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## The gall midge complexes (Diptera: cecidomyiidae) on spurges (*Euphorbia* spp.) in Europe

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### Abstract:

In Europe, one can find two generally similar galls on many species of spurge, a bud gall made up of many tightly folded leaves and an inflorescence gall. The bud galls act as an energy sink and always result in the death of the affected plant apex. The inflorescence galls prevent the development of seeds. Recent museum and field studies show that although both gall types occur on most spurges, each kind of gall on any particular spurge species is formed by a different gall midge.

The flower galls are made by *Dasineura capsulae* Kieffer and relatives. This complex of species, evidently restricted to spurges and kept for the present in *Dasineura*, is distinct from other species in that genus in having a bilaterally flattened ovipositor. A revision of these gall midges is in progress.

The bud galls, however, are caused by at least two and possibly three different genera of gall midges. They are the subject of a paper that I have submitted to the Annals of the Entomological Society of America. One genus, which will be described as the new genus *Spurgia*, is known from two species. *Spurgia esulae*, a new species, forms the bud gall on *Euphorbia esula* L. in Italy. The other, *S. capitigena* (Bremi) forms the bud gall on *Euphorbia cyparissias* L. in France, Switzerland, Italy, and Yugoslavia. The latter was once placed in the genus *Bayeria*, but only on the basis of superficial resemblances to that genus. *Spurgia* and *Bayeria* are not closely related. The bud gall on *Euphorbia palustris* L. in Germany and Sweden is formed by a true *Dasineura*, *D. schulzei* Rübssaamen, and that on *Euphorbia characias* L. in Sicily by *Janetiella euphorbiae* De Stefani Perez. The type specimens of this last species are lost, so specimens must

be reared again from typical galls before we can be certain that they belong in *Janetiella*. Larvae of at least the two *Spurgia* species may leave the gall to pupate or form a cocoon in the bud and remain there. Many multivoltine gall midges are only partially so: some specimens of the spring generation pupate directly, while some drop to the ground, where they remain as larvae until at least the following spring (Gagné, 1989). After the larvae of *Spurgia* leave the galls or build cocoons in the galls, the galls begin to rot.

Another gall midge, *Macrolabis lutea* Rübsaamen, can be found as an inquiline or secondary inhabitant in both bud and inflorescence galls. This species is known from The Netherlands, France, Germany, and Hungary, and evidently attacks the galls after the gallmaker larvae have induced gall development. The white larvae of the *Macrolabis* can be found with dead or dying yellow larvae of *Spurgia*. Galls with *Macrolabis* gall midges apparently cause the gall to last longer than normal.

This research shows that many species of gall midges from similar galls on spurge remain to be tested for possible introduction. Because species not yet studied are likely to be new, their identity has to be confirmed and each has to be tested separately for host discrimination.

It is desirable to have a species name for a new introduction, but there may be times when a species might have been tested and should be introduced, but has not yet been described. Rather than wait for a name, which may be delayed by preparation of the description and the publication process, I recommend, assuming that voucher specimens are checked and retained, that introduction not be delayed for lack of a species name and that the species temporarily be designated by the plant species and locality the specimens were from, e.g., *Dasineura* sp. near *capsulae* from *Euphorbia esula* from Pisa. This has been done for a gall midge now in quarantine.

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# Leafy spurge cell culture as the base of an artificial diet for *Aphthona flava*

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## Introduction

*Aphthona flava* is currently one of the insects being most actively studied as a biological control agent for leafy spurge. It has been released in field sites in five Canadian providences as well as in the United States (Harris *et al.*, 1985). The adults feed on the leaves of leafy spurge, and the larvae mine the primary and secondary roots and the root hairs, thereby disrupting the vascular tissues and depleting the carbohydrate reserves of the plant (Leafy Spurge News, 1989). APHIS and the USDA-ARS are cooperating on a project to develop an artificial diet for the mass rearing of the insect. This approach is an alternative to expensive and time-consuming collection of the insects from their native habitat in Europe and their subsequent processing (to remove parasites and diseases) in quarantine facilities. Successful mass rearing in the laboratory could provide the numbers of insects required for large releases in spurge-infested rangelands.

## Background research

Initial experiments on the development of an artificial diet were done last year at the Western Regional Research Center in Albany, California and reported on by Dr. Gary Manners at the Leafy Spurge Symposium in Rapid City last July. Briefly, eleven established diets for chrysomelid beetles did not support growth of *Aphthona flava* larvae. Chemical extracts of leafy spurge roots or leafy spurge root powders incorporated into an agar base were also completely ineffective in supporting growth or maturation of the larvae (Tables 1 and 2).

**Table 1. Effect of extracts of leafy spurge on survival of larvae of *Aphthona flava*\*.**

EXTRACT	YIELD (% dry wt.)	SURVIVAL (Mean no. of days)
HEXANE	10.9	1.6 <sup>a</sup>
ACETONE	1.4	1.4 <sup>a</sup>
METHANOL	4.4	1.8 <sup>a</sup>
WATER	0.6	0.8 <sup>a</sup>
HEXANE + ACETONE		0.6 <sup>a</sup>
METHANOL + WATER		0.6 <sup>a</sup>
CONTROL DIET		3.8 <sup>b</sup>

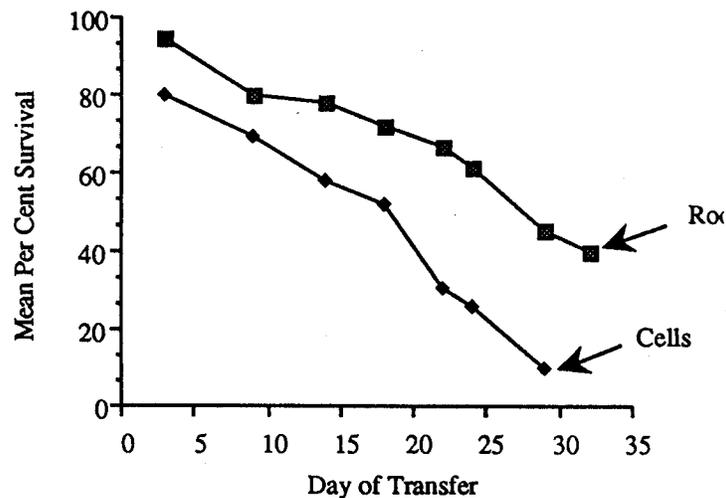
\*2nd Instar Larvae, 10 replicates/treatment of 2 larvae/container.

**Table 2. Effect of leafy spurge root powders in agar\* on survival of larvae of *Aphthona flava*.**

CONC. (mg root powder/ml)	SURVIVAL (Mean no. of days)
100	4.2 <sup>a</sup>
50	3.8 <sup>a</sup>
10	3.0 <sup>a</sup>
5	2.2 <sup>b</sup>

\*Freeze-dried secondary roots in 1.7% agar.

Significant survival of first instar larvae was observed last year on freeze-dried leafy spurge roots over the course of one month (Fig. 1). Preliminary experiments with vacuum-filtered fresh suspension culture cells of leafy spurge tissue showed similar duration, although a lower percentage of larval survival when fed unamended cultures. This was the point in time at which molting of the head capsule and entrance into the second instar stage was expected, but never observed. The larvae did eat the suspension culture cells and grew to be 3-5 times the length of the neonates.



**Figure 1. Survival of larval transfers of *Aphthona flava* on freeze-dried leafy spurge roots vs. cell suspension cultures.**

## Current research

Preliminary results this season show that larvae will also feed on freeze-dried (lyophilized) cell suspension cultures, presented on 0.8% agar containing 0.5% chloramphenicol. The protocol we have devised provides sterile freeze-dried cells, which are subsequently spread on 9 cm chloramphenicol agar plates. The agar provides enough moisture for the cells to rehydrate themselves sufficiently, without becoming so wet that the neonates drown. This limited moisture environment reduces the possibility of fungal contamination. The eggs are sterilized in 1% bleach, followed by 5% sodium thiosulfate and sterile water. Hatched larvae (50-72%) are transferred to fresh cells every three to five days.

Good survival rates have been obtained using the protocol described. Larvae can be maintained on a nutrient-rich, sterile environment in which they burrow, feed, and grow. Larvae survive 20-30 days on unamended freeze-dried leafy spurge suspension culture cells. The latest experiments have been aimed at defining a potential unknown factor(s) responsible for inducing larval molt. Amendment experiments have been set up using commercially available insect molting hormone (20-hydroxyecdysone) and chemical extractives from fresh leafy spurge roots. These amendments are added back to the freeze-dried cells at known concentrations and the effect on larval feeding and survival is observed.

Thus far, survival and significant growth has been observed on 20-hydroxyecdysone, presented at up to 10 ppm in freeze-dried cells. The oldest larvae have survived for 30 days on this treatment, but no molting has yet been observed.

## Cell-cultured based diet

The current focus of the project is to develop a successful artificial diet for *A. flava* using cell suspension cultures of the host plant tissues as the basic dietary constituent. This approach differs in a number of ways from the development of a classical empirical insect diet (Table 3):

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- Leafy Spurge News. Volume XI, Issue 1, March 1989. Russell Lorenz (editor), (insert on Biological Control of Leafy Spurge - pg. 1 of 2).

**Table 3. Comparison of classical vs. cell culture-based insect diets.**

<b>Classical Diet</b>		<b>Cell Culture-Based Diet</b>	
1)	usually in an artificial support base (i.e. agar blocks). <i>A. flava</i> will not burrow into agar.	1)	uniform, natural environment. Allows larvae to follow burrowing instinct.
2)	more labor intensive to produce large amounts and not as easy to extract for chemical analysis.	2)	technology in place to generate large quantities.
3)	based on nutrient requirements for generalist feeders.	3)	may be chemically analyzed for phagostimulants attractive to specialist feeders such as <i>A. flava</i> .
4)	artificial system - no means to examine nutritionally important products of plant metabolism.	4)	living system - can incorporate precursors to important nutrients and determine effect of metabolism on available compounds.
5)	introduction of phagostimulants by trial-and-error experiments.	5)	chemical nature of cells may provide clues as to phago stimulants. Optimization of the levels of these compounds may provide a more effective diet.
6)	eleven formulations have failed to support <i>A. flava</i> larvae.	6)	a novel approach, with positive data from initial experiments.

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## **Sheep management while grazing spurge**

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### **Abstract:**

Sheep will selectively and extensively utilize leafy spurge, and therefore it can be classed as a forage species under summer use by sheep. Production levels of sheep grazing, primarily leafy spurge, are similar and in many instances exceed those grazing more typical ranges. Although sheep will not eradicate the weed, with a good management system, they will keep it from spreading. The amount of resource inputs that a sheep enterprise will entail is entirely dependent on the type of production desired. If sheep are used to selectively graze leafy spurge, some sort of diet training may be necessary. Sheep seem to respond to good or bad management to a greater extent than other livestock species.

### **Introduction**

The addition of a sheep enterprise to the total ranch program has several advantages other than weed control. Lamb and wool are usually marketed at a different time of the year than calves. Therefore, sheep can improve the monthly cash flow of the total ranching operation. Also, an individual can schedule labor-intensive activities within the sheep enterprise during slack periods and make more efficient use of ranch labor. A third benefit is that, often, by grazing sheep and cattle together, the existing forage can be more efficiently utilized.

The amount of time and effort that a sheep enterprise will entail is entirely dependent on the type of production desired. If sheep are viewed just as a method of weed control and little production is expected, then they will probably require very little extra effort. On the other hand, if the sheep enterprise is viewed as a source of extra income, one must be prepared to make a commitment toward the sheep operation.

## Diet selection

Producers have experienced varied results when using sheep to control leafy spurge. This may in part be the result of a preconceived conclusion that it will not work. However, on the other hand, it may have something to do with diet training or imprinting.

Sheep encounter a variety of potential foods on rangelands. One question that has always puzzled researchers is how do they learn what and what not to eat. The genetic relationship between a mother and her young is similar enough that they should respond similarly to cues provided by, and consequences associated with, foods. The mother might also influence food habits of her young by transmitting odors and tastes from foods through her milk (Galef, 1976; Madsen, 1977).

Sheep reared in different habitats often prefer different foods when foraging in the same area (Arnold, 1964; Arnold and Maller, 1977). Arnold and Maller (1977) found that sheep from different environments persistently selected diets of different botanical composition and that diet selection was greatly influenced by previous dietary experiences. Dietary experiences in early life had more effect on later dietary habits than when these experiences occurred at older ages.

Food imprinting, should it exist, is a type of learning that occurs during a sensitive period. Behavior learned during this period persists throughout life (Immelmann, 1975). Should food imprinting occur in livestock, the logical time would be during weaning (Martin, 1984). During weaning the developing animal must make a major transition from complete dependence (Galef, 1981). Young lambs initially learn about foods during this period, and can learn from their dams.

In a study conducted at MSU (Bartz *et al.*, 1985) ewes were placed on pastures containing light (19%), moderate (24%) and heavy (41%) leafy spurge infestations. Diets of ewes grazing leafy spurge-infested pastures indicate that initial consumption of leafy spurge was low for the first one to three weeks of the grazing period. Following this initial period, leafy spurge consumption gradually increased to where it comprised 40 to 50 percent of the sheeps' daily dry matter intake.

It was also noted that as the amount of leafy spurge in the diet increased, the consumption of the grass and other forb components decreased. This shift in diet composition is not uncommon. Cook and Harris (1968) found that sheep diets were largely composed of grass in early summer and mostly forbs in late summer because animals tend to prefer greener plant material. The shift in diet composition of sheep in this grazing study may be explained by leafy spurge's ability to provide more succulent, green plant material during later stages of the growing season. In addition, daily observations of sheep grazing leafy spurge-infested pastures indicated all phenological stages of leafy spurge growth were selected, with the inflorescence being selected first in mature plants and the entire top growth being consumed in immature plants.

If learning affects food recognition and ingestion rates, sheep which are reared in an environment free from leafy spurge and then moved to a spurge-infested pasture cannot be expected to selectively graze the spurge plants. If the area does contain an abundant supply of grasses and forbs, which the sheep are familiar with, one would expect at least initially that the sheep would prefer those grasses and forbs. However, if the sheep have

previously been exposed to and consumed leafy spurge they should selective graze the spurge. Food imprinting in theory could also be accomplished by initially placing the sheep in an area where the spurge infestation is fairly extensive forcing some consumption of the spurge. This will force the sheep to relearn what and how to eat (Zimmerman, 1980).

## **Animal performance**

A three-year study (Bartz *et al.*, 1985) examining the value of leafy spurge as a range forage for ewes and lambs indicated that animal performance was equal to or greater than animals grazing grass pastures. In this study researchers reported higher ( $P < 0.05$ ) average daily gains (ADG) in ewes and lambs grazing leafy spurge-infested pastures than those consuming only grass and increased ( $P < 0.05$ ) ADG's in lambs grazing in heavy vs. light leafy spurge-infested pastures. Breeding and lambing was not affected following summer use of leafy spurge-infested pastures.

## **Leasing concerns**

Once the decision is made to use sheep to control leafy spurge an alternative which is often pursued is to lease the spurge-infested area to a sheep producer at a reduced rate. On the surface this may seem like an arrangement that is beneficial to both the landowner and the sheep producer. However, this is not always the case.

The sheep enterprise in most sheep operations is a long-term enterprise and should be managed as such. The number of ewes run should be balanced with the available long-term feed resources. Unless the sheep producers normal range is in poor condition and would benefit from non-use, there would be no economic advantage to lease spurge-infested pasture instead of utilizing his normal summer range. In the case where forest service land is utilized for summer grazing it may be difficult to maintain the permit if it is not utilized for several years. Unless a long-term commitment (5 plus years) on the part of the landowner can be made, leasing quite often may not be economically advantageous to the sheep producer.

Another factor to consider when adding a sheep enterprise in an area that has not traditionally been grazed by sheep is predators. Predators, both coyotes and domestic dogs can be devastating. If a sheep enterprise is to be feasible, predation must be minimized. In some areas predator may be large enough of a problem and effective control not feasible that utilizing that area with sheep is unrealistic. A visit with the local animal damage control officer during the planning stages may be extremely beneficial.

## **Conclusion**

Sheep will selectively and extensively utilize leafy spurge, and therefore this plant can be justifiably classified as a forage species under summer use by sheep. It is recommended that moderate to heavy stocking rates be utilized for the control of this noxious plant species. This is especially true during grazing periods when sheep can be observed utilizing leafy spurge as a principle component of their diet. It should be cautioned that

with this requirement for moderate to heavy stocking rates it may prove difficult to utilize small areas of leafy spurge within large pastures. Where sheep utilize leafy spurge as a forage at the conclusion of the grazing period, sheep should be placed in a holding area for one week to allow for voiding of leafy spurge seed, which may have retained its viability.

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## **Propagation of *Euphorbia esula* L. for leafy spurge biocontrol agents**

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Leafy spurge biocontrol agents presently require live plant material to complete their life cycle in the greenhouse. To mass rear insects, the optimum leafy spurge growing conditions in the greenhouse must be determined. The purpose of this research was to determine the optimum temperature, fertilizer rate and timing of application, growth media, and water quality to maximize leafy spurge shoot and root growth.

The plants were propagated by cutting 65 to 75 mm of stem from the apex portion of the plant. All but the upper 4 to 5 leaves were stripped from the stem. Then cuttings were dipped into 0.2% powdered NAA and planted into conical tubes (1.5 inch in diameter by 8 inches long) containing a mixture of peat and perlite. The plants were misted for 10 days. Then the plants were selected for uniformity prior to each experiment.

The plants were harvested and stem height, diameter, and dry weight were determined 36 days after treatment. An electronic caliper was used to measure the stem diameter about 20 mm from the stem cut. Roots were carefully washed to remove soil, and root diameter and dry weight were determined. The diameter of the largest true root and not the callus of the stem was measured. Each experiment was repeated.

Leafy spurge growth at 20, 23, 27, and 30° C was determined in separate greenhouses. There were 24 plants per treatment. The stem height and dry weight increased as temperature increased but root growth declined (Table 1). Root dry weight averaged 240 mg/plant at 20° C compared to only 190 mg/plant at 30° C. The optimum growth of both shoots and roots was 27° C and plants in all subsequent experiments were grown at this temperature.

Watering plants with tap or distilled water generally did not affect growth, although shoot dry weight was higher when tap water was used (Table 1). Plants were watered with tap water in all subsequent experiments.

For the two fertilizer experiments, water-soluble commercial fertilizer mixtures of 23-19-17, 15-30-15, and 36-6-6 were used. Each fertilizer was applied at 0, 100, 200, 400, 800, and 1600 lb N/A (100 lb/A equals 12.8 mg N/plant) in the rate study. No plants grew when fertilized at 1600 lb/A (Table 1). There was an increase in shoot and root growth with all three fertilizer types up to N rate of 200 lb/A, then growth declined. The time of

application study consisted of three fertilizers applied either 100 lb N/A once at the beginning of the experiment, 50 lb/A twice (at the beginning and 2.5 weeks later) or 20 lb/A weekly. All plants except the control received 100 lb N/A by the end of the experiment. Plant growth was similar regardless of the timing of application (Table 1).

**Table 1. Effect of temperature, water source, nitrogen rate, timing of nitrogen application, and potting media on leafy spurge growth in the greenhouse.**

Growth parameter	Height	Shoot diameter	Dry weight	Root diameter	Dry weight
Temperature	(mm)		(mg)	(mm)	(mg)
(C)					
20	87	1.2	160	1	240
23	88	1.2	150	0.9	200
27	100	1.3	210	0.9	230
30	109	1.3	210	0.9	190
LSD (0.05)	10	0.1	21	0.1	23
<b>Water source</b>					
Tap	130	1.4	290	0.9	220
Distilled	130	1.3	230	0.8	200
LSD (0.05)	NS	0.1	31	0.1	NS
<b>Nitrogen rate</b>					
(lb/A)					
0	80	1.3	170	0.7	180
100	170	1.8	450	1	310
200	190	1.9	530	1	310
400	180	1.8	490	1	240
800	85	1.7	170	0.3	80
1600	0	0	0	0	0
LSD (0.05)	27	0.2	78	0.1	48
<b>Timing of N application</b>					
(lb/A)					
0	60	1	90	0.8	80
100 once	160	1.7	450	1.1	360
50 twice	160	1.6	470	1.1	350
20 weekly	160	1.6	450	1.1	360
LSD (0.05)	14	0.1	49	0.1	79
<b>Potting media</b>					
Potting soil	180	1.7	490	1.0	330
Sunshine mix	150	1.4	360	0.9	290
Sand	80	0.9	150	0.7	140
Peat moss	0	0	0	0	0
LSD (0.05)	22	0.2	56	0.2	48

The effect of media on leafy spurge growth was determined using a commercial potting soil mix, a peat and perlite mixture (Sunshine Mix No.1, Fisons Western Corporation, Downers Grove, IL), sand, and peat moss. All plants were fertilized weekly with a 15-30-15 fertilizer at 20 lb N/A. The plants in sand were kept in the mist chamber during the entire experiment but did not grow vigorously. No plants grew in the peat moss. The

plants growing in potting soil were taller with more shoot and root biomass than those in the peat and perlite mixture (Table 1). The cost of the soil was approximately 2.8 cents/tube compared to 1.4 cents/tube with the peat and perlite mixture.

In summary, the leafy spurge plants grew best in the greenhouse at 27°C with 200 lb N/A and in a potting soil mixture.

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## Status of screening activities for new insect and pathogen natural enemies of leafy spurge

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Leafy spurge, *Euphorbia esula* (L.) is an aggressive perennial weed that currently infests over one-half million acres in Montana alone and threatens countless more (Lacey, *et al.*, 1985). The weed's extensive underground root system, high capacity for vegetative reproduction, and prolific seed production allow it to effectively out-compete many desirable forbs and grasses (Nowierski and Harvey 1988). Leafy spurge has been found to reduce forage production, wildlife habitat, and cause extensive monetary losses to the livestock industry (Derscheid and Wrage 1972, Lacey, *et al.*, 1985, Messersmith and Lym 1983, Reilly and Kaufman 1979, Selleck 1962, and Watson 1985). Each year, leafy spurge costs the cattle industry of Montana an estimated \$1.4 million loss in forage production, plus \$2.5 million for chemical control (Lacey *et al.*, 1985).

Control of leafy spurge using conventional management approaches has not been satisfactory (Watson 1985). The plant's extensive and persistent root system enables it to survive and spread despite repeated chemical treatments and tillage operations, and it occurs where limited economic returns restrict the amount of money which can be justified for spurge control (Watson 1985). Thus, more cost-effective methods of weed management, such as biological control, are currently being developed for the management of extensive infestations of the weed on rangelands of low economic return.

In this paper, I report on the status of new insect and pathogen natural enemies of leafy spurge, currently undergoing screening by: Agriculture Canada, Regina, Saskatchewan, Canada; the USDA-ARS (Biological Control of Weeds Laboratory, Rome, Italy; Rangeland Weeds Laboratory, Bozeman, MT); and the Commonwealth Institute of Biological Control (CIBC), Delemont, Switzerland.

Funding of the screening effort has been provided by the Leafy Spurge Consortium (LSC: Agriculture Canada; Canada National Defense, Manitoba; Montana Department of Agriculture [MDA]; and North Dakota Department of Agriculture); Alberta, Canada; USDA-ARS; and USDA-APHIS-PPQ. Table 1 provides a summary of the leafy spurge insects and plant pathogens currently undergoing screening for release in Canada and the U.S.

**Table 1. Natural enemies of leafy spurge currently undergoing screening by CIBC and USDA-ARS.**

Species	Funding Source(s)	Completion Date
<i>Pegomya curticornis</i> - root boring fly	Alberta MDA, APHIS	1991
<i>Dasineura</i> sp. - flower gall midge	USDA-ARS	1990?
<i>Chamaesphecia crassiformia</i> - root boring moth	LSC	1992
<i>Chamaesphecia hungarica</i> - root boring moth	LSC	1989
<i>Lobesia euphorbiana</i> - leaf tying moth	Agric. Can.	?
<i>Minoa murinata</i> - defoliating moth	Agric. Can.	?
<i>Oxycesta geographica</i> - defoliating moth	LSC, USDA-ARS	1989
<i>Simyra dentosa</i> - defoliating moth	USDA-ARS	?
<i>Acyrtosiphon cyparissiae</i> - leaf aphid	Agric. Can.	1989
<i>Aphis esulae</i> - stem aphid	Agric. Can.	1989
<i>Aphthona abdominalis</i> - flea beetle	USDA-ARS	?
<i>Aphthona lacertosa</i> - flea beetle	LSC	1989
<i>Eurytoma euphorbiae</i> - seed gall wasp	LSC, USDA-ARS	1994
<i>Uromyces</i> sp. - systemic rust	USDA-ARS, LSC	?

One of the most promising early season natural enemies of leafy spurge is the root-boring fly, *Pegomya curticornis*. This insect emerges in early spring and attacks leafy spurge shoots while the night temperatures are still below freezing. Feeding from the fly larvae kills individual stems and weakens the plant (Harris 1989a). The fly failed to establish on spurge from Canadian releases in 1988, but apparently bred successfully on leafy spurge near Regina, Saskatchewan in 1989 (Harris 1989ab).

Although *P. curticornis* is approved for release in Canada, additional host plant testing will be required before approval to release in the U.S. is granted by the Technical Advisory Group (TAG), a regulatory group under the auspices of the Animal Plant Health Inspection Service (APHIS). Initial screening of this fly for release in Canada was funded by Alberta. Additional host plant testing for release of the fly in the U.S. will be conducted by CIBC, with funds provided by MDA and APHIS.

A second fly species is currently undergoing screening by USDA-ARS. As the name implies, larvae of the flower gall midge, *Dasineura* sp. form galls in the flower-producing region of leafy spurge. The fly appears to be part of a species complex (Gagne 1989). Additional taxonomic studies and screening tests may be necessary before approval to release the fly in Canada and the U.S. is granted by TAG.

Six moth species are also being considered for release against leafy spurge in the U.S. These include: two root-boring moths, *Chamaesphecia crassiformia* and *Chamaesphecia hungarica*; one leaf-tying moth, *Lobesia euphorbiana*, and three defoliating moths, *Minoa murinata*, *Oxycesta geographica*, and *Simyra dentosa*.

Larvae of *C. crassiformia* and *C. hungarica* mine the root system of leafy spurge. Screening of these two insects by CIBC is expected to be completed by 1992 and 1989, respectively via funding by the LSC.

Larvae of the leaf-tying moth, *L. euphoriana* tie the terminal leaves of leafy spurge together and feed within. This insect was approved for release in Canada, and success-

fully reared in a field cage in Regina. However, the insect has yet to establish in the field (Harris 1989a).

