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Travel to the USSR to collect biological control agents of weeds

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BLM, Montana State Office

This was the second year that a group of American and Italian scientists visited the USSR to find natural enemies of Eurasian native plant that became noxious weeds in North America. This trip was through a Joint 0perating Agreement on Biological Control Research between the Agricultural Research Service (ARS), U.S. Department of Agriculture and the Zoological Institute USSR Academy of Sciences, Leningrad, USSR. Total travel time was 36 days.

Team members that represented the Agricultural Research Service were:

- Dr. Rick Bennett, Research Plant Pathologist, USDA, ARS, Biological Control of Weeds Laboratory-Europe, Montpellier Substation, Montpellier, France.
- Dr. Sara Rosenthal, Research Entomologist, USDA, ARS, Rangeland Weeds Laboratory, Biological Control of Weeds Research Unit, Bozeman, Montana.
- Mr. Massimo Cristofaro, Research Entomologist, USDA, ARS, Biological Control of Weeds Laboratory-Europe, Rome, Italy.
- Hank McNeel, Weed Scientist, USDI, Bureau of Land Management, Montana State Office (931), Billings, Montana.

The ARS team traveled with Dr. Oleg F. Kovalev at the Zoological Institute Academy of Sciences, Leningrad, USSR and Natashia Edel, interpreter from Leningrad. The group first traveled from Leningrad to the Tashkent, Galiya-Aral, and Samarkand area. This area is known as the Russian Central Asia (Uzbekistan) Region. This area is classified as Arid with a typical sub-continental climate with hot summers and cold winters. The desert and steppe are dominant in this region. The elevation varies from 732 to 2,362 feet above sea level. Mean temperature is 33 to 48º F. Precipitation varies from 4 to 13 inches per year. Soils are generally gravelly, sandy to silty clay loam. There are more than 130 species of weeds in this area. In some locations there are 18 to 23 weeds per square foot. In many pastures, 75% of the vegetation are weeds in this area.

The group collected biological control agents of weeds from Russian knapweed (*Acroptilon repens*), Cornflower (*Centaurea cvanus*), Purple starthistle, (*Centaurea calcitrapa*), Field bindweed (*Convolvulus arvensis*), Scotch thistle (*Onopordum acanthium*), Slender thistle (*Carduus tenuiflorus*), Yellow starthistle (*Centaurea solstlitialis*), Tamarisk (*Tamarix* spp.), Common mullein (*Vervascum thapsus*), Curley dock (*Rumex crispus*), and Camelthorn (*Onabrychis pulchella*). We did not find any leafy spurge (*Euphorbia esula*) in this area. However, a small spurge was found but no biological control agents were found on it.

During the time we were in the Galiya-Aral and Samarkand areas we traveled with Dr. Mark Volkovich, insect taxonomist of the Zoological Institute. Mark was very helpful.

The group then traveled to the Stavropol area. This area, known as real temperate zone, is humid with warm summers and cold winters. Elevation varies from 1,788 to 2,203 feet above sea level. Mean temperature is 32 to 42º F. Precipitation varies from 19 to 25.5 inches per year. Snow depth during winter months varies from 4 inches to 5.5 feet. Soils in this area are known as black soils.

The group collected biological control agents of weeds from Musk thistle (*Carduus nutans*), Scotch thistle (*Onopordum acanthium*), diffuse knapweed (*Centaurea diffusa*), Canada thistle (*Cirsium arvense*), field bindweed (*Convolvulus arvensis*), houndstongue (*Cynoglossum officinale*) and leafy spurge (*Euphorbia esula*).

Massimo found two flea beetle populations, one in the open and one in shady areas (probably *Aphthona* species), the root gall fly *Pegomya* species, the bug *Dicranocephalus* species, the larvae of the leaf feeding moth *Oxicesta geographica* and *Lobesia* species, galls of the gall midge Baveria (*Spurgia* ?) species and *Dasineura* species.

Rick found different pathogens like *Uromyces* species, *Melampsora* species and a root disease (probably *Fusarium* species).

The group traveled with Dr. Alfred (Alec) Sitdikov, insect taxonomist from the Zoological Institute. He was also very helpful while in the Stavropol area.

The results of the trip were very promising and the potentials of those areas need to be developed. For this reason it is recommended to continue for the next few years to develop a survey exploration in both areas that could produce good candidates for the biological control of several weeds and future weeds in the United States.

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1989 redistribution of insects attacking leafy spurge

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Abstract:

During 1989, the USDA-Animal, Plant, Health, Inspection Service, Plant Protection Quarantine, (APHIS-PPQ) Bozeman Bio-Control Facility expanded their year old release program to include six introduced biological control of weeds insects attacking the exotic rangeland weed leafy spurge, *Euphorbia esula* L. The purpose of this release effort was to establish domestic field insectary sites (FIS) for future collection and redistribution activities. All six agents had been previously screened, approved, and released by the USDA's Agricultural Research Service (ARS). One species, *Spurgia esulae*, was established in sufficient numbers for ARS to supply in limited numbers for APHIS domestic redistribution. Other species were collected from European and Canadian sources. Domestic pass-through quarantine screening for parasites and diseases was completed by APHIS personnel in quarantine facilities located in Texas and California prior to FIS releases.

During 1989, APHIS and state level cooperators established a total of thirty FIS in the states of Colorado (1), Idaho (2), Minnesota (2), Montana (10), Nebraska (2), North Dakota (6), Oregon (2), South Dakota (2), Washington (1), and Wyoming (2). For all states, the total number of individuals released was: *Aphthona cyparissiae* (root boring flea beetle) 1,228 adults; *A. czwalinae* (root boring flea beetle) 180 adults; *A. flava* (root boring flea beetle) 2,157 adults; *A. nigriscutis* (root boring flea beetle) 16,051 adults; *Spurgia esulae* (shoot-tip gall midge) 455 galls; and *Oberea erythrocephala* (stem and root boring longhorn beetle) 40 adults.

In 1990 FIS monitoring of 1989 release sites for population establishment and development will continue. During the 1990 field season an additional 50 FIS locations in 10 states will be initiated.

