the sheep were in the small pastures for only three days.

Leafy spurge and Idaho fescue plants were harvested in mid-June, late July, and mid September, separated by plant part (leaves, stems, flowers), and stage (mature growth, regrowth), dried, ground, and analyzed for crude protein, fiber content (ADF and NDF), and in vitro dry matter digestibility.

Results and discussion

Plant response

We only have measures from one complete cycle, May 1992 through May 1993, thus it is too early to conclude what effects one year of repeated grazing may have on leafy spurge and associated perennial bunchgrasses. Visually, in May 1993 grazed areas did not appear to have any more or less leafy spurge than ungrazed areas.

Behavior

In early June, experienced yearlings spent more time grazing leafy spurge than naive yearlings, although neither group grazed it much (5% for experienced versus 0.6% for naive). In early July, the time that the yearlings grazed leafy spurge increased considerably for both groups (45% for experienced versus 31% for naive), although the experi-

enced yearlings still grazed it more than the naive yearlings. Walker *et al*. (1992) also found that lambs that had been exposed to leafy spurge consumed more of it than lambs that had not been exposed to the plant. The 45% of their grazing time spent on leafy spurge supports Landgraf *et al*.'s (1984) findings that sheep will consume leafy spurge up to 50% of their diet. In early August, both groups of sheep were grazing leafy spurge equally (40% experienced, 42% naive). There were insignificant differences between the minimal amount of time spent grazing leafy spurge in September (1.4% experienced, 3.4% naive).

Is a sheep a sheep, or might some sheep prefer leafy spurge more than one of their conspecifics? If there are individual differences, a sheep producer could cull sheep that tend to avoid leafy spurge, a trait they may pass onto their young, but keep sheep and their young that tend to prefer leafy spurge. In the early June period, one or two sheep had a much higher preference for leafy spurge compared with other members of their group. In the early July period, there were no strong differences within a group, although time spent grazing leafy spurge by the experienced yearlings was usually higher and much more variable, within a group than time spent grazing by naive yearlings.

During the early June and July observation periods, bite counts on the grasses ranged between 24 and 27 bites per minute. In early June, the naive yearlings averaged around 2 bites per minute on leafy spurge, the experienced group averaged around 8 bites per minute. In early July, these groups averaged 18 and 20 bites of leafy spurge per minute, respectively. The low biting rates in early June, especially for the naive group, could reflect that this was their first exposure to this “new” food, and their inability to efficiently handle it.

In mid-June, crude protein (CP) and digestibility (IVDMD) of leafy spurge leaves and flowers were higher than those for Idaho fescue (leaves 18% CP vs. 6% CP, flowers 17% CP vs. 4% CP). In late July, crude protein and digestibility of mature and regrowth of leafy spurge were higher than Idaho fescue (mature 9% CP vs. 5% CP, regrowth 22% CP vs. 12% CP). These trends were maintained in the September collection. Thus, throughout the grazing season, leafy spurge provided a higher quality diet for sheep than Idaho fescue.

Our results indicate that if an individual has a large scale infestation of leafy spurge, that he or she would be at a slight advantage if they bought or leased sheep that had previous experience with leafy spurge. If possible, it would also be to a producers’ advantage to identify individual sheep that avoid leafy spurge. By avoiding leafy spurge, these sheep will selectively graze other, more desirable plant species, thus placing these plants at a competitive disadvantage to leafy spurge.

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New quarantine resources for APHIS weed biological control implementation programs

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The Mission Biological Control Laboratory is part of the biological control effort of the U.S. Dept. of Agriculture, Animal & Plant Health Inspection Service, Plant Protection and Quarantine. Included with the Mission laboratory are the Bozeman Biocontrol Facility and the Niles Biological Control Laboratory. These three units comprise the working laboratories of the Biological Control Operations group. The Mission laboratory facilities have included a small quarantine for a number of years. Due to the increased workload of the weed biological control projects and the addition of new projects, the quarantine facility has been taxed beyond its design capabilities. The new quarantine offers adequate holding, rearing and laboratory space which should be sufficient for the agency's needs. Functions and program enhancements of the new APHIS quarantine include:

- Improved quarantine facility for weed and insect biological control projects, including quarantine greenhouse space.
- Provide quarantine service to cooperators involved with agency biological control projects.
- Provide quarantine rearing of pest insects for production of natural enemies.
- Allow for additional space for cooperators to perform quarantine studies and screening work.
- To provide quarantine services where no primary quarantine facility is available (Sweetpotato Whitefly).

