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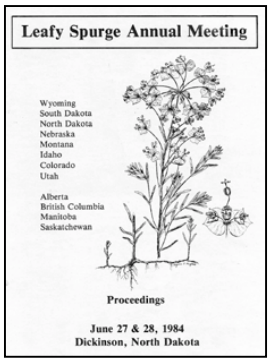

1984 Leafy Spurge Symposium

June 27-28, 1984, Dickinson, ND

Introduction / Program / Meeting minutes

Introduction

RUSSELL J. LORENZ

1984 Chairman GPC-14

This Proceedings was prepared to document the third Leafy Spurge Symposium sponsored by GPC-14 since its establishment in 1981. GPC-14 is one of the Coordinating Committees of the Great Plains Agricultural Council (GPAC). Committee meetings don't kill leafy spurge. However, since the first Leafy Spurge Symposium held in 1979, improved control technology through coordinated research efforts and increased awareness of the problem and how to deal with it through extension education, there has been a tremendous increase in coordinated control efforts. People working together at County, State/Province, National, and International levels have turned the corner toward an eventual acceptable economic level of control of leafy spurge in North America.

For those of you not familiar with GPAC and its committees, I present the following information:

- The main purpose of the GPAC is to provide an organization for effective cooperation among member agencies, in a voluntary, coordinated attack on problems of the Plains. For more information about GPAC, contact Dr. O. Wendell Holmes, Executive Secretary GPAC, 205 Filley Hall, East Campus, University of Nebraska, Lincoln, Nebraska 68583-0922.
- GP Coordinating Committees are established by the Research Committee of the GPAC when new research is not yet outlined, or new research may not be needed, but coordination of existing work is deemed desirable. The GPC Committees provide the forum for coordination of ongoing work and for coordination of planning of new work needed to properly address a particular problem.
- The objectives of the GPC-14 "Leafy Spurge Committee" as established in 1981 are:

> "To develop and evaluate techniques for weed control and land management to control leafy spurge in the field; to demonstrate through extension and other educational efforts the methods of leafy spurge control and land management to improve the productivity of agricultural and public lands; to increase the knowledge of leafy spurge biology and physiology through basic and applied research; and to coordinate the leafy spurge research and extension program efforts of the cooperating agencies." Note: The framework of the committee is such that it could be revised to address any other weed problem should the need arise.

A brief history of the development of the leafy spurge research, education and coordinated control efforts in the Northern Great Plains will help the readers of this Proceedings understand how the GPC-14 Committee came to be and what is being done to attain its objectives.

For about 15 years, a number of concerned farmers, ranchers, land managers, educators, and scientists tried to bring public attention to the insidious leafy spurge problem. In desperation, this group appointed a Steering Committee which organized the first Leafy Spurge Symposium. It was held in Bismarck, ND in June of 1979. The Bismarck symposium was the first official coordinated attempt to draw lines around the leafy spurge problem and to identify needs. About 125 people attended and it was very successful in meeting its objectives.

Following the Bismarck Symposium, a committee was established to prepare a research project proposal and a request for funding was submitted to the Old West Regional Commission (OWRC). The project entitled "Leafy Spurge Control Using the Integrated Management Systems" was approved by the OWRC with funding of $123,684 for the period March 1, 1981 to February 28, 1982. Research conducted with the OWRC funds provided the basis for additional research, funded primarily by re-direction of existing resources at state and federal research facilities, supplemented with some funding specifically appropriated for leafy spurge control research.

The Bismarck June 1979 Symposium was followed by a conference in Billings, Montana in December of 1979. The Billings Conference provided the impetus for the next step toward an active research, extension and coordinated control program by providing an action-oriented program. This conference was also very well attended and was very successful. A proceedings was published and a *pro tem* steering committee was named which then appointed a Leafy Spurge Working Committee. The Working Committee was given several assignments, one of which was to recommend to the Steering Committee a plan for keeping the leafy spurge effort moving and to insure continuity and coordination among the federal, state and province agencies concerned with the problem. The working committee explored the possibility of GPAC establishing a GPC Committee. The Steering Committee accepted the suggestion. An organizational meeting was held in Fargo, North Dakota in January of 1981, The GPC-14 Committee was approved by GPAC at their Annual Meeting in Garden City, Kansas on June 9-11, 1981, and the first annual meeting of GPC-14 was held in Fargo on June 29-30, 1981. GPC-14 has been very active. Following the Annual Meeting in Fargo in 1981, annual meetings have been held in June at Bozeman Montana in 1982; Sundance Wyoming in 1983; and at Dickinson, North Dakota in 1984. Proceedings of each of the Symposia held at the last three meetings have

been published, and in 1981 and 1982 summary annual reports were published covering research and extension efforts of the member agencies. The 1982 and 1983 symposia addressed survey of the problem and advances in chemical and cultural control. The 1984 symposium and the Proceedings presented herein cover various aspects of biological control of leafy spurge and of taxonomy as it relates to all types of control efforts.

For more information on any of the topics, please contact the author of the paper of interest.

I wish to thank all of the Committee members for their endless effort this past year. On behalf of the Committee members I wish to thank all of those non-members who contributed to the 1984 meeting by presenting a paper or by participating in the very productive discussion sessions particularly the farmers, ranchers, land managers, county weed board members, and all others who are applying the control technology in what seems to be an endless effort. It was you who made the 1984 Symposium a success.

GPC-14 Meeting and Program
Dickinson, ND

June 26

6:00 p.m.	Tour of leafy spurge plots.

June 27

6:30 a.m.	Group Breakfast
8:00	Welcome and Introductory Remarks - Russell Lorenz
8:15	Leafy Spurge Taxonomy - Alena Stahevitch, Moderator
8:20	Alena Stahevitch, Ag. Can. -
8:40	Jurgen R. Schaeffer, MSU - Cytotaxonomic studies of the leafy spurges.
9:00	David Davis, NDSU - The chemotaxonomy program on leafy spurge in Fargo, ND and the confusion regarding numbering of plant collections.
9:20	Paul Mahlberg, Indiana - Chromatographic analyses of taxonomic affinities between leafy spurge.
9:45	Coffee Break
10:05	Don Galitz, NDSU - Physiological variants among leafy spurge.
10:25	John Evans, Utah State - Biochemical evaluation of the complex.
10:45	Taxonomy Discussion Groups
12:00-1:00	Noon lunch
1:00	Bob Nowierski, MSU - The status of biological control of leafy spurge in Montana.
1:20	Norm Rees, USDA, MSU - Matching proper bioagents to the proper leafy spurge.
1:40	R. J. Lavigne, Wyoming
2:00	Robert Carlson, NDSU - Preliminary studies in preparation for release of biocontrol agents.

2:20	Larry Littlefield, NDSU - Plant pathogenic fungi as potential biocontrol agents for leafy spurge.
2:40	Peter Harris, Ag. Can. -
3:00	Bob Nowierski, MSU - Oregon and Idaho biological control updates.
3:15	Break
3:30	Biological Control Discussion Groups
5:30	No Host Social Hour
6:30	"Banquet" - speaker, Robert Nowierski

June 28

6:30-7:45	Group Breakfast and Business Meeting
8:00	Reports from Discussion Group Leaders
9:00-11:30	Chemical and Cultural Control Update by State and Agencies
11:45-12:45	Group Lunch
1:00-6:00	Bus tour to Spurge Research and Problem areas on the plains and in the Badlands.
6:00	Cookout in Medora

Minutes of the GPC-14 Meeting, 28 June 1984
Ramada Inn, Dickinson, ND

The meeting was held in conjunction with a group breakfast and was brought to order by President Russell Lorenz at 7:05 a.m. Other members of the executive committee present were Dr. Peter Fay, Vice President, Dr. Rod Lym, Secretary, and Dr. Don Anderson, Administrative Advisor. The reading of the minutes from the 1983 meeting in Sundance, WY was dispensed with and there was no old business to discuss.

The first item of new business was a proposal to create a multiple state leafy spurge extension monograph or bulletin. The entire group felt this publication would be a useful and needed source of information. Dr. Calvin Messersmith felt the printing should be done by the state extension service in Montana, North Dakota, and Wyoming. Dr. Fay proposed the publication should be in the form of an extension bulletin and should be completed by June, 1985. His proposal was passed and charged to the executive committee for completion.