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Successful laboratory mass rearing of the gall midge, *Spurgia esulae* Gagne, for field release

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Previous attempts by this laboratory to mass rear the shoot tip gall midge, *Spurgia esulae* Gagne, over an extended period of time failed after the 8th generation in culture. Plant quality was suspected to be a major contributing factor to the decline of the colony. A new attempt was initiated in 1989 with starter material collected with the permission of USDA-ARS at their establishment site in Bozeman, MT. High quality host plant material is maintained by use of an integrated-greenhouse pest management program utilizing predatory mites and whitefly parasites in conjunction with soap sprays. The gall midge colony is maintained in an environmental chamber with temperature range of 18º C to 24º C, a humidity range of 68% to 90% and a 15-hour light period. Results were improved over the previous year with an average yield of 364% over starting numbers for 15 generations. Some individual cages yielded over a 20-fold increase with one cage having a 30-fold increase. Material from this colony is being field released at several locations. The colony, now in its 17 generation, will be changed out with newly collected field material later this year.

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The biological control of leafy spurge-1990

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Leafy spurge in eastern Montana and western North Dakota is a weed of grave public interest. There is strong public support for any type of control effort. Working through Montana's Northeastern Weed Association, 11 eastern Montana counties contributed (coupled with funds from the Montana Weed Trust Fund) to Canada's biological weed control research program. Canada, in turn, has permitted the 11 counties to obtain *Aphthona* species in Canada.

Successful insect introductions have resulted in the requirement for technology to maximize insect numbers through research on release site variables which include: soil type, plant biotype, microclimate, *Aphthona* species, time of release, effects of multiple releases and number of insects released. These data are to be combined with data from Canada and other U.S. research for the development of a larger database for analysis. These data will provide a foundation for the utilization of *Aphthona* species for leafy spurge control and an indication of the needs for additional biocontrol agents for use in areas where the present species are weak. An optimum site is one that will result in a >3% survival rate. The table shows the theoretical increase in insect numbers under varying conditions. Calculations were based upon the bionomics of *Aphthona* species.

(Table found on next page.)

Theoretical increase in insect numbers given different initial release numbers.

Year	50 adults	500 adults	500 adults 8% survival/ generation	1,000 adults	1,500 adults
1990	50	500	500	1,000	1,500
1991	180	1,876	5,000	3,750	5,826
1992	703	7,031	50,000	14,063	21,094
1993	2,637	26,367	500,000	52,734	79,102
1994	9,888	98,877	5,000,000	137,764	296,631
1995	61,798	617,981	50,000,000	1,236,962	1,263,943
1996	646,733	5,497,333	500,000,000	10,314,670	14,222,000
1997	6,083,249	50,832,430	5,000,000,000	121,665,000	182,497,400
1998	83,644,360	836,446,500	50,000,000,000	1,672,283,000	2,503,330,000
1999	1,358,225,000	13,592,250,000	5E+11	27,134,510,000	40,776,760,000
2000	25,435,470,000	3E+11	5E+12	5E+11	3E+11
2001	6E+11	5E+12	5E+13	1E+13	2E+13
2002	1E+13	1E+14	5E+14	3E+14	4E+14
2003	3E+14	3E+16	5E+16	7E+15	1E+16
2004	1E+16	1E+17	5E+17	2E+17	3E+17
2005	3E+17	3E+18	5E+18	6E+18	9E+18

Calculated at 250 eggs/female and 50:50 sex ratio, except for the 8% labeled column; all insects given 3% survival rate for generations 1-3, then it increases to 5%.

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Development of *Aphthona flava* larvae on a leafy spurge cell suspension culture diet

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Laboratory mass rearing of biological control insects using artificial diets could replace the need for foreign importation by providing large numbers of insects for field releases. Success in diet formulation for specialist feeders has been limited and there are presently no artificial diets available for the biological control agents of leafy spurge. We now report the successful maintenance of *Aphthona flava* larvae through all larval instars (three) on a diet consisting solely of leafy spurge suspension culture cells.

Six-day-old leafy spurge suspension culture cells were vacuum filtered and lyophilized under sterile conditions. Lyophilized cells were placed on agar plates containing treatment concentrations of antimicrobial compounds. Unamended cells supported the growth and development of the larvae for an average of 18 days. Individual larvae were found to survive up to 72 days, while 35% of the larvae molted into the second instar and 5% survived through all larval instars.

Current efforts are aimed at manipulation of the cell culture-based diet to obtain higher percentages of third instar larvae and ultimately to induce pupation. The refinement of the technique developed in this study could provide a reliable method for the mass production of *Aphthona* species for the biological control of leafy spurge and for similar mass production of other specialist feeder insects. Cell culture-based diets could be especially important for rearing biological control agents for use in situations where chemical weed control is impractical, expensive and/or ecologically unsound.

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Progress report on field and quarantine research toward biological control of leafy spurge with phytophagous insects

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Abstract:

Seven species of insects have been released and monitoring of establishment/efficacy is continuing: *Hyles euphorbia*, a defoliating moth, has been released throughout several states, but is infected with NPV so no further work, other than opportunistic observation, is anticipated. *Oberea erythrocephala*, a stem-and-root-boring beetle, was released in 1982 in Montana, and is established in only one site where interaction with goats is under study; more have been requested from Rome. *Spurgia esulae*, a gall-fly, was released in 1985 in Montana and is established in one site now serving for life-history studies and as a limited insectary for APHIS and Canada; additional flies were received from Rome in 1990. *Aphthona* (root-boring flea beetle) species were released as follows: *A. czwalinae* released prior to 1989 in Montana, but apparently failed to establish; *A. flava* – released prior to 1989 in Montana and was established at four sites, only one of which could be confirmed in 1989 – that site will be monitored for efficacy in 1990 and will serve as a limited insectary for APHIS; *A. cyparissiae* – released prior to 1989 in Montana with probable establishment at two sites – monitoring and life history studies will continue; *A. nigriscutis* – Dr. Peter Harris of Canada has demonstrated successful reduction of leafy spurge biomass with concomitant replacement of the spurge by native vegetation on open sandy sites – Dr. Harris allowed collection of *A. nigriscutis* adults in Manitoba for 12 releases in 1989 (8 in MT, 2 in NE, 1 in ND, and 1 in ID) – plans are in place for an expanded multiagency cooperative test of various numbers and patterns of release of this insect in 1990 at 6 sites over 5 states (2 in MT, 1 each in ND, NE, CO, and ID). Four additional species of insects have been received from Rome and are

in the Montana State University Insect Quarantine Laboratory at various stages toward petitioning for release: *Dasineura* sp. nr. *capsulae*, a gall-midge, – petition for release has been approved and the environmental assessment (now required) has been written and submitted for approval by the Technical Advisory Group (TAG); *Simyra dentinosa* and *Oxicesta geographica*, defoliating moths, and *Chamaesphecia crassicornis*, a root- and stem-boring moth. Anticipated soon are: *Eurytoma euphorbiana*, a Seed wasp; *Aphthona abdominalis* from Europe; and *A. seriata* plus *A. chinchini* from China.