Funding for the initial screening of this insect was provided by Agriculture Canada. Additional host specificity testing will be required before approval to release this insect against leafy spurge in the U.S. is granted. Preliminary results suggest that the moth's host range may be too broad to justify release of the insect in the U.S. at present.

The defoliating moth, *Minoa murinata* was screened by Agriculture Canada and first released in 1988, however establishment has not yet been observed in the field (Harris 1989a). Canadian provinces targeted for release in 1989 included Saskatchewan, Manitoba, Ontario, and British Columbia. Additional host specificity testing possibly will be required before approval for release in the U.S. is granted.

The second defoliating moth or webworm, *Oxycesta geographica* is currently undergoing screening by CIBC. Host specificity testing, funded by the LSC and USDA-ARS is scheduled to be completed in 1989. Larvae of this insect form a web on the leafy spurge plant and feed on the foliage within.

The third defoliating moth, *Simyra dentosa* is currently being screened by USDA-ARS. Larvae of the moth feed on leaves of leafy spurge. The time frame for completion of the host specificity testing of this insect has yet to be determined.

Two aphid species, *Acyrtosiphon cyparissiae* and *Aphis esulae*, are currently being screened by CIBC via funds provided by Agriculture Canada. *A. cyparissiae* feeds on the leaves of leafy spurge, while *A. esulae* feeds on the stems. Host specificity testing for both aphid species is scheduled to be completed in 1989.

To date the flea beetle, *Aphthona nigriscutis*, screened by Agriculture Canada, has been the only insect to demonstrate the ability to affect leafy spurge density. Since the beetles release in 1983, spurge biomass (near the original release sites) at a number of locations in Canada has declined and grass biomass has increased (Sturko pers. comm. 1989). Approval for release of this insect in the U.S. was granted by TAG in 1989, and numerous releases were made in the U.S. by USDA-ARS, USDA-APHIS, and University personnel.

Two additional flea beetles are currently being screened for release against leafy spurge in the U.S. and Canada. These include *Aphthona abdominalis* and *Aphthona lacertosa*. Adults of the flea beetles feed on the foliage, while the larvae feed on the roots.

*A. abdominalis* is currently being screened by USDA-ARS, however a timetable for completion of the screening has not yet been determined. CIBC is conducting the host specificity testing of *A. lacertosa*, via funding provided by the LSC. It is anticipated that the screening of this insect will be completed in 1989.

The seed gall wasp, *Eurytoma euphorbiae*, screened initially by CIBC, is currently undergoing host specificity testing by USDA-ARS. Screening of this insect is scheduled to be completed in 1994. Funding for the screening effort has been provided by the LSC and USDA.

The systemic rust, *Uromyces* sp. is a promising and extremely host specific complex of rust species that have good potential for damaging leafy spurge in North America. This complex of fungi typically causes premature shoot death in leafy spurge and effectively shuts down seed production in infected shoots. Initial biological research on *Uromyces* sp. was conducted by the Institute fur Phytomedizin, Zurich, Switzerland via funds from the USDA-ARS. Additional taxonomic studies and host specificity testing will be necessary before approval to release this rust(s) in North America is granted. Taxonomic studies and screening tests are currently being conducted by CIBC via funds provided by the LSC.

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## Collection of native spurges, for screening potentially new biological control agents of leafy spurge

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An item of major concern in the importation of new biological control agents against introduced weeds is the potential adverse impact of the agents upon the native flora, especially plants which are threatened, endangered or being considered for such status. Many of the considerations and possible test species, which were being employed in the USDA-ARS studies of leafy spurge, have been presented by Pemberton, 1984.

To facilitate the screening process, USDA-APHIS has contracted with the Center for Plant Conservation (CPC) for the collection and propagation of various rare and endangered plant species. When sufficient materials are available these plant species will be provided to various researchers and cooperators who are performing the screening research.

The CPC is a private nonprofit organization affiliated with 19 botanical gardens scattered across the United States. The Center is dedicated to conserving rare and endangered plants. Through its affiliated gardens the CPC is presently collecting and propagating several rare endemic species of the genus *Euphorbia*. Species being secured for screening purposes include *E. discoidalis*, *E. exserta*, *E. hooveri*, *E. purpurea*, and *E. telephioides*. These and other related species will be utilized for screening purposes in regards to endangered and or threatened endemic *Euphorbia* species.

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# History of the biological control of leafy spurge (*Euphorbia esula* L.) in the United States

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## Abstract:

A summary of the release history for each of the eight leafy spurge (*Euphorbia esula* L.) biological control agents was presented. Utilizing release records provided by the USDA-ARS Biological Control of Weeds Research Laboratory, Albany, CA and the USDA-APHIS-PPQ Bozeman Bio-Control Facility, all known releases were mapped by state and county.

The species, date of U.S. clearance, and the number of counties and states (in parentheses) where releases were made was:

*Hyles euphorbiae* (L.), 25 May 1965, 36 (9), (Figure 1);

*Chamaespechia tenthrediniformis* (Schifferrmuller), 4 Aug. 1971, 2 (2), (Figure 2);

*Oberea erythrocephala* (Schrank), 30 Nov. 1979, 18 (5), (Figure 3);

*Bayeria capitigena* (Bremi), 16 May 1985, 9 (4), (Figure 4);

*Apthona flava* Guill., 9 June 1985, 13 (5) (Figure 5);

*A. cyparissiae* (Koch), 16 May 1986, 15 (7), (Figure 6);

*A. czwalinae* (Weise), 23 July 1987, 4 (2), (Figure 7); and,

*A. nigricutis* Foudras, 31 May 1989, 18 (10) (Figure 8).

Momentum towards the biological control of leafy spurge is exemplified by ARS clearance of five agents since 1985 and APHIS development of domestic field insectary sites for propagation and redistribution since 1988 (Figure 9).

# Hyles Euphorbia

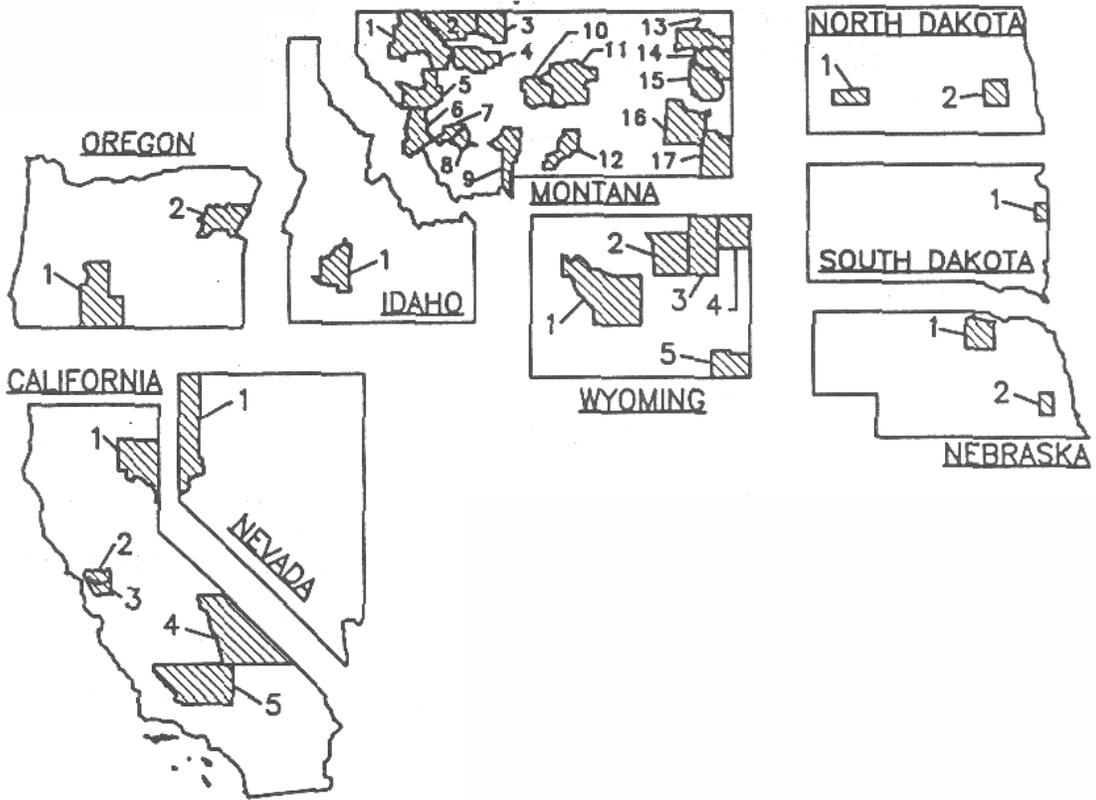


Figure 1. Releases of *H. euphorbiae* by county, 1966-1989.

Source: USDA-ARS-BCWRL, Albany, CA

# Chamaesphecia tenthediniiformis

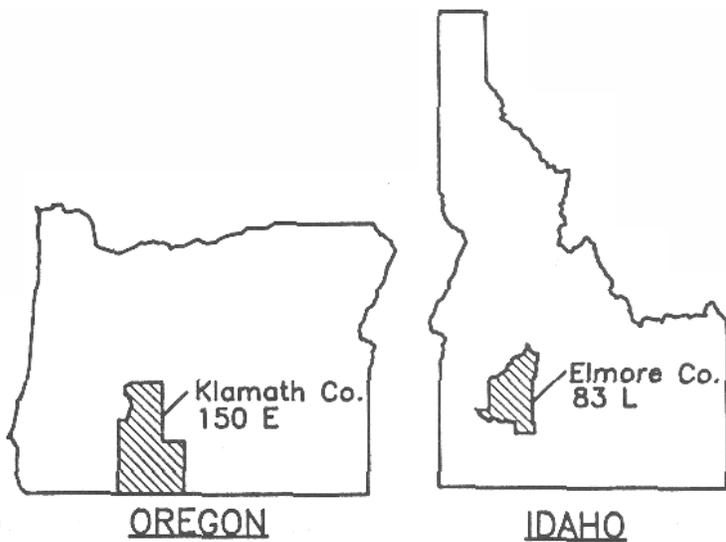


Figure 2. Releases of *C. Tenthrediniiformis* eggs (E) and larvae (L) by county, 1975-1989.

Source: USDA-ARS-BCWRL, Albany, CA

# Oberea erythrocephala

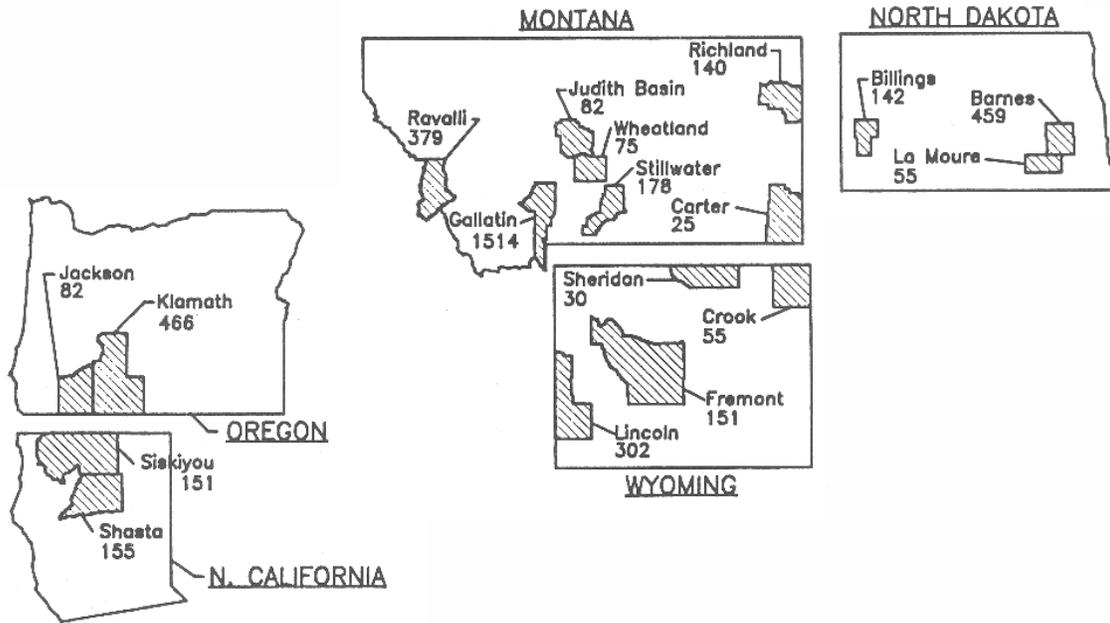


Figure 3. Releases of *O. erythrocephala* adults by county, 1980-1989.

Sources: USDA-ARS-BCWRL, Albany, CA and USDA-APHIS-PPQ-BBCF, Bozeman, MT.

# Bayeria capitigena

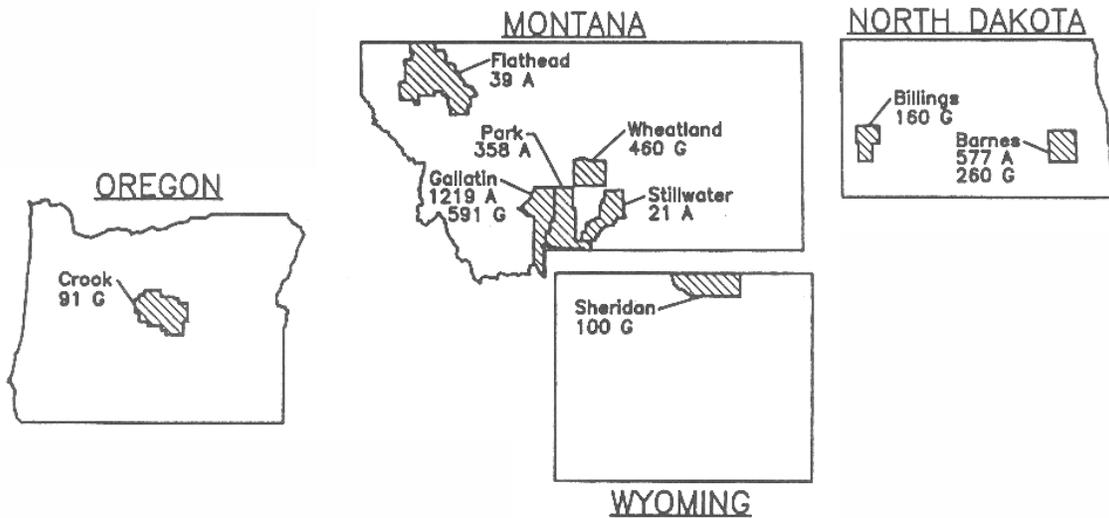


Figure 4. Releases of *B. capitigena* adults (A) and galls (G) by county, 1985-1989.

Sources: USDA-ARS-BCWRL, Albany, CA and USDA-APHIS-PPQ-BBCF, Bozeman, MT.

# *Aphthona flava*

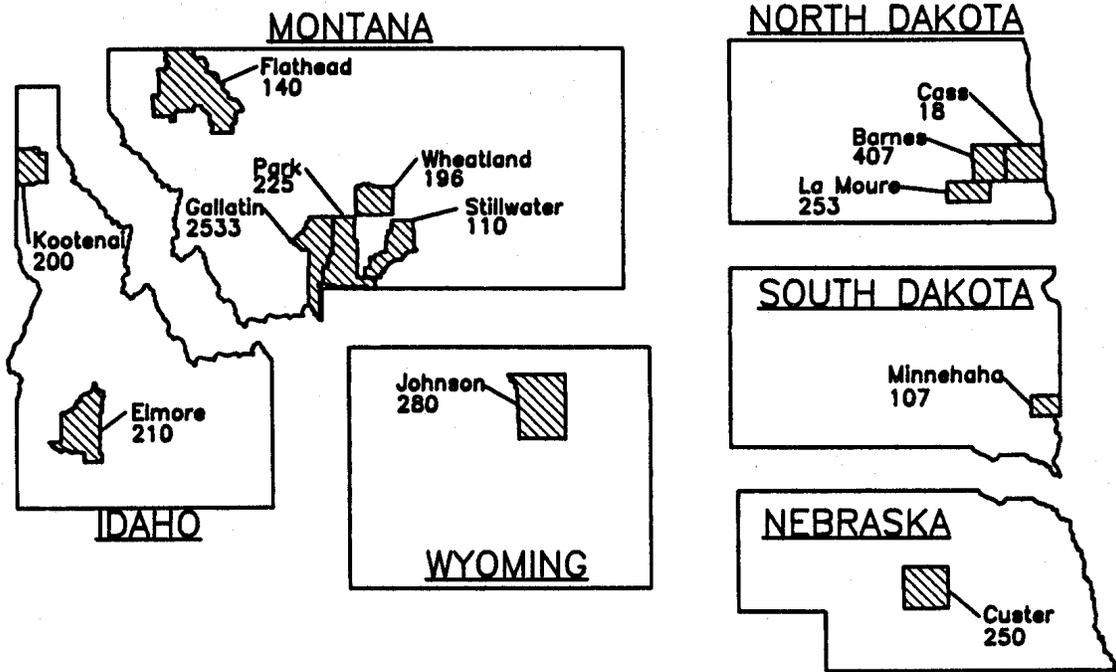


Figure 5. Releases of *A. flava* adults by county, 1985 - 1989.

Sources: USDA-ARA-BCWRL, Albany, CA and USDA-APHIS-PPQ-BBCF, Bozeman, MT

# *Aphthona cyparissice*

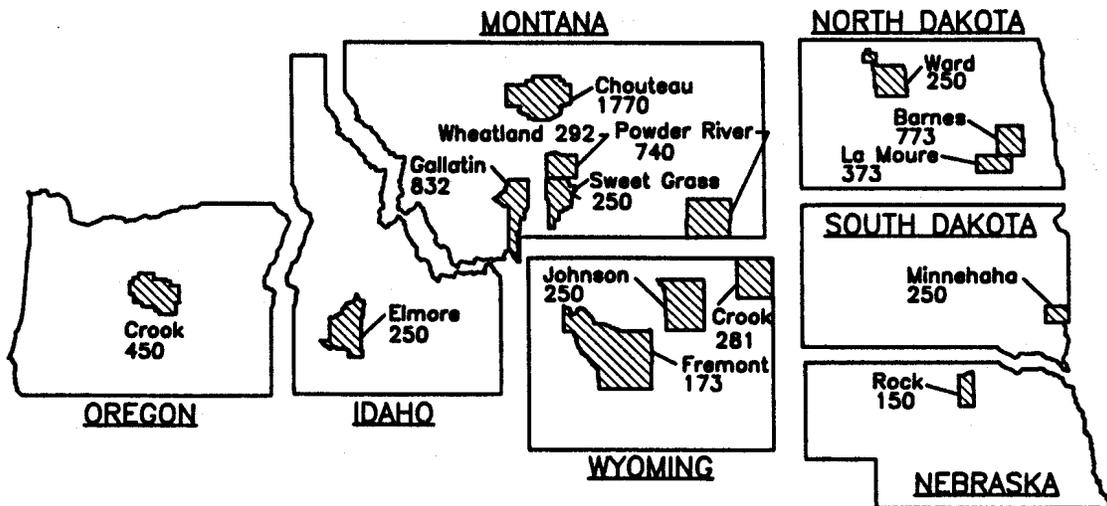


Figure 6. Releases of *A. cyparissice* adults by county, 1986 - 1989.

Sources: USDA-ARS-BCWRL, Albany, CA and USDA-APHIS-PPQ-BBCF, Bozeman, MT

# *Aphthona czwalinae*

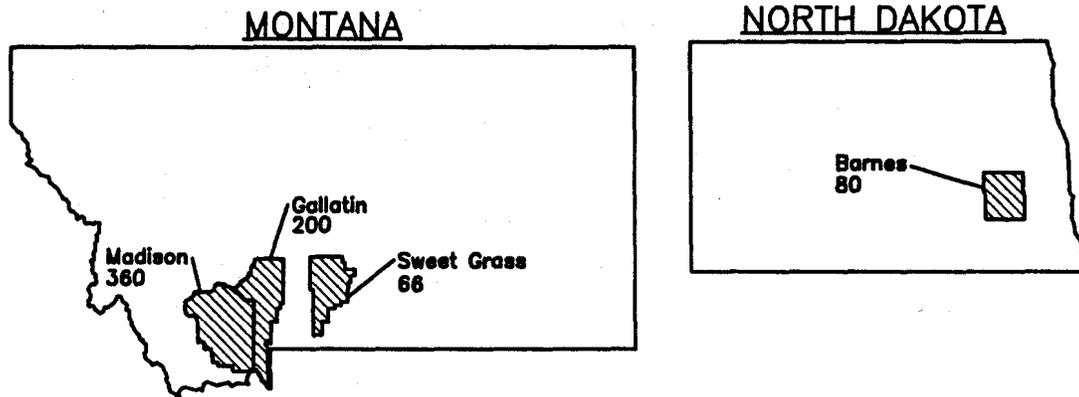


Figure 7. Releases of *A. czwalinae* adults by county, 1987 - 1989.

Sources: USDA-ARS-BCWRL, Albany, CA and USDA-APHIS-PPQ-BBCF, Bozeman, MT

# *Aphthona nigriscutis*

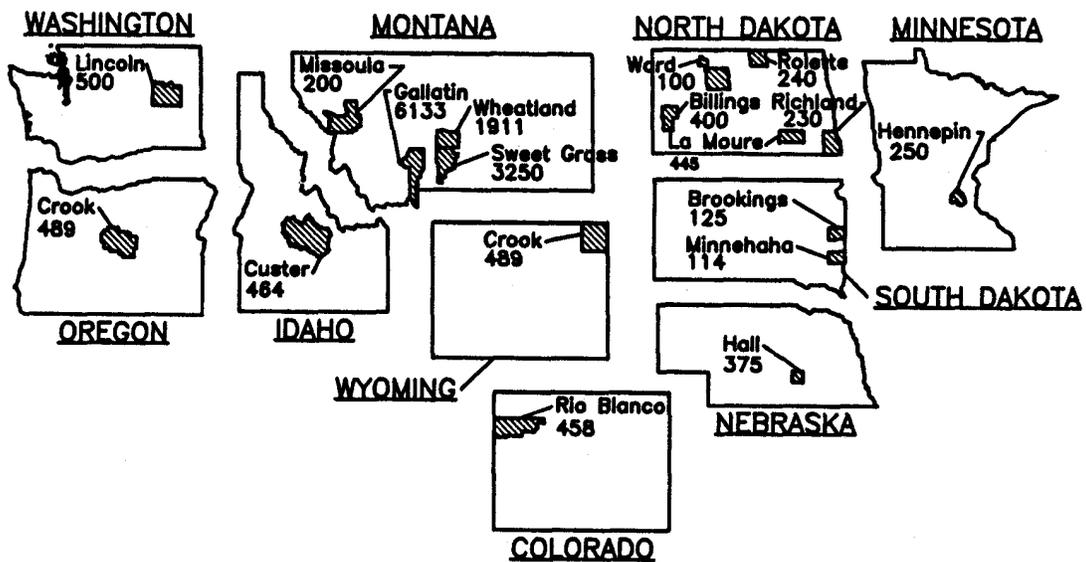


Figure 8. Releases of *A. nigriscutis* adults by county. 1989.

Source: USDA-APHIS-PPQ-BBCF, Bozeman, MT

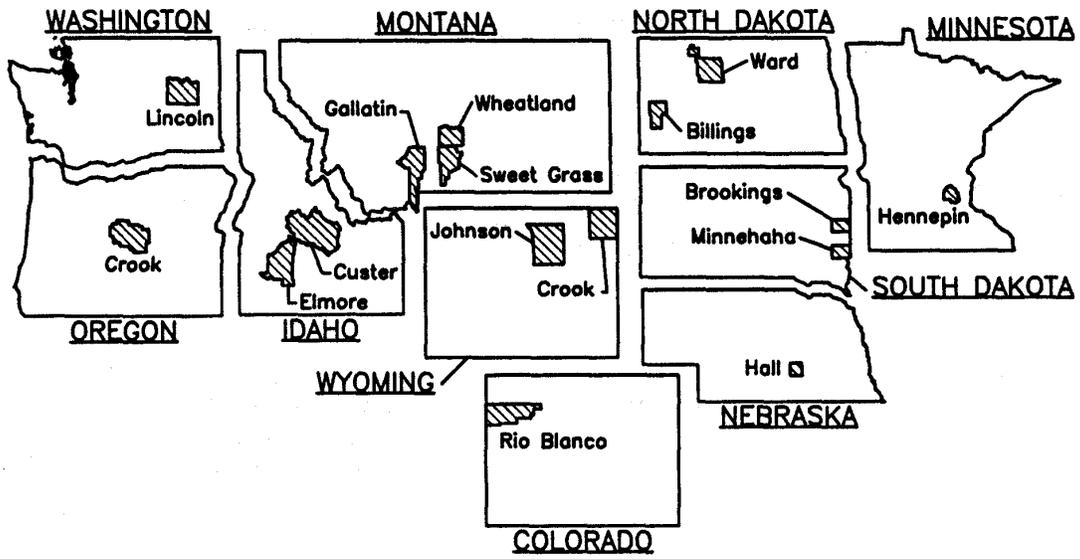


Figure 9. USDA-APHIS-PPQ Bozeman Bio-Control Facility field insectary site locations for the biological control of leafy spurge.

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## **Interagency participation in biological control of leafy spurge: APHIS assistance**

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### **Abstract:**

The United States Department of Agriculture (USDA), Animal Plant Health Inspection Service (APHIS), Biological Control Facility was established in 1986. The goal of this facility is to implement, on a national level, biological control of diffuse and spotted knapweeds and leafy spurge. Biological control of leafy spurge has become a sought-after option for state, federal, and private land stewards. APHIS has augmented existing biological control programs where existing projects were underway and through collection and redistribution of approved agents from Europe and Canada has provided the opportunity for several states to begin biological control efforts on leafy spurge. In the 1989 field season, APHIS provided biological agents, which were released in MT, NE, ND, SD, OR, WA, ID, MN, CO, and WY. Six species of approved biological agents were released. These releases were accomplished to provide the initial steps for the establishment of field insectary sites, which may provide populations of the agents for future collection and redistribution. Cooperating agencies that received agents supplied by APHIS were USDA, Agriculture Research Service (MT); USDA, Forest Service (MT(3)) (ND); USDA, Soil Conservation Service (NE); USDI, Bureau of Land Management (ID) (CO); USDI, National Fish and Wildlife Service (ND) (MN); USDI, National Park Service (ND); State Department of Agriculture (SD) (OR) (CO) (WY); Montana State University (MT); North Dakota State University (ND); Private Land Owners in cooperation with Weed District Supervisors (CO) (WA) (MT); MT Department of Fish, Wildlife, and Parks (MT); and U.S. Army Corp of Engineers (ND).