The second item concerned how often the GPC-14 should meet and where. Dr. Messersmith suggested a fall meeting at least every other year because meetings in June interfere with university leafy spurge research and extension programs. Dr. Lym suggested the meeting should be held every other year, since little new research data is generated from year to year. Mr. George Hittle suggested the meeting be held in Canada, whenever the group meets again. Dr. Anderson stated the purpose of GPC was to keep scientists meeting and comunicating and suggested the group meet annually but stay in Montana, Wyoming or North Dakota. He felt a Canadian meeting site may prevent many people from attending. Dr. Fay proposed the GPC-14 meeting to be held in Bozeman in June

1985 to be hosted by Montana State University. . The motion passed and the executive committee was urged to consider meeting in Canada in 1986.

The third item concerned the Leafy Spurge Newsletter. The newsletter has been published by Montana State University since 1980 and the present editor, Celestine Lacey, will graduate in December 1984, at which time MSU would like someone else to take over as editor. Most people present felt the newsletter was a useful method of disseminating new leafy spurge research data, meeting information, field days, etc. and want it continued. However, after a lively debate, no person or state agency volunteered to take over the editorship of the newsletter.

The last item was the election of a new secretary. Dr. Dave Davis of the USDA in Fargo was nominated and elected. Dr. Fay became the President and Dr. Lym the Vice President for 1984-85.

The meeting adjourned at 8:05 a.m.

Respectfully submitted,

Rodney G. Lym
GPC-14 Secretary

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Morphology and cytogenetics of leafy spurge

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There is a wide range of taxonomic opinion as to the number of species which make up the leafy spurge group (*Euphorbia esula* and its allies). The species concept in this group is discussed from a historical perspective. Field and greenhouse observations on morphological variation are discussed. Cytological observations indicate that most of the material examined was $2n = 60$ (*esula* type) or $2n = 40$ (*cyparissias* type). One narrow-leafed population from lower Austria had $n = 23$ and two lagging chromosomes. Some material from eastern Ontario also exhibited laggards. The most abnormal population was from Willow Creek, Teton Co. ($n = 30$) which exhibited a high proportion of multivalent formation. A survey of pollen stainability from herbarium sheets in the Department of Agriculture herbarium indicated that stainability was high.

Thirty crosses have been carried out involving *E. esula* (broad-leaved), *E. esula* (narrow-leaved), and *E. cyparissias*. Capsule development appears normal. Meiosis in the F_1's will be analyzed.

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Cytotaxonomic studies of the weedy *Euphorbia* species

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A greenhouse collection of 82 ecotypes of the weedy *Euphorbia* species collected in Washington, Oregon, Nevada, Montana, Wyoming, North Dakota, Nebraska, Pennsylvania, New Jersey, Maryland, Canada, Austria, Hungary, Switzerland, and Italy has been established at Bozeman, Montana for cytogenetic analysis.

A survey of the literature shows chromosome numbers for *E. esula* of 2n=16, 60, and 64; for *E. virgata* 2n=56; and for *E. cyparissias* 2n=20, 36, and 40. We found chromosome numbers of 2n=56 and 60 for *E. esula*, 2n=40 to ±80 for *E.* × *pseudo-virgata*, and 2n=36, 40, and 42 for *E. cyparissias*. Our study of 254 cells in 42 plants revealed a high degree of somatic instability, mixoploidy, or mosaicism considered by some to be an indication for interspecific hybridization. The nature of such somatic instability was contributed by Nielsen and Nath (1961) to possible unbalanced nucleoprotein systems that resulted from the combination of distantly related gametes in the formation of such interspecific hybrids.

A map of ecotypes collected from Montana, Washington, and Wyoming shows that average 2n chromosome numbers range from 53.3 in Flathead Co., Montana, to 61 in Sweetgrass Co., Montana. Nearly all plants in this area exhibited some degree of somatic instability. This confirms earlier hypotheses (Croizat, 1945; Radcliffe-Smith, 1981) that this material originates from introgressive hybridization between two or more species, one of which is probably *E. esula*. This is also reflected in the composite idiograms of *E. esula* (2n=60) and *E.* × *pseudo-virgata* (2n=60) which show a resemblance of chromosome morphology in these species. Our morphological studies of leaf characteristics indicated that genetic material of *E. esula*, *E. virgata*, *E. cyparissias*, and *E. uralensis* can be suspected in this complex species group.

Five major types of nucleolus organizer chromosomes (I-V) were identified in this study. Confirmation of their existence was given through the study of the nucleoli formed by them. Preliminary counts showed from 1 to 6 nucleoli per cell with 33% having 5 nucleoli. Polymorphism was reflected in the number of nucleolus organizer chromosomes per plant. *E. pseudo-virgata* showed all 5 nucleolus organizer chromosome types with an average of 3.3 pairs per cell, *E. esula* showed types I, II, IV, and V, with an average of 3.8 per cell, and *E. cyparissias*, had types II and III with 2 pairs per cell.

Segmental allopolyploidy is suggested at the tetraploid and hexaploid chromosome levels as well, with genome formulas AABBCC for *E.* × *pseudovirgata* (2n=60) and *E.*

esula (2n=60), and AABB for *E. cyparissias*, with A, B, and C chromosomes resembling each other closely morphologically.

Meiosis in *E.* × *pseudo-virgata* was normal with only about 40% of the cells showing one univalent.

Literature cited

Croizat, L. 1945. "*Euphorbia esula*" in North America. Am. Midl. Nat. 33:231-243.

Nielsen, E. L. and J. Nath. 1961. Somatic instability in derivatives from *Agroelymus turneri* resembling *Agropyron repens*. Am. J. Bot. 48:345-349.

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The chemotaxonomy program on leafy spurge in Fargo, ND and the confusion regarding numbering of plant collections

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Chemical constituents of leafy spurge have been used as criteria in attempts to determine taxanomic relationships between North American and European plants grown under identical environments. In 1983, Manners reported at the Leafy Spurge Symposium in Sundance, Wyoming, on the possible use of epicuticular wax constitutents to distinguish different leafy spurge biotypes (accessions, collections, etc.) collected from North America and Europe. From his results, it was concluded that the North American biotypes could be grouped together as having similar wax compositions, but slight differences were found in the wax composition of *Euphorbia esula* collected from near Krems, Austria (Manners and Davis, in press; Phytochemistry). The biotypes selected were from a wide geographical location, and of quite variable leaf and shoot morphology. However, only four North American and one Austrian biotype were compared; consequently definitive relationships were not established.

More recently, Davis, Galitz, Manners, Pleszczynska and Mahlberg (Submitted to American Journal of Botany) studied the shoot latex triterpenoids from these same biotypes and from several other North American biotypes in an effort to corroborate and extend these studies. This work will be presented in detail by Dr. Mahlberg in this symposium, and at the meetings of the Botanical Society of America. A gas chromatography method of fairly low resolution was used. The results indicated that the relationships between the various spurges tested appear to be more complex than those proposed by Manners and Davis in their wax study. At least three different groupings of leafy spurge appeared to be possible from that analysis. These differences between the two studies need to be resolved, and correlated with cytological observations underway by several of the people in this symposium. Also, preliminary high resolution gas chromatographic analyses of these same latex constituents lead us to conclude that the relationships are more complex than hoped.

A single analysis of root latex and shoot latex from one plant by Dr. Manners (USDA, Berkeley, California) resulted in different triterpenoid profiles (gas chromatography). If true, this result contradicts the concept that the laticifer in leafy spurge is a single continuous cell with a uniform distribution of triterpenoids throughout the plant. This needs to be investigated further, if the triterpenoids are to be considered for chemotax-

onomic classifications. Perhaps other constituents of the laticifer might be used, as well as the triterpenoids.

Dr. Manners is presently looking for chemical constituents of leafy spurge that can be used as taxonomic tools and/or allelopathic agents. He has found at least one compound of interest extracted from roots of flowering leafy spurge collected in the sandhills of North Dakota in May, 1983. He is characterizing that compound, and will be testing it as the possible irritant factor on the skins of cattle. He has also indicated that the compound appears to be in a class of compounds reported in the literature obtained only from three members of the Euphorbiaceae (two in Japan). This compound may be a potential taxonomic marker, and the European spurges should be checked for its presence or absence. He is following this up, and will extract roots from non-flowering plants as well as shoots from flowering and non-flowering material to determine whether it is organ specific and transient. It exists at a concentration of $2x10^{-4}$% which is quite high. He may report on these results next year.

In summary, it appears that a great deal more work needs to be done to determine whether the laticifer contents or other chemical constituents can be used to separate taxonomic relationships amongst the various collections of leafy spurge. Dr. Mahlberg will discuss this at a greater length, in this symposium.