Acknowledgments

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Potential control of leafy spurge by Vesicular-arbuscular mycorrhizae fungi

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Leafy spurge (*Euphorbia esula* L.), a noxious weed infesting millions of acres of range and farmland in the north central United States, is infected with Vesicular-arbuscular mycorrhizae (VAM) fungi. These fungi, which inhabit the plant's fine roots, are widely accepted as beneficial organisms in the majority of plant families. The objectives of this work are to ascertain how VAM fungi impact the growth and development of leafy spurge and determine if such information can be used to generate a strategy for control of this noxious weed. Plants harvested from various sites in Wyoming exhibit varying degrees of infection levels, and plants grown in the greenhouse have variable infection levels depending, in part, on the plant's age. Results show plants infected with VAM fungi are slower in regrowth when clipped, compared to noninfected clipped plants. The VAM fungi use the plant's stored carbohydrates for energy, which may explain the decreased regrowth. Attempts to eradicate the VAM fungi with the fungicide Tilt® (1-((2-(2,4-dichlorolphenyl)-4-propyl-1,3-dioxolan-2-yllmethyl-lH-1,2,4,-triazole) proved ineffective. Inoculation of leafy spurge with 13 VAM fungi endophytes shows *Glomus mosseae* Colorado isolate, *G. mosseae* Arizona isolate, *G. etunicatum*, and *Acaulospora spinosa* having the greatest levels of infection. The effectiveness of the fungicide metalaxyl (N-(2,6-dimethylphenyl)-N-(methoxyacetyl) alanine methyl ester) for VAM fungi eradication will be evaluated, and plant biomass reduction will be determined utilizing a clipping study with VAM inoculated and noninoculated plants and analyzing root total nonstructed carbohydrate levels.

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Differential enzymatic glucosylation of hydroquinone in tissue cultures of small everlasting and leafy spurge: An allelochemical detoxification mechanism

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Natural phytochemical interactions (allelopathy) offer potential new approaches to weed management and the development of new, ecologically safe herbicides. Traditional allelopathy studies have concentrated on the isolation and identification of putative phytotoxic agents, with minimal consideration of the underlying biochemical mechanisms. We now report a differential allelochemical detoxification mechanism that may play a prominent role in the observed dominance of the low-growing, noncompetitive forb, small everlasting, over the deep-rooted, noxious weed, leafy spurge.

Small everlasting has been observed to be allelopathic toward leafy spurge in the field. The mechanism of this allelopathic interaction was speculated to be based upon differing abilities of leafy spurge and small everlasting to metabolize hydroquinone: a phytotoxic phenolic compound isolated from small everlasting plant material (Manners and Galitz, 1985) and tissue cultures (Hogan and Manners, 1990). Hydroquinone is glucosylated to its corresponding monoglucoside, arbutin; a non-toxic, water-soluble compound, in callus and suspension cultures of small everlasting and leafy spurge. The glucosyltransferase enzyme responsible for the detoxification of hydroquinone was continuously detected in cell-free extracts of small everlasting callus tissue. This enzyme was biosynthesized only when hydroquinone was added to leafy spurge suspension culture cells and the enzyme activity in leafy spurge was found to be six-fold lower than that measured in small everlasting. This differential ability of leafy spurge and small everlasting to detoxify hydroquinone provides a biochemical basis for the observed allelopathic interaction between these two species.

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Sunn hemp is allelopathic to leafy spurge

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Our research over several years has focused on using crop plants for allelopathic weed control. Recently, we discovered that sunn hemp (*Crotalaria juncea*) seeds contain a potent phytotoxin. The water-soluble compound(s) extracted from the sunn hemp seeds inhibited the growth of *Lemna obscura* in bioassay by 50%, when applied at a concentration of 50 ppm (freeze dried weight).

Leafy spurge was established in a sand-nutrient culture. When the plants were near the flowering stage, they were cut at soil level and two, four, or eight sunn hemp seeds were planted in the pots. The regrowth of leafy spurge in pots with sunn hemp was only 37% of the control plants after five weeks. When the leafy spurge was again cut after five weeks, and the emerged sunn hemp severed at the cotyledonary node, the leafy spurge growth over three weeks was 25 and 12% of controls with two and eight sunn hemp seeds, respectively. In soil culture, significant inhibition of leafy spurge was achieved using 12 or more sunn hemp seeds per pot. A crude water extract of the sunn hemp seeds at 10,000 ppm applied as a spray reduced leafy spurge growth by 54%. With further development and testing, we hope to use sunn hemp and/or its phytotoxin as an integrated biological control strategy for leafy spurge.

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Optimum growing conditions for propagation of leafy spurge (*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. To mass rear insects, the optimum leafy spurge growing conditions in the greenhouse have been investigated. Previously we determined that the optimum growing conditions are 27º C with 250 lb/A nitrogen using a complete 15-30-15 fertilizer, at pH 7 with a 16-hour day-length. The purpose of this research was to determine the effect of increased photoperiod, chilling the roots prior to planting, the ratio of N:P:K, and plant growth regulators on leafy spurge growth.

The plants were propagated by cutting 65 to 75 mm of stem apex from the plant. All but the upper 4 to 5 leaves were stripped from the stem. Then cuttings were dipped into 0.2% powdered NAA, were planted into conical tubes (1.5 inch diameter by 8 inches long) containing a mixture of peat and perlite, and were misted for 10 days to maximize root development. 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 2 cm 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 during a 10, 12, 14, 16, 18, and 20 hour photoperiod was determined. Plants from 10- or 20-day-old cuttings or regrown from roots were grown inside black plastic tents with natural light supplemented with metal halide lamps to achieve the correct photoperiod. The plants were fertilized at 100 lb N/A using a mixture of 23-19-17 water-soluble fertilizer. Optimum day-length for both shoot and root height and diameter was 16 to 20 hours and for shoot and root dry weight was 18 to 20 hours (Table, see page 3).