The new quarantine resources provide enlarged and previously unavailable facilities at Mission, TX including quarantine greenhouse space comprising of 5 separate houses for a total of 630 sq. ft. of space. Three laboratory areas comprising over 860 sq. miles of space provide ample space for routine laboratory work and visiting scientists. Specific areas are dedicated to shipment receiving, identification functions and cold storage. The focal point of the facility space constitutes 12 walk-in environmental rearing chambers with over 750 sq. ft. of total space. Special features of the facility include a high capacity effluent treatment system with drowning baffle tank and automatic chlorine treatment tank. Emergency power generation provides a back up in case of electrical power failure.

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USDA-APHIS and Colorado Department of Agriculture, an active partnership in biological control of leafy spurge

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A partnership has been developed between the USDA, APHIS and the Colorado Department of Agriculture to control leafy spurge in Colorado. Since 1988, efforts have been directed at importing and establishing a complex of exotic bioagents that attack all growing stages of leafy spurge. All beneficial organisms have been supplied by APHIS while CDA has been responsible for locating release sites. A total of 39 field insectary sites have been selected in 15 counties of Colorado in which four species of leafy spurge predators have been released. Since the start of the project, 18,142 specimens have been relocated to these insectary sites. The current species that are being utilized in this program include: *Aphthona nigriscutis*, *A. cyparissiae* and *A. flava*, which are root-mining flea beetles and *Oberea erythrocephala*, a root and stem boring beetle. Additional species to be supplied by APHIS and released in future years include: *Aphthona czwalinae*, a rootfeeding flea beetle, and *Spurgia esulae*, a shoot tip gall midge, along with other species as they become available. As these species become established and begin to thrive in the Colorado field insectary sites, collection and redistribution to other leafy spurge infestations will be accomplished by the Colorado Department of Agriculture.

An establishment survey conducted during the 1992 growing season by the Colorado Department of Agriculture using procedures described by APHIS revealed successful recoveries in 15 of the 25 field insectary sites. Several survey dates were selected in an effort to determine peak predator emergence, which will make timing for collection and redistribution more accurate.

In addition to the import and release program, soil samples have been collected from all of the field insectary sites to determine if certain soil types favor individual species. At this time, all that has been determined is that *Aphthona nigriscutis* and *Aphthona cyparissiae* have been recovered at releases that exhibit wide range of soil types.

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Genetic diversity of the leafy spurge chloroplast genome

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DNA markers from the chloroplast genome of leafy spurge have been used to assess the amount of genetic variation within leafy spurge populations in North America. DNA samples from 124 individuals in five North American populations were analyzed. Mung bean chloroplast DNA probes were used to detect restriction fragment length polymorphisms from EcoRI, EcoRV, and HindIII digests of the DNA. Genotypes were determined by scoring individuals for the presence or absence of each restriction fragment. Twenty-two chloroplast genotypes were identified among the samples. Three of these genotypes included 68% of the individuals and could be used to distinguish four out of the five populations. One population did not have a predominant genotype, but was a mix of several types. Band difference and principle component analysis were used to describe the degree of difference between each genotype. This study indicates that large sample sizes are required to adequately describe the genetic makeup of leafy spurge populations, and that it is possible to distinguish genetically different populations. Correct assessment of the amount of genetic variability in a population of leafy spurge may be an important factor in the development of an effective biocontrol strategy.