In Fargo, tissue cultures of several biotypes have been established, and significant differences in the characteristics of the cultures have been observed. One biotype has been regenerated, another appears to be amenable to regeneration, but five others have shown little evidence of being capable of regeneration. Dr. Galitz (North Dakota State University) has compared some of these cultures in their response to the herbicide dicamba, and Dr. Frear (USDA, Fargo, ND) has compared their abilities (and those of intact plants of the same biotype) to metabolize dicamba. A striking difference was found in the metabolism of dicamba by one biotype or selection. Dr. Schaeffer (Montana State University) made an assessment to determine whether the cell cultures might be a good source of material to study the karyotypes of these materials. He has indicated that the cultures do <u>not</u> appear to be useful for his work, for technical reasons.

A problem that should be addressed by this group in this meeting is in the reporting of information obtained from different leafy spurge collections. A concensus of opinion by this group hopefully will eliminate confusion in the literature. First, what should these collections of leafy spurge be called? We have used the term <u>biotype</u> for our own convenience. The advisability of using the term <u>accession</u> was discussed by some in this group at a meeting in Spokane, Washington this past winter. The term <u>collection</u> has been used by Bruckhart at Frederick, Maryland. No matter what term we use in our own research, it would be most useful to be consistent in published articles.

Secondly, a consistent and useful numbering system would be helpful, especially when material is exchanged between locations, as is being done frequently. At Fargo, the material collected was simply numbered consecutively as it was collected in the field or obtained from Dr. McCarty (USDA, Lincoln, Nebraska). Last November, Ebke and McCarty published their results on the taxonomy of their collection based on leaf characteristics, using their numbering system; again, theirs was a numberical system with *E. esula* from Austria being numbered 1-4, and the remaining numbers were consecutive

according to the order of collection. Their numbers include different species of spurge as well as different variations of leafy spurge. This numbering system works well for an individual location, or for one or two publications, but can cause complications later when material is moved from one location to another. In McCarty's case, his nursery has been moved out of Lincoln, with duplicate root stock being taken to Bozeman, Montana and mailed to Fargo, North Dakota. Whose numbering system do you then use? McCarty's or your own?

Several people in this meeting have already been contacted regarding the numbering system. And, of course, several solutions have been proposed. One such solution was to retain numbers 1-100 for McCarty's original collection, 101-200 for the collection at Fargo, 201-300 for a collection in Montana, etc. for other states.

Dr. Messersmith suggests using a two letter zip code (e.g. ND01, ND02, etc.) according to the state, province, or country followed by a numerical sequence of collection. Pros and cons of this system were discussed by Messersmith. Some confusion might arise. For example, would MN be Minnesota or Manitoba? Eileen Sutker and Dr. Bruckhardt (USDA, Frederick, Maryland) are using such a zipcode. They use BC for British Columbia, and Eileen suggests CM for Manitoba (Canada Manitoba). Again, possible confusion arises, since they also use the first letter to designate species other than *Euphorbia*, e.g. CMT = *Cyparissas* from Montana. They use IC to refer to *Euphorbia esula* from Italy, Campito (the town from which the plant was collected). Eileen also recommends using letters rather than just numbers for the pertinent and practical reason that numbers often get lost on pot tags in the greenhouse, Letters seem to be easier to retain and see when you are working with them.

If Dr. Galitz collects plants in North Dakota, and Dr. Lym does also, who's numbers should be used, and how is the information communicated quickly enough to be useful and avoid unnecesary problems? These appear to be minor points, but they are a nuisance when you want to get on with a research program and write up results without being bothered by a lot of interruptions and complication of details. All of the above points merit serious discussion here because many of the people involved in leafy spurge research in the U.S. and Canada are here, and it probably affects us all.

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Chromatographic analyses of taxonomic affinities between North American and European populations of leafy spurge (*Euphorbia*, Euphorbiaceae)

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North American leafy spurges (*Euphorbia* spp.) can be separated into potentially different taxonomic groups on the basis of the qualitative and quantitative analyses of acetone-soluble triterpenoids in the latex exudate from shoots. Three distinct groups, Group I, II, and III, were identified from nine different plant populations (biotypes) of North American and Europe. The gas-liquid chromatographic profile, or fingerprint, for each population was stable under different conditions of growth. The major triterpenoids varied from five to seven in each population. Groups I and II were distinguished from each other by the ratio 3:1 to 5:1 for the two peaks with retention times (RT) 14.1 and 14.5 min, respectively. The two major components, identified as the triterpenols, cycloartenol (RT 15.7) and 24-methylene cycloartenol (RT 16.5), occurred in a ratio of 1.8 to 2.9 and represented 77-85% of the total triterpenoids. The triterpenol, euphol (RT 14.1), was identified as one of the other components in the profile. The profile of Group III differed both qualitatively and quantitatively from the other groups in possessing two new triterpenoids, lacking two triterpenoids present in the other groups, and possessing a ratio of 1:1 for cycloartenol and 24-methylene cycloartenol which together composed 63% of the total triterpenoids. Because of the qualitative similarities of their profiles, Groups I and II were interpreted to be closely related, whereas Group III was considered distantly related to Groups I and II because it differed both qualitatively and quantitatively from Groups I and II. This study has demonstrated that the triterpenoid composition of the laticifer can supplement other criteria for interpreting the taxonomic relationships within the leafy spurge complex as well as may have broader applications in defining nomenclatural relationships within the genus, *Euphorbia*.

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Physiological variants amongst leafy spurge

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Leafy spurge (*Euphorbia esula* L.) is a perennial weed which infests millions of acres of uncultivated land across the northern tier of the United States and Canada and which continues to spread in spite of present control practices. Its economic impact has become staggering because the costs of present control practices surpass economic returns of these marginally productive lands.

Vegetative differences amongst field grown leafy spurge plants have been observed and recorded. In some instances these differences have been due to environmental factors, for this plant is found to grow under widely diverse conditions. However, some of these differences are genetic and these different plant forms have been termed "Biotypes" or "Ecotypes". Several investigators are conducting research on spurge plants which have been propagated from some of the same original stock biotypes collected and grown in nurseries for experimental purposes. Such stock plants have been characterized, their origin noted and have been assigned "Accession numbers" for reference purposes.

Proposed system 0001-0100

A. Numbers 1-100 are reserved for the collection originally made by Dr. Melvin McCarty and maintained at Lincoln, Nebraska from 1978 to 1983. This material has been described in detail by Ebke and McCarty, Weed Science, 31(6):861-865, 1983.

   This material has been removed from Nebraska and is being maintained in outdoor nurseries at Fargo, North Dakota and Bozeman, Montana.

B. Numbers 101-200 are reserved for the North Dakota collection initiated by Dr. Donald Galitz and currently maintained by the Agronomy Department, North Dakota State University, Fargo, N.D.

C. Numbers 201-300 -Montana collections 0201-0300

D. Numbers 301-400 –Wyoming collections 0301-0400

E. Numbers 401-500 -Washington collections 0401-0500

F. Numbers 501-600 -Utah collections 0501-0600

G. G. Numbers 601-700 -South Dakota collections 0601-0700

H. H. Numbers 701-800 -Idaho collection 0701-0800

I. I. Numbers 801-900 -Oregon collection 0801-0900

J. J. Numbers 901-1000 Collections from other 0901-1000

1001 -1100 States as needed 1001-1100

1101-1200 -

etc.

to 2000

K. Numbers in the 2000 range will be reserved for collections from Canada.

2001-2100 British Columbia

2101-2200 Alberta

2201-2300 Saskatchewan

2301-2400 Manitoba

2401-2500 Ontario

2501-2600 Quebec.

Vegetative characteristics which appear to vary from biotype to biotype include leaf surface area and the number and distribution of stomata on both adaxial and abaxial leaf surfaces. It has also been observed that biotype 0007 (collected near Weiser, Idaho) is extremely sensitive to a powdery mildew, a couple others are slightly susceptible, while the rest of the biotypes are apparently resistant to this mildew.

Rooting propagules were used to study the relative sensitivities of biotypes to different herbicides. Varying degrees of response were observed amongst biotypes but questions regarding the quantities of herbicide taken up by each biotype, because of differences in leaf characteristics, made interpretation difficult.