Plants were fertilized with various ratios of N:P:K (Table). In general there were no differences in plant growth regardless of the N:P:K ratio. In a second study, four water-soluble commercial fertilizers with a mixture of 15-30-15, 19-24-18, 20-20-20, and 23-19-17 N:P:K: were applied to 20-day-old leafy spurge plants at 200, 250, 300, and 350 lb N/A. Plant growth was similar regardless of fertilizer. Based on these and previous studies, the optimum growth of leafy spurge is achieved when at least 250 lb N/A is applied monthly regardless of the N:P:K ratio.

The effect of age of plants when first fertilized was evaluated. Cuttings 20, 30, 40, and 50 days old were fertilized using a fertilizer mixture of 15-30-15 at 250 lb N/A and harvested when 80 days old. Fertilization when the plants were 20 days old resulted in the best growth with almost double the stem and root dry weight compared to application when the plants were older (Table).

The effect of chilling roots to 2º C prior to planting was evaluated. Roots were chilled 48 hours and 1, 2, 4, and 8 weeks prior to planting. The roots were replanted and fertilized weekly with 20 lb N/A using a mixture of 15-30-15. Roots that had been chilled tended to grow more rapidly than the control (data not shown).

Plant growth regulators were applied as a soil drench at 1, 10, 100, 1000 ppm (Table). The plants were fertilized using a mixture of 15-30-15 at 100 lb N/A five days before treatment. GA increased shoot height and decreased root diameter and dry weight (Table). Ethephon (Ethrel), IAA and flurprimidol (Cutless) decreased growth. NAA at 1000 ppm was toxic to plants. Maleic hydrazide and chlormequat chloride (Cycocel) decreased shoot length slightly.

In summary, leafy spurge plants grew best in the greenhouse at 27º C, fertilized when 20 days old at a rate of 250 lb N/A, at pH 7 with a 16-hour photoperiod.

Table. Effect of day-length, N:P:K ratios, water-soluble fertilizers, age of plant at first fertilizer application, and plant growth regulators on leafy spurge growth.

Growth parameter	Shoot Height	Shoot Diameter	Shoot Dry wt.	Root Diameter	Root Dry wt.
	(mm)	(mm)	(mg)	(mm)	(mg)
Day-length (H)					
10	139	1.52	192	0.95	176
12	133	1.53	215	0.97	220
14	148	1.65	320	0.96	244
16	172	1.77	392	1.03	278
18	170	1.81	441	0.99	297
20	182	1.84	477	1.05	315
LSD (0.05)	12	0.07	39	0.08	36
N:P:K ratio[a]					
2:2:1	165	2.02	567	1.16	368
2:1:2	154	1.90	486	1.13	367
4:2:1	171	2.00	571	1.21	362
4:1:2	164	1.97	543	1.12	345
6:2:1	165	1.89	543	1.20	365
6:1:2	165	1.94	569	1.22	396
4:4:2	179	2.00	595	1.20	385
4:2:4	164	1.95	522	1.19	343
6:6:3	193	2.08	624	1.23	360
6:3:6	165	1.89	558	1.23	341
LSD(0.05)	22	NS	NS	NS	NS
Fertilizer[b]					
15-30-15	168	2.01	510	1.15	277
19-24-18	201	2.12	696	1.19	348
20-20-20	182	2.10	641	1.20	401
23-19-17	182	2.25	681	1.20	374
LSD (0.05)	NS	NS	NS	NS	NS
Age at fertilization (days)					
20	163	2.03	715	1.47	808
30	129	1.89	356	1.27	435
40	120	1.98	444	1.04	383
50	119	1.68	378	0.76	257
LSD (0.05)	27	0.20	159	0.27	135
Plant growth regulators[c]					
GA	442	2.09	459	0.74	103
Ethephon	113	1.64	250	1.18	173
IAA	144	1.73	414	1.10	248
Flurprimidol	54	1.64	123	0.57	297
NAA	0	0	0	0	0
Maleic hydrazide	50	1.46	185	0.48	86
Chlormequat chloride	111	1.75	427	1.27	382

[a]1 equals 50 lb/A

[b]Applied at 250 lb N/A.

[c]1000 ppm.

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Evaluation of genetic diversity in leafy spurge (*Euphorbia esula*) using chloroplast DNA sequence variation

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Understanding the genetic diversity within the North American leafy spurge complex is essential for increasing the success of biocontrol efforts. Multiple introductions of leafy spurge into North America has led to a population of intra-specific hybrids or biotypes often having distinct morphological characteristics. Defining specific North American biotypes and relating them to Eurasian progenitors would be useful in biocontrol efforts. Restriction fragment length polymorphorism (RFLP) analysis of chloroplast DNA (cpDNA) was used to determine the existence and extent of cpDNA sequence variation among collections of morphogically, and geographically diverse *Euphorbia* spp. Accessions from Russia, Italy, Austria, Nebraska and Montana were used in the initial phase of this research. Total DNA was extracted and digested with restriction endonucleases Eco RI, Hind III, Pst I and Xho I. DNA fragments were then separated by electrophoresis on agrose gels. Southern blot analyses were performed using labeled mung bean cpCNA probes. Preliminary data suggests that cpDNA sequence variation does exist among the accessions examined and could provide a means of determining relationships between North American biotypes and Eurasian progenitors.