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## **Sheep prosper on spurge**

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In this paper, we report the use of sheep in managing leafy spurge. Sheep were found to effectively control the rate of spread of leafy spurge. In doing so, wasteland, formerly heavily infested with leafy spurge, was turned into profitable grazing land.

The control area used on the Talcott ranch had a total of 503 acres in it. Sheep had not been used in the area before, but some of the land had been treated chemically in 1979 with limited results. Two hundred twenty-six ewes with lambs were placed in the area in April of 1987. In mid-July, with an average age of 90 days, 305 lambs were sold with a total weight of 22,450 pounds.

The grasshopper infestation that year may have kept the weight down some. The gross income was \$19,750, and the unshorn lamb wool incentive brought the total receipts for the lambs to \$20,749 or about \$41.25 gross profit per acre. This figure does not reflect the wool or incentive payment from the ewes.

No less than 200 acres of the 503 acres were covered with old, well-established spurge plants, and, if chemically controlled, would have to be sprayed at the higher recommended rate of 2 quarts per acre with Tordon 22K. The cost per acre to treat the infested areas would be \$45.50 for chemicals. The cost averaged over the total area would be \$18.09 per acre. Some areas could never be sprayed because of the shallow water table, and the closeness of the spurge to the water's edge. It would appear that the cost differential in controlling spurge with chemical versus using sheep the first year would be \$59.34.

If the land was untreated by chemical or sheep, the 503 acres would provide winter pasture for 200 cows for about 6 weeks without supplement, until the spurge eventually infested the total area.

Having experimented with sheep on spurge for six years, Ron Talcott feels that the stocking rate should be decreased the second year after the spurge plants are weakened. Fewer sheep can then control the spurge.

In the spring of 1988, the sheep numbers were reduced by 50% because of the drought conditions. The weight of the lambs was very good and the spurge was totally utilized.

This year in April of 1989, the Talcotts stocked the control area at a higher rate than in 1988. This being a good grass and spurge year in most areas, they found the spurge in a weakened condition in comparison with spurge plants in adjacent pasture not being grazed by sheep. By June 15, the Talcotts will have the sheep moved to another area that is becoming quite heavily infested with spurge.

Mr. Talcott has come to the conclusion that some river-bottom pastures are nearly inaccessible for chemical control, the spray cannot be used near the river or high water table areas, it takes out non-target plants and shrubs and is costly and ineffective. In these areas that are heavily infested with spurge and difficult to control by other means, he has found this noxious weed to be a good high-quality forage for sheep. While the sheep have not completely eradicated the spurge, they have certainly controlled its spread. The wasteland can be turned into the most profitable grazing land on the ranch!

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## **Leafy spurge control with chlorflurenol tank-mixes**

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### **Abstract:**

Experiments were conducted to assess the effects of tank-mixing chlorflurenol with picloram, dicamba, sulfometuron, and fluroxypyr for leafy spurge control. Control with picloram, dicamba, and sulfometuron at 0.25, 1.0, and 0.09 lb ai/A, respectively, and both fluroxypyr rates did not differ with or without chlorflurenol additions the year of application. Leafy spurge control was greater during the year of application when picloram and dicamba, at 0.5 and 2.0 lb ai/A, respectively, were tank-mixed with chlorflurenol. The year following application, control with picloram, sulfometuron, and fluroxypyr at 0.5, 0.09, and 0.25 lb ai/A, respectively did not differ with or without chlorflurenol additions. Also, dicamba at 1.0 lb ai/A did not differ with or without the addition of 0.125 lb ai/A of chlorflurenol. When this treatment was mixed with 0.07 lb ai/A of chlorflurenol, leafy spurge control improved. Leafy spurge control was improved with both chlorflurenol rates when mixed with picloram, dicamba, and fluroxypyr at 0.25, 2.0, and 0.125 lb ai/A, respectively, when compared to these compounds sprayed by themselves.

## **Introduction**

Chlorflurenol is a morphactin possessing growth regulator properties. Depending on dose and plant species, the compound can stimulate or inhibit growth and development. CF125 and Maintain CF125 are commercial products that have been used to retard herbaceous and woody plant growth. Curbiset induces cucumbers to set large numbers of parthenocarpic fruits and Multiprop induces additional vegetative growth of pineapple slips.

In 1973, Ilays (1973) observed that foliar applications of chlorflurenol to cauliflower caused numerous shoots to develop from roots. Baradari, *et al.* (1980), conducted Canada thistle experiments with <sup>14</sup>C-chlorflurenol, <sup>14</sup>C-dicamba tank-mixed with chlorflurenol,

and field studies with chlorflurenol + dicamba tank-mixes. Studies with labeled chlorflurenol indicated that the compound showed strong acropetal movement and weak basipetal movement in Canada thistle. Labeled dicamba mixed with chlorflurenol displayed twice the absorption, lowered acropetal movement, produced a ten-fold increase in root label, and four-fold increase in label exuded from roots compared to dicamba applied alone. In field studies, no differences occurred between dicamba applied alone compared to tank-mixes with chlorflurenol. However, these researchers indicated that more consistent control was observed when dicamba was applied with chlorflurenol at 0.5 + 0.5 lb ai/A compared to dicamba alone at the same rate. Non-published research conducted at Colorado State University indicated that a split application of clopyralid + chlorflurenol (0.25 + 0.25 lb ai/A) in spring followed by dicamba, + chlorflurenol (0.25 + 0.25 lb ai/A) in fall provided 97% Canada thistle control in July of the year following application. Clopyralid (0.25 lb ai/A) and dicamba (0.25 lb ai/A) applied in spring provided 24 and 0% control, respectively, the year following application. No split applications of clopyralid in spring and dicamba in fall without chlorflurenol were made.

## Materials and methods

A field study was established in 1988 near Meeker, CO to assess the effects of tank-mixing chlorflurenol with several different herbicides for leafy spurge control. The experiment was designed as a randomized complete block with four replications. Picloram (0.25 and 0.5 lb ai/A), dicamba (1.0 and 2.0 lb ai/A), sulfometuron (0.09 lb ai/A), and fluroxypyr (0.125 and 0.25 lb ai/A) were applied with and without chlorflurenol at 0.07 and 0.125 lb ai/A. Additionally; chlorflurenol was applied alone at 0.07 and 0.125 lb ai/A. All treatments were applied using a CO<sub>2</sub> pressurized backpack sprayer calibrated to deliver 24 GPA at 15 psi through 1103LP flat fan nozzles. All herbicides except fluroxypyr were applied on June 10, 1988 when leafy spurge was flowering. Fluroxypyr was applied on August 2, 1988 during leafy spurge seed set. Other application data and information are presented in Table 1. Visual evaluations comparing control in treated plots to non-sprayed check plots were taken on August 2, August 30, September 28, 1988 and on July 5, 1989.

**Table 1. Application information for leafy spurge control with chlorflurenol tank-mixes.**

Environmental data			
Application date		June 30, 1988	Aug 2, 1988
Application time		2:00 pm	6:00 pm
Air temperature, C		28	30
Cloud cover, %		0	80
Relative humidity, %		18	58
Wind speed/direction, mph		0 to 2/SE	0 to 2/SE
Soil temperature (2 in), C		18	24
Weed data			
Application date	Species	Growth Stage	Density (plt/ft <sup>2</sup> )
Jun 30, 1988	EPHES	flowering	3 to 10
Aug 2, 1988	EPHES	seed set	3 to 10

## Results and discussion

Leafy spurge control with picloram, dicamba, and sulfometuron at 0.25, 1.0, and 0.09 lb ai/A, respectively, and both fluroxypyr rates did not differ with or without chlorflurenol additions the year of application (Table 2). However, there was a tendency for greater control with these treatments by tank-mixing with chlorflurenol. Leafy spurge control was greater during the year of application when picloram and dicamba, at 0.5 and

**Table 2. Leafy spurge control with chlorflurenol tank-mixes.**

Herbicide	Rate (lb ai/A)	Timing	Leafy spurge control			
			8-2-88	8-3-88	9-28-88	7-5-89
			(% of Check)			
picloram	0.25	flower	23	26	30	29
picloram	0.5	flower	31	34	30	70
dicamba	1.0	flower	15	11	14	4
dicamba	2.0	flower	4	5	5	20
chlorflurenol	0.07	flower	19	15	15	20
chlorflurenol	0.125	flower	6	4	9	0
sulfometuron	0.09	flower	21	25	20	15
fluroxypyr	0.125	seed set	0	61	63	8
fluroxypyr	0.25	seed set	0	79	86	54
chlorflurenol	0.07					
+ picloram	0.25	flower	20	46	50	61
chlorflurenol	0.07					
+ picloram	0.5	flower	51	58	60	75
chlorflurenol	0.125					
+ picloram	0.25	flower	26	33	44	69
chlorflurenol	0.125					
+ picloram	0.5	flower	60	69	76	78
chlorflurenol	0.07					
+ dicamba	1.0	flower	29	28	33	44
chlorflurenol	0.07					
+ dicamba	2.0	flower	46	51	58	45
chlorflurenol	0.125					
+ dicamba	1.0	flower	19	20	26	16
chlorflurenol	0.125					
+ dicamba	2.0	flower	60	70	68	60
chlorflurenol	0.07					
+ sulfometuron	0.09	flower	13	15	19	16
chlorflurenol	0.125					
+ sulfometuron	0.09	flower	13	21	25	23
chlorflurenol	0.07					
+ fluroxypyr	0.125	seed set	0	71	75	48
chlorflurenol	0.07					
+ fluroxypyr	0.25	seed set	0	92	95	54
chlorflurenol	0.125					
+ fluroxypyr	0.125	seed set	0	76	82	43
chlorflurenol	0.125					
+ fluroxypyr	0.25	seed set	0	92	95	70
check			0	0	0	0
LSD (0.05)			17	21	25	20

2.0 lb ai/A, respectively, were tank-mixed with chlorflurenol. On the September 28 evaluation, leafy spurge control with picloram at 0.5 lb ai/A was increased by 30 and 46% when mixed with chlorflurenol at 0.07 and 0.125 lb ai/A, respectively. Control was increased with dicamba at 2.0 lb ai/A by 53 and 63% when mixed with chlorflurenol at 0.07 and 0.125 lb ai/A, respectively.

Data was somewhat different the year following application. Leafy spurge control with picloram, sulfometuron, and fluroxypyr at 0.5, 0.09, and 0.25 lb ai/A, respectively, did not differ with or without chlorflurenol additions (Table 2). Also, dicamba at 1.0 lb ai/A did not differ with or without the addition of 0.125 lb ai/A of chlorflurenol; however, when this treatment was mixed with 0.07 lb ai/A of chlorflurenol, leafy spurge control was improved by 40%. Leafy spurge control was improved with both chlorflurenol rates by 32, 40, 25, 40, 40, and 35% when mixed with picloram, dicamba, and fluroxypyr at 0.25, 2.0, and 0.125 lb ai/A, respectively, when compared to these compounds sprayed by themselves.

Chlorflurenol additions promote better leafy spurge control with picloram, dicamba, and fluroxypyr, depending upon morphactin and herbicide rate compared to these compounds applied alone. Leafy spurge control with sulfometuron was poor and never showed increased control when tank-mixed with chlorflurenol. Chlorflurenol tank-mixes with picloram, dicamba, and fluroxypyr warrant further investigation. If a lower amount of herbicide can be used to gain the same or better leafy spurge control, economic and environmental advantages could be realized.

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## **Influence of temperature on <sup>14</sup>C-sulfometuron and <sup>14</sup>C-fluroxypyr absorption and translocation in leafy spurge**

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Fluroxypyr and sulfometuron have shown potential for controlling leafy spurge in various field studies. However, the optimum growth stage and environmental conditions for herbicide application are not well understood. The absorption and translocation of <sup>14</sup>C-fluroxypyr and <sup>14</sup>C-sulfometuron in leafy spurge as influenced by temperature and time were evaluated in separate studies.

The experiments were conducted in a randomized complete block design with four replications and were conducted three times. All experiments were conducted using stem cuttings from leafy spurge accession 84-ND 001. The plants were 2 months old, with 6- to 8-inch shoots and two lateral roots with bud development. Plants were over-sprayed with a 2 oz ae/A rate of the respective herbicide using a greenhouse pot sprayer delivering 14 GPA prior to <sup>14</sup>C-herbicide application. The treated leaf was protected during the whole plant treatment with a wax-paper sleeve. Then the sleeve was removed and an appropriate amount of <sup>14</sup>C-labeled and unlabeled herbicide plus 0.25% surfactant WK was applied for a total rate of 2 oz herbicide/A.

Plants were maintained in growth chambers with 24/20° C or 18/14° C day/night temperature regimes and a 15-hour photoperiod with 60% relative humidity. Plants were harvested 24, 48, 96, and 168 hours after treatment by sectioning into the stem and leaves above the treated leaf, stem and leaves below the treated leaf, and roots. Unabsorbed <sup>14</sup>C-herbicide was washed from the treated leaf and the plant material was frozen, dried, and weighed. The plant material was combusted with a biological material oxidizer, and the <sup>14</sup>C-fraction was collected in scintillation cocktail fluor and assayed using liquid scintillation spectrometry.

<sup>14</sup>C-fluroxypyr absorption was similar over time and averaged 41 and 47% of applied herbicide at 8 and 24° C, respectively. Most of the herbicide absorbed by the plant remained in the treated leaf. <sup>14</sup>C-fluroxypyr translocation was greatest to the growing point above the treated leaf at 24° C but to the root at 18° C and averaged 3 and 1.4% of applied herbicide, respectively. Translocation of absorbed material to the root averaged

47% at 18° C compared to 27% at 24° C 24 hours after treatment (HAT) with no increase over time at either temperature.

<sup>14</sup>C-sulfometuron absorption increased from 32% to 47% of applied from 24 to 168 HAT at 24° C but was similar over time at 18° C and averaged 34%. Most of the absorbed herbicide remained in the treated leaf. Translocation of absorbed herbicide to the shoot above the treated leaf was greater at 24° C than 18° C (2 and 0.8%, respectively). Translocation of absorbed herbicide to the root averaged 39% at 18° C compared to 22% at 24° C.

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## **Leafy spurge (*Euphorbia esula* L) control with fluroxypyr**

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### **Abstract:**

Early reports from Oregon and Wyoming indicated that fluroxypyr (4-amino-3, 5-dichloro-6-fluro-2-pyridyloxyacetic acid) has activity on leafy spurge. Three field studies were conducted near Devil's Tower, in north-eastern Wyoming, to study the activity of fluroxypyr, alone and in combination with other herbicides, for the control of leafy spurge.

A first field study was established to compare the efficacy of initial treatments of fluroxypyr, retreated with dicamba (3,6-dichloro-2-methoxybenzoic acid), 2,4-D LVE ((2,4-dichlorophenoxy) acetic acid), picloram (4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid), and fluroxypyr on the control of leafy spurge. Visual weed control evaluations prior to retreatment applications, showed the leafy spurge to be in a stunted condition with very little flowering. Visual weed control evaluations one year after retreatments showed picloram applied late summer at 0.5 lb ai/a to be the only retreatment that resulted in control, however, this control (approximately 40%) was inadequate.

A second field study involved the use of picloram and fluroxypyr with and without surfactant (X-77) to compare the efficacy of these treatments for the control of leafy spurge. The surfactant, X-77, was not effective in increasing the activity of either picloram or fluroxypyr.

A third field study involved fluroxypyr applied as a tank mix with picloram, dicamba, and 2,4-D LVE for leafy spurge control. No treatments were effective in long-term control of leafy spurge.

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## Introduction

Leafy spurge is a deep-rooted, herbaceous plant 20 to 100 cm tall. It reproduces by seeds and numerous root and crown buds (Hanson and Rudd 1933). As seed chambers ripen they dehisce explosively, throwing the seed up to 4.5 meters from the parent plant (Bakke 1936). This allows the plant to spread rapidly and form dense infestations of up to 205 shoots per M<sup>2</sup> (Selleck *et al.*, 1962). Control is difficult because of an extensive underground root system containing large amounts of carbohydrate reserves (Bakke 1936) and reproductive buds (Coupland and Alex 1955). It also can tolerate various habitats and environmental conditions (Selleck *et al.*, 1962).

Leafy spurge has caused scours and weakness in cattle and sheep and may lead to death (Johnston and Peake 1960, Kingsbury 1964, Muenscher 1940). However, sheep have also been reported to graze readily on leafy spurge with no apparent harmful effects (Helgson and Thompson 1938; Landgraf, *et al.*, 1984). Cattle grazing capacity may be reduced by as much as 75% due to the competitive effect of leafy spurge (Reilly and Kaufman 1979). Cattle also avoid consumption of leafy spurge and will not eat palatable forages in areas of high leafy spurge density (Lym and Kirby 1987).

Herbicide research to control leafy spurge in Wyoming began in 1952 with 2,4-D. Picloram which became available in 1963 has proven to be the most reliable and effective herbicide for controlling leafy spurge (Vore and Alley 1982). However, long-term herbicide control is either ineffective and/or too expensive (Alley and Messersmith 1985, Messersmith 1979).

Wyoming has over 46,949 acres infested with leafy spurge with infestations present in all 23 counties (Hittle 1983). Although it is primarily a problem on noncultivated land, its presence and control are costly. Wyoming has projected the overall cost of managing 48,619 acres of leafy spurge to be over \$10 million (Hittle 1983).

New herbicides must continually be evaluated for activity on leafy spurge in the hope that a more effective and economical means for control might be discovered. Fluroxypyr is a readily translocatable non-phenoxy herbicide showing activity to a large spectrum of broad-leaved plants when applied post-emergence (The Dow Chemical Company). Early reports from Oregon (Whitson 1985) and Wyoming (Whitson and Ferrell 1988) indicated that fluroxypyr has activity on leafy spurge. The purpose of this research was to study the activity of fluroxypyr, alone and in combination with other herbicides, for the control of leafy spurge.

## Materials and methods

Three field studies were conducted near Devil's Tower, in northeastern Wyoming, to study the activity of fluroxypyr, alone and in combination with other herbicides, for the long-term control of leafy spurge.

Initial applications of fluroxypyr with retreatments of various herbicides for leafy spurge control. A first field study was established to compare the efficacy of initial treat-

ments of fluroxypyr, retreated with dicamba, 2,4-D LVE, picloram, and fluroxypyr on the control of leafy spurge.

Three areas, each 90 ft by 120 ft, were treated with initial applications of fluroxypyr at 3/8, 1/2, and 5/8 lb ai/a. After initial treatments were applied, the areas were divided into plots 9 by 30 ft. with four replications, to which spring and late summer retreatments were applied. The initial treatments were applied broadcast with a CO<sub>2</sub> pressurized six-nozzle knapsack sprayer delivering 30 gpa at 35 psi August 12, 1986 (air temp. 96° F, soil temp. 0 inch 115° F, 1 inch 93° F, 2 inch 83° F, 4 inch 78° F, relative humidity 27%, wind south at 5 mph, sky clear). The leafy spurge was 14 inches tall and most of the seed had been shed 4 weeks earlier. 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. Spring retreatments were applied May 28, 1987 to a dense stand of leafy spurge 8 to 12 inches tall (air temp. 65° F, soil temp. 0 inch 70° F, 1 inch 60° C, 2 inch 60° F, 4 inch 55° F, relative humidity 63%, wind calm, sky clear). Late summer treatments were applied August 27, 1987 to high-density leafy spurge 10 to 14 inches tall (air temp. 57° F, soil temp. 0 inch 75° F, 1 inch 70° F, 2 inch 65° F, 4 inch 60° F, relative humidity 77%, wind calm, sky clear).

**Picloram and fluroxypyr with and without surfactant for leafy spurge control.** A second field study involved the use of picloram and fluroxypyr with and without surfactant (X-77) to compare the efficacy of these treatments for the control of leafy spurge.

Plots were 10 by 27 ft. with four replications arranged in a randomized complete block. Treatments were applied broadcast with a CO<sub>2</sub> pressurized six-nozzle knapsack sprayer delivering 30 gpa at 35 psi Picloram treatments were applied May 28, 1987 when leafy spurge was in the full bloom stage and 8 to 12 inches high (air temp. 60° F, soil temp 0 inch 60° F, 1 inch 55° F, relative humidity 75%, wind west at 5 mph, sky cloudy). Fluroxypyr treatments were applied July 7, 1987 when leafy spurge plants were setting seed and 10 to 14 inches high (air temp. 80° F, soil temp. 0 inch 95° F, 1 inch 80° F, 2 inch 75° F, 4 inch 70° F, relative humidity 75%, wind south at 5 mph, sky partly 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. Infestations were heavy throughout the experimental area. Visual weed control evaluations were made June 8, 1988.

**Fluroxypyr in combination with various herbicides for leafy spurge control.** A third field study involved fluroxypyr applied as a tank mix with picloram, dicamba, and 2,4-D LVE for leafy spurge control.

Plots were 10 by 27 ft. with four replications arranged in a randomized complete block. The herbicide treatments were applied broadcast with a CO<sub>2</sub> pressurized six-nozzle knapsack sprayer delivering 30 gpa at 35 psi May 28, 1987 (air temp. 60° F, soil temp. 0 inch 60° F, 1 inch 55° F, relative humidity 75%, wind west at 5 mph, sky 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 8 to 12 inches high. Infestations were heavy throughout the experimental area. Visual weed control evaluations were made June 8, 1988.

## Results and discussion

**Initial applications of fluroxypyr with retreatments of various herbicides for leafy spurge control.** Visual weed control evaluations made May 28, 1987, prior to re-treatment applications, showed the leafy spurge to be in a stunted condition with very little flowering. Visual weed control evaluations were also made June 8, 1988 to evaluate the retreatments. Picloram applied late summer at 0.5 lb ai/a was the only retreatment that resulted in control, however, this control was inadequate (Table 1).

**Table 1. Initial applications of fluroxypyr with retreatments of various herbicides for leafy spurge control. Crook County, 1988.**

Retreatment <sup>a</sup>	Rate (lb ai/a)	Percent shoot control <sup>b</sup>		
		Fluroxypyr initial treatment lb ai/a <sup>c</sup>		
		3/8	1/2	5/8
		(%)		
(Spring)				
dicamba	2.0	0	0	0
2,4-D LVE	2.0	0	0	0
picloram	0.5	0	0	0
fluroxypyr	0.5	0	0	0
check	0.0	0	0	0
(Late summer)				
dicamba	2.0	0	0	0
2,4-D LVE	2.0	0	0	0
picloram	0.5	43	40	40
fluroxypyr	0.5	0	0	0
check	0	0	0	0
LSD (0.05)		2	2	6

<sup>a</sup>Spring retreatments applied May 28, 1987. Late summer retreatments applied August 27, 1987.

<sup>b</sup>Visual evaluations June 8, 1988.

<sup>c</sup>Initial treatments applied August 12, 1986.

**Picloram and fluroxypyr with and without surfactant for leafy spurge control.** The surfactant, X-77, was not effective in increasing the activity of either picloram or fluroxypyr (Table 2).

**Fluroxypyr in combination with various herbicides for leafy spurge control.** No treatments were effective in controlling leafy spurge (Table 3).

The results of these three studies indicate that fluroxypyr does not provide long-term control of leafy spurge alone or in combination with other herbicides.

**Table 2. Picloram and fluroxypyr with and without surfactant for leafy spurge control. Crook County, 1988.**

Treatment <sup>a</sup>	Rate (lb ai/a)	Control <sup>b</sup> (%)
picloram	0.25	3
picloram + X-77	0.25	6
picloram	0.5	10
picloram + X-77	0.5	8
picloram	0.75	30
picloram + X-77	0.75	38
picloram	1.0	43
picloram + X-77	1.0	28
picloram	1.25	38
picloram + X-77	1.25	43
picloram	1.5	50
picloram + X-77	1.5	58
picloram	1.75	58
picloram + X-77	1.75	51
picloram	2.0	61
picloram + X-77	2.0	56
fluroxypyr	0.125	0
fluroxypyr + X-77	0.125	0
fluroxypyr	0.25	0
fluroxypyr + X-77	0.25	0
fluroxypyr	0.5	0
fluroxypyr + X-77	0.5	0
Check	0	0
LDS (0.05)		23

<sup>a</sup>Picloram treatments applied May 28, 1987. Fluroxypyr treatments applied July 7, 1987. X-77 applied at 0.25% v/v.

<sup>b</sup>Visual evaluations June 8, 1988.

**Table 3. Fluroxypyr in combination with various herbicides for leafy spurge control. Crook County, 1988.**

Treatment <sup>a</sup>	Rate (lb ai/a)	Control <sup>b</sup> (%)
fluroxypyr + picloram	0.5 0.25	20
fluroxypyr + picloram	0.5 0.5	18
fluroxypyr + dicamba	0.5 1.0	0
fluroxypyr + dicamba	0.5 2.0	0
fluroxypyr + 2,4-D LVE	0.5 2.0	0
fluroxypyr + 2,4-D LVE	0.5 4.0	0
picloram	0.25	0
picloram	0.5	13
dicamba	1.0	0
dicamba	2.0	0
2,4-D LVE	2.0	0
2,4-D LVE	4.0	0
fluroxypyr	0.5	0
Check	0	0
LSD (0.05)		12

<sup>a</sup>Treatments applied May 28, 1987.

<sup>b</sup>Visual evaluations June 8, 1988.

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Sponsored by: Montana Agricultural Experiment Station, Montana State University, Bozeman, MT.

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## Iron toxicity in leafy spurge (*Euphorbia esula* L.)

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Many heavy metals are toxic to plants, but iron is usually regarded as having little direct effect on plants. Most problems with iron occur in acid soils and are related to changes in soil pH. In calcareous soils, commonly found in much of Montana, most of the iron is precipitated and unavailable to plants.

During the course of growing *Euphorbia esula* in the greenhouse as food for a biological control insect, the plants developed chlorosis, which led us to suspect iron deficiency. When chelated iron was applied to the plants in an attempt to alleviate the chlorosis, all plants that received the application either died or were severely injured within two weeks. As a consequence, a study was initiated to test whether *Euphorbia esula* was sensitive to iron.