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Angora goat grazing as a biological control for leafy spurge: A three-year summary

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Leafy spurge (*Euphorbia esula*) continues to be a serious problem in North Dakota, infesting over 1.5 million acres of land, predominately rangeland. Chemicals continue to be the primary method for control and attempts for eradication. Biological control methods have received much interest in recent years. Grazing with sheep has been a control method since the 1930's, but utilized sparingly (Helgeson and Thompson 1939, Helgeson and Longwell, 1942). A new animal receiving much attention today is the angora goat. Studies conducted on the use of goats as a leafy spurge control method have been limited, but have shown consistent results (Hanson and Kirby 1993, Sedivec and Maine 1993).

Angora goats were introduced to the 560 acre Hawk's Nest Butte area in southeastern Wells County as a biological control method for leafy spurge. Hawk's Nest Butte has been grazed by cattle in the past, but has provided little available forage due to a heavy infestation of leafy spurge.

Project objectives were to determine 1) if angora goats will significantly reduce the stem and herbage density of leafy spurge, 2) if angora goat grazing will stimulate the growth of graminoids through reducing the leafy spurge canopy, and 3) recommended stocking rate of angora goats on leafy spurge infested pastures.

Study area and methods

The study was conducted on the 560 acre Hawk's Nest Butte located approximately 10 miles southwest of Carrington, North Dakota. The butte is situated in Sec. 26, T. 145 N., R. 68 W., 5th Principal Meridian. Hawk's Nest Butte is classified as a morainic coteau of hills situated in front of the Missouri Plateau. Vegetation on the Hawk's Nest Butte was typical of the mixed grass prairie of the Missouri Coteau prior to the introduction of leafy spurge.

Leafy spurge stem counts were conducted prior to the introduction of angora goats on June 1, 1990 to achieve initial stand counts. Stems were counted using 1 ft. × 1 ft. frames on two line transects in the spring of the year prior to grazing in 1990, 1991, 1992, and 1993. A control site was developed in 1991, which has been ungrazed throughout the duration of the study. Stem counts were collected on about June 1, 1991, 1992 and 1993 on

the ungrazed control site. The paired plot technique was used comparing grazed and non-grazed sites using 2.5 ft. × 5 ft. cages to determine forage production potential and degree of use for leafy spurge, graminoids, shrubs, and other forbs.

Stocking rate was 567 animal unit months (AUMs) from June 1 to Sept. 20 (900 angora goats), or 1.01 AUMs/acre in 1990. The stocking rate was 505 AUMs from May 20 to Oct. 5 (660 angora goats), or 0.90 AUMs/acre and 637 AUMs from May 15 to Oct. 1 (828 angora goats), or 1.13 AUMs/acre in 1991 and 1992, respectively.

Leafy spurge stem counts were tested for significant ($P<0.05$) main effects using multi-response permutation procedure (MRPP) (Biondini *et al.* 1988).

Results and discussion

Initial leafy spurge stem counts for June 1, 1990 on the grazed transects was 38.7 stems per square foot. After one year of angora goat grazing, stem counts were collected on May 15, 1991. Leafy spurge stem density was reduced to 36.2 stems per square foot or a reduction of 6.3 percent ($P>0.05$) (Table 1).

Leafy spurge stem counts were collected on May 15, 1992, that period following two years of grazing. Stem densities were 30.4 stems per square foot in 1992, a significant reduction of 21.3 percent ($P<0.05$) (Table 1). May 20, 1993, leafy spurge stem density counts were collected to achieve three years of grazing response. Leafy spurge stem density counts were 29.6 stems per ft^2, a significant reduction from 1990 and 1991 (Table 1).

Table 1. Stem density counts of leafy spurge, forbs and shrubs in 1990 through 1993 at the Hawk's Nest Butte, near Carrington, ND.