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Control of leafy spurge along rights-of-way with burning and herbicides

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The emphasis of our program for the last several years has been to explore new methods of leafy spurge control that limit the use of herbicides. In cooperation with the Minnesota Department of Transportation, experimental research plots were established in the fall of 1988 along a severely infested, sloping right-of-way in the city of Eden Prairie, MN. The experimental design was a randomized complete block with six replications of each treatment. Individual plot size was 3 m x 4 m. Evaluations were taken as percent reduction of the original population density on each plot. Herbicides were applied by hand using a backpack sprayer. Burning was accomplished by lighting the downwind edges of plots with a propane torch. The herbicides used in this study were picloram (4-amino-3,5,6-trichloropiclinic acid), 2,4-D amine (2,4-dichlorophenoxyacetic acid), picloram + 2,4-D, and glyphosate (N-(phosphonomethyl) glycine). Treatments were 1) late fall application of 0.10 lb ai/A picloram followed by burning, 2) application of 0.25 lb ai/A picloram followed by burning, 3) late fall application of 0.25 lb ai/A picloram + 1.0 lb ai/A 2,4-D followed by burning, 4), late fall application of 4.0 lb ai/A glyphosate followed by burning, 5) late fall burning only, and 6) unburned controls. All plots burned in fall of 1988 were again burned in the fall of 1989. Picloram + 2,4-D followed by burning resulted in 100% control after 2 years. Treatments in order of effectiveness were picloram at 0.25 lb ai/A + 2,4-D at 1.0 lb ai/A + burning>picloram at 0.25 lb ai/A + burning > picloram at 1.0 lb ai/A + burning > glyphosate + burning > burning alone. The annual grasses *Setaria glauca* and *Panicum capillare* tend to dominate the burned plots after 2 years.

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Imidazolinone herbicides for leafy spurge control in Nebraska

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Experiments were conducted to determine the effectiveness of selected imidazolinone herbicides to control leafy spurge (*Euphorbia esula* L.) in subirrigated meadows in the Nebraska sandhills. Imazapyr (ARSENAL) applied at 0.8 kg ai/ha in the spring of 1988 provided 100% control of leafy spurge 18 months following application. ARSENAL applied at this rate severely injured desirable forage grasses [smooth brome (*Bromus inermis* Leyss.), redtop bent (*Agrostis stolonifera* L), timothy (*Phleum pratense* L.), little bluestem (*Schizachyrium scoparium* (Michx.) Nash), big bluestem (*Andropogon gerardii* Vitman), and indiangrass (*Sorghastrum nutans* (L.) Nash)]. Application of ARSENAL at rates of 0.8, 0.6, and 0.3 kg ai/ha in the fall of 1988 resulted in better than 95% control of leafy spurge 12 months after treatment, but injured associated herbaceous vegetation. In contrast, Imazethapyr (PURSUIT) applied at 0.2 kg ai/ha in the fall of 1988 provided greater than 85% control of leafy spurge with no apparent injury to desirable forage grasses 8 months after treatment. Additional experiments were initiated in the spring and fall of 1989 to determine if rates of ARSENAL and PURSUIT could be reduced to 0.2 or 0.1 kg ai/ha and still provide adequate control of leafy spurge and, where ARSENAL is applied, reduce injury of desirable forages to an acceptable level.

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Leafy spurge control with glyphosate compounds

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Experiments were established at Ft. Collins and Parker, CO to evaluate the effectiveness of prepackaged mixes of glyphosate + 2,4-D or dicamba for leafy spurge (*Euphorbia esula* L.) control. Leafy spurge and downy brome were the dominant plant species at Ft. Collins. The Parker site was a pasture where leafy spurge and Kentucky bluegrass (*Poa pratensis* L.) were the dominant species.

Different glyphosate + 2,4-D formulations were included in the experiment. Landmaster II and Landmaster BW contain 1.2 + 1.0 and 1.2 + 1.9 lb of glyphosate + 2,4-D per gallon of product, respectively, both herbicides formulated as their isopropylamine salts. Fallowmaster, 1.5 + 0.6 lb of glyphosate + dicamba per gallon of product, respectively, also was evaluated. Glyphosate and dicamba were formulated as their isopropylamine salts. Picloram and dicamba were included for comparison.

Landmaster II was applied at 0.94 lb ai/A (0.51 + 0.43 lb glyphosate + 2,4-D, respectively), Landmaster BW at 1.36 lb ai/A (0.53 + 0.83 lb glyphosate + 2,4-D, respectively), Fallowmaster at 0.73 lb ai/A (0.52 + 0.21 lb glyphosate + dicamba, respectively), picloram at 1.0 lb ai/A, and dicamba at 2.0 lb ai/A. Treatments were applied in spring at flowering or in fall to regrowth on May 22 and October 10, 1989, respectively at Ft. Collins, and on June 14 and October 20, 1989, respectively at Parker. All treatments were applied with a CO_2 pressurized backpack sprayer at 15 psi. All treatments containing glyphosate were applied through 1100lLP flat fan tips at 10 gpa, whereas picloram and dicamba were applied through 11003LP flat fan tips at 24 gpa. In spring at Ft. Collins, leafy spurge was 12 to 15 inches tall, flowering and there were 1 to 5 plants/ft^2; in fall, leafy spurge was 12 to 16 inches tall, vegetative, and there were 5 to 10 plants/ft^2. Downy brome was the only grass present at Ft. Collins, therefore, grass injury data were not taken. In spring at Parker, leafy spurge was 13 to 18 inches tall, flowering with some plants setting seed, and there were 2 to 5 plants/ft^2; in fall leafy spurge was 10 to 16 inches, vegetative, and there were 2 to 5 plants/ft^2. In spring at Parker, Kentucky bluegrass was 4 to 6 inches tall and vegetative; and in fall it was 3 to 5 inches tall and vegetative.

At Ft. Collins, both Landmaster formulation rates provided rapid leafy spurge shoot burn-down with spring applications, but control did not persist during the year of application (Table 1). Spring applied Fallowmaster provided poor control the year of application. Excellent season-long control occurred with spring applied picloram whereas, dicamba provided poor season-long control. Fall treatments were evaluated 17 days after application and at that time, Landmaster II and Landmaster BW provided 79 and 73% leafy spurge control, respectively (data not shown). Fall applied Fallowmaster only provided 59% control. Fall applied picloram and dicamba provided 80 and 73% leafy spurge control, respectively.

At Parker, both Landmaster formulation rates applied in spring provided fair control 30 DAT, but again, control did not persist (Table 2). Grass injury caused by these compounds was evaluated 90 DAT; 45 and 29% injury occurred with Landmaster II and Landmaster BW, respectively. Spring applied Fallowmaster provided poor season-long control. Grass injury caused by Fallowmaster was 48% 90 DAT. Picloram applied in spring provided only 68% control 30 DAT, but increased to 83% 90 DAT. Dicamba provided 40% control 30 DAT, and control still was poor (58%) 90 DAT. Low grass injury occurred with spring applied picloram and dicamba (10 and 17%, respectively, 90 DAT). Fall applied treatments were not evaluated in 1989 at Parker.