*Euphorbia esula* was grown in sand culture with four treatments employed: 1) Control - a standard Hoagland's nutrient solution, 2) Fe-EDTA - a Hoagland's solution with 100 times the normal iron in the chelated form, 3) FeCl<sub>3</sub> - a Hoagland's solution with 100 times the normal iron in the form of iron chloride, and 4) EDTA - a Hoagland's solution with the normal level of iron but 100 times the level of chelating agent (the EDTA). For each treatment, 50 root crowns were planted in 4-inch square pots filled with sand and placed in a 10 by 15 by 1 dm galvanized tray on the greenhouse bench. Each pot was numbered and each tray was randomly assigned to a treatment. Trays and pots within trays were systematically rotated weekly to reduce variance due to location. Watering was applied thrice weekly by pouring 20 liters of nutrient solution into each tray and letting the pots stand in the solution for 4-6 hours after which time the solution was drained from each tray. The height of each plant was recorded weekly.

High levels of iron in either the chloride or chelated form significantly depressed growth within 21 days ( $P < 0.01$ ). Fe-EDTA reduced growth greater than FeCl<sub>3</sub>, but not significantly ( $P > 0.50$ ). This may have resulted from some precipitation of iron in the FeCl<sub>3</sub> treatment, effectively reducing the dosage received. In both treatments, bronzing (typical of iron toxicity) was abundantly evident, particularly in the older leaves. Plants in the Fe-EDTA treatment also had a very pale yellow chlorosis that was most pronounced in the youngest leaves and diminished with leaf age. Inflorescences were not produced in either iron treatment.

The EDTA treatment was included to eliminate the possibility that the chelating agent was the cause of the necrosis and death of *E. esula* plants we originally observed. EDTA did indeed reduce growth, but took longer to show an effect (28-31 days). EDTA treated plants had the pale yellow chlorosis observed in the Fe-EDTA treatment, but had no bronzing.

The results demonstrated that *E. esula* was indeed sensitive to the concentration of available iron. A second experiment was conducted to determine a general threshold level of iron that would elicit major injury and/or death to *E. esula*. The experimental design was as before, except that the four treatments were: 1) 1X - Control - a standard Hoagland's nutrient solution, 2) 3X Fe - Hoagland's solution with 10 times the normal iron in the unchelated form ( $\text{FeCl}_3$ ), 3) 10X Fe - Hoagland's solution with 10 times the normal iron in the unchelated form, 4) 100X Fe - Hoagland's solution with 10 times the normal iron in the unchelated form. The height of the tallest plant in each pot and any evidence of plant injury were recorded weekly. Six categories were recognized as evidence of injury to plants: death, general chlorosis, chlorosis of apical leaves, leaf bronzing, leaf drop, and leaf curling.

High levels of iron (10X and 100X) significantly depressed growth within 21 days ( $P < 0.01$ ), and in the case of the 100X treatment, arrested growth within 14 days. However the 3X treatment actually stimulated growth as compared to the control treatment.

Death of root crowns did not occur in either the control or the 3X treatment, occurred rarely in the 10X treatment, and in more than half the pots in the 100X treatment. The control plants rarely had generalized chlorosis or leaf abscission, occasionally had leaf bronzing or leaf curling, and a few times had apical leaf chlorosis. In the 3X treatment, apical leaf chlorosis and leaf bronzing occurred more often than in the controls, but there was less leaf curling and no leaf abscission. In the 10X treatment leaf curling was uncommon, general and apical chlorosis occurred frequently, and leaf bronzing and abscission occurred in more than half the pots. In the 100X treatment apical leaf chlorosis was impossible to assess because leaf abscission was so prevalent that only apical leaves were present on the plant. The leaves that remained were generally chlorotic and had bronzing as well. Leaf curling was rare.

Since a single root crown has several stems that arise from separate crown buds, in those pots that had live plants we also counted the total number of stems that had grown from the root crown and the number of living stems at the end of the seven-week experiment. The total number of stems that were initiated by root crowns were not significantly different for any treatment, although the two lower iron concentrations had more stems than the two higher iron treatments. Only in the high iron treatment was the number of live stems significantly lower.

A third experiment was conducted to determine the effect of these same concentrations of iron on range grasses. The experimental design was as in the previous experiment, except that ten pots each of five grass species were grown in the four iron concentrations. The five grasses were *Agropyron smithii* Rydb., *A. spicatum* (Pursh) Scibn. & Smith, *Bromus inermis* L., *Festuca idahoensis* Elmer, and *Poa pratensis* L.

The pattern of grass growth in the same iron concentrations was similar to *E. esula*, except that the growth reduction (as compared to the control) in the 10X treatment was

proportionally less than for *E. esula* in all five grasses. All grasses in the 100X treatment died within five weeks.

Injury symptoms for grasses were not evident in the control or 3X treatments. Occasional bronzing and chlorosis of leaf tips occurred in the 10X treatment although grasses in most pots appeared quite healthy looking. *P. pratensis* had more injury symptoms than any of the other grasses.

The results demonstrate that *E. esula* is indeed sensitive to the dosage of available iron. Low levels of iron may even increase the growth of *E. esula*. Grasses seem less sensitive to iron at medium concentrations, and in particular grasses grown in the 10X treatment appeared to be less damaged than did *E. esula*. This presents the possibility that application of iron in the field may significantly stress *E. esula* while at the same time allowing significant grass growth.

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Sponsored by: Montana Agricultural Experiment Station, Montana State University, Bozeman, MT.

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## Control of leafy spurge with natural chemical products

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### Abstract:

Bioassays were used to evaluate the phytotoxicity of natural chemicals contained in extracts of plants and fungi for possible use as control agents of leafy spurge (*Euphorbia esula* L.). Sunnhemp (*Crotalaria juncea* L.) seeds contained a water-soluble substance that was highly phytotoxic in a Lemna bioassay, and that inhibited the growth of leafy spurge by 65%. Planting sunnhemp in pots with growing leafy spurge also inhibited this weed by 75 to 85%. *Alternaria angustiovoidea* (Simmons) growing in liquid culture produced substances that were phytotoxic to Lemna, but showed limited effects on leafy spurge.

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## Introduction

Among several strategies to control the proliferation of leafy spurge is the use of natural chemical products. This concept is not new and suggested exploitation has taken several forms including the use of these products as natural herbicides/plant growth regulators, as the structural basis for herbicides, or enhancement of the production of these chemicals in the producing organism (Einhellig and Leather, 1988). Research at this laboratory has demonstrated that selected crop plants can be used to reduce weeds (Leather, 1983) and, in certain situations, can control weeds to the same extent as applied synthetic herbicides (Leather, 1987).

Biological control (in the classical sense) of leafy spurge has had limited success, but with additional research has good potential (Carlson and Littlefield, 1983). The use of pathogenic fungi for leafy spurge control is currently under investigation (Littlefield, 1985), and a prospective *Alternaria* sp. has been identified (Krupinsky and Lorenz, 1983; Simmons, 1986). However, the requirements for infection with the fungus and the possi-

ble diversity of biotypes of leafy spurge (Yang *et al.*, 1988), suggest that alternative approaches are necessary.

The objectives of this research were to determine the potential for using the palatable legume plant *Crotalaria juncea* (sunhemp) and/or its chemical products for leafy spurge control and to identify any toxins produced by *Alternaria* sp. that may be used in a similar manner. There have been many toxins identified from *Alternaria* sp. and a few are host-specific (Nishimura and Kohmoto, 1983). With the development and use of highly sensitive bioassays (Leather and Einhellig, 1988), we hope to identify naturally produced chemicals that may be useful for the control of leafy spurge.

## Materials and methods

### Fungal culture

*Alternaria angustiovoidea* was grown on potato-dextrose agar (PDA). A block of agar with mycelium was cut from the margins of three- to five-day old cultures on PDA with a No. 3 cork borer and transferred to a 250 ml flask containing 50 ml of Fries medium (liquid). A total of 20 flasks were used on two occasions. The flasks were incubated in darkness at 25°C for two weeks.

### Extraction

After the culture period, the fungal mycelium was separated from the culture medium by filtration through a 0.25 µm membrane filter, both freeze-dried and stored at 20°C until extracted. For sunhemp tissues were ground in a Wiley Mill at 20 mesh and stored at -20°C until extracted.

The extraction procedure was the same for the *Alternaria* and sunhemp. The freeze-dried material was extracted at 6°C for 24 hours with 80% methanol/water. The slurry was centrifuged and the pellet discarded. The supernatant was partitioned with an equal volume of hexane three times. The hexane fraction was discarded after it was determined by bioassay to be void of active materials. The methanol/water fraction was evaporated at 35°C under vacuum until only the water remained. The water extract was freeze-dried and an aliquot was bioassayed for phytotoxicity.

Three grams of the freeze-dried extract were reconstituted in water and layered on a pre-activated Amberlite XAD-4 column. The extract was eluted with 200 ml water and collected in 50 ml fractions followed by 200 ml MeOH also collected in 50 ml fractions. The water fractions were freeze-dried, and the MeOH fractions were reduced to 2 ml. All fractions were stored at -20°C until used for bioassay.

### Bioassays

The primary bioassay for phytotoxicity was the *Lemna* (duckweed) bioassay described by Einhellig *et al.*, (1985), using *Lemna obscura* (Leather and Einhellig, 1985). The bioassay employed a 24 well tissue culture plate with 1.5 ml of growth medium and

a beginning 4-frond *Lemna* plant. A 3 mg sample of each freeze-dried fraction was reconstituted in 100 ml of water and 5 ul of the solution was placed in a well giving a final concentration of 100 ppm. A 5 ul aliquot of the concentrated MeOH fractions was used directly for assay. After 7 days incubation, *Lemna* growth was determined by counting the fronds and measuring dry weight. Anthocyanin production was also determined when phytotoxicity was not evident. Each treatment was replicated four times.

The *Alternaria* fractions were also bioassayed by placing a 10 ul droplet of the solutions upon a puncture made by a needle through a detached leafy spurge leaf. The detached leaves were maintained on moist filter paper in a closed 100 by 15 mm petri plate and incubated at 28°C under constant light. Each treatment was replicated four times.

Greenhouse assays of the fractions were accomplished by determining the regrowth of leafy spurge. Leafy spurge plants, growing in sand/Hoagland and Arnon's (1950) solution were severed 1 cm above the crown. After 3 days, when the regrowth of leafy spurge was evident, 10 ml of the reconstituted MeOH/HOH extract (1000 ppm) was used as a crown drench for each plant. The drench was repeated after a five-day interval. Each treatment was replicated six times.

A second greenhouse test was used to evaluate the influence of germinating and growing sunnhemp on the growth of leafy spurge. Two, four or eight sunnhemp seeds were distributed in pots around a single 6-week-old leafy spurge plant growing in sand culture. The sunnhemp seeds were covered with vermiculite and watered. Growth of the leafy spurge was determined after 7, 13, 24, and 35 days by the change in height measured from the sand surface. After 35 days the leafy spurge was severed 1 cm above the crown and dry weight determined for each plant. On day 35 the sunnhemp was also severed 0.5 cm above the cotyledonary node. The pots were maintained an additional 21 days and the dry weight of the leafy spurge regrowth was again determined. Each treatment was replicated six times.

## Chromatographic analysis

Active fractions of the sunnhemp and *Alternaria* were further separated by High Performance Liquid Chromatography (HPLC) with a reverse-phase column and using ultraviolet absorption detector set at 254 nm. The mobile phase was water isocratic for 5 minutes; a linear gradient to 90% MeOH in 25 minutes; isocratic at 90% MeOH for 5 minutes; and a linear gradient to water in 5 minutes.

## Results and discussion

Results of the primary bioassays using duckweed indicated that all tissue extracts of sunnhemp contained phytotoxic compounds. Extracts of the seed, however, contained a compound(s) that was highly toxic to the duckweed. Further separation indicated the toxic material was water-soluble and was eluted from the XAD-4 column in the first water fractions. HPLC analysis indicated that the fraction contained one major compound with a few minor peaks. The major peak eluted during the first 5 minutes while the mobile phase was at 100% water.

Extracts of the *Alternaria* mycelia and culture medium were also active in the duckweed bioassay; the mycelium had the greater phytotoxicity. Further separation on the XAD-4 column indicated activity was not confined to a single fraction but eluted with both water and methanol. HPLC analysis confirmed a number of ill-defined peaks that were present in most fractions. Further research is needed to separate the active component(s) into cleaner fractions.

In the leafy spurge bioassay, the mycelium extracts did not produce a phytotoxic reaction. The extracts of medium, however, caused discoloration and/or necrosis 1 to 5 mm around the puncture in the leafy spurge leaf.

In greenhouse experiments, only the sunnhemp extract was phytotoxic to leafy spurge (Table 1). The dry weight of the leafy spurge regrowth was less than 50% of that of control plants. In addition to a stunted appearance, the sunnhemp extract caused a loss of chlorophyll (yellowing) and twisting and curling of the leaves and stem.

**Table 1. Effect of natural compounds on the regrowth of leafy spurge in greenhouse culture.**

Treatment	3 wk regrowth <sup>a</sup> (mg dry wt.)
Control	282.3 b
<i>Alternaria angustiovoidea</i> extract	255.3 b
<i>Crotalaria juncea</i> extract	132.0 a

<sup>a</sup>Means followed by the same letter do not differ significantly at the p=0.05 level.

Sunnhemp seeds and seedlings in pots with leafy spurge reduced the growth of the spurge plants, and this was significant after 13 days (Table 2). It was interesting that only two sunnhemp seeds were required to produce maximum growth inhibition of the leafy spurge. Overall inhibition after 35 days was 50 to 60% as determined by height. The dry weight of leafy spurge (Table 3) also reflects the overall inhibition after 35 days.

**Table 2. Influence of sunnhemp on the growth of leafy spurge in sand culture.**

Treatment Seeds/pot 1-7	Change in height <sup>a</sup> Days			
	7-13	13-24	24-35	0-35
	(cm)			
0	4.2 a	3.9 b	5.9 b	7.8 b 21.8 b
2	3.2 a	1.4 a	4.2 a	4.1 a 12.9 a
4	3.8 a	1.5 a	3.9 a	3.0 a 12.1 a
8	3.2 a	1.5 a	4.2 a	2.8 a 10.8 a

<sup>a</sup>Means in a column followed by the same letter do not differ significantly at the p=0.05 level.

**Table 3. Dry weight of leafy spurge five and eight weeks after seeding with sunnhemp.**

Treatment Seeds/pot	Leafy Spurge <sup>a</sup>	
	5 weeks	8 weeks <sup>b</sup>
	----- (mg dry wt.) -----	
0	1042.5 b	738.7 b
2	368.2 a	188.5 a
4	510.0 a	126.7 a
8	337.0 a	86.0 a

<sup>a</sup>Means in a column followed by the same letter do not differ significantly at the p=0.05 level.

<sup>b</sup>Leafy spurge was severed 1 cm above crown and allowed to grow for 3 weeks after the initial 35-day growing period.

The sunnhemp plants were severed after 35 days to eliminate the possible influence of competition for light. Leafy spurge begins regrowth within 3 days of cutting while the sunnhemp requires 2 weeks before regrowth is visible. The dry weight of the leafy spurge growing with eight sunnhemp was 12% of that of control plants, while that growing with two sunnhemp was about 25% of controls. We suggest that chemicals leached from the seeds of sunnhemp, which from other bioassay data indicates high phytotoxicity, inhibit the growth of leafy spurge and that inhibition continues with the presence of the sunnhemp plant.

Initial results from this ongoing research suggest that sowing of sunnhemp on leafy spurge infestations could be an important factor in the biocontrol and management of this weed. Results also indicate that once identified, the phytotoxic component of the sunnhemp seed could be an important natural herbicide. Although there are phytotoxic compounds in the growth medium and the mycelium of *A. angustiovoidea*, further research is needed to determine the nature of those compounds and whether they are important in disease expression of this fungus on leafy spurge.

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*Sponsored by: Montana Agricultural Experiment Station, Montana State University, Bozeman, MT.*

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## **Picloram translocation to leafy spurge roots over the growing season<sup>1</sup>**

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Picloram applied at 1 to 2 lb ae/A generally provides 70 to 90% leafy spurge control for 18 to 36 months. However, control from picloram can be inconsistent and occasionally has given only 5% or less control 2 months after application even when properly applied at 2 lb/A. The purpose of this research was to evaluate picloram absorption and translocation in leafy spurge over the growing season.

<sup>14</sup>C-picloram was applied to leafy spurge plants in a series of greenhouse and field experiments. The data from 204 plants in 11 experimental trials were combined to produce an overview of the absorption and distribution of picloram in leafy spurge following foliar application. The amount of picloram found in various plant sections was averaged and was converted from percent of applied <sup>14</sup>C-picloram found in each plant section to dollars per acre (\$/A) based on a \$20/A treatment.

For the field study, roots of leafy spurge accession 1984 ND 001 were divided and planted into pots in July of 1983 or 1984. The pots were buried in the soil to a depth of 7 inches and allowed to grow until the following growing season. <sup>14</sup>C-picloram was applied weekly from mid-May until mid-October in 1984 or 1985 and the plants were harvested 72 hours after treatment. <sup>14</sup>C-picloram translocation to the roots (12 inches deep) was determined and is presented as dpm/gm.

Only \$7.40 of chemical from a \$20/A treatment is absorbed into leafy spurge (Figure 1). Of this, \$6 remains in the stems and leaves and only \$1.40 is translocated to the roots. Approximately 60% of the picloram reaching the roots leaks into the soil leaving only \$0.60 of the original \$20/A treatment to control leafy spurge roots.

Long-term leafy spurge control with picloram is best when applied when the true flower and seeds are developing or in early to mid-September after the stems have developed fall regrowth (Figure 2). Maximum <sup>14</sup>C-picloram translocation to the roots occurred during the flowering and seed-set growth stages with nearly five times as much picloram reaching the roots at this time compared to any other growth stage (Figure 3). It is impor-

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<sup>1</sup> Summary of a poster presented during the field tour portion of the meeting.

tant to apply picloram during this growth stage to maximize the cost-effectiveness of the treatment. The increased control obtained from fall-applied compared to spring- or mid-summer-applied treatments must be due to factors other than  $^{14}\text{C}$ -picloram translocation to the roots since there was no increase in translocation to the roots in the fall. Perhaps fall-applied treatments disrupt carbohydrate translocation to the roots and thus decrease water hardiness.

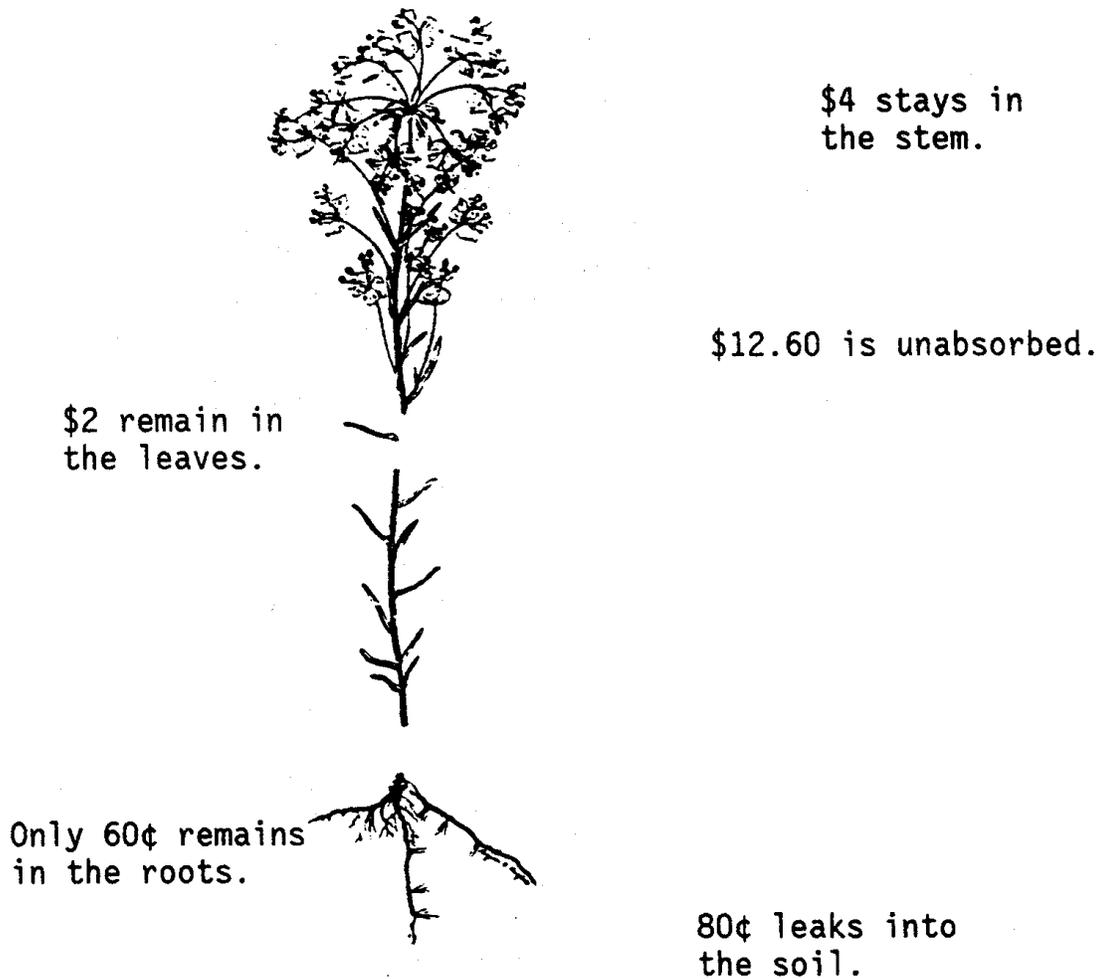


Figure 1. Distribution of picloram in leafy spurge following a \$20/A foliar application.

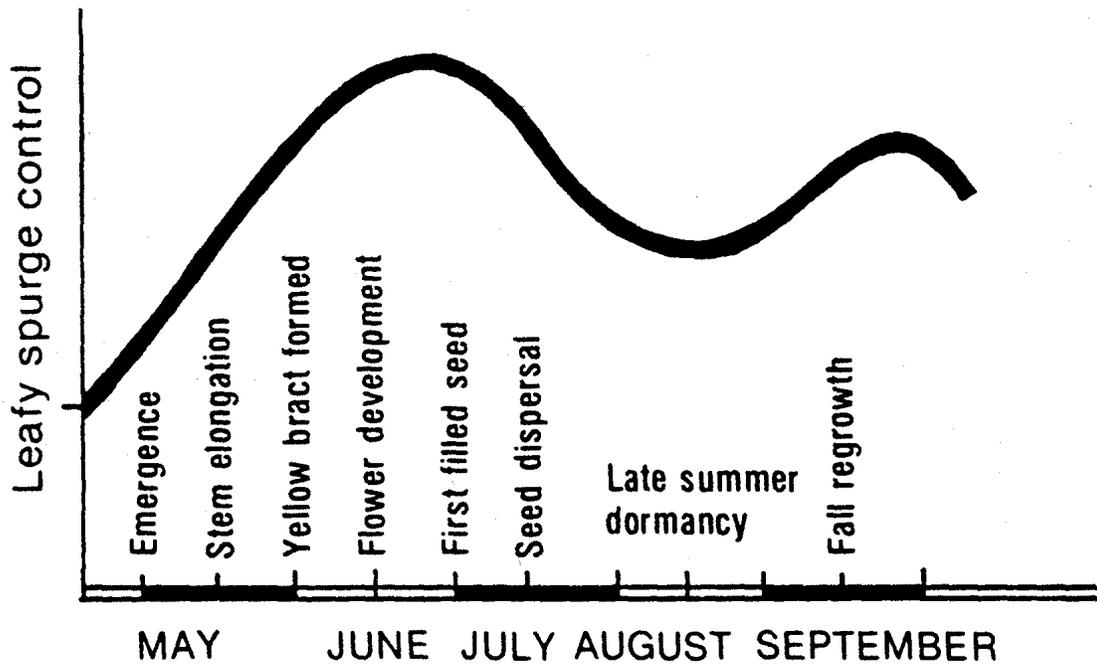


Figure 2. Susceptibility of leafy spurge to picloram applied at different times during the growing season.

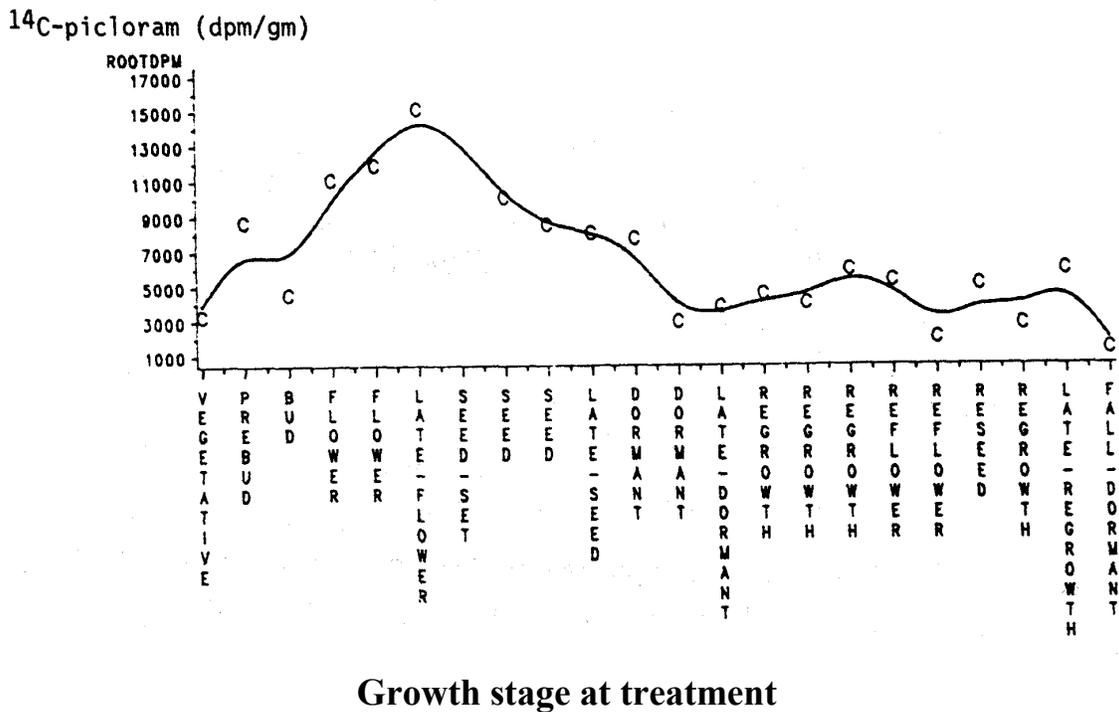


Figure 3. Picloram translocation to leafy spurge roots.

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## **Leafy spurge control in North Dakota - 1989**

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Evaluation of sulfometuron alone and applied with auxin herbicides, of mixtures of various auxin herbicides and of spray additives with picloram for leafy spurge control have been the primary emphases of the field program in 1989. Fluroxypyr amine formulations, BAS-514 and various glyphosate plus 2,4-D combinations are also being evaluated for leafy spurge control.

