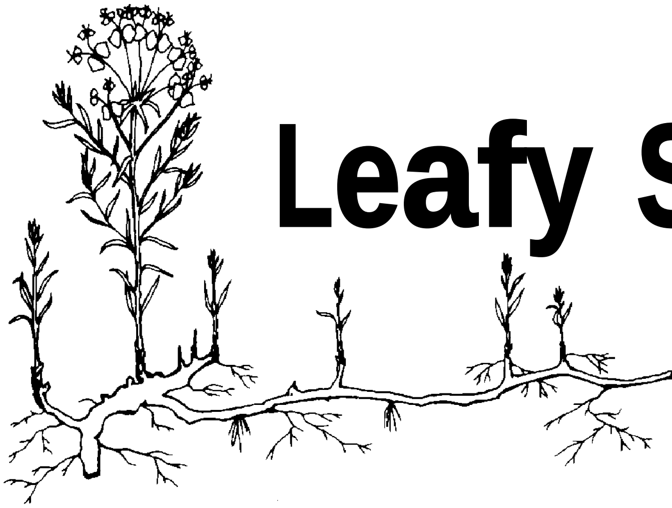

Leafy Spurge *News*

Agricultural Experiment Station
NDSU Extension Service
North Dakota State University, Fargo, ND 58105

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From The Editors Desk

By the time you receive this issue of **Leafy Spurge *News*** I hope that all of you who plan to attend the Leafy Spurge Symposium in Gillette, Wyoming have sent in your registrations and made your reservations. For those of you that have not been there, a flyer is enclosed with the information you need.

In keeping with the new format of honoring a person in the state or area where we are having our symposium; our honoree is Dr. Harold P. Alley, who unfortunately died six years ago. I was fortunate to have served with him on that important steering committee; and found him to be a very enthusiastic and dedicated person. We have come a long way since 1979, but the battle against leafy spurge is not yet over. That is the reason for the 1997 Leafy Spurge Symposium. I am looking forward to seeing many of you in Gillette in July.

I got an interesting news release from Nebraska as follows: The Nebraska Leafy Spurge Working Task Force announced recently that they will be giving away 50 containers of 10,000 root feeding flea beetles at their annual meeting in North Platte, NE, on August 7, 1997. A total of 500,000 insects will be given away as door prizes with delivery of the beetles scheduled for June 1998. Depending on availability and if someone purchased the insects from private suppliers, these 500,000 insects would be worth $300,000 to $500,000. To be eligible for the drawing you must attend the annual meeting and be present to win.

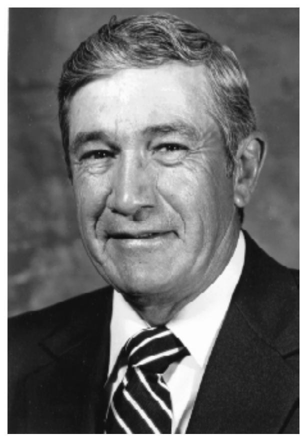
This insect give-away will be just part of a 10th anniversary celebration held in conjunction with their annual meeting Aug 6-7, 1997. For more information contact the North Central Nebraska RC&D Office, P.O. Box 130, Bassett, NE or phone (402) 684-3346.

Congratulations on this very innovative approach and hope that you will have a very successful 10th anniversary celebration.

C. H. Schmidt, Editor
1827 N 3rd Street
Fargo ND 58102-2335
(701)293-0365, Fax (701) 231-8474

Leafy Spurge Honoree

Harold P. Alley 1924-1991



Harold Alley is a native of Wyoming. He spent his early boyhood on a homestead ranch on Rabbit Creek about 20 miles south of Cokeville, in the southwest part of the state.

Harold enrolled in the University of Wyoming, graduating in 1949. He began work as a Vocational Agriculture Instructor and coach at LaGrange, Wyoming. Then he accepted a faculty position at the University of Wyoming and pursued an M.S. degree, which he obtained in 1955. Following this, Harold decided to obtain a Ph.D. at the University of Nebraska and moved to Lincoln the summer of 1958. But because of an extended illness, he had to drop out of school. He returned to the University of Wyoming, where he worked for another 5 years.

He then went to Colorado State University and completed course work for his Ph.D. in 2 years and finished his research and dissertation while back on the job at the University of Wyoming. He directed the research of 36 graduate students and was responsible for research, teaching, and extension programs in Weed Science and Technology.