Consequently tissue culture techniques were employed to obtain data on the relative susceptabilities of different spurge biotypes to dicamba treatment. Cell suspension cultures were obtained from callus tissue formed by young spurge stem segments that were grown on a commercial B5 culture medium containing lppm 2,4-D. Dicamba, at final concentrations of 10^{-9}, 10^{-6} and 10^{-3} molar, added to the culture medium proportionately decreased growth of the cell suspension cultures during a 15 day growth period. At 10^{-3}M there was 100% inhibition of cell suspension growth. Although growth curve responses to dicamba concentrations were not significant till 5 to 6 days growth after subculturing, metabolic indicators of herbicide stress exhibited responses at 2 to 3 days after treatment. Primary effects observed were decreased protein, total acid soluable nucleotide and ribonucleic acid content of the cultures as expressed on a per gram fresh weight basis. Nitrate is the primary nitrogen source for cells growing on a B5 medium and the activity of the enzyme nitrate reductase was shown to decrease as the dicamba concentration of the medium increased. No change in the conductivity of the nutrient medium of dicamba treated cells was observed indicating little change in cell membrane function with dicamba treatment. Low concentrations of the herbicide stimulated while high concentrations inhibited the generation of ethylene by the spurge cultures. At this time no evidence of

dicamba degradation or metabolism by the cultures has been defected. Response curves indicate as much as ± 50% variation in the sensitivity of cultures from different spurge biotypes when treated with dicamba.

Cell suspension cultures have provided a mechanism for studying the cellular basis for the response of a plant species to herbicide treatment and has generated additional evidence supporting the concept that there are genetic differences between biotypes.

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Spectral analysis of eight leafy spurge (*Euphorbia esula* L.) accessions

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This study was undertaken to measure the visible (350-750nm) spectral reflectance curves from the leaves of eight accessions of *Euphorbia esula* from Gallatin County, Montana. The objectives were to determine: 1) if differences exist in the relative percentage of reflectance between accessions, and 2) if there is a relationship between the relative percentage of reflectance and habitat.

It was found that the overall mean spectral reflectance values for five of the eight leafy spurge accessions exhibited differences in their percentage of reflected radiation. In addition, it was those accessions collected from the uppermost sites in elevation which exhibited the greatest percentage of reflected radiant energy (Table 1).

The conclusion that differences exist between accessions and that these differences may relate to habitat differences (e.g. elevation and possibly water availability) is very important. It would be valuable to continue these measurements beyond the visible waveband and examine if differences continue into the infrared region of the spectrum.

Table 1. Overall mean spectral reflectance values for eight leafy spurge (*Euphorbia esula* L.) accessions.

ACCESSION #	OVERALL MEAN REFLECTANCE VALUE*
1	10.46a
2	9.40b
3	9.32b
4	9.30b
5	9.23b
6	8.95c
7	7.28d
8	8.47e

↑ Increasing elevation

* Means when followed by a different letter are significant at LSD of 0.01.

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The status of biological control of leafy spurge in Montana

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The leafy spurge hawk moth, *Hyles euphorbiae*, has become well established on leafy spurge NE of Bozeman, in Gallatin County, Montana. Although the moth has been released in eight other Montana counties in addition to Gallatin over the past 17 years, the Bozeman site represents the only known established population of the moth on leafy spurge in North America. The moth has reportedly become established on cyprus spurge, *Euphorbia cyparissias* in Ontario, Canada (P. Harris pers. comm.) and on cypress spurge infestation in New York.

The most recent release of the moth in Gallatin county was made back in 1974 and hawk moth larvae were first observed by the author during summer in 1982 when approximately 60 larvae were collected. The hawk moth population at Bozeman has since expanded to thousands of individuals and the moth has dispersed and colonized sites a number of miles from the original release site. We are currently conducting research on the moth to determine field population levels, rates of growth and development, fecundity, and intrinsic and extrinsic mortality factors. Approximately 2000 larvae have been collected this summer for additional research and redistribution throughout Montana, surrounding states, and Canada.

The stem and root boring beetle, *Oberea erythrocephala* was released in 1982 in field cages 3 miles NE of Bozeman, Montana by Norman Rees, Research Entomologist with the USDA Rangeland Insect Laboratory, Bozeman, Montana. Initial findings showed that approximately 40% of the leafy spurge roots were attacked and contained viable larvae. After three years the beetles are still in the larval stage, which Mr. Rees attributes to poor adaptation to the type of leafy spurge they were released on. Mr. Rees also released *Oberea* at two sites in Stillwater County, Montana in 1983. The beetle is apparently well adapted to the type of leafy spurge found near Columbus, Montana (Stillwater Co.) as he has recovered adults from the release site that were able to complete their development in one year.

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Results of releases of *Oberea erythrocephala* against leafy spurge in Wyoming

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The stem-root boring beetle, *Oberea erythrocephala*, imported from Italy, has been released at five (5) different sites in Wyoming during the period 1980-1982. Two locations were in the northeast (Crook County), two in central Wyoming (Fremont County) and one in the southwest (Lincoln County). Unfortunately because of overzealous ranchers and/or weed & pest district personnel, the spurge at three (3) of the five (5) sites were treated with herbicides within one to two years following the release (Table 1). At the 4th site, in Lincoln County, the original site was sprayed in the spring following the release with the exception of a small center plot in which the release had been made. The 5th site and the oldest (Fremont County) has not been sprayed.

Prior to the spraying, all plots were examined periodically for presence of beetles or evidence of oviposition and/or stem boring. No adults have been observed at any of the sites in any year following the release. With the exception of the two sites in central Wyoming, neither larvae nor evidence of stem boring was encountered. Both larvae and stem tunneling were found the year of release and the following year in plots in Fremont County suggesting a two (2) year life cycle under Wyoming conditions. In the subsequent two years nothing has been found during non-destructive sampling. At Devil's Tower (Crook Co.) adults caged on plants in 1980 died within 3 days suggesting that they were unable to survive on that "variety" of spurge. Consequently, there is little hope that the species of beetle has established itself in Wyoming.

Table 1. Fate of *Oberea erythrocephala* release sites in Wyoming 1980-1984.

County	Site	Date of release	Condition
Crook	Devils Tower	VII-23-80	sprayed, fall 1982
Crook	Devils Tower	VI-2-81	sprayed, fall 1982
Fremont	Lander	VII-13-81	sprayed, fall 1983
Fremont	Sinks Canyon	VII-24-80	extant
Lincoln	Rte. 126	VII-4-82	sprayed, spring 1983
Sheridan	Clear Creek	VI-2-81	sprayed, July 1983

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Native insects associated with leafy spurge and potential impacts on biological control efforts

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The biological control program at NDSU is currently pursuing two lines of investigation. The first is a continuing survey of the fauna associated with leafy spurge which was begun last year. The second is a study of *Sparganothis sulfureana*, a native tortricid moth whose larvae tie up spurge foliage and flowers and feed within the protective "nest."

The survey, which includes sampling at over 100 sites carried out primarily during flowering of spurge, has resulted in identification of 275 insects to genus or species. Represented are species that were considered to have potential importance in any biological control efforts such as spurge-feeders, parasites, and predators. The abundance of parasitic and predacious forms during the period of flowering would seem to indicate that the leafy spurge flowers are providing a desirable and plentiful supply of food for nectar-feeding adult insects; in response, many predacious insects are taking advantage of the presence of an increase in their potential food resources. Many of the parasitic species of Hymenoptera are known to have adult forms which are nectar-feeders. Adult parasite activity on the spurge plants represents a threat to any insect which might be feeding on spurge foliage, including any exotic forms that might be released. In the same vein, predators, attracted by the high level of activity around the plants, represent a similar threat.

Twenty-two species of Hymenoptera have been identified which are known to utilize Lepidoptera larvae as hosts. Eleven of the Hymenoptera species which were collected are known parasites of Coleoptera. Some of these parasites may be host specific, but many have broad host ranges. Since many of the species which are being considered for release for biological control of leafy spurge are lepidopterans and coleopterans, it is possible that successful establishment of any release may depend on the particular insect's ability to escape parasitization and/or predation. Thus, successful establishment may require the "management" of parasitic and predacious insects through timing of release of biological control agents or some method of reducing the numbers present at the time of release.

The second line of investigation, studies on *Sparganothis sulfureana*, centers around two areas of interest:

1. Population densities and the insect's potential as a contributor to the overall reduction of spurge infestations.

2. Its usefulness as an indicator organism in studying biological and ecological factors that may impact on successful establishment of other foliage-feeders that might be released for control of leafy spurge.

Populations of *S. sulfureana* which have been observed in the field did not appear to have much impact on the leafy spurge. The heaviest infestation recorded in 1983 was 13 larvae per 100 plants. In the greenhouse, however, we were able to "flood" plants in cages with larval densities sufficient to kill the plants. It is unlikely that such population levels could be achieved in the field, but it does indicate that the insect has the potential to contribute at least some degree of stress on the plant. Such a contribution could be important is a multi-species biological control strategy.

As a biological indicator, *S. sulfureana* illustrates the potential range of parasites that a foliage-feeder on spurge must cope with. Eight species of parasites were reared from various stages of host development. Although no quantitative estimate of parasite-caused mortality has yet been obtained, it seems possible that low population levels in the field are at least partially due to the parasites.