A GPC-14 regional screening trial of sulfometuron applied alone and with various herbicides in the spring and fall was planned by 14 cooperators following the 1987 meeting in Fargo (Table 1). The experiment was begun in 1988, which was a hot, dry year region-wide but especially in Minnesota and North Dakota. Picloram at 16 oz/A provided the best control of the spring-applied treatments; averaging 91% 3 MAT (Table 2). Sulfometuron plus 2,4-D at 3 plus 16 oz/A and sulfometuron plus picloram at 1.5 plus 8 oz/A averaged 71 and 69% control, respectively. Sulfometuron at 3 oz/A averaged 24% grass injury but one location reported 78%. An identical experiment was established in the fall of 1988. Initial evaluation indicated good control with all treatments but grass injury was also very high (80 to 100%) with all sulfometuron treatments (data not shown).

An experiment to evaluate leafy spurge control from sulfometuron plus auxin herbicides applied annually spring or fall was established on very mature leafy spurge stands near Chaffee and Dickinson, ND. Treatments were applied in spring (June) or fall (late-August to early September) in 1986, 1987, and 1988 (Table 3). Leafy spurge was in the true flower or fall regrowth stages when the original treatments were applied but was in the vegetative growth stage when the treatments were reapplied the following growing season.

Leafy spurge control averaged 49 and 90% at Chaffee and Dickinson, respectively, following three spring applications of sulfometuron plus an auxin herbicide (Table 3). Sulfometuron at 1 or 2 oz/A alone resulted in only 11% control after three applications averaged over both locations. Control following the spring treatment was greatest with picloram or dicamba applied with sulfometuron and there was little grass injury.

Leafy spurge control was higher when sulfometuron was applied annually in the fall compared to the spring (Table 2). Control in the fall averaged 98% following three applications of sulfometuron plus picloram – or sulfometuron plus dicamba. Sulfometuron

alone or applied with 2,4-D generally provided poor leafy spurge control. Grass injury also was higher following fall compared to spring sulfometuron applications and averaged 92%.

Sulfometuron applied with picloram or dicamba in the fall has provided better leafy spurge control than either auxin herbicide applied alone at similar rates. Grass was injured severely when sulfometuron was fall applied but did recover by the following growing season in 1987 and 1988. Sulfometuron in combination with an auxin herbicide could be used in a leafy spurge management program to provide initial control but probably should not be applied annually because of the potential for grass injury. Previous research at North Dakota State University has shown an annual treatment of picloram plus 2,4-D for two years following the sulfometuron treatment provided nearly 100% leafy spurge control and the grass species recovered (data not shown).

Picloram plus 2,4-D at 0.25 to 0.5 plus 1 lb/A has become the primary treatment in the leafy spurge control program in North Dakota. The herbicides applied together provide 20 to 40% better leafy spurge control than either herbicide applied alone. The addition of dicamba to this tank mixture or a specific type of 2,4-D formulation may also increase leafy spurge control. Thus, the effect of dicamba and/or various 2,4-D formulations applied with picloram on leafy spurge control was evaluated in a three-year experiment (Table 4). Treatments were applied annually in the spring or fall in 1986, 1987, and 1988.

In general, leafy spurge control was similar with all 2,4-D formulations in combination with picloram and dicamba (Table 4). However, the 2,4-D mixed amine formulation occasionally did provide better short-term leafy spurge control in a combination treatment than the alkanolamine or ester formulations. Leafy spurge control with picloram improved by adding dicamba in the fall treatments at Dickinson only.

Various additives applied with picloram and picloram plus 2,4-D are being evaluated for leafy spurge control. Many of the commonly used additives were phytotoxic to leafy spurge and apparently decreased rather than increased absorption. Compounds that appeared to increase picloram activity (absorption) in the greenhouse experiments are being field tested in 1989 (Table 5). Initial evaluations indicate the additives are also increasing picloram activity in the field.

Fluroxypyr ester has shown limited phytotoxicity on leafy spurge. The ester formulation may cause too rapid leaf kill for optimum herbicide absorption and translocation. Two fluroxypyr amine formulations XRM-5196 (diisopropylamine) and XRM-5195 (triisopropylamine) are being field tested in 1989. Visual observations 7 DAT indicated 70 to 80% less phytotoxicity to the leaf with the amine compared to the ester formulation.

BAS-514 (Facet) is an auxin-like herbicide with soil residual activity. BAS-514 showed similar phytotoxicity to leafy spurge as picloram plus 2,4-D in greenhouse evaluations and is being field-tested in 1989. Glyphosate plus 2,4-D has provided good leafy spurge control when applied in late July and August.

**Table 1. Cooperators, state, and application data for 1988 GPC-14 regional study.**

Cooperator	State	Code	Estab- lished	Air temp.	Relative humidity	Cloud cover	Soil				Spray water pH	Plot size
							Temp 1-2 in.	pH	Organic matter (%)	Sand- silt-clay (%)		
George Beck	CO	01	8 June	72 (F)	45 (%)	30%	54	7.5	4.0	29-32-39	7.9	10x30
Peter Fay	MT	02	22 June	80	70	40%	58	—	—	—	—	10x30
Robert Callihan	ID	03	—	—	—	—	—	—	—	—	—	—
Ann Henson	CO	04	7 June	88	—	—	72	—	—	—	—	10x40
Rod Warner	MT	05	—	—	—	—	—	7.2	3.4	22-48-30	—	—
Galen Schroeder	ND	06	—	—	—	—	—	—	—	—	—	—
Tom Whitson	WY	07	14 May	—	—	—	—	6.8	1.8	22-58-20	7.2	10x36
Ron Frank	NO	08	15 June	—	—	—	—	—	—	—	—	—
Mark Peterson	SD	09	17 June	88	51	Clear	87	—	—	—	—	16x40
Deane Finnerty	MN	10	30 May	71	45	Clear	60	6.8	2.1	Sandy Loam	—	20x25
Tim Chicoine	SD	11	15 June	68	62	Clear	48	6.1	4.1	44-44-12	—	15x40
David Vos	SD	12	3 June	80	68	Clear	65	7.9	4.2	16-45-39	—	10x30
Bob Stougaard	NE	13	1 June	—	—	—	—	5.8	1.7	Valentine sand 97%	—	15x50
Jack Evans	UT	14	—	—	—	—	—	—	—	—	—	—
Roger Becker	14N	15	7 June	88	50	Clear	82	6.9	4.7	Sandy Loam	—	15x30
Rod Lym	ND	16	2 June	89	42	Clear	82	6.7	9.4	43-41-16	7.8	15x50
Gus Foster	CO	17	—	—	—	—	—	—	—	—	—	—

**Table 1 (Continued).**

Code	Evaluation date			Leafy spurge		Grass species present
	1 MAT	3 MAT	12 MAT	Growth stage	Height (in)	
01	7 July	8 Sept		Flower	—	Downy brome, few grass spp present
02	11 July	—		Mid-flower	—	Timothy and Kentucky bluegrass both headed
03	—	—		—	—	
04	8 July	8 Aug		Flower		
05	15 Aug	—		—	—	
06	—	—		—	—	
07	—	—		—	—	Island bluegrass and intermediate wheatgrass
08	8 Aug	—		Flower	—	Variety of native spp.
09	28 July	7 Sept		Seed set	36	Mostly smooth brome
10	1 July	25 Sept		Flower	18	Smooth brome and Kentucky bluegrass
11	28 July	—		30% flower	12	Bluegrass spp.
12	—	—		Flower	—	Brome grass
13	7 July	7 Sept		Flower	—	Kentucky bluegrass, prairie junegrass <sup>b</sup> Smooth brome
14	—	—		—	—	
15	25 July	21 Sept		Late-flower	24	Bluegrass, brome grass, all severe drought stress
16	18 July	23 Aug		Flower	24	Bluegrass spp., western wheatgrass
17	—	—		—	—	

<sup>a</sup>Prairie sandreed, little bluestem and sand bluestem also present.

**Table 2. Leafy spurge control and grass injury 1 and 3 months after treatment, GPC-14 regional summary, November 1988.**

State	Code	Sulfometuron 1.5		Sulfometuron 3		Sulf. + 2,4-D 1.5 + 16		Sulf. + 2,4-D 3 + 16		Sulf. + Pic. 1.5 + 8		Sulf. + Dic. 1.5 + 32	
		Control	Inj	Control	Inj	Control	Inj	Control	Inj	Control	Inj	Control	Inj
%													
1 MAT													
CO	01	3	—	3	—	85	—	85	—	8	—	17	—
MT	02	0	0	0	0	53	0	53	0	22	0	12	—
ID	03	—	—	—	—	—	—	—	—	—	—	—	—
CO	04	28	21	62	34	95	33	95	33	87	22	78	28
MT	05	15	0	10	0	77	0	97	0	53	0	63	0
ND	06	—	—	—	—	—	—	—	—	—	—	—	—
WY	07	—	—	—	—	—	—	—	—	—	—	—	—
ND	08	13	0	27	0	93	0	95	0	81	0	68	0
SD	09	0	3	0	12	88	3	88	7	57	3	70	7
MN	10	8	5	10	7	92	10	77	10	53	5	38	5
SD	11	7	0	7	0	93	3	98	8	65	7	73	10
SD	12	40	3	53	7	92	27	93	37	89	23	85	20
NE	13	22	19	28	19	83	21	88	17	82	12	58	13
UT	14	—	—	—	—	—	—	—	—	—	—	—	—
MN	15	18	3	28	27	99	12	99	31	78	8	79	38
ND	16	5	0	5	0	75	0	93	7	49	0	13	0
CO	17	—	—	—	—	—	—	—	—	—	—	—	—
Trt.	mean	13	5	19	9	85	9	88	13	60	7	55	10
3 MAT													
CO	01	13	—	37	—	82	—	93	—	65	—	42	—
MT	02 <sup>a</sup>	—	—	—	—	—	—	—	—	—	—	—	—
ID	03	—	—	—	—	—	—	—	—	—	—	—	—
CO	04	7	10	13	5	89	27	85	23	92	17	47	20
MT	05	—	—	—	—	—	—	—	—	—	—	—	—
ND	06	—	—	—	—	—	—	—	—	—	—	—	—
WY	07	10	0	12	0	17	0	13	0	33	0	15	0

State	Code	Sulfometuron 1.5		Sulfometuron 3		Sulf. + 2,4-D 1.5 + 16		Sulf. + 2,4-D 3 + 16		Sulf. + Pic. 1.5 + 8		Sulf. + Dic. 1.5 + 32	
		Control	Inj	Control	Inj	Control	Inj	Control	Inj	Control	Inj	Control	Inj
%													
Experiment over sprayed and lost following initial evaluation.							Table is continued on the following pages.						
ND	08	—	—	—	—	—	—	—	—	—	—	—	—
SD	09	0	3	18	5	33	17	84	27	70	15	13	10
MN	10	20	0	17	47	50	0	33	37	63	25	50	35
SD	11	—	—	—	—	—	—	—	—	—	—	—	—
SD	12	40	7	43	7	96	27	94	37	90	15	82	27
NE	13	10	18	23	35	12	16	50	19	53	9	47	25
UT	14	—	—	—	—	—	—	—	—	—	—	—	—
MN	15	38	38	46	78	65	57	84	86	75	65	61	83
ND	16	13	11	10	34	40	10	75	48	65	24	15	13
CO	17	—	—	—	—	—	—	—	—	—	—	—	—
Trt.	mean	17	10	25	24	57	17	71	31	69	18	44	24
<b>I MAT</b>													
CO	01	83	—	10	—	32	—	13	—	9	—	31	—
MT	02	65	0	13	0	25	0	5	0	14	NS	25	0
ID	03	—	—	—	—	—	—	—	—	—	—	—	—
CO	04	82	0	80	0	88	4	77	0	12	15	70	16
MT	05	93	0	57	0	80	0	77	0	27	NS	97	0
ND	06	—	—	—	—	—	—	—	—	—	—	—	—
WY	07	—	—	—	—	—	—	—	—	—	—	—	—
ND	08	62	0	88	0	97	0	67	0	24	NS	63	0
SD	09	88	0	59	5	94	20	37	7	42	10	53	6
MN	10	88	7	37	22	63	18	35	8	25	12	46	9
SD	11	80	3	83	0	93	0	65	5	15	8	60	3
SD	12	90	13	81	10	88	23	87	23	14	13	73	17
NE	13	75	0	83	0	85	0	78	3	22	9	62	10
UT	14	—	—	—	—	—	—	—	—	—	—	—	—
MN	15	93	40	54	7	84	25	64	43	17	33	63	21
ND	16	29	0	44	0	97	0	20	0	19	NS	39	0
CO	17	—	—	—	—	—	—	—	—	—	—	—	—
		—	—	—	—	—	—	—	—	—	—	[61	[41

State	Code	Sulfometuron 1.5		Sulfometuron 3		Sulf. + 2,4-D 1.5 + 16		Sulf. + 2,4-D 3 + 16		Sulf. + Pic. 1.5 + 8		Sulf. + Dic. 1.5 + 32	
		Control	Inj	Control	Inj	Control	Inj	Control	Inj	Control	Inj	Control	Inj
Trt.	mean	77	5	57	4	77	8	52	7	6	4	—	—
%													
3 MAT													
CO	01	18	—	72	—	90	—	45	—	19	—	51	—
MT	02 <sup>a</sup>	—	—	—	—	—	—	—	—	—	—	—	—
ID	03	—	—	—	—	—	—	—	—	—	—	—	—
CO	04	70	0	81	0	97	0	50	0	24	12	57	9
MT	05	—	—	—	—	—	—	—	—	—	—	—	—
ND	06	—	—	—	—	—	—	—	—	—	—	—	—
WY	07	8	0	33	0	80	0	10	0	14	NS	21	0
ND	08	—	—	—	—	—	—	—	—	—	—	—	—
SD	09	22	0	40	0	80	12	17	0	28	NS	34	8
MN	10	30	0	48	8	86	0	22	3	26	23	38	14
SD	11	—	—	—	—	—	—	—	—	—	—	—	—
SD	12	90	13	84	10	88	27	81	30	12	12	72	18
NE	13	7	0	35	7	99	0	25	2	29	15	35	12
UT	14	—	—	—	—	—	—	—	—	—	—	—	—
MN	15	51	10	68	0	97	0	29	0	20	28	56	37
ND	16	0	0	50	8	96	0	9	0	24	22	34	14
CO	17	—	—	—	—	—	—	—	—	—	—	—	—
Trt.	mean	36	2	60	4	91	4	36	4	7	6	[61	[51

<sup>a</sup>Experiment over sprayed and lost following initial evaluation.

**Table 3. Leafy spurge control with sulfometuron applied annually either alone and in combination with dicamba, picloram, and 2,4-D applied in the spring and fall at two locations in North Dakota.**

Treatment and application date <sup>a</sup>	Rate (oz/A)	Chaffee June 1989 Grass		Dickinson June 1989 Grass	
		Control	injury	Control	injury
		(%)			
Spring					
Sulfometuron	1	0	53	18	25
Sulfometuron	2	5	78	20	21
Sulfometuron + picloram	1 + 8	70	44	63	25
Sulfometuron + dicamba	1 + 32	50	39	58	16
Sulfometuron + 2,4-D	1 + 16	28	40	28	16
Fall					
Sulfometuron	1	22	94	39	88
Sulfometuron	2	49	99	57	92
Sulfometuron + picloram	1 + 8	98	99	98	100
Sulfometuron + dicamba	1 + 32	95	98	95	95
Sulfometuron + 2,4-D	1 + 16	53	99	81	56
Picloram	32	99	50	100	44
LSD (0.05)		22	34	22	25

<sup>a</sup>Applied annually in 1986, 1987, and 1988.

**Table 4. Leafy spurge control with picloram applied with dicamba and various formulations of 2,4-D applied annually since 1986 for leafy spurge control.**

Application date/ treatment	Rate (lb/A)	Location/1989 evaluation date		
		Valley City June	Dickinson June	Sheyenne June
		(%) control		
Spring				
2,4-D mixed amine <sup>a</sup> + dicamba + picloram	2 + 1 + 0.25	63	43	—
2,4-D mixed amine <sup>a</sup> + dicamba + picloram	2 + 0.5 + 0.25	68	44	—
2,4-D mixed amine <sup>a</sup> + picloram + dicamba	1 + 0.5 + 0.12	55	37	—
2,4-D alkanolamine+ dicamba + picloram	2 + 1 + 0.25	54	56	—
Dicamba + picloram	1 + 0.25	68	44	—
LSD (0.05)		NS	NS	
Fall				
2,4-D mixed amine <sup>a</sup> + dicamba + picloram	2 + 1 + 0.25	91	73	98
2,4-D alkanolamine+ dicamba + picloram	2 + 1 + 0.25	81	—	98

Table 4 continued on following page.

Application date/ treatment	Rate (lb/A)	Location/1989 evaluation date		
		Valley City June	Dickinson June	Sheyenne June
2,4-D mixed amine <sup>a</sup> + dicamba + picloram	4 + 2 + 0.5	98	97	99
2,4-D ester <sup>b</sup> + 2,4-DP +picloram +dicamba	2 + 2 + 0.5 + 0.25	94	43	98
2,4-D ester <sup>b</sup> + 2,4-DP +picloram +dicamba	2 + 2 + 0.5 + 0.5	98	86	99
2,4-D alkanolamine+ dicamba + picloram	4 + 2 + 0.5	99	90	99
Dicamba + picloram	2 + 0.5	98	96	99
Picloram	0.5	97	59	98
LSD (0.05)		16	21	NS

<sup>a</sup>Mixed amine salts of 2,4-D (2:1 dimethylamine: diethanolamine)-EH 736.

<sup>b</sup>2,4-D isooctyl ester:2,4-DP butoxyethanol ester: dicamba (4:4:1)-EH 680.

**Table 5. Spray additives field-tested with picloram and picloram plus 2,4-D in 1989.**

Compound	Manufacturer	Remarks
Soybean oil + Atplus 300F	ICI	Only veg. oil not phytotoxic to leafy spurge
Emulpher ON-877	GAF	Polyoxy ethylated fatty alcohol
GAFAC RA-600	GAF	Emulsifier detergent, lowers pH
GAFAC RS-710	GAF	Free acid of phosphate ester complex
MAPEG 400 MOT	Mazer	Monotallate
MAPEG 200 MOT	Mazer	Monotallate
MAPEG 400 DO	Mazer	Dioleate
X-77 + NH <sub>4</sub> SO <sub>4</sub>	Chevron	Various free fatty acids
Silwett L-77	Union Carbide	Silicone copolymer
Igepal CO-530	GAF	Nonionic coupling agent
Triton CS7	Rohm and Haas	Blended surfactant
SCI-40	Sorber	Lowers pH
LI-700	Loveland	Lowers pH
Inhance	MCA Labs	Blended surfactants + fertilizer

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# Control of leafy spurge with picloram and fluroxypyr

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## Abstract:

Leafy spurge (*Euphorbia esula* L.) control with picloram (4-amino-3,5,6-trichloropicolinic acid) and fluroxypyr (4-amino-3,5-dichloro-6-fluro-2-pyridyloxyacetic acid) was evaluated at a site near Carrington, ND. Initial treatments were fluroxypyr at 0.25 and 0.5 lb ae/A, picloram at 0.5 lb ae/A and an untreated check. Retreatments included fluroxypyr at 0.125 and 0.25 lb ae/A; picloram at 0.25 lb ae/A; and combinations of picloram at 0.125 and 0.25 lb ae/A with either fluroxypyr at 0.25 lb ae/A, fluroxypyr at 0.125 lb ae/A or 2,4-D (2,4-dichloro-phenoxyacetic acid) ester at 1.0 lb ae/A. Treatments containing fluroxypyr gave a very rapid control of leafy spurge top growth. However, considerable regrowth occurred in the fall following spring applications of the initial treatments. By June of 1988 control with any treatment was 25% or less. Control in 1988 and 1989 after application of retreatments followed a pattern similar to that noticed with the original treatments. However, the combination of picloram at 0.25 ae/A with fluroxypyr at 0.25 lb ae/A provided control which was significantly better than that achieved with other retreatments; yet this retreatment still only achieved 26% control 11 months after treatment when averaged across the initial treatments. The poor control of leafy spurge in this study may be in part due to the extremely hot and dry conditions, which occurred during application, especially at the time of retreatment.

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## Introduction

Fluroxypyr is a non-phenoxy growth regulator herbicide which is currently under development by the Dow Chemical Company in the United States as a methyl-heptyl ester formulation. It is active on a number of broadleaf species while having little effect on

grasses. The objectives of this study were to examine the potential of fluroxypyr to reduce leafy spurge infestations and to see whether fluroxypyr could be an effective "set-up" treatment or tank-mix partner for picloram.

## Materials and methods

The study was established on a non-cropland site near Carrington, ND. The experimental design was split block with three replications. Initial treatments consisting of fluroxypyr at 0.25 and 0.5 lb ae/A, picloram at 0.5 lb ae/A and an untreated check were applied July 7, 1987 when leafy spurge plants had set seed. Retreatments consisting of fluroxypyr at 0.125 and 0.25 lb ae/A, picloram at 0.25 lb ae/A, picloram at 0.125 lb ae/A plus fluroxypyr at 0.125 and 0.25 lb ae/A, and picloram at 0.25 lb ae/A in combination with either fluroxypyr at 0.25 lb ae/A or 2,4-D (2,4-dichloro-phenoxyacetic acid) ester at 1.0 lb ae/A were applied perpendicularly to the initial treatments on July 6, 1988, after seed set. All treatments were applied with a CO<sub>2</sub> backpack sprayer set at a pressure of 28 psi with TeeJet 8002 nozzles to deliver a spray volume of 15 gpa at 3.0 mph. Leafy spurge stand reduction was evaluated visually on a 0 to 100 scale with 0 = no stand reduction and 100 = complete stand reduction as compared to the untreated check.

## Results and discussion

Treatments containing fluroxypyr gave a very rapid control of leafy spurge top growth (data not shown). However, considerable regrowth occurred in the fall following spring applications of the initial treatments. As is shown in Table 1, regrowth of leafy spurge in fluroxypyr-treated plots had reduced control to 40% or less. Control with picloram at that time was 60%. By June of 1988 control with any treatment was 25% or less. Normally, 0.5 lb ae/A of picloram would provide better control than that achieved in this case. This may be due to the fact that the treatments were applied at seed set, which is past the optimum timing for picloram application. Control after application of retreatments followed a pattern similar to that noticed with the original treatments. Leafy spurge control 2.5 months after application of the retreatments was 30 to 50% for most treatments (Table 2). However, control with the combination of picloram at 0.25 ae/A with fluroxypyr at 0.25 lb ae/A was superior to that achieved with other retreatments. Control with this retreatment averaged across initial treatments was 69%. Yet this retreatment still only achieved 26% control 11 months after treatment when averaged across the initial treatments (Table 3). The poor control of leafy spurge in this study may be in part due to the extremely hot and dry conditions, which occurred during application, especially at the time of retreatment. There was no significant difference between the initial treatments in terms of retreatment performance, although control was generally lower where no initial treatment was applied.

In summary, leafy spurge top growth is rapidly controlled by application of fluroxypyr. However, this rapid action may limit translocation to crowns and roots since substantial regrowth usually occurs shortly after treatment. Fluroxypyr was not better than picloram as a "set-up" in a retreatment program. In this study, tank-mixes of piclo-

ram and fluroxypyr were more effective than mixes of picloram and 2,4-D ester when picloram was applied at 0.25 lb ae/A.

**Table 1. Leafy spurge control from initial fluroxypyr and picloram treatments.**

Treatment	Percent Control	
	9/30/87	6/16/88
Fluroxypyr 0.25	40	7
Fluroxypyr 0.5	33	20
Picloram 0.5	60	25
Untreated	0	0
LSD (.05)	11	24

**Table 2. Leafy spurge control 2.5 months after application of retreatments. Carrington, ND.**

Retreatment		Initial Treatment			
		Fluroxypyr 0.25	Fluroxypyr 0.5	Picloram 0.5	Untreated
Fluroxypyr	0.125	32	33	30	20
Fluroxypyr	0.25	27	31	30	10
Picloram	0.25	25	30	34	26
Picloram	0.125+	32	38	54	12
Fluroxypyr	0.125				
Picloram	0.125+	38	43	40	10
Fluroxypyr	0.25				
Picloram	0.25+	65	68	75	43
Fluroxypyr	0.25				
Picloram	0.25+	42	48	53	37
2,4-D ester	1.0				
Untreated		12	10	22	0
LSD (.05) = 22					

**Table 3. Leafy spurge control 11 months after application of retreatments. Carrington, ND.**

Retreatment		Initial Treatment			
		Fluroxypyr 0.25	Fluroxypyr 0.5	Picloram 0.5	Untreated
Fluroxypyr	0.125	7	12	5	0
Fluroxypyr	0.25	3	3	0	0
Picloram	0.25	3	3	10	0
Picloram	0.125+	3	7	15	0
Fluroxypyr	0.125				
Picloram	0.125+	3	7	10	0
Fluroxypyr	0.25				
Picloram	0.25+	22	17	40	5
Fluroxypyr	0.25				
Picloram	0.25+	0	7	10	0
2,4-D ester	1.0				
Untreated		0	0	0	0
LSD (.05) = 14					

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## **Effects of combined herbicides and various seeded grass species on leafy spurge**

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Single herbicide applications do not provide long-term leafy spurge control. This study was conducted near Sundance, WY to determine long-term effects provided by combinations of herbicides and grass species competition. Before seeding perennial grasses, two applications of glyphosate at 0.75 lb ai/A were broadcast applied with a truck-mounted sprayer delivering 15 gpa at 35 psi on June 2, 1986, temperature: air 69°F, soil surface 65°F, 1 inch 64°F, 4 inches 63°F with 58% relative humidity and calm wind. A post-emergence broadcast application of pentimethalin at 2.0 and 0.5 ai/A were applied May 16, 1988, temperature: air 73°F, 1 inch 68°F, 2 inches 67°F, 4 inches 64°F with 64% relative humidity and wind 2 to 3 mph NW, with a tractor-mounted sprayer applying 20 gpa at 35 psi. Plots were arranged as a split plot, 60 by 9 feet, with four replications, one-half of the plot tilled, the other half left untilled. Tilling was done with a roto-tiller on August 11, 1986, and grasses were seeded into a silt loam soil (22% sand, 58% silt, 20% clay with 1.8% organic matter and a 6.3 pH) with a powertill drill August 12, 1986.