Harold was very active in the fight to control leafy spurge, and was involved in chemical control studies since the mid 1950's. In fact he could be called Mr. Leafy Spurge of Wyoming as he was instrumental in getting the "Leafy Spurge Control Act of 1978" adopted by the Wyoming Legislature. That same year he helped put in place Wyoming's leafy spurge program to provide a way for weed districts, farmers and ranchers to control leafy spurge. The importance and significance of the leafy spurge infestations in Wyoming can be borne out by the fact the the 44th Wyoming legislature appropriated $1.4 million for the treatment of this weed. Although $1.4 million appeared to be a very large sum, and the program over ambitious and doomed for failure by some, Harold said that it was a shame this same foresight was not prevalent some 20 years ago. Then the infestations could have been isolated, controlled or even eradicated when the acreage infested was only a fraction of what it was in 1979, 36,000 acres!

He was one of the six members of a Steering Committee whose charge was to develop a total program for a needed attack on leafy spurge involving research, extension-education and improved coordination. As part of this committee he was involved in organizing the First Leafy Spurge Symposium held in Bismarck, ND June 26-27, 1979, and the Northern Regional Leafy Spurge Conference held in Billings, MT, Dec 17-18, 1979. When Harold spoke, people really listened!

He published over 500 articles and received many honors. He retired from the University in 1984, but continued working for about 4 years as a consultant with Dow Chemical. Harold died at age 67 in Omaha on July 25, 1991, after an extended illness.

Manitoba Weed Supervisor's Association Past, Present and Future?

- The development of Manitoba Weed Districts and specifically the role of Manitoba Weed Supervisors from the inception of the Weed District program to the present.
- Status of Weed Districts and Supervisors – Then and Now.
- The relationship between Manitoba Agriculture and the Manitoba Weed Supervisor's Association and how it has changes in recent years in the areas of Program Planning, Funding and Training.
- Manitoba Weed Supervisor's Association special projects (eg. Bio-control, Weed Resistance, Purple Loosestrife, etc).
- The evolution of the Manitoba Weed Supervisor's Association as it has worked to become self driven and how the Association and Weed Districts have coped with funding problems. Looking to the future.

R. Reimer
Southeast Weed District
Box 2201 , Steinbach, Manitoba ROA 2AO
(204) 326-3922

Reproductive Status of Established Black Dot Flea Beetle (*Aphthona nigriscutis*) Populations in South and North Dakota

Black dot flea beetles, *Aphthona nigriscutis* Foudras, are used for biological suppression of leafy spurge, *Euphorbia esula L.* Thousands of adults are collected annually from insectaries in several states for redistribution to other spurge infested areas. However, little is known about the reproduction biology of black dot flea beetles. The purpose of this research was to monitor the reproductive status of black dot flea beetles under field conditions.

In 1995, adults were collected weekly from an insectary near Pollock, SD by sweep net sampling from June 26 through August, when flea beetle adults were no longer present in the samples. One hundred adults were examined and gender ratios determined. The spermatheca of 25 females were removed from the abdomen and analyzed for the presence of sperm. Oocyte maturation in these females also was determined by rating oocyte development. Males were rare in sweep net samples and did not exceed 4% of the adult population throughout the summer. The insemination rate of females on July 6 was 44% and was significantly ($P<0.01$) higher than previous or subsequent sampling periods. Following July 18, only about 20% of adult females from samples were inseminated. Oocyte maturation gradually increased to a peak on July 25. Adults were rare at the Pollock site on August 3 and were not present on August 22.

In 1996, the study was expanded to include two North Dakota populations (Theodore Roosevelt National Park, and Valley City). Adults began to emerge at the three sites between June 14 to 26. The percentage of males in the Pollock, Valley City and Theodore Roosevelt National Park samples (from the first week) was 6.9%, 14% and 20% respectively. Analysis of weekly samples is continuing and additional results will be reported. These data are useful in determining the optimum period for collection of reproductively viable individuals. These data could also be used as an index for numbers of individuals needed to be released in redistribution sites.

M Brinkman
Plant Sci. Dept, SDSU
Brookings, SD 57007 (605) 688-5082

Summary of Biocontrol of Leafy Spurge in Wyoming During 1996

During 1996, 1,209 releases of natural enemies were made for leafy spurge, as seen in Table 1. Several agencies worked together to redistributed 1,022,100 *Aphthona lacertosa*. A majority of the *lacertosa* was collected in North Dakota.

Table 1. Bioagents Released for Leafy Spurge in 1996

Species	Number of Sites	Number Released
Aphthona lacertosa\czwalinae	134	1,022,100
Aphthona nigriscutis	1,008	2,729,600
Oberea erythrocephala	12	1,623
Spurgia esulae	55	5,310
Total	1,209	3,758,633

Since 1990, the number of insects redistributed has increased, as seen in Table 2. In the last seven years 6,579,633 insects for leafy spurge have been redistributed in Wyoming.