Date Collected	Leafy Spurge[1]	Forbs	Shrubs
	per sq. foot		
May 31, 1990	38.7[a]	3.4	1.0[a]
May 15, 1991	36.2[a]	1.6	0.3[a]
May 15, 1992	30.4[b]	2.5	0.5[a]
May 20, 1993	29.6[b]	1.7	0.6[a]
Percent reduction from 1990 to 1993	23.5	50.0	40.0

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

Data collected from the paired plots were collected in mid-August, 1990 and 1991, and late August, 1992, that time period when forage production tended to peak in North Dakota (Whitman *et al.* 1951). Forage production on the nongrazed plots of the paired plots had a leafy spurge-to-grass density ratio of 56.5:43.5% in 1990 (Table 2).

Herbage production using the paired plots technique was collected for all years during peak production. After one year of grazing, the ratio of leafy spurge-to-grass density was 37.1:62.9%, an increase in grass density of 44.6% and reduction of leafy spurge density of 37.1%. Herbage production following two years of grazing had a ratio of leafy spurge-to-grass density of 31.6:68.4%, an overall increase in grass density of 57.2% and reduction of leafy spurge density of 44.1%.

Table 2. Percentage change in canopy cover from 1990 to 1992 at the Hawk's Nest Butte near Carrington, ND.

	Percent of Canopy Cover by Weight			
Year	Percent[1] Leafy Spurge	Percent Change	Percent[1] Grass	Percent Change
1990	56.5[a]		43.5[a]	
1991	37.1[ab]	- 34.3	62.9[ab]	+ 44.6
1992	31.6[b]	- 14.8	68.4[b]	+ 8.7
Percent total change		- 44.1		+ 57.2

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

Degree of use on the leafy spurge and grass species was determined in 1991 and 1992. Over 84 and 65% of the leafy spurge was utilized in 1991 and 1992, respectively, with the remaining percentage almost completely standing stems (Table 3). Grass utilization was plus 16.6 and 11.1% in 1991 and 1992, respectively, thus an increase in grass production occurred on the grazed plots as comparing with the nongrazed plots. As the leafy spurge canopy was removed through grazing, grasses increased in production faster than the goats could utilize it.

Table 3. Herbage production and degree of use on leafy spurge and graminoid species by angora goats in 1990, 1991 and 1992.

Treatment	Herbage Production lbs/acre					
	Leafy Spurge			Graminoid Species		
	1990	1991	1992	1990	1991	1992
Ungrazed	960	1277	501	739	2165	1084
Grazed		201	176		2526	1206
Degree of use		- 84	65		+ 17	+ 11

Summary

Angora goats did an excellent job in controlling the spread of leafy spurge. Leafy spurge stem counts were significantly reduced after two years of grazing while grass production increased due to the removal of the leafy spurge canopy. As the canopy of leafy spurge is opened, forage is improved for cattle grazing during summer and fall months.

Recommended angora goat stocking rates as it correlates to leafy spurge control was difficult to measure based on our data. Stocking rates ranged from 505 to 637 AUMs, or 5.3 to 6.7 goats/acre/month. These rates achieved our goal in reducing leafy spurge production and density without over-utilizing the graminoid species. Recommendations would be at least 6.7 goats/acre/month or 1.5 goats per acre for 4.5 months due to a more efficient leafy spurge utilization earlier in the grazing season while acheiving little utilization of the graminoid species in North Dakota. The lighter stocking rates allowed a

zation of the graminoid species in North Dakota. The lighter stocking rates allowed a larger percentage of leafy spurge plants to flower and set seed.

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Membrane perception of 2,4-D as the initial interaction leading to phytotoxicity in leafy spurge

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The shoots of leafy spurge are controlled satisfactorily by auxinic herbicides such as 2,4-D, but crown and root bud regrowth is not readily controlled. Leaf absorption and translocation of 2,4-D to the roots occur but the herbicide is lost very rapidly from the root tissues. To improve crown and root bud control, 2,4-D absorption by leafy spurge shoots and translocation to roots must be increased concomitantly in leafy spurge. The mechanism of influx and efflux of 2,4-D from leafy spurge cells is unknown at present. The involvement of either passive and/or active mechanisms in the influx and efflux of 2,4-D may determine if the loss of translocated 2,4-D from root tissues can be regulated or modulated. The objectives of our research were to increase foliar absorption of 2,4-D and translocation to roots of leafy spurge and to investigate the mechanism of loss or efflux of the herbicide from leafy spurge cells.