All herbicide treatments combined with tillage treatments resulted in greater grass establishment with greater production per acre than the untilled areas (Table 1). Pubescent wheatgrass and big bluegrass were the only two grasses to establish adequately on areas without tillage. Mountain rye and bluebunch wheatgrass were the only two grasses failing to establish adequately in tilled areas. Leafy spurge control levels above 88% in no-tilled areas were found in plots seeded with pubescent wheatgrass and big bluegrass. Leafy spurge control was less than 83% in tilled areas seeded to mountain rye and bluebunch wheatgrass, the other nine grasses had control greater than 91%.

**Table 1. Control of leafy spurge using glyphosate, competition from various grass species and two tillage practices.**

Grass Species (Variety)	% Grass established Tilled	% Grass established No-tilled	% Leafy spurge control Tilled	% Leafy spurge control No-tilled	Lbs. grass (D.M./Acre)	Lbs. grass (D.M./Acre) No-tilled
Pubescent wheatgrass (Luna) <sup>3</sup>	90 <sup>4</sup>	70	97	84	572	274
Crested wheatgrass (Ephraim)	83	55	95	79	474	218
Mountain Rye	18	05	79	58	368	224
Big bluegrass (Sherman)	74	79	96	89	594	336
Hybrid wheatgrass (RS1)	74	13	94	60	518	142
Smooth bromegrass (Lincoln)	80	18	92	68	294	152
Intermediate wheatgrass (Oahe)	71	16	97	68	652	152
Bluebunch wheatgrass (Secar)	64	15	83	64	194	128
Western wheatgrass (Rosana)	76	26	91	65	464	174
Russian wildrye (Bozoisky)	83	30	97	63	552	160
Thickspike wheatgrass (Critana)	81	29	.94	70	484	210

<sup>1</sup>Research/demonstration conducted by: T. D. Whitson, U. W. Extension Weed Specialist, D. W. Koch, U. W. Extension Agronomist and A. E. Gade, U. W. Crook Co. Agricultural Agent, M. E. Ferrell, U. W. Extension Pesticide Specialist.

<sup>2</sup>Study location: U. W. leafy spurge research area, Crook Co., WY.

<sup>3</sup>Grasses were seeded August 12, 1986.

<sup>4</sup>Evaluated September 14, 1988.

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*Sponsored by: Montana Agricultural Experiment Station, Montana State University, Bozeman, MT.*

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## **Burning as a tool in the management of leafy spurge**

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In the Fall of 1988, Western Conservation Services, Inc. (WCS) began to implement controlled burning of leafy spurge prior to the application of Tordon 22K. Tordon 22K was applied to the soil surface after a burn and prior to rain.

Our burning objectives were to remove the living and dead vegetative material (shadow effect) which prevents the Tordon 22K from getting to the soil surface. Additional benefits are:

- 1) allows for a consistent “blanket” of herbicide to be applied and eliminates “shadow effect”;
- 2) the application equipment can move across a field much easier after a burn;
- 3) the burned area is a very visible announcement that a leafy spurge control project is underway; and
- 4) follow-up management is made easier by the removal of non-target vegetation, i.e., brush, shrubs, etc.

In the last five years, WCS has successfully used prescribed burning to enhance our control efforts on spotted knapweed, common tansy, cheat grass and other undesirable types of vegetation. “Cowboy” analysis of our control efforts lead us to believe that the burning enhanced our overall control objectives.

In September and October of 1988, WCS implemented three leafy spurge prescribed burns in three different areas of Gallatin County. These leafy spurge infestations were from less than one acre to over 50 acres in size. Project areas were chosen to represent various conditions, such as slope, aspect, spurge density, native grass cover, etc.

Prescribed burning of leafy spurge requires a great deal of control work prior to the main burn. Leafy spurge is the hottest burning of any plant material that we have worked with in this area. Two factors affect the intensity of the burn: the sheer amount of dead plant material found in these spurge patches, and the plant latex sap. The intensity of the burn is a benefit, in that, there is very little vegetation left on the site to interfere with the equipment applying the herbicides.

After burning, Tordon 22K was applied to the soil surface at approximately 112 lb/acre, prior to or during rain, to wash the Tordon 22K into the soil profile. Our intention was, that by locking the Tordon 22K into the soil, a “cap” would be created that would prevent the leafy spurge from growing through. The results have shown that any spurge that does get through the “cap”, does so where the herbicide concentration is weak due to misapplication.

This fall (1989), WCS will continue to implement prescribed burning on approximately 140 acres of leafy spurge-infested ground. We feel that controlled prescribed burning is an enhancement tool in the management of leafy spurge. Burning alone will do little good; herbicides alone have less than a perfect track record. The combination of the two steps gives us better initial suppression, and longer term control of this, the most difficult to control noxious weed.

We recommend extreme caution when using prescribed burning. If control of a fire is lost, you will probably do more harm to your leafy spurge control efforts than you gain in the long run. Additional information about the use of prescribed burning can be obtained from WCS.

### **Update, Fall 1989 (Grandview Subdivision):**

The overall control of the leafy spurge in areas that were burned in the fall of 1988 and treated with Tordon 22K at 1 qt/acre was significant (60-90%). Areas that did not receive Tordon 22K treatment after burning showed no significant setback of the spurge. The spurge plants that did manage to emerge through the Tordon 22K "cap", were small, spindly and did not produce seeds. A large percentage of these plants dried up earlier in the fall than untreated plants. The grass has responded well to the reduced competition and has begun to fill in the base spots.

Fall management included burning those areas that had heavy plant regrowth. The entire project area was then treated with Tordon 22K at 1 qt/acre on October 14-18, 1989. In the future, 2,4-D will be used to manage any regrowth.

### **Update, Fall 1989 (Brass Lantern Estates):**

WCS completed the burning of an additional 100 acres of leafy spurge infestation, northeast of Bozeman. This area is just below the “M” at the south end of the Bridger Mountains.

Prior to the initiation of this burn, no management steps had been taken to control the spurge on this land since it was first established in the 1940's. Consequently, the spurge was very thick, and we were concerned that no amount of water would carry the Tordon 22K to the soil surface. The actual burning took place September 18 and 19. Again, the heat intensity of the fire was very hot and gave us a clean burn with very little vegetation left standing. Within two weeks of the burn, “greenup” of the desirable grasses had begun, with most regrowth occurring in the timothy, brome and Kentucky blue grasses. Orchard grass seems to be set back more by burning than these other grasses. Idaho fescue

was found to be the only grass that survived in the heaviest spurge infestations. This grass also seems to respond to the elimination of the spurge competition.

In addition to the usual afterburning application of Tordon 22K at 1 qt/acre, this year we will utilize new information out of the University of Wyoming. Dr. Tom Whitson, Extension Weed Specialist with the University of Wyoming, Laramie, has been using Roundup to control spurge followed by reseeding with a variety of perennial grasses. We are setting aside approximately 20 acres of the burned-off ground and will let the spurge regrow next spring. In the spring of 1990, we will implement the Roundup/reseeding technique to see if it offers an alternative to the use of Tordon in the areas where the spurge competition has eliminated most of the desirable grasses.

In conclusion, I believe that burning of leafy spurge prior to the application of Tordon 22K is beneficial to the long-term control of this plant. Burning is beneficial because it:

- 1) removes plant material that interferes with a consistent amount of the herbicide getting to the ground;
- 2) releases nutrients that are utilized by desirable grasses;
- 3) allows equipment to work more efficiently;
- 4) makes follow-up work easier; and
- 5) is a very visible announcement that something is being done.

From the standpoint of community support, this alone may be doing more to help with our overall control efforts than anything else.

Western Conservation Services, Inc. would like to thank the property owners of the Grandview Subdivision for their individual support, advice and criticism; the Gallatin County Weed Board for their encouragement; Mr. Gene Surber, Gallatin County Extension Agent, for his guidance; the Montana Noxious Weed Trust Fund for their 1989 financial support; and Mr. Reeves Petroff, Gallatin County Weed Supervisor, for his patience, his willingness to try something new and his determination to "never give up."

*Reprinted with permission from: 1989 Leafy Spurge Symposium. Bozeman, MT. July 12-13, 1989. pp. 76-80.*

*Published by: Montana Agricultural Experiment Station, Montana State University, Bozeman, MT.*

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# **Leafy spurge control: Reflections on 17 years of research**

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## **Introduction**

My specific involvement with leafy spurge began in 1972 when I established a research project for perennial weed control with emphasis on leafy spurge. Leafy spurge was first reported in the northern Great Plains in the early 1900's; 1909 in North Dakota. As I reviewed the literature, it was evident that plant scientists as early as the 1930's recognized that leafy spurge probably would become a serious weed problem. Reports in the 1930's on leafy spurge biology and control were by H. C. Hanson and V. E. Rudd in North Dakota and A. L. Bakke in Iowa and on grazing by sheep by E. A. Helgeson in North Dakota.

Following World War II, the most extensive research was by Canadians in the 1950's and 1960's; the names R. T. Coupland, J. F. Alex, G. W. Selleck, and M.V.S. Raju were especially common. In the United States, L. A. Derscheid in South Dakota and M. K. McCarty in Nebraska had modest programs for leafy spurge control. Many people recognized the importance of leafy spurge control and "bootlegged" research, i.e., conducted a limited number of leafy spurge control experiments as an adjunct to their specified research responsibilities. The results of most of these experiments were reported only in annual research reports in their state or in a regional publication.

My first knowledge of cooperative political action came in 1978 when I spoke at the Montana State Weed Control Conference and learned of their efforts to obtain legislative support for leafy spurge control. Near the same time, I learned of the Wyoming Leafy Spurge Control Act passed in 1978 that provided state funding for leafy spurge control.

## **Cooperative regional program**

### **Ad hoc program**

The current coordinated regional research effort began with the Leafy Spurge Symposium, June 26 and 27, 1979, Bismarck, ND. The innovator was Dan McIntyre, Supervisor, Custer National Forest, U.S. Forest Service, Billings, MT. He visited H. Ronald Lund, Director of the North Dakota Agricultural Experiment Station, Fargo, and the outcome of the discussion was establishment of a steering committee to conduct a symposium in Bismarck; Edwin H. Amend, Associate Director of the North Dakota Cooperative Extension Service, was chairman. About 125 educators, scientists, land managers, farmers, ranchers, legislators, and concerned citizens attended the symposium. A follow-up meeting, the Northern Regional Leafy Spurge Conference, was held in Billings, MT, on December 17-18, 1979, with a similar total attendance as the Bismarck symposium.

The administrators of several key agencies, e.g., Directors of the Agricultural Experiments Stations, Area Directors for the USDA-ARS, Supervisors for the U.S. Forest Service formed an ad hoc committee to sustain the momentum for enhanced leafy spurge control. These people made some personnel and funding changes within their own administrative units to support the effort. They appointed a Regional Leafy Spurge Working Committee, Russ Lorenz, USDA-ARS, Mandan, ND, chairman, as a group of research and extension scientists to develop a plan of action.

### **Permanent program**

One outcome of the plan was approval by the Great Plains Agricultural Council of a research committee, GPC-14 Leafy Spurge Control in the Great Plains, as a recognized organization to facilitate program coordination. The first GPC-14 meeting was held in June 1981, Fargo, ND, and annual meetings have been held since then.

1982-Bozeman, MT

1983-Sundance, WY

1984-Dickinson, ND

1985-Bozeman, MT

1986-Riverton, WY

1987-Fargo, ND

1988-Rapid City, SD

1989-Bozeman, MT

### **Enhanced funding**

An immediate objective adopted at the Bismarck symposium was to submit a request to the Old West Regional Commission for research funding. A cooperative project of the Agricultural Experiment Stations of all five states, Montana, North Dakota, Nebraska,

South Dakota, and Wyoming, with North Dakota as the lead state, was funded from March 1981 through February 1982. To provide continuity, the USDA-ARS, through the Metabolism and Radiation Research Laboratory in Fargo, ND, established separate cooperative agreements with Montana, North Dakota, and Wyoming that provided funding for various durations during 1981-1985.

Grant funds supported most of the initial research. However, the major boost to the program was through redirection and enhancement of research efforts by the Agricultural Experiment Stations and by the USDA, initially by the ARS and in the last couple years by APHIS. To use North Dakota as the example, enhancement occurred when Director H. R. Lund immediately committed \$100,000 at the Bismarck symposium to fund a non-tenure research associate position that subsequently was adopted by the 1983 Legislature as a tenure-track position, and redirection occurred when a position in the Entomology Department was converted to biocontrol of leafy spurge. Several similar examples could be cited, especially in the Montana Agricultural Experiment Station and the USDA-ARS and APHIS.

An early cooperative effort was the Leafy Spurge News, a newsletter initially edited and published by the Montana Agricultural Experiment Station. Publication began in April 1980, and there have been 3 or 4 issues per year with up to 1200 recipients per issue since then. Editors have been Clare Barreto, Bruce Maxwell, and Celestine Lacey of Montana State University and currently by Dr. Russ Lorenz, North Dakota State University.

## **Chemical control**

Herbicides have been the backbone of control efforts to date, because they are the most available and effective developed technology. However, many refinements have been made in the past 10 years. For example, the paper on chemical control presented at the 1979 symposium refers to “light rates” of 2,4-D as 2 to 6 lb/A and “heavy rates” as 20 to 40 lb/A, and picloram was used frequently at 2 lb/A. Now, picloram usually is applied at 0.25 to 0.5 lb/A in combination with 2,4-D at 1 to 2 lb/A. Also, we understand that the most effective time of treatment is during true flower development with a secondary peak for control during the fall when leafy spurge has established regrowth.

Among other herbicides, dicamba has provided better results in the Intermountain states than further east but is less effective than picloram. Glyphosate can be used under trees, on cropland, and near water, but may cause too much injury to be acceptable on grazing land. Many other herbicides have been evaluated; especially fluroxypyr, sulfometuron, fosamine, triclopyr, and clopyralid, but none have provided control comparable to the older herbicides.

Several other generalizations about herbicide use have been developed in the past 10 years. Wipe-on applicators, e.g., roller and pipe-wick, can be used to apply picloram to leafy spurge, but control generally is not improved over a broadcast application of picloram plus 2,4-D. Withdrawal of the granular formulation of picloram from the market meant loss of one tool for leafy spurge control, especially for spot treatment of small (usually new) patches of leafy spurge. Herbicides generally provide longer-term control

in drier areas but more grass injury occurs also. Awareness of adverse effects of herbicides, especially of picloram, on the environment has increased, so they are being applied at lower rates and with more care. Despite the advances, most herbicide treatments for leafy spurge control are not economical.

## Cultural control

Options for cultural control of leafy spurge are limited. Leafy spurge is occurring more frequently on tilled land now due to reduced tillage. Mowing and burning haven't been effective for reducing leafy spurge, except they may result in uniform regrowth that can be treated more timely with herbicides. Nitrogen fertilization in combination with herbicide treatment has not resulted in improved control. There may be differences in competitive ability of forage species with leafy spurge, but they will not eliminate the weed.

Sheep and goats can be considered as a means of cultural control. The cases where sheep or goats are an economical alternative to raising cattle or to using other control methods are limited, but they can be used to fill special niches.

## Biocontrol with insects

Insects for biocontrol have been considered a viable research goal for many years. The spurge hawk moth (*Hyles euphorbiae*) was released as early as 1966 and 1973 in Gallatin Co., Montana. Spurge hawk moth introductions frequently have not survived, and when they do, they provide too little control too late in the growing season. A root borer, *Oberea erythrocephala*, was released in 1979 in Canada and in 1980 in Wyoming and Oregon. Additional releases of this insect have since been made in several other states. However, establishment at the release sites has been inconsistent, and no demonstrable impact by this insect has yet been realized on leafy spurge.

Through increased research, primarily by Agriculture Canada and the USDA-ARS, several insects have been screened and approved for release on leafy spurge. For example, two flea beetles, *Aphthona flava* and *A. cyparissias*, were released in Saskatchewan in 1982 and in Montana in 1985. A gall midge, *Bayeria capitigena*, was released in Montana in 1985. Releases of several other insects and establishment of many more release sites have been reported at this meeting. This currently is the most rapidly growing area of research activity.

## Biocontrol with diseases

One native disease, *Alternaria tenuissima* f. sp. *euphorbiae*, has shown the most virulence on leafy spurge. It effectively controls leafy spurge when infection occurs, but environmental conditions are not favorable over a broad area to provide effective control. Other species, especially *Melampsora* spp. and *Uromyces striatus*, have been evaluated

as potential biocontrol candidates, but effective strains that can be reproduced effectively have not been identified. At this point, better organisms are needed before diseases can contribute to leafy spurge biocontrol.

## **Plant physiology and basic research**

The general morphology, anatomy, and ecology of leafy spurge has been studied fairly extensively. Although many unanswered questions remain, the knowledge base in these areas probably are not the limiting factors to developing better weed control programs. Conversely, the basic understanding of leafy spurge physiology and genetics is limited.

Taxonomic studies indicate that leafy spurge is a genetically diverse species. Although some taxonomists divide this plant complex into several species, most scientists believe it is one species, *Euphorbia esula* that is diverse. Studies of chemotaxonomy, allelopathy, and natural product chemistry indicate this diversity exists, but the role of these compounds in leafy spurge physiology or how to use specific chemical characteristics to improve leafy spurge control remains largely unknown.

Physiological characteristics of the roots and of latex are not well understood. Picloram and 2,4-D are released rapidly from roots. The carbohydrate content of roots fluctuates rapidly from day to day; for example, soluble carbohydrate content of roots changes within hours and varies inversely with temperature. Starch storage in the latex is irreversible.

Physiological characteristics regulating bud growth and survival are not well understood. Crown buds that begin growth in the fall stop development apparently when exposed to light. Crown buds and root buds differ in susceptibility to freezing temperatures. Whether bud growth can be altered to increase winterkill is not known.

## **Miscellaneous observations**

### **Benefits of leafy spurge**

Latex with its high hydrocarbon content has been evaluated as an alternate source either for fuel or rubber. Neither alternative is of high enough quality to be economically viable at this time. Honey from leafy spurge is of low quality for human preferences. However, it is desirable for bees, because it is an early season food source and it does not granulate easily which is desirable for over winter-feeding. As somewhat “tongue in cheek” benefits, many of us are employed to provide leafy spurge control and sometimes leafy spurge competition is less detrimental to survival of native plant species than overgrazing by cattle.

## **Miscellaneous projects**

Unique attempts at control have included using high voltage to electrocute plants or paired rollers to pull plants. Neither alternative was effective.

## **Public awareness**

Many meetings, newspaper articles, extension bulletins, radio and television reports have been presented to the public. Leafy spurge control was the impetus that led to formation of the North Dakota Weed Control Association and has been a goal of similar associations in Montana, Wyoming, and probably other states. All of these efforts have led to state and federal legislative action including financial support for some new research positions and buildings in the agricultural experiment stations and the USDA, cost-sharing for chemical control in several states, and projects like USDA-APHIS programs to establish insectaries to expedite redistribution of biocontrol agents.

Overall there probably is as much public awareness of leafy spurge as any other weed, although we all recognize that more must be done. Because so many people in North Dakota are aware of the adverse impact of leafy spurge, they responded immediately when they were informed of the possible adverse impact of spotted knapweed in the state.

## **Future for leafy spurge control**

In the next 5 to 10 years, herbicides will remain the backbone of the leafy spurge control program. Any improvements in the efficacy of control with herbicides will be small, although there may be advances either in minimizing adverse environmental impacts or reducing the cost of control.

Biocontrol with insects includes many promising leads, so a widespread distribution program and localized visible success should be accomplished in the next 10 years. A breakthrough in using diseases for biocontrol apparently is not imminent; perhaps diseases as biocontrol agents can advance in 10 years to the point where insects are today.

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*Sponsored by: Montana Agricultural Experiment Station, Montana State University, Bozeman, MT.*

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## **A realistic approach to managing leafy spurge, or: Toto, I don't think were in Kansas anymore**

REEVES PETROFF

*Supervisor, Gallatin County Weed Control District, Bozeman, MT 59715.*

Besides spotted knapweed, leafy spurge is an extremely troublesome noxious weed in Gallatin County. Herbicides have been the mainstay of our control program with good results in some areas and poor results in others. Generally, infestations greater than two years old defy treatment with herbicides. What we are currently developing is an integrated, realistic approach to leafy spurge control. We realize that spurge is here to stay. We just have to manage it to a certain level of infestation and learn to live with it.

Successful “control” of leafy spurge is seldom achieved with a one-shot control effort. Variety of terrain and environmental constraints where the spurge grows are factors in deciding which control method we attempt to use. We have opted for no control in some areas and intensive chemical control in others and a combination of techniques for the “in-between” spots. The many different aspects that go into developing a successful management program include:

1. Adopting a realistic approach to spurge control. Look at the big picture rather than just a part. Education can be used effectively when adopting a multi-faceted approach to spurge management.

2. Being aware of the proper stage of growth most susceptible to control measures used (herbicides, tillage, sheep, goats, biological control, etc).

3. Recognizing the levels of control that are possible and how they are achieved. (i.e., eradication, top-kill only, reduction of vigor or competitive ability, prevention of formation of reproductive structures, damaging underground structures, no control).

4. Knowing when and how to evaluate the level of control or management (e.g., shoot kill vs. root kill, amount of burn down achieved, grass injury, long term control).

5. Understanding the probable effect of individual control measures and management practices on leafy spurge.

6. Tie it all together and be willing to accept or develop an integrated, multi-step control program. You will probably come under fire for adopting this approach. Many people will see you as ineffective and slow. Obviously, these people have never dealt with leafy spurge. Stick to your guns.

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## Phytosterol content of leafy spurge roots and cells from suspension cultures

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### Abstract:

Neonate larvae of *Aphthona flava* were fed on either cells obtained from a suspension culture or on intact roots of leafy spurge. Root-fed larvae grew and molted to the 2nd instar within 2 weeks. Cellfed larvae grew equivalently to that of root-fed larvae, but failed to molt and eventually died after 25-30 days of feeding. We are investigating if the failure to molt is a result of qualitative or quantitative deficiencies in the supply of phytosterols in the cells.

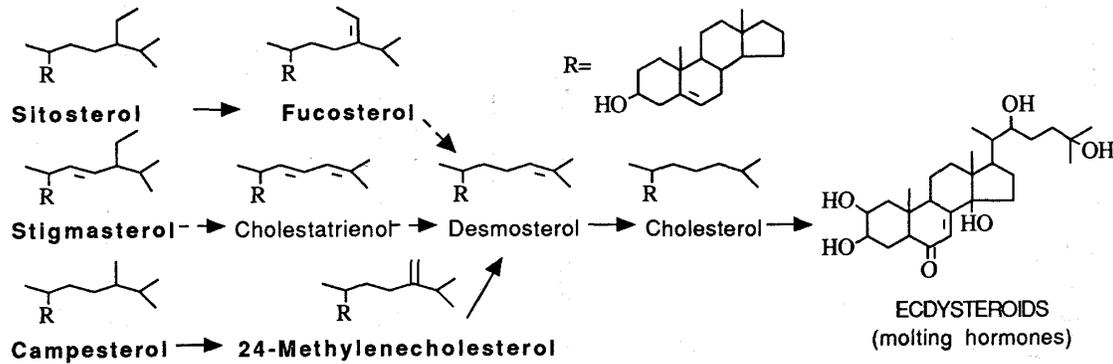
The roots contained two major phytosterols, campesterol and  $\beta$ -sitosterol, and minor amounts of stigmasterol and isofucosterol. The cells contain 4 major sterols, 24methylenecholesterol, campesterol,  $\beta$ -sitosterol and isofucosterol, and minor amounts of 3 unidentified sterols.

The cells possess an adequate supply of phytosterols to fulfill the sterol-requisites of most phytophagous insects. Further studies are in progress to characterize the sterol metabolism of *Aphthona larvae* and to determine if the cells possess an inhibitor of sterol metabolism.

### Molting and sterols

Insects are incapable of the *de novo* biosynthesis of sterols (Bloch, *et al.*, 1956). Sterols are required by insects as components of cellular membranes, for reproduction and as precursors for the biosynthesis of molting hormones (*viz.*, ecdysteroids) (Karlson and Hoffmeister, 1963). Most studies to date have shown that phytophagous insects synthesize ecdysteroids from the C27 sterol, cholesterol, through a number of hydroxylating and reducing steps. Cholesterol, in turn, is derived from C28 and C29 phytosterols by dealky-

lation at the C24 position of the side-chain (Figure 1) (Svoboda and Thompson, 1983). These phytosterols must be procured by these insects from their respective host-plants. Conversely, inadequate procurement of phytosterols and/or problems in their bioconversion to ecdysteroids may lead to the abrogation of molting.



**Figure 1. Metabolic pathway used by most insects for the conversion of C<sub>28</sub> and C<sub>29</sub> phytosterols (bold print) to cholesterol and eventually to ecdysteroids.**

## Diet for *Aphthona* larvae

### Artificial formulations and roots

The flea beetle, *Aphthona flava*, and its congenitors, show promise as biological control agents of leafy spurge (Maw, 1981). Over the past two years, we have been attempting to formulate an artificial diet for the mass-rearing of these insects. Attempts to rear *Aphthona* larvae on over 10 different diets formulated for various other chrysomelids (Singh, 1976) have all failed (Manners, *et al.*, 1988). Addition of various chemical extracts of leafy spurge to these diets to stimulate feeding by *Aphthona* larvae were ineffective (Manners, *et al.*, 1988). In general, *Aphthona* larvae failed to feed on any of these diets and the larvae usually died within 1 or 2 days.

We have successfully reared *Aphthona* larvae to the pupal stage on freshly chopped roots of leafy spurge. This was achieved if the roots were either placed on filter paper or suspended in agar (Manners, *et al.*, 1988). However, this method for the mass-rearing of *Aphthona* has inherent drawbacks. Firstly, expansive greenhouse facilities are required to produce enough root material. Secondly, the roots must be harvested and processed. Lastly, in order to find the larvae, the roots must be ground or chopped. Once the roots are ground or chopped, they degrade very quickly, within days, and fresh root material must be replaced continuously.