Control longevity and grass injury carryover were evaluated at 13 and 12 months after treatment (MAT) at Ft. Collins and Parker, respectively. At both locations, all products containing glyphosate provided poor control the season after spring treatments were applied (Tables 1 and 2). Fall applied Landmaster products provided poor to fair control the season after treatments were applied. Fall applied Fallowmaster provided only 8% control 12 MAT at Parker; however, at Ft. Collins, 70% leafy spurge control occurred with this treatment 13 MAT. Picloram provided good to excellent control at Ft. Collins and excellent control at Parker the season after treatments were applied, regardless of timing. Spring applied dicamba provided poor control at both locations (37 and 59% control at Ft. Collins and Parker, respectively) in 1990. However, 84% leafy spurge control occurred at both locations with fall applied dicamba.

Grass injury persisted at Parker (Table 2). Spring applied Landmaster II caused 47% grass injury 12 MAT whereas, Landmaster BW caused only 13% injury. Grass injury from spring applied Fallowmaster persisted and was 29% 12 MAT. No grass injury occurred with either fall applied Landmaster treatments but 20% grass injury persisted with fall applied Fallowmaster. Fall applied picloram caused greater grass injury to carryover into the following growing season compared to the spring treatment. Dicamba caused 13 and 21% grass injury to persist 12 MAT with the spring and fall applied treatments, respectively.

Herbicide treatments containing glyphosate + 2,4-D generally caused rapid leafy spurge shoot burn-down, but weed recovery ensued before the end of the growing season. Only the glyphosate + 2,4-D at 0.94 lb and the Fallowmaster at 0.73, applied in fall, provided comparable control to picloram, and only to picloram applied in spring.

Using glyphosate + 2,4-D mixtures is most likely best suited for areas where herbicides with soil activity presents a potential hazard. Repeat applications will be necessary for adequate leafy spurge control where glyphosate + 2,4-D is used and grass injury may occur. Sequential applications of glyphosate + 2,4-D and split applications of 2,4-D in spring followed by fall applied glyphosate + 2,4-D are being evaluated.

Table 1. Leafy spurge (EPHES) control with glyphosate + 2,4-D, glyphosate + dicamba, picloram, and dicamba applied in spring or fall at Ft. Collins, Colorado.

Treatment	Rate	Timing	EPHES control		
			30 DAT	90 DAT	13 MAT
	(lb ai/A)		% of check		
Glyphosate + 2,4-D	0.51 + 0.43	flower	94	55	51
Glyphosate + 2,4-D	0.53 + 0.83	flower	95	60	59
Glyphosate + dicamba	0.52 + 0.21	flower	51	36	38
Picloram	1.0	flower	96	99	82
Dicamba	2.0	flower	54	55	37
Glyphosate + 2,4-D	0.51 + 0.43	fall	-	-	64
Glyphosate + 2,4-D	0.53 + 0.83	fall	-	-	53
Glyphosate + dicamba	0.52 + 0.21	fall	-	-	70
Picloram	1.0	fall	-	-	99
Dicamba	2.0	fall	-	-	84
LSD (0.05)			15	15	19

Table 2. Leafy spurge (EPHES) control and Kentucky bluegrass (POAPR) injury with glyphosate + 2,4-D, glyphosate + dicamba, picloram, and dicamba applied in spring or fall at Parker, Colorado.

Treatment	Rate	Timing	EPHES control & POAPR injury				
			30 DAT	90 DAT		12 MAT	
			EPHES	EPHES	POAPR	EPHES	POAPR
	(lb ai/A)		% of check				
Glyphosate + 2,4-D	0.51 + 0.43	flower	65	54	45	52	47
Glyphosate + 2,4-D	0.53 + 0.83	flower	71	52	29	56	13
Glyphosate + dicamba	0.52 + 0.21	flower	40	43	48	66	29
Picloram	1.0	flower	68	83	10	90	14
Dicamba	2.0	flower	40	58	17	59	13
Glyphosate + 2,4-D	0.51 + 0.43	fall	-	-	-	0	0
Glyphosate + 2,4-D	0.53 + 0.83	fall	-	-	-	8	0
Glyphosate + dicamba	0.52 + 0.21	fall	-	-	-	9	20
Picloram	1.0	fall	-	-	-	95	33
Dicamba	2.0	fall	-	-	-	84	22
LSD (0.05)			8	10	8	14	23

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Leafy spurge control in North Dakota - 1990

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Evaluation of spray additives with picloram, various glyphosate plus 2,4-D combinations, and screening of new herbicides for leafy spurge control have been the primary emphases of the research program in 1990. Also, fluroxypyr amine formulations, BAS-514, and various auxin herbicides applied with insecticides are being evaluated for leafy spurge control.

Many of the commonly used spray additives were phytotoxic to leafy spurge when evaluated in the greenhouse and apparently decreased rather than increased absorption. Compounds that appeared to increase picloram absorption in greenhouse experiments were field tested in 1989. The additives Emulphor ON-877, GAFAC RA-600, MAPEG 400 MO, X-77, L-77, and LI-700 increased leafy spurge control when applied with picloram and all except LI-700 when applied with picloram plus 2,4-D all at 0.5% (v/v), compared to the herbicides applied alone. Triton CS7 also increased leafy spurge control when applied with picloram plus 2,4-D. More additives were evaluated in the greenhouse in the winter 1989-90. The best additives will be field tested in 1990 including several Triton formulations, Surftac, Pluronic L64, Tetronic 50R4, and a combination of X-77 plus L-77.

A GPC-14 regional screening trial of various glyphosate plus 2,4-D combinations was planned by six cooperators following the 1989 meeting in Bozeman. Glyphosate plus 2,4-D or dicamba were applied as commercial formulations on August 15 and September 15, 1989. Glyphosate was applied at 0.38 or 0.76 lb/A while the auxin herbicides ranged from 0.34 to 1.3 lb/A. Leafy spurge control in June 1990 by glyphosate at 0.38 lb/A applied in August and September averaged 29 and 52%, respectively, at the North Dakota location. Control averaged 60% when glyphosate was applied with 2,4-D or dicamba regardless of application date. Picloram at 0.5 lb/A and picloram plus glyphosate plus 2,4-D provided similar control averaging 87% when applied in August and 99% when applied in September. Grass injury averaged over all glyphosate treatments was 28 and 84% when applied in August and September, respectively. Grass was drought stressed when treated in August but was actively growing after a good rain when treated in September, which probably accounts for some of the difference in injury between dates.