All eight of the parasite species, according to the literature, have relatively wide host ranges, and *S. sulfureana* is a new host record for five of the species.

Our survey and studies on *S. sulfureana* are continuing in 1984. In addition, it is hoped that some imported species may be available for study this year. Montana State researchers have indicated that some spurge hawk moth larvae will be available soon. We will be testing these on greenhouse-grown spurge and in cages over natural infestations of spurge in eastern North Dakota.

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Plant pathogenic fungi as potential biocontrol agents for leafy spurge

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During the past year numerous plant pathogenic fungi have been studied for the purpose of determining their potential for use in biological control of leafy spurge, *Euphorbia × pseudovirgata.*

The rust fungus, *Uromyces striatus*, occurs sporadically on leafy spurge in eastern North Dakota. It is a systemic rust that remains in the plant for several years and appears in the early spring as pycnia and aecia following emergence of plants from the soil. Typically all above ground portions of the host are killed by early summer, but the systemic mycelium remains viable in the crown or roots and initiates symptoms in the following year's growth. Studies are underway to determine the rate of spread from diseased to healthy plants via the connecting horizontal roots. The repeating uredial stage and the telial stage of *U. striatus* occurs on alfalfa. That fact plus the low rate of spread of the rust within spurge populations precludes its use in biological control. Other rusts, e.g. *Uromyces dictyosperma* on *E. spathulata*, *U. scutellatus* on *E. cyparissias*, *Melampsora euphorbiae* on *E. virgata*, and others are being studied at North Dakota State University or by personnel at the USDA/ARS Plant Disease Research Laboratory, Ft. Detrick, MD. Procedures for inoculation and propagation of those rusts are still being perfected, thus conclusions regarding their biocontrol potential on leafy spurge are not yet available.

Infections of powdery mildew, *Sphaerotheca euphorbiae*, occur occasionally in moist, protected habitats but do essentially no damage to the infected plants. Some leafy spurge ecotypes appear to be more susceptible to powdery mildew than others.

Two soil borne fungi have been studied but they also have serious limitations. *Sclerotinia sclerotiorum* will cause rapid wilting and death of leafy spurge when inoculated into vermiculite in which spurge is growing in a greenhouse. However, healthy plants subsequently grow from crown buds and replace those shoots previously killed. Also, this fungus has an extremely wide host range that includes the most economically important broadleaf crops in North Dakota, e.g. sunflower, potato and dry beans. Research at Montana State University by Dr. David Sands indicates that possibly the wide host range of *S. sclerotiorum* can be limited by genetic manipulation. If that is confirmed and the resulting narrowed host range remains stable, *S. sclerotiorum* might become useful in biocontrol of leafy spurge. Extreme caution, of course, is exercised in such research and field release studies. Another fungus that has a wide host range, i.e. *Sclerotium rolfsii*, has

been studied. Similar to *Sclerotinia*, this fungus rapidly kills shoots of inoculated plants, but those are soon replaced by healthy shoots that arise from crown buds.

To date, the most promising yet very tentative results have come from the foliar pathogen, *Alternaria tenuissima* f. sp. *euphorbiae*, originally reported by Dr. Joseph Krupinsky, USDA/ARS, Mandan, ND. This pathogen occurs commonly on leafy spurge growing in somewhat protected habitats that have longer periods of moisture retention, e.g. within shelterbelts or under shrubby trees. It is not commonly found in open prairie habitats. In greenhouse inoculation with 5.5×10^6 conidia per ml in 0.5% DuPont WK wetting agent caused extensive stem, leaf and floral organ infection and subsequent death. Growth of new shoots from lateral buds below the killed portions of stems provided some replacement for the plant portions killed. Field inoculations made during the recent weeks of May and June 1984 indicated that leafy spurge control in the field with this fungus is much less successful than that obtained in a greenhouse.

A recent collecting trip to eastern Europe (Yugoslavia, Hungary, and Romania) yielded several rusts and Alternaria infections that will be tested for their effects on leafy spurge in the USA.

Successful biocontrol of leafy spurge with pathogens remains an elusive goal at best.

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Biological control of leafy spurge in Canada

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Two institutions in Canada are involved in work on the biological control of leafy spurge, the Regina Research Station of Agriculture Canada and the Alberta-Environmental Centre at Vegreville, Alberta. Studies on potential biocontrol agents in Europe are carried out on behalf of both these organizations by the European Station of the Commonwealth Institute of Biological Control (CIBC) at Delemont, Switzerland. All introductions into Canada are made through the quarantine facility of the Regina Research Station.

To date 6 insect species from Europe have been cleared for release in Canada as biocontrol agents for leafy spurge, and studies are under way on 6 more. Two other species were imported but proved unsuitable as control agents for Canadian leafy spurge. The current status of' these 14 insects is summarized below.

Hyles euphorbiae (L.) (Lepidoptera: Sphingidae)

The spurge hawk moth is established on *Euphorbia cyparissias* L. (cypress spurge) in Ontario but not on leafy spurge. A new strain of this species collected on leafy spurge in Europe is now in culture at Regina and Vegreville and attempts will be made to establish it.

Chamaesphecia tenthrediniformis (Denis & Schiff) (Lepidoptera: Sesiidae)

This root-boring moth was introduced from Europe where it attacks *Euphorbia esula*, but failed to establish in Canada as the Canadian biotypes of leafy spurge proved toxic to it.

Chamaesphecia empiformis Esp. (Lepidoptera: Sesiidae)

This moth, also a root-borer, was imported from cypress spurge in Europe and released against leafy spurge. Leafy spurge has been shown to be toxic to it and it has not been established.

Oberea erythrocephala (Schrank) (Coleoptera: Cerambycidae)

This stem- and root-boring beetle is established in small numbers at one site in Saskatchewan. A release was made at Cardston, Alberta, where initial indications of attack were promising, but the site was later burnt and sprayed. All larvae were presumably destroyed. Further material of this species is to be obtained from Europe for field releases.

Aphthona cyparissiae (Koch) (Coleoptera: Chrysomelidae)

Several species of this genus have been investigated as possible biocontrol agents for leafy spurge. All feed on the roots of the plant in the larval stage, in which they overwinter, and on the foliage as adults. *A. cyparissiae* was released in Alberta and Saskatchewan in 1982 and 1983. One colony at Maxim, Saskatchewan, has completed a generation and is thriving, and it has also survived and bred at Cardston, Alberta.

Aphthona flava Guill. (Coleoptera: Chrysomelidae)

This species, similar in biology to *A. cyparissiae*, was also released in Alberta and Saskatchewan in 1982 and 1983. It has overwintered successfully at Cardston, Alberta and at Mortlack, Saskatchewan.

Aphthona czwalinae Weise (Coleoptera: Chrysomelidae)

This species has a considerable more northerly and easterly distribution in Europe than the two preceding *Aphthona* species. It is hoped that it will extend the range of Canadian leafy spurge sites subject to *Aphthona* attack. Screening tests have been completed in Europe by CIBC on behalf of the Alberta Environmental Centre, and clearance is now awaited to release this species in Canada.

Aphthona nigriscutis Foudras (Coleoptera: Chrysomelidae)

Host-specificity screening of this beetle is being initiated in Europe by CIBC on behalf of Agriculture Canada. It has a similar biology to that of other members of the genus, but appears to show a stronger field preference for cypress spurge on dry sites.

Lobesia occidentis Falk. (Lepidoptera: Tortricidae)

Screening tests on this species at Regina indicate that it is specific to *Euphorbia amygdaloides* and it did not survive on Canadian leafy spurge. Thus it is of no interest as a biocontrol agent for use in North America.

Lobesia euphorbiana Frr. (Lepidoptera: Tortricidae)

The larvae of this moth are leaf-tiers on the tips of leafy spurge shoots. It has been screened and cleared for release in Canada, and is in culture at Regina and Vegreville. Releases are being made in Saskatchewan in 1984.

Minoa murinata (Scop.) (Lepidoptera: Geometridae)

The larvae of this moth are also defoliators, which feed on older leaves than those favoured by *L. euphorbiana* and thus may complement its action. It is well adapted to cool conditions and can complete its development at 12°C. Host-specificity screening of this species is almost complete in the quarantine facility at Regina.

Pegomya argyrocephala Meigen (Diptera: Anthomyiidae)

This fly had been recorded in the literature from cypress spurge in Europe, but has now also been found in the field attacking leafy spurge. The eggs are laid at the shoot tip and the larvae mine down through the shoot to the root. The shoot is usually killed by the larval mining and there is little regrowth from attacked shoots in the following year. On cypress spurge a gall is formed at the base of the attacked shoot, but on leafy spurge the larvae appear to leave the root without gall formation and pupate in the soil. Biological and host-specificity studies on this species are currently being carried out by CIBC on behalf of the Alberta Environmental Centre.