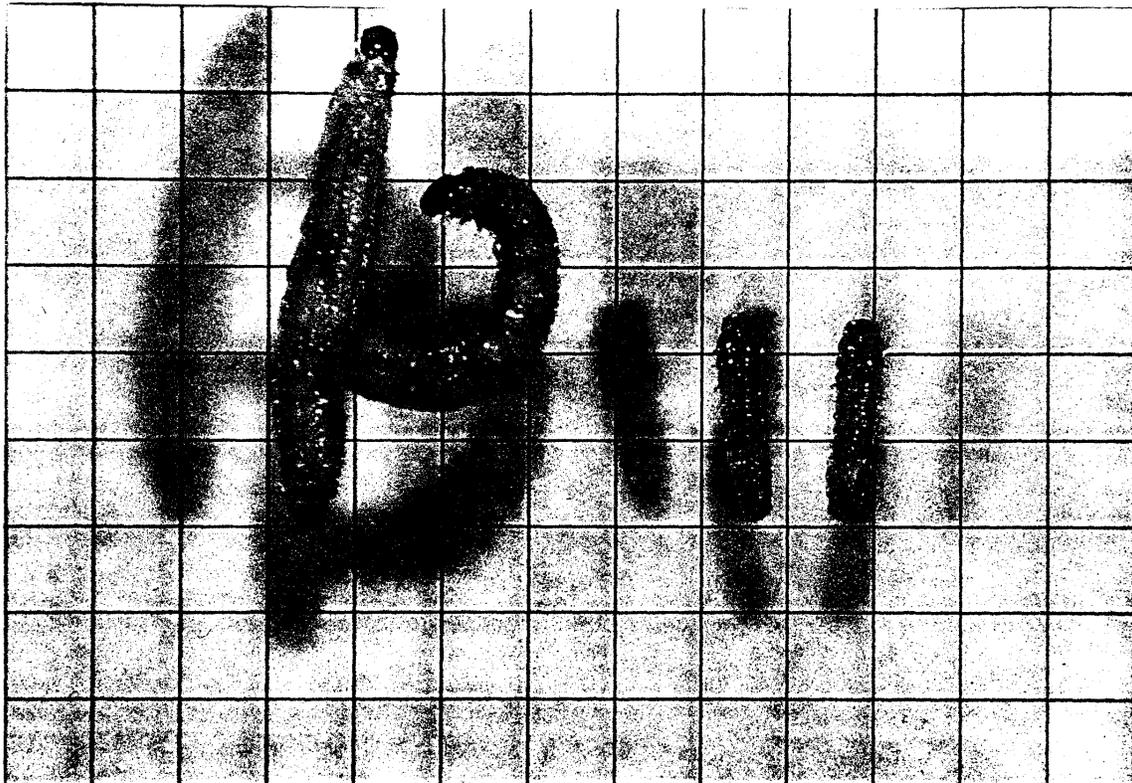
### Suspension culture cells

Over the past year, we have been researching the possibility of mass-rearing *Aphthona* larvae on leafy spurge cells grown in a suspension culture (Hogan and Manners,

1989). This approach, if successful, shows great promise mainly from the standpoint that a virtually unlimited supply of diet material will be readily available and simple to harvest.

Unlike the other diet formulations tested, *Aphthona* larvae feed and grow on the suspension culture cells of leafy spurge. Moreover, the larvae can be sustained on these cells for almost a month. Whereas, larvae placed on the other diet formulations die within 1-2 days (Hogan and Manners, 1989).

Although the larvae feed and grow on these cells, they fail to molt to the 2nd instar. The larvae grow from neonates of .1 mm in length to > 3 times that length. Head capsule width, however, remains at  $\approx 160\mu\text{m}$  throughout this growth period (Figure 2). Examination of hundreds of larvae fed on suspension culture cells failed to show any larvae with a head capsule width equivalent to that of 2nd instar larvae (i.e.,  $\approx 320\mu\text{m}$ ) even after 3-4 weeks of growth (Hogan and Manners, 1989). This lack in head capsule width is accentuated by the fact that overall length and size of these larvae exceeded that of 2nd instars.



**Figure 2.** Photograph showing the overall growth of *Aphthona* larvae reared on suspension culture cells. The larvae on the left are 28 days old, those on the right are 8 days old. Note that head capsule width is the same between both groups while overall body length has increase >3X in the 27-day-old group (0.5 mm grid).

## Ecdysteroids and molting

We are currently investigating if there are nutritional deficiencies in the suspension culture cells. Nutritional inadequacy of an insect diet may explain the failure of *Apthona* larvae to molt when fed on these cells. One possibility we are investing is that this failure to molt may involve complications in normal sterol metabolism and/or ecdysteroid biosynthesis.

Most insects synthesize a prohormone form of ecdysteroid in the prothoracic gland. During normal insect development, the synthesis of the prohormone is activated approximately a week prior to molting (Redfern and Bownes, 1985). This synthesis is generally reflected by elevated titers of a more hydroxylated form of the ecdysteroid in the hemolymph 5-6 days before molting (Figure 3). The occurrence of this hormone in the hemolymph triggers a cascade of physiological events that eventually results in ecdysis (Smith, 1985).

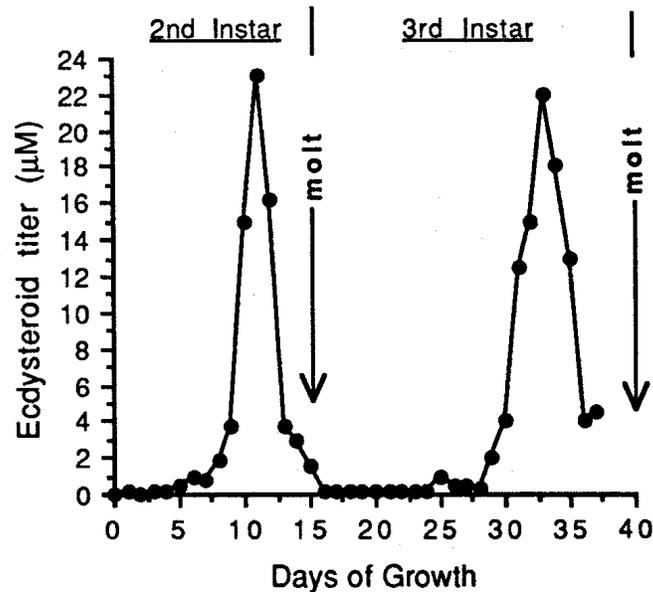


Figure 3. Titer of ecdysteroids in the hemolymph of a representative insect during larval development. Note that peak titers occur 5-6 days before molting (adapted from Smith, 1985).

## Phytosterols in leafy spurge

### Composition of roots vs. cells

The phytosterol content of leafy spurge cells, grown in suspension culture, was compared to that of roots from greenhouse grown plants. Undifferentiated parenchymal cells of leafy spurge were grown as outlined by Hogan and Manners, 1989. Cells were con-

tinuously harvested, freeze-dried and stored at  $-20^{\circ}\text{C}$  until extraction. The methods used for extraction, partial purification and identification of phytosterols were similar to those described by Campbell and Nes (1983). Briefly, subsamples ( $\approx 1$  g) of freeze-dried cells or roots were extracted with chloroform by Soxhlet for 4 hours. The extract was dried *in vacuo* and redissolved in a small volume of benzene. A  $100\ \mu\text{l}$  aliquot was streaked onto a silica gel TLC plate. The plate was developed in benzene:diethyl ether (9:1; v:v). The region of the TLC plate which cochromatographed with free cholesterol was scraped from the plate, eluted with diethyl ether, filtered and redissolved with  $100\ \mu\text{l}$  benzene.  $5\alpha$ -cholestane ( $1\ \mu\text{g/ml}$ ) was added as an internal standard.

Sterols were preliminary identified by capillary gas-chromatography. Underivatized samples ( $0.2\ \mu\text{l}$ ) were applied to 30m, J + W DB-1 and DB-5 columns (13:1 split). Relative retention times to cholestane of unknown peaks were compared to that of standard sterols (Figure 4). Identity of the sterols was later confirmed by gas chromatography/mass spectroscopy (GC-MS).

**Table 1. Relative percent phytosterol composition of leafy spurge cells and roots.**

Phytosterol	Cells	Relative Percent Roots
24-Meihylenecholesterol	7	tr*
Campesterol	23	15
Stigmasterol	tr	1
Unknown (500 mw)	3	tr
$\beta$ -Sitosterol	44	79
Isofucosterol	18	5
Unknown (414 mw)	5	tr

\*tr - trace or undetectable amounts

The phytosterol compositions and relative percents of each sterol in the cells and roots are outlined in Table 1. In general, the phytosterol composition of leafy spurge roots was notably simple. Almost 80% of the sterol in the roots consists of the common phytosterol,  $\beta$ -sitosterol. Another common phytosterol, campesterol, was present at 15%. The remaining sterols found in the roots were isofucosterol (5%) and a minor amount of stigmasterol (1%).

The phytosterol profile of the cells, conversely, was markedly more intricate than that of the roots. The roots featured essentially one major sterol,  $\beta$ -sitosterol. The cells, however, possessed 3 major phytosterols. These were  $\beta$ -sitosterol (44%) and 2 other prominent phytosterol, campesterol (23%) and isofucosterol (18%). In addition to these, the cells also contained 3 minor sterols that were either absent or present in trace quantities in the roots. These minor sterols included 24-methylene cholesterol and two other unknown sterols. The structures of these unknown sterols are being determined. They have molecular weights of 400D and 414D according to molecular ions detected by mass-spectroscopy.

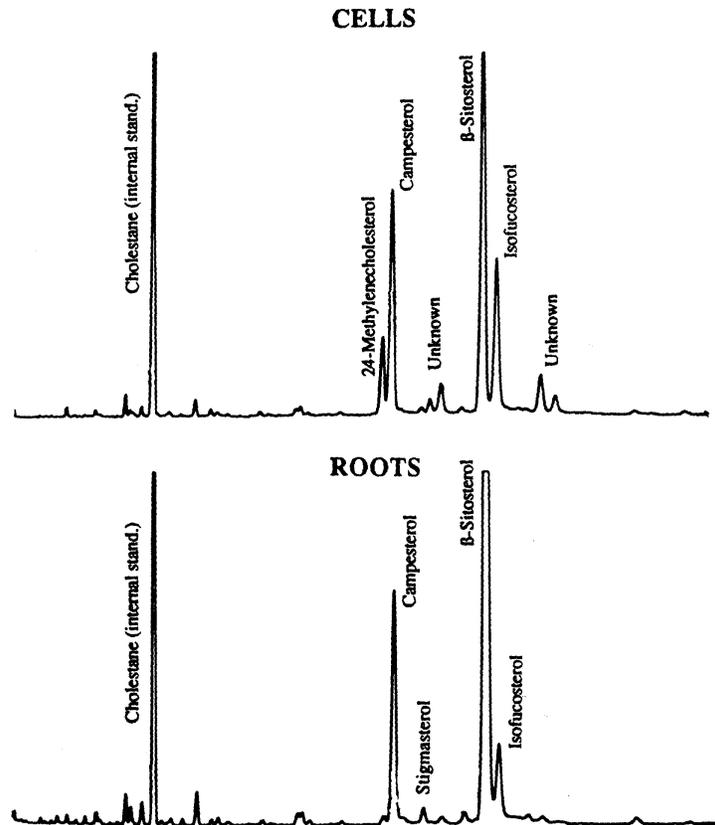


Figure 4. Gas chromatograms of phytosterols in leafy spurge cells (top) and roots (bottom). Sterols were identified by GC-MS. Cholestane was used as the internal standard.

## Impact on growth of *Aphthona* larvae

The phytosterol composition of intact leafy spurge roots compared to that of cells grown in suspension culture is obviously different. Does this different composition of phytosterols in the cells account for the lack of molting by *Aphthona* larvae fed on these cells? Without the results of metabolic studies, currently in progress, no certain answer can be provided.

However, the major phytosterols identified in the cells (i.e.,  $\beta$ -sitosterol, campesterol, isofuco sterol and 24-methylenecholesterol) can generally be converted to cholesterol by insects (Figure 1). It is also likely that *Aphthona* larvae can dealkylate these phytosterols and convert them to cholesterol. Otherwise, how would these larvae be able to survive on leafy spurge roots whose chief sterol is  $\beta$ -sitosterol?

The possibility exists that one or more of the unknown sterols in the cells, or perhaps some other components, are inhibiting sterol metabolism of the larvae. The dealkylation of phytosterols to desmosterol is necessary for their bioconversion to cholesterol by insects. Moreover, there are a number of known substances, both manmade and natural,

which inhibit the ability of insects to convert desmosterol to cholesterol (Svoboda, 1984). However, the prospect that sterol metabolism of *Aphthona* is disrupted by some component in the cells can only be speculated at this time.

Currently, we are attempting to answer some of the questions associated with sterol metabolism and lack of molting by *Aphthona* larvae fed on cells. Some of the experiments which are currently in progress include: 1) rearing larvae through one instar on roots and transferring them onto cells (the larvae could procure any essential precursors required for molting from the roots), 2) treating larvae and cells with 20-hydroxyecdysone in an effort to trigger molting, and 3) examining the sterol compositions of *Aphthona* larvae fed on roots and cells to determine if the amount of cholesterol in these larvae is different (this would indicate if a component in the cells is disrupting sterol metabolism).

There is a possibility that a nutritional component other than sterols is affecting larval growth of *Aphthona* fed on the cells (e.g., certain essential fatty acids). We will pursue this possibility if our current research on sterols does not ascertain the basis for the lack of larval molting.

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Sponsored by: Montana Agricultural Experiment Station, Montana State University, Bozeman, MT.

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## Organogenesis of *Euphorbia esula* L. from hypocotyls

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### Abstract:

Studies of organogenesis from leafy spurge were undertaken to identify tissues or organs that would consistently produce roots and shoots for use in bioassays for herbicides and to determine those factors that influence the regenerative potential.

Regeneration of leafy spurge occurred from all parts of germinated seedlings without the use of exogenous growth regulators on B5 medium (Gamborg *et al.*, 1968) containing 2% sucrose. Isolated whole roots, hypocotyls, and the shoots of germinated seedlings (13 to 19 days old) produced both roots and shoots, with the intact hypocotyl producing five times as many shoots as roots. Segments of hypocotyls also formed shoots more readily than roots, but the reverse was true for the isolated root segments. Short hypocotyl segments (< 7 mm) produced relatively few organs, whereas segments up to 15 mm long formed both organs readily. Treatment with IAA increased root formation greatly, with a threshold value of about 0.04 mg/L (0.23  $\mu$ M). Shoot formation was inhibited at 0.2 mg/L (1.1  $\mu$ M) IAA. Low concentrations of cytokinins had little effect on shoot formation, but inhibited root formation at the higher concentrations used (up to 0.9  $\mu$ M).

Root formation on hypocotyl segments in the presence of exogenous auxin was extensive in B5 medium with the salts and vitamins diluted to 0.1X normal concentration (constant 2% sucrose). At 0.01X B5, roots appeared on nearly 20% of the hypocotyls and less than 10% formed shoots. Omission of phosphate from the B5 medium did not affect root or shoot formation; presumably because of inadvertent phosphate (0.29 mM) already present in the agar used to solidify the medium, as well as reserves in the isolated hypocotyl segments (0.29 ug/mg fresh weight).

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## Introduction

The control of leafy spurge is likely to be a result of a combination of biocontrol methods and the application of specific herbicides that interfere with plant development. Such growth regulators must either translocate long distances from the point of application to other parts of the plant that are capable of regeneration, or induce the plant to synthesize messengers that in turn will initiate the biosynthesis of endogenous inhibitors in other organogenic parts of the plant. Therefore, it is important to learn the potential regenerative capacity of the plant and to understand the factors that promote or inhibit organ formation.

In previous work in this laboratory using leafy spurge cell suspensions (Davis *et al.*, 1988), it was discovered that regeneration of plants from cultures obtained from one specific accession was possible by removing all exogenous growth regulators from the culture medium. Cultures initiated from other accessions were not capable of organogenesis, and even the regenerable accession was unpredictable in its response to various manipulations *in vitro*. A more consistent system for regenerations studies has been established by using isolated hypocotyl segments.

## Materials and methods

Field-collected seeds were used because leafy spurge will not produce seeds in a greenhouse or growth chamber. Seeds were sterilized 2 minutes in 70% (v/v) ethanol followed by 20 minutes in 60% (v/v) bleach (5.25% Ca hypochlorite). Isolated plant parts from dark grown sterile seedlings (13 to 19 days old) were placed onto B5 medium with 2% (w/v) sucrose and 0.7% (w/v) agar in 6 cm plastic petri dishes wrapped with parafilm. Experiments were conducted in darkness (dishes wrapped with aluminum foil) at 26°C. Replicates contained eight hypocotyl segments per dish with five dishes per treatment in each experiment. Visible roots and shoots were recorded with time up to 60 days (earlier experiments) or 28 to 30 days (later experiments). Data were expressed as percentages of the hypocotyl (or root) segments that produced at least one root or shoot visible under a dissecting microscope. Phosphorous was determined spectrophotometrically at 820 nm on ashed samples treated with acidified ascorbic acid-ammonium molybdate reagent (Chen *et al.*, 1956).

## Results

All parts of germinating seedlings produced both roots and shoots without exogenous growth regulators when they were tested as unsegmented parts (i.e., both roots and shoots contained apical meristems). Unsegmented hypocotyls produced numerous shoots (average of 12.1) but only a few roots (average of 2.2). The other two parts of the seedlings (unsegmented roots and cotyledons plus apex) formed about two of each organ per plant part.

One cm segments of roots and hypocotyls (neither of which contained apical meristems) were organogenic without the need for exogenous growth regulators. Both roots and shoots were produced to varying degrees. Hypocotyl segments produced shoots more readily than roots segments whereas; the reverse was true for isolated root segments. Typically, about 60 to 90% (or sometimes greater) of the hypocotyl segments formed shoots and 20 to 40 percent formed roots. Root segments typically formed visible adventitious roots in about 30 to 40% of the segments while 20 to 30% formed shoots.

Hypocotyl segments shorter than about 7 mm produced few roots or shoots and the orientation of the hypocotyl had little influence. One cm segments from the basal, middle or cotyledonary end of the hypocotyls behaved similarly in that approximately the same percentage of all three segments produced both roots and shoots at about the same rate.

Formation of visible roots was stimulated by the addition of IAA to the culture medium, with a threshold concentration of about 0.04 mg/L (0.23  $\mu$ M) (i.e., 0.04 to 0.2 mg/L IAA induced nearly equal numbers of hypocotyl segments to form roots). The threshold concentration had no effect on shoots, but IAA at 0.2 mg/L (1.1  $\mu$ M) inhibited shoot formation.

Visible root formation was inhibited by cytokinins at concentrations of 0.1 mg/L or greater. Shoot formation appeared to be slightly stimulated, but the results were significant ( $p < 0.05$ ) only with the highest concentration of zeatin riboside (0.2 mg/L, 0.56  $\mu$ M).

Root formation on hypocotyl segments was similar in B5 medium containing 0.1 times the normal salt and vitamin concentration as full strength B5 medium. Further dilutions of the salts resulted in formation of fewer roots, but did not totally suppress them. Shoot formation was reduced by a greater amount, but even at 0.01X salt concentration a few shoots were formed. In other experiments organogenesis occurred in the absence of B5 salts and vitamins with 2% sucrose as the only nutrient. In one experiment 48% of hypocotyls produced roots and 3% formed shoots, in the dark.

Reduction of phosphate in the medium had only minor effects on organogenesis. Elimination of P<sub>04</sub> from the medium had no effect on root formation, which remained close to 100% and 30% in the presence and absence of IAA, respectively. Shoot formation was lowest when phosphate was eliminated in the presence of IAA, but in the absence of IAA no effect was observed on shoot formation. However, analysis of the agar used to solidify the medium revealed the presence of approximately 0.29 mM phosphorous. This value is 26% of that contained in full strength B5 medium. The hypocotyl segments also contained an average of 0.2  $\mu$ g of phosphorous per mg fresh weight. There appears to be ample phosphorous for good root and shoot development when all other factors are optimum.

## Discussion

Leafy spurge is an extremely versatile perennial weed capable of regeneration at a fairly high level from a variety of tissues and organs. Apical dominance was evident when unsegmented hypocotyls were isolated and found to develop about five times as

many adventitious shoots as roots. Hypocotyl segments one cm or greater in length formed both shoots and roots when grown on agar medium containing dilute salts and vitamins or sucrose alone. Hypocotyl segments less than 7 mm in length may have had insufficient reserves of nutrients or growth regulators to develop roots or shoots to any great extent. Shoot formation was more sensitive to the reduced nutrient concentrations than were the roots, presumably because shoots are more complex than roots. Roots formed readily on hypocotyl segments cultured at 0.1X the normal B5 salts and vitamins, provided 2% sucrose was available; whereas shoots required higher salt concentration for full expression. The phosphate contained in hypocotyl tissues and added inadvertently with the agar to solidify the medium was sufficient for both root and shoot formation.

The response of leafy spurge hypocotyls to auxin treatment appears to follow the traditional classical pattern in that auxin stimulates root formation and inhibits shoot initiation. Likewise, the response of the hypocotyl segments to exogenous cytokinins was somewhat predictable. Because shoot formation was already quite high, only slight stimulation occurred at higher concentrations of cytokinins. Visible root formation was inhibited by cytokinins, possibly due to reduced growth as much as to reduced root initiation, since the root is, initiated internally and the data reported here is for visible roots.

The formation of organs by leafy spurge tissue may be regulated by the ratio of the auxins to cytokinins as indicated by Skoog and Miller (1957) working with tobacco callus. Since both of these growth regulators occur naturally in higher plants the endogenous levels of both of these classes of compounds must first be established. In experiments with externally applied growth regulators the different solubilities as well as different steric configurations of the cytokinins and their respective relative activities within the tissues must be considered. If the relative rates and interactions of the processes influenced by each of the growth regulators are the sole controlling mechanisms, it will be difficult to design specific herbicides for leafy spurge, or possibly any other, perennial weed because of the lack of sufficient specificity.

This report covers only some of the very basic physiology of leafy spurge hypocotyls. Other studies are being conducted in our laboratory to establish unique features of leafy spurge that can be utilized to ultimately control the weed. Whether that program is successful or not, the hypocotyl system has potential for use as a screening system for herbicides that interfere with organogenesis.

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## Technical abstract

Studies of leafy spurge organogenesis were done to establish a bioassay for herbicides and determine factors that alter regeneration. Regeneration occurred from all parts of germinated seedlings on B5 medium without exogenous growth regulators. Intact hypocotyls produced five times many shoots as roots. Isolated hypocotyl segments formed shoots more readily than roots, but the reverse was true for the isolated root segments. Hypocotyl segments < 7 mm long produced few organs, but segments 8-15 mm long formed both organs readily. IAA increased root formation greatly, with a threshold value of about 0.04 mg/L (0.23  $\mu$ M). Shoot formation was inhibited at 0.2 mg/L (1.1  $\mu$ M) IAA. Cytokinins (up 0.2 mg/L) had little effect on shoot formation, but inhibited root formation. Hypocotyl segments formed roots readily (in the presence of IAA) when the salts and vitamins were diluted to 0.1X normal concentration (constant 2% sucrose). At 0.01X B5, roots appeared on nearly 20% of the hypocotyls but less than 10% formed shoots. Omission of phosphate from the medium did not affect organ formation; presumably due to mobilization of sufficient phosphate reserves from the isolated hypocotyl segments and inadvertent phosphate contained in the agar used to solidify the medium.

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Sponsored by: Montana Agricultural Experiment Station, Montana State University, Bozeman, MT.

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# The impact of introgressive hybridization on the weediness of leafy spurge

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## Abstract:

Root tip cells of *Euphorbia* accessions, collected from three Canadian provinces, fourteen U.S. states, and five European countries were analyzed according to chromosome number and morphology. Extensive chromosome instability was discovered to be caused by introgressive hybridization between species with different ploidy levels. Introgression between diploid *E. virgata* Waldst. & Kit. ( $2n = 20$ ) and *E. cyparissias* L. ( $2n = 20$ ), tetraploid *E. virgata* ( $2n = 40$ ) and *E. cyparissias* L. ( $2n = 40$ ), and hexaploid *E. esula* ( $2n = 60$ ) has led to the establishment of chromosome races in introgressive *E. X pseudovirgata* (Schur) Soó which range in predominant chromosome numbers from  $2n = 46$  to 64. This genetic variability complicates biological and chemical control of leafy spurge and helps explain the variability in vegetative morphology within and among plants of *Euphorbia X pseudovirgata*.

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## Introduction

Long-term chemical control of leafy spurge has so far proven ineffective (Alley and Messersmith, 1985; Messersmith, 1979) and biological control is only in the developmental stages (Harris, 1979). Alley and Messersmith stated that new herbicides for control of leafy spurge are not available at the present time, and that there is little promise of more in the future. Harris (1979) mentioned that the establishment of a biocontrol agent will not automatically reduce the density of leafy spurge. The difficulty is that insects, for instance, may or may not have much impact on the population dynamics of their host plants. Chromosome instability within and among plants may be one of the causes of the present ineffectiveness of leafy spurge control (Schulz-Schaeffer and Gerhardt, 1987, 1989).

Chromosome instability within plants, termed mosaicism, was reported in plants from Fergus County, MT ( $2n = 40$  to  $60$ ), Flathead County, MT ( $2n = 52$  to  $56$ ), Gallatin County, MT ( $2n = 58$  to  $60$ ), and Teton County, MT ( $2n = 62$  to  $65$ ) (Schulz-Schaeffer and Gerhardt, 1987). Mosaicism is frequently found in allopolyploids or segmental allopolyploids like *E. X pseudovirgata* (Schulz-Schaeffer and Gerhardt, 1989). Our morphological studies -of leaf characteristics (Schulz-Schaeffer and Gerhardt, 1987) indicated that genetic material of *E. esula*, *E. virgata*, *E. cyparissias*, and *E. uralensis* Fisch. ex Link may be involved in this complex species group. Variability in vegetative morphology, particularly among leaves, has been noted both within-site and within-plant by Bakke (1936), Dunn and Radcliffe-Smith (1980), Groh (1935), Moore (1958), Radcliffe-Smith (1981), and Harvey *et al.*, (1988). Harvey *et al.*, (1988) have been unable to satisfactorily assign North American field grown *E. X pseudovirgata* plants to a specific taxon, using current identification keys. They found evidence of morphological expression of several apparent nominate taxa to be present at most sites.

Chromosome instability among plants of the same population was found in 62 of 107 leafy spurge accessions (Schulz-Schaeffer and Gerhardt, 1987). This kind of instability is believed to be caused by introgressive hybridization between species of different ploidy levels. Introgressive hybridization of two or more species in weedy leafy spurges has been postulated by Croizat (1945) and Radcliffe-Smith (1985). Dunn and Radcliffe-Smith (1980) adopted the name *Euphorbia X pseudovirgata* (Schur) Soó indicating its interspecific hybrid nature. They have shown how widespread this plant type is in the United States in relation to other members of this species aggregate, bearing out Croizat's contention that this is the aggressively invasive entity which has become naturalized and has spread rapidly in Montana, the Dakotas, Nebraska, Kansas, Minnesota, and Iowa. Similar plant material has subsequently been seen from Wyoming, Colorado, Wisconsin, and Michigan. It is also present in some of the northeastern states like New Hampshire, Massachusetts, New York, and New Jersey, but does not appear to be troublesome there. *E. X pseudovirgata* has also been reported in every province in Canada from British Columbia across to Nova Scotia.