Table 2. Increase in the Number of Insects Released for Leafy Spurge

Year	Number of Insects
1990	26,960
1991	60,759
1992	32,845
1993	131,357
1994	1,010,213
1995	1,558,750
1996	3,758,633

Kiana Zimmerman
Cooperative Agricultural Pest Survey (CAPS)
Department of Plant, Soil and Insect Sciences
University of Wyoming
P.O. Box 3354, Laramie, WY 82071
(307)766-5278 e-mail: caps@uwyo.edu
WWW: http://plains.uwyo.edu/~caps/caps.html

2,4-D Reversal of DFMO-Induced Inhibition of Adventitious Root Formation in Leafy Spurge (Euphorbia esula L.) In Vitro

Indoleacetic acid (IAA) stimulates adventitious root formation and partially reverses the effects of difluoromethylornithine (DFMO), a specific suicide inhibitor of putrescine biosynthesis, in etiolated aseptically-grown hypocotyl segments of the perennial weed, leafy spurge. DFMO also is a strong inhibitor of adventitious root formation in the hypocotyl segments. Dichlorophenoxyacetic acid (2,4-D) has auxin-like activity and was also tested to determine if it could counteract DFMO-induced inhibition of root formation. When 2,4-D was applied at 45 to 450 nM in the nutrient medium, root formation was inhibited. Unexpectedly, 450 nM 2,4-D applied simultaneously with 500 pM DFMO reversed the DFMO-induced inhibition of adventitious root formation, forming root numbers similar to controls. This reversal occurred under growth conditions with complete B5 nutrient medium or with B5 medium containing salts and vitamins reduced to 10% of their normal concentrations. This action of 2,4-D resembles that of indoleacetic acid (IAA), although 2,4-D did not stimulate root formation in the absence of DFMO, as did IAA (at 230 to 1100 nM).

D. Davis
USDA-ARS Biosciences Res. Lab.
Fargo, ND 58105-5678 (701) 239-1247

Cloning and Characterization of a Genomic Clone of the root Bud Growth-Response Gene Gro7 from Leafy Spurge (Euphorbia esula L.)

One of the key features of leafy spurge is the presence of a large number of below ground shoot meristems located along the roots of the plant (referred to as root buds) which remain dormant until they are separated from the aerial portion of the plant. Previous results indicated that loss of contact to the aerial plant parts results in changes in gene expression in the cells of the root buds. We have reported cloning of a partial cDNA for one of the genes, Gro7. Here, we report the cloning of a partial genomic clone of the Gro7 gene. A genomic library (of 2 x 106 primary pfu with an average insert size of 6 kilobases) was constructed. This library was screened by hybridization to the Gro7 cDNA clone. Eight out of 500,000 plaques hybridized to the cDNA. Since the Gro7 gene is a single copy gene, this result indicates that the leafy spurge genome is roughly 3.75 x 10^8 bp/haploid genome. One of the genomic clones was further purified and characterized by restriction mapping. This clone is currently being sequenced in hopes of gaining information concerning the function of this gene.

D. Horvath
USDA-ARS Biosciences Res. Lab.
Fargo ND 58105-5674 (701) 239-1246

Imazameth is Safe on Grass; Leafy Spurge is Becoming Resistant to Picloram; and Other Myths?

Imazameth (AC 263,222) has been shown to provide good leafy spurge control with acceptable grass tolerance for warm-season species in Nebraska. However, cool-season grass injury was severe when imazameth was evaluated in North Dakota. Leafy spurge control in June 1996 increased as application rate increased and averaged 80% to 100% when imazameth was applied from 0.125 to 0.5 lb/A, respectively, on September 18, 1995. Unfortunately, grass injury to cool-season species ranged from 10% to 65%. Imazameth is currently being evaluated at lower rates, alone and with additives, and as a spring applied treatment in an effort to obtain good leafy spurge control with minimal grass injury in North Dakota.

Glyphosate plus 2,4-D plus 0.6 lb/A will provide 70% to 90% leafy spurge control after one treatment but can cause severe grass injury with repeated applications. A series of experiments was established at three locations to compare cost and efficacy of glyphosate plus 2,4-D as part of a long-term management program for leafy spurge control. The initial treatments of glyphosate plus 2,4-D or picloram plus 2,4-D were applied in late June of 1993 and were retreated with the same or an alternate treatment in 1994 and 1995. Visual evaluation were taken annually from 1993 to 1996. Glyphosate plus 2,4-D provided 75% leafy spurge control 12 months after treatment (MAT) compared to 30% for picloram plus 2,4-D. All treatments provided similar control when annually applied for 3 yr but the total treatment cost was variable. Glyphosate plus 2,4-D applied three consecutive yr provided 73% control with only 10% grass injury and cost $27/A. Glyphosate plus 2,4-D applied in 1993 and 1995 with picloram plus 2,4-D applied in 1994 averaged 80% control and cost $31/A. Three annual applications of picloram plus 2,4-D provided 70% control and cost $40/A. There was no significant grass injury for any treatment.