Acyrthosiphon cyparissiae (Koch) (Homoptera: Aphididae)
Aphis euphorbiae (Kltb.) (Homoptera: Aphididae)

These two aphids are currently under study in the quarantine facility at Regina. *A. cyparissiae* feeds mainly on the leaves of leafy and cypress spurge while *A. euphorbiae* feeds on the stems.

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Status report on the host specificity testing of leafy spurge insects at the USDA, Albany, CA, laboratory[1]

ROBERT PEMBERTON

Albany has the responsibility of completing the host specificity of candidate leafy spurge feeding insects, intended for release in the United States. The purpose of the host specificity testing is to predict what the potential host ranges of biological control insects could become if the agents were released. Our goal is to discover insects with broad enough host ranges to accept (and damage) the leafy spurge hybrids and yet narrow enough to avoid use (and damage) of economic and native plants. The overseas (Switzerland, Rome and Canada) screening programs identify leafy spurge insects which are host specific to the genus level. Usually few or no native spurges are tested overseas, and thus little is known concerning the abilities of the insects to use native North American spurges (of which there are 113 species, including 14 under review for legal protection as endangered species).

At Albany we have attempted, often with the help of cooperators, to collect and grow a number of representative native spurges to use as test plants. These plants include species from the different North American subgenera, some endangered species and some bridging species (which are sympatric with both leafy spurge and endangered species and which could carry insects onto endangered species). The emphasis is on species belonging to the subgenus esula which contains the native species most subject to attack, since this is the group to which leafy spurge belongs. The subgeneric concept is not only useful in organizing the large number of *Euphorbia* species, it also appears to be a natural grouping reflecting true relationships. Many *Euphorbia*-feeding insects respond to these subgenera, perhaps accepting as host plants, most of the species in one subgenus while rejecting the species in the other subgenera.

Lobesia euphorbiana is one of three candidate insects currently being tested in the Albany quarantine. This tortricid moth feeds within and kills the shoot tips of its host plants. To date this moth has completed its development on members of the subgenera chamaesyce, agaloma and esula, which represent all but 3 of the native *Euphorbia* species. The species utilized included small annual plants as well as large perennial species. In oviposition tests, the moth laid on all of the spurges which were offered to it. Depending on the availability of plants, we plan to test *Lobesia* against the following subgenus esula species: *E. incisia*, *E. robusta, E. telephiodes* and *E. purpurea*. At this point, *Lobe-*

[1] June 1984

sia euphorbiana's potential host range appears to be too broad to recommend its release in the United States.

Aphthona flava is a chrysomelid flea beetle whose larvae feed on the roots of *Euphorbia* species. It is one of several *Aphthona* species (*A. czwalinae* and *A. cyparissiae* are the others) which are under study as candidate biological control agents for leafy spurge. *A. flava* appears, at this point, to be specific to plants belonging to the subgenus esula. There are 21 species of these esula *Euphorbia* species native to the United States, including 3 endangered species (*E. telephiodes, E. purpurea* and *E. roemeriana*). Of the 3 species belonging to the subgenus esula, which have been tested, *E. robusta* and *E. spatulata* supported oviposition and development while *E. telephiodes* did not. Although a single *E. spatulata* appears too small for complete development of an *A. flava* larva to occur, larvae moving and feeding within clumps of plants probably could complete development. We plan to test *E. incisia, E. purpurea* and *E. palmeri* during the 1984 season. *Aphthona* species are difficult to work with, being univoltine insects with quite poor laboratory rearing rates, even on preferred hosts. For these reasons, it may take some time to complete the work on *A. flava* and the other *Aphthona* species.

Bayeria capitigena is a cecidomyiid gall midge, which galls the apical tips of its host euphorbias. Tips which are galled usually fail to produce flowers. The plants that have been accepted for both oviposition and development thus far have been members of the subgenus esula. No subgenus chamaesyce (58 native species), 1 subgenus agaloma (26 native species) and no native esula (21 native species) euphorbias have been tested. During 1984, we plan to test the following species: *E. spatulata, E. purpurea, E. incisa, E. robusta, E. telephiodes, E. palmeri, E. corallata, E. maculata* and *E. supina*. *Bayeria* is a multivoltine insect that does quite well in the laboratory. For this reason, we expect to finish the testing of this species in the near future.

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Some basic aspects of biological weed control[1]

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Biological Control is defined as the action of parasites, predators and pathogens (viruses, fungi, and bacteria etc.) in maintaining another organism's density at a lower average level than would occur in their absence (Debach, 1964). The practice of biological control has been used effectively against both weeds and insects in the U.S. and other parts of the world.

Some examples of successful biological control of weeds include the control of prickly pear cactus, *Opuntia* sp., by the moth *Cactoblastis cactorum*, the control of skeleton weed *Chondrilla juncea* in Australia, by the rust, *Puccinia chondrillina*, the control of St. John's-wort, *Hypericum preforatum*, in California by the defoliating beetle *Chrysolina quadrigemina*, and the successful reduction of musk thistle densities in Montana by the action of the seed-head weevil, *Rhinocyllus conicus*.

Chemical and cultural weed management methods have played a vital role in controlling weeds in crop and rangeland in the past. But, because of the marginal economic aspects of much of our rangeland, the increased cost of petroleum derived chemicals, the development of resistance of some weeds to herbicides, the inaccessability of many rangeland areas to herbicide application, and the restrictions on herbicide use along waterways and some Park and Forest Service land, additional control methods that are more practical and economically feasible in rangeland, such as the use of biological control agents, will find greater utility in the management of weeds in these areas in the future. Furthermore, in a newly developing technology, plant pathogens hold great promise for controlling weeds of cropland in the future because of their quick kill potential, and relative ease of culturing and broadcasting into the field.

In contrast to conventional chemical and cultural weed management practices, which have traditionally been used to solve immediate weed problems, the practice of using biological control agents has generally not been used for short term control purposes but rather a more long term management of the weeds is the goal. Developing a successful biological control program for a given weed generally takes a number of years and is dependent on the biology of the weed, the success in finding effective and safe natural enemies that have the ability to adapt to a new release area, the number of economically important and/or native plants in potential conflict with the introduced natural enemies

[1] Introductory remarks made during the banquet speech.

(which may restrict the number of agents sanctioned for release and increase the number of host range and host-specificity tests required for each control agent), environmental, political, and other factors.

The protocol for developing a biological control program generally includes: 1) determining the suitability of a weed for biological control (i.e., does it have few economically important or native plant relatives, or perhaps conventional control measures in some areas are not economically feasible or physically impossible); 2) conducting a survey for natural enemies in the place of origin of the weed as well as for native or "naturalized" control agents that might already be present in the introduced area; 3) ecological studies of the weed and natural enemies, preferably in the area of origin of the weed, to determine the potential of the natural enemies in regulating the weed; 4) screening studies to determine the host range and specificity of natural enemies and ascertain their safety; 5) approval by the working group on Biological Control of Weeds, our USDA governing committee that determines the safety of the release agents and sanctions their release; 6) collection/colonization, release, establishment, and redistribution of the natural enemies; and 7) evaluation of the natural enemies effectiveness on the weed (Schroeder, 1984).

There are many advantages in utilizing biological control agents for weed management, particularly for rangeland. Among them are: 1) the application of the practice to economically marginal land where the use of herbicides or cultural management may be too expensive or impossible, such as up steep mountain draws, or along waterways and Park/Forest Service lands with restrictions on the use of chemicals; 2) permanency – once these control agents are established they become a permanent fixture in the environment and year after year they reappear to have an impact on the weed and thus savings accrue year after year; 3) environmental safety – there are no toxic residues associated with these agents or their associated feeding; 4) specificity – the sanctioned biological control agents only attack the weed in which they are purposely released against or, at most, a few close relatives, otherwise they are not given the okay for release; 5) cost-effectiveness – because biological control agents, once they are established and having an impact on the weed, tend to increase on their own, disperse, and find new weed infestations, savings in control costs accrue year after year, which makes biological control a very cost-effective weed management approach, particularly in rangeland; and 6) the potential for integration of biological control with chemical and cultural weed management strategies – there have been numerous weed management programs that have successfully utilized all feasible control methods in a complementary fashion to successfully manage weed problems (i.e., Integrated Weed Management). If the chemical and/or cultural weed control measures are properly timed so that they have a minimal impact on the natural enemies and still control the weeds, then the control strategies will be complementary in their impact on the weed and the rancher will get a "double punch" for his money, so to speak.