Three ^{14}C-labeled and unlabeled, variably hindered esters of 2,4-D (methyl, *sec*-butyl, and *t*-butyl) were synthesized for our studies. Unlabeled 2,4-D and its three esters were sprayed separately on 6-week-old leafy spurge plants at 0.04kg\ha acid equivalent per compound. The 2,4-D acid was most phytotoxic with the methyl and *sec*-butyl esters slightly less phytotoxic and *t*-butyl ester showing very little herbicidal activity.

Excised leaf sections (0.5 cm) were used to determine uptake, metabolism and accumulation of 2,4-D in leafy spurge. Maximum uptake of all compounds were complete within 4 hours with loss from tissues occurring with all compounds except *t*-butyl ester over the next 20-hour period. Only 6% of applied 2,4-D was absorbed within 4 hours whereas about 25% of the three esters were absorbed over the same period. Total uptake of the compounds in leaf tissues after 4 hours expressed as nmol\g f.w. (in parenthesis) was: 2,4-D (64), three esters (ave. of 162). Rapid hydrolysis of the methyl and *sec*-butyl esters to 2,4-D occurred in leaf tissues with only limited hydrolysis of the *t*-butyl ester. At 4 hours after treatment, the accumulation of 2,4-D in nmol\g f.w. of leaf tissue treated with 2,4-D and its esters were: (a) 2,4-D (48); (b) methyl ester (157); (c) *sec*-butyl ester (133); and (d) *t*-butyl ester (29). Very little metabolism of 2,4-D other than the hydrolysis of its esters occurred over a 24-hour period when severe herbicidal response to the herbicide was observed in whole plants.

The results described above indicated that 2,4-D acid is the herbicidal molecule. In whole plants, the least absorbed form (2,4-D) causing very little accumulation of the active molecule in leaf tissues is most phytotoxic to leafy spurge. The effects on the plasma membrane potential in root cortex cells from the zone of elongation indicated that leafy spurge cells appear to recognize or perceive the acid form as the biologically active molecule and not the esters of 2,4-D. An interaction between the plasmalemma and the 2,4-D acid during influx of the herbicide may be the physiologically significant process in the herbicidal action of 2,4-D.

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Potential benefits of establishing a reference map of the chloroplast genome of leafy spurge

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We have pursued a Southern blotting strategy, utilizing mung bean clones in order to obtain a reference (or physical) map of the leafy spurge chloroplast genome. A reference map is a figure that represents a particular genome and the location of restriction sites within that genome. Once completed a map of this type can be used to develop more specific screening tests (i.e. Southern blotting or PCR) of leafy spurge populations. A completed reference map can also aid in determining the nature of distinctive polymorphisms, as they could be the result of site mutations, addition/deletion events, or inversions. At this point we have mapped a portion of the leafy spurge chloroplast genome. Several key polymorphisms appear to be located within this newly mapped region, therefore, we are currently attempting to determine their nature.

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The effects of organosilicone surfactants on herbicide absorption by leafy spurge (*Euphorbia esula* L.)

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Abstract

Leafy spurge (*Euphorbia esula* L.) is a serious problem weed on rangeland in the Northern Great Plains. In field studies, imazethapyr has been determined to provide good control of leafy spurge similar to 2,4-D/picloram treatments. In laboratory experiments imazethapyr uptake by leafy spurge foliage was limited to only 18% of the actual amount of herbicide applied. Previous reports of laboratory experiments also indicate low picloram and 2,4-D absorption by leafy spurge. This study was conducted to (1) identify adjuvants that improve uptake of imazethapyr, 2,4-D amine, and picloram by leafy spurge; (2) determine imazethapyr and adjuvant performance when applied to plants grown in the field; (3) evaluate uptake of imazethapyr as effected by abaxial or adaxial application.