Picloram is one of the most effective herbicides for leafy spurge control. Previous research at North Dakota State University has shown that picloram plus 2,4-D at 0.25 plus 1 lb/A will provide approximately 85% control or better after 3 to 5 years of annual treatment. Picloram alone at 1 to 2 lb/A will provide acceptable leafy spurge control for 18 to 24 months in North Dakota. Recently at field tours and educational meetings land managers have expressed concern that picloram provides less leafy spurge control then they have come to expect. To determine if leafy spurge was becoming resistant or tolerant to picloram, the average leafy spurge control with picloram and picloram plus 2,4-D treatments applied from 1963 to 1982 (historical) was compared to the same treatments applied from 1983 to 1985 (present). The average control was less with present day treatments for every picloram treatment regardless of application rate, if applied alone or with 2,4-D, or in the spring or fall. For example, picloram at 0.5 lb/A alone historically averaged 85% control 12 months after treatment compared to an average of 55% control with the present day treatments. Also, picloram at 1 lb/A provided 88 and 68% control when the historical average was compared to the present treatment average, respectively.

The reason for the better control observed with the historical compared to present treatments may be due to the plant becoming resistant to picloram, or the more susceptible plants have been controlled and only the most vigorous plants remain, or the personnel conducting the evaluation have become more demanding. To determine if control indeed was decreasing with time, the average control from 1984 to 1988 was compared to the average control of the same treatments applied from 1991 to 1995. The same personnel conducted the evaluations in both time periods. The average control was similar to slightly better from 1991 to 1995 compared to treatments applied from 1984 to 1988. If leafy spurge control with picloram was declining with time, the control observed from 1984 to 1988 should have been better than that from 1991 to 1995. Thus, it is not likely leafy spurge has become resistant to picloram, or that only the most vigorous or tolerant plants remain. It is likely the expectations of control with picloram have increased and historical evaluators tended to rate control higher then present.

R. G. Lym
Plant Science Dept. NDSU
P.O. Box 5051, Fargo, ND 58105
(701) 237-8996

Differential Tolerance Toward Oxidative Stress Between Immature and Mature Leafy Spurge Leaves – An Evolutionary Mechanism for Survival Under Adverse Environmental Conditions?

The most effective method for controlling leafy spurge is to kill the crown and root buds or to break bud dormancy and kill the newly emerging shoots. However, crown and root buds did not break dormancy and emerge unless the entire shoot or above ground portion of the plant was excised or killed with 2,4-D. The apical leaves are able to survive severe conditions such as moisture stress, pathogen invasion (mildew), herbicide (diclofop-methyl), etc., whereas more mature leaves were killed rapidly and abscised. The various dissimilar stresses mentioned above are known to have a common lethal mechanism, i.e. the induction of active oxygen species (AOS)(free radicals) to cause rapid cell destruction and death. A consequence of A0S formation is the secondary induction of ethylene which is not the lethal factor.

Diclofop-methyl (DM) was used as a physiological probe to induce oxidative stress on leafy spurge. Significant increases in ethylene was induced by DM and 2,4-D over control tissues in mature and immature leafy spurge leaves. However, only mature leaves were killed by DM whereas all leaves were killed by 2,4-D. AVG (aminoethoxyvinyl-glycine) inhibited completely ethylene induction by both 2,4-D and DM. Conversely, vitamin E (tocopherol) inhibited DM induction of ethylene by approximately 50% of control but it had no effect on ethylene induction by 2,4-D. Therefore, the action of the inhibitors on ethylene production indicates that DM induces death of mature tissues by a free radical oxidation mechanism in contrast to 2,4-D which induces ethylene by an alternative mechanism not involving AOS. Tocopherol, an effective scavenger of AOS, protected leafy spurge hypocotyls from damage by DM in tissue culture. It is hypothesized that apical tissues may survive oxidative stress due to more active anti-oxidation systems than mature tissues and, therefore, prevent root buds from emergence and damage under adverse environmental stress conditions.