Many labeled and unlabeled herbicides were evaluated for leafy spurge control in greenhouse experiments. Compounds that reduced or eliminated regrowth from leafy spurge roots are being field tested in 1990. These compounds include CGA-136,872

(Beacon), DPX-V9360 (Accent), EPTC, imazethapyr (Pursuit), imazaquin (Scepter), and quizalofop (Assure).

Fluroxypyr ester has shown limited phytotoxicity on leafy spurge. The ester formulation may cause too rapid leaf kill for optimum herbicide absorption. Two fluroxypyr amine formulations XRM-5196 (diisopropylamine) and XRM-5195 (triisopropylamine) at 0.25 to 1 lb/A were evaluated for leafy spurge control. The amine formulations provided 70 to 80% less leaf phytotoxicity than the ester formulation but did not control leafy spurge. Control across rates averaged 23 and 63% 3 months after application and 0 and 10% 12 months after application with the amine and ester formulations, respectively.

BAS-514 (Facet) is an amine-like herbicide with soil residual activity. BAS-514 applied at 1 lb/A in June or July averaged 41 and 17% control 12 months after treatment which was similar to picloram plus 2,4-D at 0.25 plus 1 lb/A. The herbicides imazethapyr, imazaquin, sulfometuron, 2,4-D, picloram, and dicamba applied with various insecticides may provide better leafy spurge control than the herbicides applied alone. These combination treatments are being field evaluated in 1990.

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Absorption and translocation of ^{14}C-fluroxypyr and ^{14}C-sulfometuron in leafy spurge (*Euphorbia esula* L.)

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Leafy spurge control with fluroxypyr and sulfometuron in the field has been variable. Several studies were conducted in the greenhouse to evaluate how plant growth stage and application with auxin herbicides affect the absorption and translocation of ^{14}C-fluroxypyr and ^{14}C-sulfometuron in leafy spurge.

Leafy spurge plants from a single biotype (accession 1984 ND 001) were propagated and grown in the greenhouse. Plants were selected for both shoot and root growth uniformity. The experiments were in a randomized complete block design with four replications, were conducted three times, and were combined for statistical analyses.

Plants were oversprayed with a 2 oz/A rate of the respective herbicide. A leaf midway on the stem was protected during the whole plant treatment and then treated with the appropriate ^{14}C-herbicide solution. Enough ^{14}C-herbicide and unlabeled herbicide with 0.25% surfactant WK was applied to obtain field application rates. The plants were harvested 72 hours after treatment and sectioned into treated leaf, stem and leaves above the treated leaf, stem and leaves below the treated leaf and roots. The treated leaf was dipped in scintillation fluor to remove unabsorbed herbicide. Plant sections were dried, weighed, and combusted in a biological matter oxidizer. Radioactivity was assayed using liquid scintillation spectrometry.

^{14}C-labeled and unlabeled sulfometuron was applied to leafy spurge plants in the vegetative, flowering, and postflowering growth stages. More ^{14}C-sulfometuron was absorbed in leafy spurge at the vegetative and flowering growth stages (approx. 22%) than the postflowering growth stage (approx. 9%). ^{14}C-sulfometuron translocation was low regardless of growth stage, averaging less than 2% of applied. The majority of the ^{14}C-herbicide remained in the treated leaf. Translocation to the above-treated-leaf section was greatest during the flowering stage compared to the vegetative and postflowering growth stages. More ^{14}C-sulfometuron translocated to the root during the vegetative stage than the flowering and post-flowering stages.

When sulfometuron plus picloram or 2,4-D were applied to leafy spurge in the vegetative stage, absorption and translocation of ^{14}C-sulfometuron applied alone and in combination with picloram or 2,4-D was similar. Absorption and translocation of

^{14}C-picloram were not affected by adding unlabeled sulfometuron. ^{14}C-2,4-D absorption was reduced, averaging 46% of applied herbicide when applied alone compared to 30% when applied with sulfometuron.

Absorption and translocation of ^{14}C-sulfometuron in leafy spurge was low regardless of plant growth stage or the addition of auxin-type herbicides. Most of the ^{14}C-sulfometuron remained in the treated leaf.

^{14}C-labeled and unlabeled fluroxypyr was applied to leafy spurge plants in the vegetative, flowering and postflowering growth stages. More ^{14}C-fluroxypyr was absorbed in the vegetative stage (39%) as compared to the flowering and postflowering stages (24%). More ^{14}C-fluroxypyr was translocated to the stem and roots in the vegetative stage compared to the other growth stages.

When fluroxypyr plus picloram or 2,4-D were applied to leafy spurge in the vegetative stage, absorption of ^{14}C-fluroxypyr was reduced. ^{14}C-fluroxypyr applied alone averaged 48% absorption compared to 35 and 24% when applied with picloram or 2,4-D, respectively. Translocation of absorbed herbicide to the above treated-leaf portion of the plant was reduced when C-fluroxypyr was applied with 2,4-D (averaged 2.8%) compared to 5% when applied alone or with picloram. In general, absorption and translocation of ^{14}C-picloram were not affected by adding fluroxypyr.

Absorption and translocation of ^{14}C-fluroxypyr applied alone to leafy spurge is better than ^{14}C-picloram, ^{14}C-2,4-D, and ^{14}C-sulfometuron alone or in various treatment combinations. The majority of the ^{14}C-fluroxypyr remained in the treated leaf, but the translocation of absorbed fluroxypyr was better than the commonly recommended herbicides.

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Selective use of glyphosate and sulfosate in perennial grass species for leafy spurge control

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Experiments conducted by Whitson in 1988 and 1989 have shown that glyphosate can be used as split applications to selectively control leafy spurge without totally controlling perennial grasses found in the understory. Two studies were conducted in 1989 and 1990 to determine tolerances perennial grasses have for glyphosate. The first study was conducted with 5 grass species which were transplanted from a field study to the U.W. greenhouse. The second study was conducted in the field with ten grass species established in 1986 at the Powell Research and Extension Center. Glyphosate, sulfosate and glyphosate plus 2,4-D were compared in the field study.