Plants were vegetatively propagated from a single source plant and were grown in the greenhouse for 3 months. Top growth was removed and root systems were chilled for 14 days before plants were transplanted into cone-tainers containing a fine, washed-silica sand. Plants were maintained in a controlled environment chamber with 16-hour photoperiod, 50% relative humidity, and PPFD of 650 $\mu E\ m^{-2}\ sec^{-1}$. Plants were watered daily and fertilized every 14 days.

Four experiments were conducted to address the objectives of the study. Adjuvants evaluated in the experiments were crop oil concentrate (COC), methylated sunflower oil (Sun-it II), non-ionic surfactant (X-77), organosilicones (Sylgard 309, Silwet L-77 and Silwet 408), ammonium sulfate, 28% urea ammonium nitrate (UAN), and 3:1 mixtures of three acetylinic diol ethoxylates (ADE's) with Silwet L-77. The ADE's have been shown to have a synergistic effect on reducing surface tension of Silwet L-77. Plants were at the full bloom growth stage when treatments were applied. Surfactants at 0.25% (v/v) were combined with a commercial formulation of imazethapyr, 2,4-D, or picloram to be applied at a rate of 0.07 kg ai ha^{-1}, 1.12 kg ai ha^{-1}, or 1.12 kg ai ha^{-1}, respectively, and delivered in a total volume of 187 l ha^{-1}. ^{14}C-radiolabelled herbicide was added to the treatment solutions so that there was a total of 50,000 DPM applied to each leaf. Five

leaves starting 5-10 cm from top of the plant were treated with 10, 0.5 µl drops per leaf in experiment 1 or 1, 1.0 µl drop per leaf in experiments 2, 3, and 4. In experiment 1, surfactants combined with or without UAN were evaluated over a 2 and 8 day time course. In experiment 2, the influence of COC and the organosilicones, with and without UAN, on field-grown plants was determined. Field-grown plants were grown outside and transferred to the growth chamber 24 hours before treatment. Experiment 3 was conducted to evaluate differences in imazethapyr absorption associated with abaxial or adaxial application. Experiment 4 was utilized to determine the effects of organosilicones and organosilicone/ADE combinations on uptake of imazethapyr, 2,4-D, and picloram. At harvest, leaves were vortexed for 30 seconds in 5 ml aqueous 10% methanol, 0.25% Tween 20. Radioactivity of the solution was determined by liquid scintillation spectrometry. Percent uptake was determined by difference of amount of radioactivity applied to amount recovered from the leaf surface.

Uptake of imazethapyr was greatest (>90%) when the herbicide was applied with UAN combined with either COC or Sun-it II as compared to surfactants applied without the addition of UAN. Imazethapyr uptake by leaves from field-grown plants was greatest (>85%) when the herbicide was applied with the combination of COC and UAN. Imazethapyr uptake was limited to less than 35% when applied with the organosilicone surfactants with or without UAN. Abaxial application provided greater uptake of imazethapyr than adaxial application for both organosilicone and COC surfactants, but only in the presence of UAN (10% avg. difference). Uptake of 2,4-D amine was greatest (>76%) when the surfactants used were Silwet 408 or the ADE 40 (40 percent ethylene oxide) / Silwet L-77 mixture. Picloram absorption was similar for all the organosilicones and ADE mixtures (13-19%). COC was ineffective when used with picloram (only 3% uptake) Based on evidence from this research, crop oils combined with UAN were superior to the organosilicone surfactants in improving imazethapyr uptake by leafy spurge. Picloram absorption by leafy spurge was not improved above levels previously reported. Uptake of 2,4-D was increased in the presence of organosilicones and some ADE mixtures.

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GIS/GPS applications in USDA-APHIS redistribution of leafy spurge biocontrol agents

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Geographic Information Systems (GIS) are computer-based systems used to store, manipulate and analyze geographic information. Global Positioning Systems (GPS) are hand-held units that utilize three dimensional position fixes by satellites to determine locations in map coordinates. USDA-APHIS-PPQ is making use of this technology to collect, store, analyze and display weed and weed biocontrol agent information in its programs on rangeland weeds.