I should mention that in every situation good range management should be practiced and competing grass and forage vegetation encouraged otherwise the biological control agents will probably have very little impact on the weeds particularly in ultimately reducing plant densities. Competing vegetation is one of the rancher's greatest resources in solving the spread of a weed and enhancing the effectiveness of biological control agents and one should take great advantage of this and not overgraze the rangeland.

I've discussed some advantages of biological control – now I will elaborate on some disadvantages. Because of the underlying risks, how ever remote, that an introduced biological control agent may attack economically important plants or other desirable flora, biological agents are necessarily subjected to an exhaustive series of tests to guarantee their safety, otherwise they are not even considered for release. The long biological control protocol mentioned above is an example of the steps one goes through to guarantee this safety. Thus, getting from the point of finding the agents to eventually releasing them in the field on some target weed may take several years. Even after the control agents have established in the field it may take 5 to 10 years, or longer, to adapt to the weed or environmental conditions and have a substantial impact on the weed. Thus, biological control is a relatively slow, complex process in contrast to conventional weed management approaches. However, in some situation such as with the biological control of prickly pear cactus in Australia and St. John's-wort in California, the effective controlling agents were able to build up their populations very quickly and have a dramatic impact within a few years in reducing weed population levels. Another disadvantage of biological control is host-specificity. In rangeland and more commonly in cropland the rancher or farmer may be faced with a complex of different weeds that necessitate control. Since the biological control agents sanctioned for release are generally only adapted to a single species of weed host or at most a few close relatives, they would not be helpful in attacking other species of weeds they can not utilize. And lastly, another disadvantage is the potential risk of these introduced agents attacking economically important plants or other desirable flora. I should mention that one of the basic premises of biological control is that the control agents, even in the most highly evolved association between a weed and natural enemy, never completely eliminate their host. Thus, it becomes even less remotely possible that a given control agent could eliminate a plant it was not adapted to. For the record, there has been no case in which a "sanctioned" control agent has been released and has caused the decline of any non-target plant species to date.

I will finish my introductory remarks on biological weed control by discussing the applicability of biological control to rangeland versus cultivated land. Biological control is most suited for rangeland situations for a number of reasons. Rangeland is a more stable agroecosystem – it tends to be less disrupted by pesticides, herbicides, and cultivational practices, and thus the weed host tends to be more readily available for attack by the natural enemies in addition to providing conditions for natural enemy build-up and perpetuation. Furthermore, the economics of much of our marginal rangeland favors non-conventional control, and slower acting weed management strategies are tolerable. In contrast, biological control agents face a tougher situation in cropland. Any insecticides applied to control insects in the cropland could be potentially lethal to insect biocontrol agents. Also, any herbicide and cultivational measures practiced could potentially kill the weed host and thus the natural enemies. In short, it is an unstable agroecosystem. Because of the generally higher cash value per unit area of agricultural products produced from cropland, the economics still favors conventional weed control. More importantly, the farmer is often faced with having to control his weed problems in a hurry or risk losing his crop – something that herbicides and/or cultivational practices can generally prevent.

Insect biological control agents, because of their relatively slower kill potential, will probably continue to have limited utility in the management of weeds in cropland. How-

ever, some plant pathogens such as fungi do have great potential for selective weed control in cropping systems (Charudattan and Walker, 1982). Because fungal plant pathogens are relatively easy to propagate and apply in the field and because of their quick kill potential, they will find increased utility for selective weed management of cropland in the future.

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Effect of original treatments, retreatments and combinations on leafy spurge control as evaluated by live shoot regrowth

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This experiment, located near Devil's Tower National Monument, was established for accumulation of original/retreatment efficacy data for control of leafy spurge. Six successive years of data have been collected since the experiment was established in the spring of 1978.

Original treatments were made May 25, 1978, when the leafy spurge was in the prebud to bloom stage of growth. Liquid formulations were applied with a garden tractor mounted spray unit delivering 128 gpa water carrier. The granular formulation was applied with a hand operated centrifugal granular spreader. Retreatments were made June 12, 1979, May 13, 1980, May 20, 1981, May 19, 1982, May 18, 1983, and May 22, 1984. The retreatments of picloram at 0.5 and 1.0 lb ai/A were terminated with the 1981 retreatment. Retreatments were made with a 13 nozzle truck mounted sprayer delivering 32 gpa water carrier in 1979, 1981 and 1982 and 40 gpa in 1980. Leafy spurge was in the bud to flower stage-of-growth and 8-14 inches in height each year that retreatments have been applied. Plots were 11 by 22 feet arranged in a split block design with two replications. Soil was a sandy loam (65% sand, 23% silt and 11% clay) with 1.5% organic matter and a pH of 7.7.

Percent shoot control is based on reduction of live leafy spurge shoots per square foot recorded from treatment plots as compared to the untreated (check) plots. The retreatments with picloram at 1.0 lb ai/A, applied over all original treatments, is maintaining 97 to 100% shoot control as evaluated in 1984. The 0.5 lb ai/A of picloram is somewhat less effective but is still maintaining 91 to 97% shoot control except where the original treatment was dicamba. The original treatments, without a retreatment program, are being reinfested to a point that retreatment programs would have to be considered. The retreatments of 2,4-D amine, dicamba and the combination of dicamba/2,4-D have not been as effective as the light rates of picloram.

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An evaluation of the activity of selected plant growth regulators, herbicides and mixtures of plant growth regulators and herbicides on leafy spurge (*Euphorbia esula* L.)

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Introduction

Leafy spurge (*Euphorbia esula* L.) is a perennial herbaceous plant which produces an extensive underground root system. It is extremely difficult to control because it develops dense stands, produces vegetative root buds, its roots contain large carbohydrate reserves and it can tolerate a wide variety of habitats and environmental conditions (2).

Wyoming currently has over 48,000 acres infested with leafy spurge. It is mainly a problem on noncultivated land, however, its presence can be very costly. Wyoming has projected the overall cost of controlling 48,618 acres of leafy spurge to be $10,501,488 (3).

Herbicide research for controlling leafy spurge began around 1952 in Wyoming with 2,4-D being the most prominent chemical tested. Many other herbicides have been developed and released since then, however, picloram which became available in 1963 has proven to be the most reliable and effective herbicide for controlling leafy spurge (4).

Regeneration of leafy spurge from viable root buds is a major problem encountered in its control. While certain herbicides have been shown to be effective in controlling shoot growth they appear to not be as effective in destroying the root systems from which new shoots can develop. While there has been considerable research involving growth regulators and their effects on plant growth, research involving growth regulator-herbicide combinations on controlling problem weeds is limited. Research involving growth regulator-herbicide combinations on leafy spurge has not yet resulted in effective control (l).

The purpose of this study was to evaluate the activity of selected growth regulators, herbicides, and mixtures of plant growth regulators and herbicides on leafy spurge shoot and root growth.

Materials and methods

A field study was established to evaluate the following growth regulators; ABG-3034, a cytokinin, (6-benzylamino-purine), mixed cytokinins, mostly zeatin-like, extracted from marine algae tissue, 2,4-D (2,4-dichlorophenoxy=acetic acid), gibberellic acid (2,4a,7-trihydroxy-1-methyl-8-methylenegibb-3-ene-1,10-carboxylic acid-1,4-lactone), glyphosate (*N*-[phosphonomethyl] glycine, NAA (1-naphthaleneacetic acid), and PP333 an experimental antigibberellin compound ([2RS,3RS]-l-[4-chlorophenyl]-4,4-dimethyl-2-1,2,4-triazol-1-yl-]pentan-3-ol) and herbicides; dicamba (3,6-dichloro-o-anisic acid) and picloram (4-amino-3,5,6-trichloropicolinic acid) on leafy spurge shoot control.

The experimental site was located 5 miles south of Hulett, Wyoming on the Terry Peterson ranch, on the first alluvial bench of the Belle Fourche River. Plots were established June 29, 1982 on a uniform infestation of leafy spurge 8-24 inches tall. Plants were in the prebud to full bloom stage of growth with densities averaging 18 shoots/sq feet. A sparse understory of blue grass and western wheatgrass 4-12 inches in height was also present.

Growth regulators and herbicides were applied by hand with a 6-nozzle knapsack spray unit in 40 gal/A water carrier. Plots were 9 by 30 feet and arranged in a completely randomized design with three replications. Soil was a clay loam (39% sand, 31% silt, and 30% clay) with 2.1% organic matter and a pH of 7.8. Subsoil moisture was good and the leafy spurge was in excellent condition. The air temperature was 75F with a relative humidity of 45%. Winds were from the northeast at 0-10 mph and skies were partly cloudy. Soil temperatures ranged from 64F at the surface to 65F at 1 in., 75F at 2 in., and 80F at 4 in. Treatment applications began at 2:00 pm and were finished at 6:00 pm MDT.