Grass species in the greenhouse study included: Western wheatgrass (Rosana), pubescent wheatgrass (Luna), crested wheatgrass (Ephraim), big bluegrass (Sherman), and Russian wildrye (Bozoisky). Grass species in the field study included: Russian wildrye (Russian), western wheatgrass (Rosana), thickspike wheatgrass (Critana), streambank wheatgrass (Sodar), slender wheatgrass (Prior), mountain bromegrass (Bromar), Russian wildrye (Synthetic A), basin wildrye (Magnar), meadow bromegrass (Regar) and crested wheatgrass (Hycrest).

The results of both studies show that differences do exist among perennial grass species for tolerances to glyphosate. In the greenhouse study glyphosate rates of 0.25, 0.5, 0.75 and 1.0 lb ae/A were applied after grasses were established and growth rates for each transplant were determined. Pubescent wheatgrass showed tolerance up to 1.0 lb ae/A, western wheatgrass up to 0.75 lb ae/A, Russian wildrye up to 0.5 lb ae/A, while crested wheatgrass and big bluegrass exhibited tolerance for levels up to 0.25 lb ae/A.

Field studies revealed that grass species are less sensitive to glyphosate and sulfosate when applied in early vegetative stages (April 11) than at the early bolt stage (May 31). The wildrye species had considerable seedhead suppression as a result of the vegetative stage application because of the early seed head initiation found within those species. Tolerance was found for all grass species for application of both glyphosate and sulfosate for rates up to 0.62 lb ae/A, at the early vegetative application. Grasses were more tolerant to sulfosate than glyphosate when applied at the same rates. Evaluations made 3 weeks after the May 31, 1990 applications of glyphosate and sulfosate indicate that tolerance levels are below 0.38 lb ae/A when applied at the early bolt stage. Therefore, when glyphosate or sulfosate are applied as selective treatments for leafy spurge control an application of up to 0.62 lb ae/A can be applied early in the season followed by successive glyphosate or sulfosate applications up to the .38 lb ae/A level.

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The control of leafy spurge (*Euphorbia esula* L.) by the integration of herbicides and perennial grasses

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Plant competition has long been recognized as an important method for control of weeds. This experiment was established near Sundance, WY, to determine the effects of establishing eleven perennial grass species on control of leafy spurge. Before seeding perennial grasses, two applications of glyphosate at 0.75 lb ai/A were broadcast with a truck-mounted sprayer delivering 15 gpa at 35 psi on June 2, 1986 (temperature: air 69º F, soil surface 65º F, 1 inch 64º F, 2 inches 63º F, 4 inches 63º F with 58% relative humidity and calm winds) and on July 1, 1986 (temperature: air 85º F, soil surface, 85º F, 1 inch 84º F, 2 inches 81º F and 4 inches 80º F with 40% relative humidity and 2 to 3 mph west winds). Soils were classified as a silt loam (22% sand, 58% silt and 20% clay) with 1.8% organic matter and 6.3 pH. A postemergent broadcast application of pendimethalin at 2.0 and fluroxypyr at 0.5 lb ai/A was applied May 16, 1988 (temperature: air 73º F, 1 inch 68º F, 2 inches 67º F, 4 inches 64º F with 64% relative humidity and wind 2 to 3 mph NW) with a tractor-mounted sprayer applying 20 gpa at 35 psi. Plots (60 by 90 feet) were arranged in a split plot design with four replications, one half the plot tilled, the other half left untilled. Tillage was performed with a rototiller on August 11, 1986 and grasses were seeded with a John Deere powertill drill on August 12, 1986. Evaluations were made September 14, 1988 and Aug. 8, 1989. In areas established with no tillage before seeding, pubescent wheatgrass and big bluegrass provided 72 and 78% control of leafy spurge and were 71 and 83% established with yields of 1,062 and 2,118 lb. dry matter (D.M.) per acre, respectively, while in treatment areas with tillage before seeding, western wheatgrass, hybrid wheatgrass, crested wheatgrass, big bluegrass, intermediate wheatgrass, pubescent wheatgrass and Russian wildrye provided 88, 89, 90, 91, 91, 93, and 93% control of leafy spurge, and were 58, 85, 86, 88, 91, 90, and 90% established with yields of 1,348, 2,886, 1,434, 2,997, 3,173, 2,074, and 1,283 lb. D.M./Acre, respectively. Yields were especially high due to considerably greater than normal rainfall at the study site in May and June.

The control of leafy spurge by the integration of herbicides and perennial grasses.

Grass Species (Variety)[1]	Grass Establishment[2]				% Leafy Spurge Control				Lb. Grass (D.M./Acre)			
	Tilled		No-Tilled		Tilled		No-Tilled		Tilled		No-Tilled	
	1988	1989	1988	1989	1988	1989	1988	1989	1988	1989	1988	1989
Pubescent wheatgrass (Luna)	90	90	70	71	97	93	84	72	572	2,074	274	1,062
Crested wheatgrass (Ephraim)	83	86	55	14	95	90	79	56	474	1,434	218	413
Mountain Rye	18	11	5	4	79	50	58	31	368	436	224	119
Big bluegrass (Sherman)	74	88	79	83	96	91	89	78	594	2,997	336	2,118
Hybrid wheatgrass (RSl)	74	85	13	10	94	89	60	33	518	2,886	142	619
Smooth Bromegrass (Manchar)	80	80	18	23	92	79	68	40	294	1,263	152	605
Intermediate wheatgrass (Oahe)	71	91	16	53	97	91	68	51	652	3,173	152	2053
Bluebunch wheatgrass (Secar)	64	64	15	2	83	76	64	35	194	968	128	169
Western wheatgrass (Rosana)	76	58	26	19	91	88	65	48	464	1,348	174	387
Russian wildrye (Bozoisky)	83	90	30	10	97	93	63	44	552	1,283	160	220
Thickspike wheatgrass (Critana)	81	61	29	15	94	78	70	29	484	1,587	210	690

[1]Grasses seeded August 12, 1986.
[2] Evaluations made September 14, 1988 and August 8, 1989.