Field personnel are currently collecting field insectary site location data in sixteen states with GPS units for use with the GIS. A map showing USDA-APHIS redistribution of three species of leafy spurge flea beetles in Colorado was displayed. Maps depicting the spread of leafy spurge over a sixty-year period in five Pacific Northwest states and a dot-density map of leafy spurge acreage in Montana and North Dakota were displayed.

GPS units are used to map sites where natural enemies of rangeland weeds have been released. The map features depicted in a leafy spurge field insectary site map were collected with GPS methods. GIS programs enable the user to determine the area of both the leafy spurge infestation and of the flea beetle impact. Percent cover of leafy spurge was reduced from over 85% to 0% at the initial point of release of the leafy spurge flea beetle *Aphthona nigriscutis.*

Personnel at the Bozeman Biocontrol Facility have developed a phenological model to predict the eclosion and peak collecting period of *Aphthona* flea beetles and to plan adult beetle sampling and collection efforts. Daily temperature data from fifteen states are referenced for the development of this model. The spatial analysis features of the GIS were used to select climatological stations within a fifteen mile radius of leafy spurge field insectary sites from thousands of possible stations. An example of this analysis for Montana and northern Wyoming leafy spurge field insectary sites was displayed. USDA-APHIS plans to utilize GIS decision making capabilities as more climatological and ecological data pertinent to biocontrol of weeds become available.

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Leafy spurge control in North Dakota - 1993

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Several experiments were established to evaluate various formulations of picloram for leafy spurge control. The compounds evaluated were XRM-5255 (picloram acid water-soluble powder), XRM-5173 (potassium salt water-soluble powder), Tordon 101 [picloram triisopropanol-amine plus 2,4-D triisopropanol-amine (1:4)], and a picloram isooctyl ester plus triclopyr butoxyethyl ester (1:2) (Access). The various formulations were compared to picloram K-salt (Tordon 22K). Leafy spurge control with XRM-5255 and XRM-5173 alone was less than picloram (Tordon 22K) 1 and 3 months after treatment (MAT) but similar 12 MAT. XRM-5255 and XRM-5173 applied with a methylated seed oil (Scoil) provided similar control to picloram (Tordon 22K). Leafy spurge control with picloram TIPA and picloram ester was similar to picloram (Tordon 22K).

An experiment to evaluate grass injury and leafy spurge control with glyphosate applied in late June was established at two locations. Glyphosate plus 2,4-D (Landmaster BW) was applied alone or with picloram in late June provided 74% or more control 12 MAT compared to 39% for picloram plus 2,4-D at 4 plus 16 oz/A. There was no grass injury 12 MAT with any treatment.

A regional experiment was established in 1992 to compare various formulations of 2,4-D to picloram for leafy spurge control. Leafy spurge control evaluated visually 12 MAT averaged 13% regardless of 2,4-D formulation. There was a slight increase in stem density when the 2,4-D amine formulation was applied compared to a slight decrease with the esters 12 MAT. The picloram treatment averaged 65% visible control and 72% decline in stem density.

A study was established in 1990 to evaluate the competitiveness of several grass species with leafy spurge. The most competitive species evaluated were ‘Rebound’ smooth brome, ‘Rodan’ western wheatgrass, and ‘Bozoisky’ Russian wildrye and which averaged 63% leafy spurge reduction in 1993. One species ‘Killdeer’ sideoats grama never established. The total grass production increased each year and averaged 1550 lb/A with the three most competitive species.

An experiment was established to evaluate leafy spurge control with goat grazing alone or combined with herbicides. Stem counts and changes in root carbohydrate and protein were used to evaluate control. There were two locations with either season-long-grazing or rotational grazing management. Overall there was a reduction in stem density when chemical treatment was combined with grazing. Season-long grazing alone reduced leafy spurge stand 12 MAT while rotational grazing resulted in a stem density increase.