R. Shimabukuro
USDA-ARS, Biociences Res. Lab.
Fargo ND 58105-5674 (701) 239-1256

Cloning and Expression of a Cold-Regulated Gene From Leafy Spurge (*Euphorbia esula L.*) In Vitro

Leafy spurge (*Euphorbia esula*) is a perennial weed which is capable of acclimatising to sub-freezing temperatures. We have used the differential display technique to identify and clone a cDNA for a cold regulated gene (*Cor103*) whose mRNA accumulates specifically during the cold acclimation process. The expression of *Cor103* was analyzed, and it has been determined that the RNA from this gene reaches maximal expression in less than 2 days following exposure of the plant to temperatures of 5°C, and remains at high levels in the plant for at least 30 days so long as the plant is left in the cold. RNA from *Cor103* return to control levels within 24 hours when the plant is returned to normal growing temperatures. *Cor103* transcript does not accumulate under conditions of drought or heat stress. The *Cor103* gene is induced in response to low temperatures in roots, stems and leaves, but is expressed at high levels in tissue culture at control temperatures.

Southern blot analysis indicates that the *Cor103* gene is a member of a gene family. Sequence analysis indicates that the 3' end of the *Cor103* gene is extremely rich in glycine, and thus shares some homology to a group of glycine-rich RNA binding proteins, some of which have also been shown to be cold regulated.

D. P. Horvath
USDA-ARS Biosciences Lab.
Fargo ND 58105-5674 (701) 239--1246

Biocontrol of Leafy Spurge with Aphthona Nigriscutis in Alberta "The Beverly Bridge Site"

There are over 130 research sites in Alberta where *Aphthona nigriscutis* beetles have been released on leafy spurge. Once beetles have established, the population is monitored and vegetation data is collected from the sites. *A. nigriscutis*, the black dot spurge beetle, is most effective on lighter soils with leafy spurge flowering heights of less than 70 cm, and on high, dry slopes or hilltops.

The Beverly Bridge in Edmonton is one of the most successful *A. nigriscutis* sites in Alberta. In 1989, 500 beetles were released at one of six sites on the Beverly bridge. The site is located on a dry, south facing railway embankment. The soil texture is 82% sand, 14% silt and 4% clay. The leafy spurge was very dense at 279 shoots/m^2, and the above ground leafy spurge biomass at the time of the release was over 170 g/m^2. The flowering leafy spurge canopy was 60 cm in height.

In 1992, the beetle population peaked , and by 1993 the leafy spurge biomass was reduced to less than 2 g/m^2, while the grass biomass increased to 43 g/m^2. The leafy spurge density and percent cover were also dramatically reduced after four years time.

There have been over 100,000 beetles collected and redistributed from this site.

K. Stromme
Alberta Agr, F&RD
Agronomy Unit, Ag Centre 2nd Fl, 6903-116 St.
Edmonton, Alberta T6H 4P2 (403)427-7098

Claude Schmidt
Agricultural Experiment Station
North Dakota State University
Fargo, ND 58105

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Department of National Defence's Involvement in Leafy Spurge Management to Date at Canadian Forces Base Shilo, Manitoba, Canada.

Control of the noxious weed, leafy spurge, has been steadily increasing since the mid 1980's at Canadian Forces Base (CFB) Shilo training ranges. The use of chemical control has changed significantly during the last decade. Currently only the outer areas of the training ranges are sprayed with 2,4-D Amine. Biological control began at Shilo in 1986 with test plots for biological agents placed in various locations throughout the training ranges. Department of National Defence has, in the past, actively participated in the research in bringing new biological agents for release by providing monetary support and research data. Research efforts at Shilo are controlled by the weather and access to the training area.

Data sheets listing the topography and vegetation at release sites have been completed and filed with pictures of the release sites. To date, CFB Shilo now has 196 biological release sites throughout the ranges. Concentration of release in the last two years has been in the southern portion of the ranges to prevent the spread of spurge into native mixed-grass prairies which dominate the southern portions. This year, the Base Environmennt Office will be determining a Standing Operating Procedure for the releasing and monitoring of biocontrol sites based on data collected during the last seven field seasons.

G. Shearer
Base Const. Eng. Br.
CFB Shilo, Shilo, Manitoba R0K 2AO (204) 765-3000

1996 Manitoba Weed Supervisors Association and the New Utility

Using a holistic approach to controlling noxious weeds we have incorporated the use of insects at bio-release sites.

The guide post to a sites success requires semi-annual avoits at the first point of release.

A GPS receiver lends itself cheaply and accurately to this end. Within minutes a GPS can establish the position of a release site. The same point can be re-established repeatedly so bio-sites can be precisely monitored.

E. Richardson
Dauphin-Ochre Weed District
Box 574, Dauphin, Manitoba R7N 2V4 (204) 638-7050