## Materials and methods

A *Euphorbia* collection was established in the greenhouse at Bozeman, Montana, for cytotaxonomic analysis. The material was collected by weed supervisors and research personnel in Colorado, Idaho, Iowa, Maryland, Michigan, Minnesota, Montana, Nebraska, Nevada, New Jersey, North Dakota, Oregon, Washington, Wyoming, Alberta, British Columbia, and Saskatchewan, and in Austria, Hungary, Italy, Switzerland, and Yugoslavia. The collection consisted of 107 accessions of the weedy *Euphorbia* species *E. X pseudovirgata*, *E. esula*, *E. virgata*, *E. cyparissias*, *E. salicifolia* Host, and *E. seguieriana* Neck. A standard leafy spurge numbering system recommended by the GPC-14 committee at the 1984 Leafy Spurge Symposium at Dickinson, North Dakota, was used for all accessions (Davis, 1985). Root tips grown on a root zone heating pad were harvested from potted plants in the greenhouse. Excised root tips were treated in 0.002 M 8-hydroxy-quinoline for 2 hours at room temperature and for 20 hours at  $1^{\circ}\text{C}$ , for 7 minutes in 0.2 N HCl, fixed in Carnoy's, squashed and stained in aceto-orcein (Gurr) or car-

bol-fuchsin, and observed under phase contrast on the microscope to conduct chromosome counts and morphological chromosome studies.

## Results and discussion

### 1. Introgressive hybridization and its effects in weedy leafy spurges.

As stated, chromosome instability in weedy leafy spurges is believed to be caused by introgressive hybridization. Introgressive hybridization is the incorporation of genes of one species into the gene pool of another species by hybridization and backcrossing (Anderson and Hubricht, 1938). Introgressive hybridization can only occur in that part of a geographic range of a species which overlaps the distribution of closely related species, and then only when the habitat provides an ecological niche for the establishment of introgressive types (Stebbins, 1950). If, therefore, the variation pattern of a species is being altered by introgressive hybridization, this pattern should contain more variability in regions where the ranges of two related species overlap than where either species grows alone. Also, this variability should be greater in newly opened and much-disturbed areas than in old, stable habitats. An example of introgressive hybridization in the U.S. is the study by Abel and Austin (1981) of introgressive types of the wild sweet potato species *Ipomoea trichocarpa* Ell. and *I. lacunosa* L. which was based on corolla width and length.

Radcliffe-Smith (1985) reported some signs of introgressive hybridization of *E. esula* with *E. virgata* in populations of *E. X pseudovirgata*. According to him, the European distribution of *E. X pseudovirgata* includes eastern Austria, southern Czechoslovakia, Hungary, Romania, Bulgaria, Yugoslavia, and Poland. He also noted that the European distribution of *E. esula* is essentially the same as that of *E. cyparissias*. It was noticed that two of the three accessions received by us from Europe designated *E. esula* had narrower leaflets than the majority of *E. X pseudovirgata* and *E. esula* accessions (Schulz-Schaeffer and Gerhardt, 1987). Since narrow leaflets are a distinct characteristic of *E. cyparissias*, these accessions probably were derived from *E. esula X E. cyparissias* hybrids. The Italian *E. esula* accession (1982 I 001) had a chromosome number range of  $2n = 48$  to  $51$  which is between  $2n = 40$  for *E. cyparissias* and  $2n = 60$  for *E. esula*. Moore (1958) described an artificial hybrid of this nature with  $2n = 50$  chromosomes which matched natural European hybrids in all significant morphological characteristics. Moore and Frankton (1969) reported three natural *E. esula X E. cyparissias* hybrids from Ontario, Canada, one of which had  $2n = 50$  chromosomes. Another one was analyzed by Parmlee (1962) as  $2n = 50$ . In Europe the *E. esula X E. cyparissias* hybrid has been reported along waterways of northern and eastern Austria (Dörfler in Moore, 1958), Czechoslovakia, eastern Germany (Reichinger, 1902; Hegi, 1930), Hungary (Dörfler in Moore, 1958), and Romania (Schur, 1866).

Introgressive hybridization in weedy leafy spurges obviously has occurred between species of three different ploidy levels. *E. virgata* and *E. cyparissias* are mainly diploids and tetraploids ( $2n = 20, 40$ ). *E. esula* and *E. X pseudovirgata* should be mainly considered to be hexaploids ( $2n = 60$ ). The hexaploids have taken up genetic material from the diploids and tetraploids in order to colonize new disturbed environments. According to

the literature it was formerly thought that the basic chromosome numbers of the weedy leafy spurge group were  $x = 8, 9,$  and  $10$  (Table 1). We believe that the reports of  $2n = 16$  for *E. esula* (Van Loon and DeJong, 1978),  $2n = 36$  for *E. cyparissias* (Zhukova, 1967),  $2n = 56$  for *E. virgata* (Hurusawa and Shimoyama, 1976), and  $2n = 64$  for *E. esula* (Gadella and Kliphuis, 1968) are aneuploid chromosome numbers based on  $x = 10$  rather than euploid multiples of  $x = 8$  and  $x = 9$  (Table 1).

**Table 1. Chromosome numbers of the weedy leafy spurge complex as reported in the literature (for references, see Schulz-Schaeffer and Gerhardt, 1987, 1989).**

Ploidy level	$x = 8$	$x = 9$	$x = 10$
Diploid	$2n = 16$ <i>E. esula</i>		$2n = 20$ <i>E. virgata</i> <i>E. cyparissias</i>
Tetraploid		$2n = 36$ <i>E. cyparissias</i>	$2n = 40$ <i>E. virgata</i> <i>E. cyparissias</i>
Pentaploid			$2n = 50$ <i>E. cyparissias</i> X
Hexaploid			$2n = 60$ <i>E. esula</i> <i>E. X pseudovirgata</i>
Heptaploid	$2n = 56$ <i>E. virgata</i>		
Octoploid	$2n = 64$ <i>E. esula</i>		

## 2. Colonization of weedy leafy spurges by means of a pivotal genome.

Zohary (1965) states that the genes of a pivotal basic genome control the preadaptive theme, while the other basic genomes of a polyploid complex provide the wide variation on the theme in the form of modified genomes. We have evidence that a pivotal basic genome of ten chromosomes exists in the weedy leafy spurge species complex. A marker chromosome (satellite chromosome II) may represent this pivotal genome (Schulz-Schaeffer and Gerhardt, 1989). This marker chromosome was most common in *E. X pseudovirgata* but was also present in *E. esula* and tetraploid *E. cyparissias* ( $2n = 40$ ). This common basic genome must have served as a buffer in the process of hybridization.

If hybridization and subsequent introgression of chromosomes of one species into the pool of another species has taken place by backcrossing between tetraploids and hexaploids, and if the hybrids have moved into habitats where the hexaploids predominate, then aneuploid forms may have arisen which were closer in chromosome number to the hexaploids. The predominant hexaploid, aneuploid, and euploid chromosome levels of *E. X pseudovirgata* ( $2n = 56$  to  $60$ ) in North America may well be the result of such development.





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*Sponsored by: Montana Agricultural Experiment Station, Montana State University, Bozeman, MT.*

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## **Progress report: Translatable mRNA's in crown and root buds of leafy spurge**

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### **Abstract:**

Leafy spurge is a problematical weed because of the manner in which it perenniates. The crown and roots of the plant produce a large number of dormant shoot buds throughout the growing season. These buds are under correlative inhibition and when the top of the plant is killed by herbicides or mowing, these buds are released from inhibition and grow rapidly. Eradication of the plant is difficult because translocation of herbicides to both the root and crown buds is often incomplete allowing the plant to re-grow soon after treatment. Factors that control dormancy and development of these buds is certainly the most under-researched area of leafy spurge biology and may hold the key to controlling leafy spurge. It is our broad objective to begin studying the molecular biology of root and crown bud development in leafy spurge.

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What evidence suggests that control of root bud and crown bud formation may be crucial for the eradication of leafy spurge? In an enlightening article, Watson (1985) presented a model to show how a leafy spurge population might decline under various methods of control. Seed production might appear to be a weak link in the reproduction of leafy spurge. However, the simulations of Watson show that even if 100% of leafy spurge seed production is controlled for 15 years, the population will not decline. This simulation also holds true for 80% and 90% control of above-ground shoots with 10 to 20% control of seed and root/crown bud production. Satisfactory control can only result, according to this model, by controlling 95-99% of the top growth of a population and 40 to 80% of root and crown bud formation. Top growth is easily eliminated with herbicide treatment, however, we know little about the control of, or growth and development of the root and crown buds.

As stated above, it is our broad objective to begin studying the molecular biology of root and crown bud development in leafy spurge. Our specific objectives are to:

- 1) Determine whether changes in gene expression, as determined by alterations in the levels of translatable mRNA's, accompany release of root and crown buds from dormancy. If we identify mRNA translational products whose abundances significantly increase or decrease shortly after release of the buds from dormancy, then our next objective is to:
- 2) Isolate several cDNA clones that are complementary to the developmentally regulated mRNA's.

In order to determine whether changes in gene expression occur following release of buds from dormancy, we have begun to isolate RNA from inhibited root and crown buds, from rapidly developing root and crown buds released from correlative inhibition by removal of shoots from growing plants, and from crown buds that have been actively growing for a significant period of time. The use of growing buds as one of the controls should allow us to screen out those RNA's that function primarily in the growth process, e.g. components of the translational and transcriptional machinery, "garden variety" cell wall components, components of the photosynthetic apparatus, etc. RNA preparations will be translated *in vitro* in the presence of a labeled amino acid, and the translation products displayed on a two-dimensional polyacrylamide gel.

Single dimension gels of total protein fractions isolated from the various buds at different stages of development have indicated that certain proteins are specific to the different buds. However, isolations of mRNA from the various buds have been problematical. Modified PAS/TNS phenol extractions of buds (Gantt and Key, 1983) do not seem to be able to remove enough contaminating proteins to be able to effectively translate isolated mRNA in our wheat germ translation system. Methods using guanidium salts are better (MacDonald *et al.*, 1987) but still have not yielded RNA of sufficient quality to allow for effective translations. Work is in progress to improve our methods for the isolation of mRNA from buds of leafy spurge.

## Acknowledgements

Financial support was provided for this study by the Plant Molecular Genetics Institute at the University of Minnesota.

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*Sponsored by: Montana Agricultural Experiment Station, Montana State University, Bozeman, MT.*

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## **Methods for estimating leafy spurge (*Euphorbia esula*) populations**

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### **Introduction**

Information reported in this paper is from a larger on-going study designed to evaluate methods for estimating the density of leafy spurge and Canada thistle populations. This was done by comparing the results obtained from various methods to an actual population density.

Many different types of methods have been used by weed scientists and ecologists to measure species density (Behrens and Strand 1979, Crete 1981, Elliot *et al.* 1977, Inouye *et al.* 1987, Mukula *et al.* 1979, Thomas 1985, Steenhagen and Zimdahl 1979, Wilson 1981). Some are simple and quick, sampling a small percentage of a survey area, while others are complicated but sample a larger area. A systematic comparison was needed to determine which method was the most accurate.

### **Materials and methods**

Three methods were chosen for comparison. First was a single 5 m line with 3 quadrats. Four sets of 20 replications were done. Each set used a different size and shape quadrat (1/2 and 1 m squares and 1/2 and 1 m circles). Next was a W pattern with 50 pace legs and 20-1/2 m square quadrats. Lastly a double sampling procedure was chosen. This used 24-30 m transects with 15-1 m circular quadrats per transect. In the first phase, transects were laid out and all sample locations visually rated as to level of infestation. This was recorded along with line number and location on the line. Next a percentage of each level was randomly selected for sampling.

After all methods were tested, the survey area was divided into 5 x 5 m squares and each individual shoot in the entire field was counted.

## Results and discussion

The single line method had high variability between replications. The comparison of quadrat shape and size determined larger quadrats to be better (Table 1). Circular quadrats are usually preferred because fewer decisions need to be made concerning boundaries.

The W pattern was also highly variable. Size limitations only allowed two replications so this method will need to be tested again. The double sampling method was the most accurate (Table 1). The large number of samples resulted in estimates with lower variability. Categorizing samples into levels insured that different levels were treated proportionately.

**Table 1. Comparison of estimated field density versus actual field density for varying quadrat size and shape and for three different methods.**

Method	Quadrat	Estimated Density <sup>1</sup>	Difference from Actual <sup>1</sup>
Single Line	1 m <sup>2</sup>	10.917	+1.05
Single Line	1/2 m <sup>2</sup>	13.333	+3.47
Single Line	1 m diameter	7.558	-2.30
Single Line	1/2 m diameter	14.966	+5.10
W	1/2 m <sup>2</sup>	17.7	+7.84
Double Sampling	1 m diameter	10.4	+0.28

<sup>1</sup>shoots/m<sup>2</sup>.

## Conclusions

It was determined that large circular quadrats were preferred and double sampling was the most accurate method. This method was costly in time and effort so modifications are being tested to increase efficiency. The final modifications will be used in competition studies by our laboratory and could be used to measure the effectiveness of biological control methods in the future.

## Acknowledgements

Financial support and cooperation of the Minnesota Department of Transportation is gratefully acknowledged.

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Sponsored by: Montana Agricultural Experiment Station, Montana State University, Bozeman, MT.

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# Inhibitory effects of smooth brome leachates on leafy spurge

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## Introduction

Biological control of leafy spurge has in large measure been directed towards the use of insects and fungal pathogens to reduce the populations of the weed (Harris *et al.* 1985). Reductions in the population of a given weed species such as leafy spurge could conceivably be achieved by the presence of another more desirable species. The competitive advantage that one species can achieve over another may occur in a number of ways, including allelopathy (cf. Putnam and Tang 1986). The material described in this paper provides a brief summary of some of the results that we have obtained from studies on the effects of root leachates on leafy spurge.

## Materials and methods

Seedlings of smooth brome (*Bromus inermis* Leyss.), timothy (*Phleum pratense* L.), and leafy spurge (*Euphorbia esula* L.) that served as the source of leachate were grown from seed and maintained in controlled environment chambers under conditions similar to those described elsewhere (Koukkari *et al.* 1984). The seeds were sown on cheese cloth suspended on plastic rafts that were kept afloat in pans, first in distilled water (about 10 days), and then in Hutner's nutrient solution (cf. Hillman 1969). Most of the root growth and development occurred in the nutrient solution that was below the cheesecloth. After about seven days, the solutions in which the roots developed, from here on referred to as leachates, were used in various experiments. The experiments were designed to test the effects of each of the leachates on *Lemna minor* L. growth and chlorophyll (Koukkari *et al.* 1984), seed germination of lettuce (*Lactuca sativa* L.) and leafy spurge, and seedling growth and development of leafy spurge.

## Results

The effects of leachates on *Lemna* fronds and chlorophyll, seed germination, and leafy spurge seedlings are illustrated in Table 1, 2, and 3. Both the smooth brome and leafy spurge leachates appeared to inhibit frond production, while only the leafy spurge leachates seemed to cause a decrease in total chlorophyll levels.

**Table 1. Effects of Leachates on *Lemna*<sup>1</sup>**

Leachate	number of fronds	chlorophyll <sup>2</sup>
None	21.20 ± 2.62	30.92 ± 4.88
Timothy	28.80 ± 3.78	32.28 ± 5.28
Brome	16.60 ± 2.27	30.25 ± 4.87
Spurge	9.89 ± 2.78	19.11 ± 4.53

<sup>1</sup>Each value = mean ± SEM of 10 replications.

<sup>2</sup>Total chlorophyll as ug/mg dry weight.

**Table 2. Effects of root leachates on seed germination.**

Leachate	% Seed Germination*	
	Leafy Spurge <sup>1</sup>	Lettuce <sup>2</sup>
None	63 ± 0.03	100
Timothy	62 ± 0.04	82 ± 0.41
Brome	61 ± 0.05	39 ± 0.14
Spurge	80 ± 0.03	64 ± 0.32

\*Each value = mean ± SEM of 8 (leafy spurge) or 4 (lettuce) replications.

<sup>1</sup>n = 50.

<sup>2</sup>n = 7.

Although leachate from all three species inhibited the germination of lettuce seeds, none of them inhibited the germination of leafy spurge. In fact, the leachate of leafy spurge appeared to enhance germination of leafy spurge seeds.

The results of a more comprehensive study are illustrated in Table 3. The leachates of brome appeared to show the greatest effects on the seedlings, and this effect was primarily on shoot height and visual rating. In many instances the shoots of the leafy spurge seedlings that were transferred to brome leachate turned brown, appearing to be dead within a few days. Experiments are being continued to study the effects of these and other leachates on leafy spurge and to determine the nature of the substances that promote the inhibition.

**Table 3. Effects of root leachates on leafy spurge seedlings<sup>1</sup>.**

Leachates	Visual <sup>2</sup> Rating of	Number Leaves	Plant Height	Root Length	Chlorophyll <sup>3</sup>
None	1.4±0.2A	4.6±0.3AB	56.9±3.4C	60.4±8.7	28.3±3.5CDE
Brome	4.7±0.2B	1.6±0.5A	4.8±4.8	57.2±10.4	9.8±3.2A
Timothy	2.9±0.7AB	3.8±0.4AB	28.7±8.6A	44.4±10.4	15.5±3.1AB
Spurge	1.1±0.1A	4.6±0.3AB	50.1±3.2BC	63.8±12.4	22.6±2.4BCD
Brome	3.0±0.6AB	3.8±0.6AB	29.6±9.0A	66.9±10.8	31.9±12.0DE
Timothy	1.9±0.5AB	5.2±0.8B	47.1±7.2BC	78.1±11.5	32.6±4.4E
Spurge	2.8±0.6AB	3.9±0.6AB	43.9±7.2B	50.4±5.3	29.2±7.5DE
Brome	3.5±0.6AB	3.3±0.4AB	19.5±7.6A	48.9±8.3	18.3±2.4ABC
Timothy	1.5±0.5A	4.9±0.5AB	46.1±7.3BC	48.5±6.8	28.3±3.7CDE

<sup>1</sup>Each value represents the mean ISEM for 8 leafy spurge seedlings and means within a column having a common letter do not differ significantly at the 5% level.

<sup>2</sup>ug/mg dry weight.

<sup>3</sup>rating scale 1-5 (1 = healthy, and 5 = leaf and stem dead).

## Acknowledgements

Support from the Minnesota Department of Transportation is gratefully acknowledged.

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*Sponsored by: Montana Agricultural Experiment Station, Montana State University, Bozeman, Montana.*

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# **1989 Leafy Spurge Symposium**

## **Bozeman, Montana • July 12-13, 1989**

### **Foreword / Business Meeting Minutes**

## **Foreword**

ROBERT M. NOWIERSKI and PETER K. FAY

*Symposium Co-Chairmen*

These proceedings resulted from the “Leafy Spurge Symposium” held July 12-13, 1989 in Bozeman, Montana. The intent of the symposium was to provide current research results and technical developments relating to the management of leafy spurge. Topics of discussion included biological control, chemical management, cultural management, integrated weed management, plant physiology, and genetics.

Researchers, educators, weed supervisors, personnel from land management agencies, and ranchers presented some interesting ideas and along with the general audience provided some lively discussion. Their thoughts, concerns, and suggestions are found in these symposium proceedings.

Appreciation is extended to Jim Cuda, Barbra Mullin, Norman Rees, Reeves Petroff, and Bob Richard for their assistance in planning the symposium. We also wish to thank Joyce Ryen, Laura Estes, and Bonnie McCallum for their help in the production of the Proceedings.

# **Minutes of the GPC-14 business meeting: Holiday Inn, Bozeman, Montana, 12 July 1989**

D. BIESBOER, Secretary

## **Introduction**

The meeting was called to order by the President, Robert Nowierski.

## **Report by Russell Lorenz, editor of The Leafy Spurge News**

R. Lorenz briefly described changes in the structure of the GPC-14 Leafy Spurge committee, (first formed in 1979), as proposed by the Great Plains Agricultural Council. These changes have been summarized by Lorenz in the Editor's Corner of the May, 1989 issue of the Leafy Spurge News. This summary is brief enough to warrant repeating in the minutes in order to keep the membership informed and up-to-date:

The name of the committee that has provided common ground for those concerned with leafy spurge has been changed. What used to be GPC-14 Leafy Spurge Committee is now the Leafy Spurge Task Force. This came to be through a reorganization of the committee system of the Great Plains Agricultural Council.

The Great Plains Agricultural Council (GPAC) is made up of the Agricultural Experiment Stations and Cooperative Extension Services of the Land Grant Universities in the Great Plains States and interested agencies of USDA. The purpose of GPAC is to provide an organization for effective cooperation and coordination in response to current and emerging issues of importance to Great Plains agriculture, including natural resources. GPAC evolved for cooperative efforts in developing special agricultural programs to combat the severe drought and economic depression during the 1930's. Since its origin, GPAC has held at least one meeting each year, and has maintained a committee system which has been very effective in addressing problems and carrying on the work of the Council.

Under the new system, six permanent committees of the GPAC are subject-matter oriented: Crops and Soils, Economics, Forestry, Range and Livestock, Water, and Wildlife. Each of these six committees can form a committee task force to address specific problems. Our complete nomenclature is now Leafy Spurge Task Force of the Crops and Soils Committee of the Great Plains Agricultural Council.

Lorenz noted that our administrative advisor for the GPAC was Donald Anderson. Lorenz also noted that he had provided the GPAC with an update on the by-laws and activities of the Leafy Spurge Task Force for the GPAC.

## Report by Donald Anderson, Administrative Advisor

Anderson began his report by saying that he has been the administrative advisor to the leafy spurge group since 1981 and has observed that the scope and diversity of research has increased tremendously since that time. After an informal meeting of 11 July with other concerned members of the Leafy Spurge Task Force, Anderson presented a recommendation for restructuring the traditional format of the Symposium. Briefly, the recommendation proposed several changes for future symposia. a) A technical research session be added to the symposium. This session would include the following aspects of integrated management of leafy spurge: biological control, chemical control, cultural control and preventative measures, and basic studies in physiology, biochemistry, and taxonomy. b) The technical session might be one day in length and be attached to the end or beginning of the regular Symposium. Otherwise the meeting would be structured as usual. c) The meeting time be changed to midwinter. This suggestion was made in order to avoid field research commitments in the summer. d) An Executive Committee would coordinate future activities of the Leafy Spurge Task Force and be composed of 9 members. The Executive Committee would be structured as follows: A Program Committee of 4 people representing these areas – Biological and Cultural Control, Chemical Control, Basic Research, and a Non-Research representative; the 3 Officers of the Task Force, namely the Chairperson, The Chairperson Elect, and the Secretary; the Administrative Advisor; and the Executive Coordinator.

A large amount of discussion followed Anderson's report. Q) What is the objective of changing the present system? A) R. Lorenz answered by saying that the objective was to preserve the scientific aspects of leafy spurge research and to provide long-term coordination of our efforts to control spurge. An important change will be to set aside a session strictly for consideration of hard scientific issues in spurge research. Q) Is there an established membership for the Leafy Spurge Task Force? A) R. Lorenz replied that the membership was not formalized and that a single individual should be designated from each representative state as its official representative. R. Nowierski pointed out that members of the Planning Committee should have 3- or 4-year overlapping terms to give continuity to session areas. Q) What time of the year should we meet? A) D. Anderson replied that the issue must be resolved by the yet unappointed Planning Committee. Several people voiced concern over the fact that the Executive Committee as proposed was so large as to be unmanageable. A motion was proposed that a six-member committee be formed composed of: 4 Program Committee members (serving terms of 4 years), the Administrative Advisor, and the Executive Coordinator. The motion was seconded. After much discussion, the motion was amended slightly to include the Chairperson (currently designated as President) as a seventh member whom would be in charge of the Symposium during the year in which he or she was chairperson. The amended motion was seconded. Eight voted against the motion, six voted for the motion, and everyone else abstained. The motion did not carry. Further discussion was tabled until next year.

## Minutes

R. Nowierski moved to have the minutes of the 1988 meeting approved. The motion was seconded and approval was unanimous.

## **Roster**

R. Lorenz noted that the roster was recently up-dated and a list was passed to members present at the business meeting to further update the roster.

## **Future symposia**

R. Nowierski asked if Symposia were still being planned for 1990 and 1991. T. Whitson confirmed that he was prepared to hold the Symposium in Gillette, Wyoming, for 1990 in the summer, and D. Biesboer confirmed that he had begun making arrangements for the Symposium in Minneapolis, MN, for 1991.

## **Election of secretary**

R. Nowierski called for nominations for Secretary. Gene Lehnert of Nebraska was nominated, immediately declined, and then suggested R. Masters of Nebraska as a nominee. He accepted, nominations were closed, and Buffalo Bob Masters was unanimously elected as the secretary for 1990. The 1992 meeting is expected to be held in Lincoln, Nebraska. Officers are now:

Tom Whitson, President. (Symposium to be held in Gillette, WY, in 1990)

Dave Biesboer, Vice-President

Bob Masters, Secretary

## **Funding for the 1990 Symposium**

It was reported by R. Nowierski that \$1750 remained in the Leafy Spurge Task Force coffers. After a short discussion a motion was made to provide \$500 of the balance to support the newsletter and that the remainder be used to support the conference next year in Gillette, WY.

## **Endorsement**

Gene Lehnert asked the Leafy Spurge Task Force to endorse a planning effort (RC & D Measure Plan #31-999-045) that will apparently be discussed at a National Grazinglands Weed Management Conference being held in Omaha, Nebraska, in 1990. The objectives of the conference are: a) to heighten national awareness of the threat that weeds pose to the nation's grazingland resources and the economic well-being of the states relying on those resources, and b) to heighten national awareness of the effect that weed management on grazinglands has on the quality and quantity of the nation's subsurface water supplies. A motion was made to endorse the plan, was seconded, and unanimously approved.

## **Leafy Spurge Newsletter**

R. Lorenz reported that the list of participants in the conference and other sources were being used to update the mailing list for the newsletter. He also announced that Billy Smith would assist him in preparation of the newsletter for the coming year.

A motion was made to adjourn, was seconded and approved.

*Respectfully submitted by D. Biesboer, Sec., Leafy Spurge Task Force, 19 July 1989.*