Growth regulators and herbicides were applied singularly and in combination at the following rates: cytokinin (BAP) at 12 g ai/A, mixed cytokinins at 1 gal of formulation/A, 2,4-D amine at 0.25 lb ai/A, gibberellic acid at 12 g ai/A, glyphosate at 1/8 lb ai/A, NAA at 12 g ai/A, PP333 at 12 g ai/A, dicamba at 1.0 and 2.0 lb ai/A, and picloram at 0.25 and 0.5 lb ai/A.

The experiment was evaluated May 19, 1983, 324 days following treatment. Evaluations were based on percent shoot control as compared to the untreated check.

Data were analyzed using an analysis of variance procedure for a completely randomized design.

Data were analyzed for significance at the 95% confidence level. Means were separated on the basis of the least significant difference (LSD) test. Due to the large number of treatments involved in this study all treatment means are not reproduced on the same page. Treatment means within the same experiment, although on different pages, are comparable using the appropriate LSD value.

Results

Significant increases or decreases in shoots/sq feet were not observed for any of the GR treated plots. However, GA at 12 g ai/A had the largest increase in shoots/sq feet at 28.0. BAP at 12 g ai/A resulted in the lowest number of shoots/sq feet with 16.9. The untreated plots had an average of 24.3 live shoots/sq feet (Table 1).

Table 1. Effects of growth regulators on leafy spurge shoot counts.*

Treatment	Rate** ai/A	Percent shoot control	Shoots/ sq ft
BAP	12 g	30	16.9
Cytokinin	1 gal	20	19.4
2,4-DA	0.25 lb	0	26.0
Gibberellic acid	12 g	0	28.0
Glyphosate	0.125 lb	16	20.3
NAA	12 g	16	20.5
PP333	12 g	0	24.4
Check	---	0	24.3
LSD (.05)			10.3
CV%			41

* Values are the average of three replications.
**Cytokinin is reported as actual formulation/A.

No combination treatments of GR's + dicamba at 1.0 lb ai/A resulted in significant decreases in shoots/sq feet. However, the mixed cytokinins + dicamba resulted in the greatest reduction at 17.2 shoots/sq feet. Dicamba applied alone at 1.0 lb ai/A had no significant effect on the number of shoots/sq feet (Table 2).

Table 2. Effect of growth regulator-dicamba combinations on leafy spurge shoot counts, dicamba applied at 1.0 lb ai/A.*

GR + dicamba at 1.0 lb/A	Rate** ai/A	Percent shoot control	Shoots/sq ft
BAP	12 g	2	23.8
Cytokinin	1 gal	29	17.2
2,4-DA	0.25 lb	23	18.6
Gibberellic acid	12 g	24	18.4
Glyphosate	0.125 lb	28	17.4
NAA	12 g	12	21.3
PP333	12 g	5	23.2
dicamba	1.0 lb	0	24.7
Check	---	0	24.3
LSD (.05)			10.3
CV%			41

* Values are the average of three replications.
**Cytokinin is reported as actual formulation.

2,4-D at 0.25 lb ai/A + dicamba at 2.0 lb ai/A and PP333 at 12 g ai/A + dicamba at 2.0 lb ai/A both resulted in significant decreases in the number of shoots with 12.9 and 13.9 shoots/sq feet, respectively. Dicamba applied alone at 2.0 lb ai/A had no significant effect on the number of shoots/sq feet (Table 3).

Table 3. Effect of growth regulator-dicamba combinations on leafy spurge shoot counts, dicamba applied at 2.0 lb ai/A.*

GR + dicamba at 2.0 lb/A	Rate** ai/A	Percent shoot control	Shoots/sq ft
BAP	12 g	18	20.0
Cytokinin	1 gal	18	19.9
2,4-DA	0.25 lb	47	12.9
Gibberellic acid	12 g	23	18.7
Glyphosate	0.125 lb	40	14.6
NAA	12 g	36	15.6
PP333	12 g	43	13.9
dicamba	2.0 lb	23	18.8
Check	---	0	24.3
LSD (.05)			10.3
CV%			41

* Values are the average of three replications.
**Cytokinin is reported as actual formulation/A.

GR's + picloram at 0.25 lb ai/A combination treatments resulting in significant reductions in the number of shoots/sq feet were glyphosate at 0.125 lb ai/A + picloram and PP333 at 12 g ai/A + picloram. The reduction to 10.2 shoots/sq feet by PP333 was highly significant, compared to the untreated check. Picloram applied by itself at 0.25 lb ai/A did not significantly reduce the number of shoots/sq feet (Table 4).

Table 4. Effects of growth regulator-picloram combinations on leafy spurge shoot counts, picloram applied at 0.25 lb ai/A.*

GR + picloram at 0.25 lb/A	Rate** ai/A	Percent shoot control	Shoots/sq ft
BAP	12 g	35	15.8
Cytokinin	1 gal	40	14.6
2,4-DA	0.25 lb	40	14.7
Gibberellic acid	12 g	38	15.1
Glyphosate	0.125 lb	53	11.5
NAA	12 g	40	14.7
PP333	12 g	58	10.2
picloram	0.25 lb	38	15.1
Check	---	0	24.3
LSD (.05)			10.3
CV%			41

* Values are the average of three replications.
**Cytokinin is reported as actual formulation/A.

All GR + picloram at 0.5 lb ai/A combination treatments resulted in highly significant reductions in the number of shoots/sq feet with the exception of BAP + picloram whose reduction to 11.3 shoots/sq feet was significant at the 95% confidence interval. Picloram applied by itself also had at highly significant reduction of 3.1 shoots/sq feet (Table 5).

Table 5. Effect of growth regulator-picloram combinations on leafy spurge shoot counts, picloram applied at 0.5 lb ai/A.*

GR + picloram at 0.5 lb/A	Rate** ai/A	Percent shoot control	Shoots/sq ft
BAP	12 g	53	11.3
Cytokinin	1 gal	89	2.7
2,4-DA	0.25 lb	82	4.3
Gibberellic acid	12 g	88	2.8
Glyphosate	0.125 lb	91	2.3
NAA	12 g	94	1.4
PP333	12 g	87	3.2
picloram	0.5 lb	87	3.1
Check	---	0	24.3
LSD (.05)			10.2
CV%			41

*Values are the average of three replications.
**Cytokinin is reported as actual formulation/A.

Discussion and summary

Growth regulators were applied to leafy spurge with hopes of enhancing the activity of the herbicides dicamba and picloram. Growth regulator screening studies were conducted both in the greenhouse and field to observe the effects of growth regulators, herbicides, and growth regulator-herbicide combinations on various parameters of leafy spurge growth.

None of the GR treatments had a significant effect on the number of shoots/sq feet. Combination treatments of GR's and dicamba at 1.0 lb ai/A also had no significant effect on the number of shoots/sq feet. However, combination treatments of GR's with dicamba at 2.0 lb ai/A did result in a significant reduction in the number of shoots/sq feet, although the best GR-dicamba combination only produced 47% shoot control which was not significantly better than dicamba applied alone at 2.0 lb ai/A.

Treatments containing GR + picloram at 0.25 lb ai/A also demonstrated significant shoot reductions. However, the largest reduction only resulted in 58% shoot control, and was not significantly better than the control obtained with picloram applied alone at 0.25 lb ai/A.

The greatest shoot/sq feet reductions were attained with GR's + picloram at 0.5 lb

ai/A treatments, with the largest reduction resulting in 94% shoot control. However, this reduction was not significantly better than where picloram was applied alone at 0.5 lb ai/A, which resulted in 87% shoot control.

The results of this field study tend to support the data of the greenhouse study indicating that the GR's evaluated in these studies seemed to have no significant effect on increasing the activity of dicamba and picloram in controlling the regeneration of leafy spurge from viable root buds.

Although none of the GR's evaluated in this study seemed to hold promise for increasing the activity of dicamba and picloram in controlling leafy spurge, there are many GR's yet to be evaluated. Continued research is necessary in this field of study if an effective GR-herbicide combination is to be found. If such a combination were to be found it would greatly aid in the effort of eliminating this persistent and expensive pest from our rangelands.

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Evaluation of mowing as a setup treatment prior to herbicide treatment for leafy spurge shoot control

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Plots were established near Hulett, Wyoming to determine the effectiveness of mowing, prior to treatment with herbicides, on controlling leafy spurge shoot regrowth.

Leafy spurge plants were mowed within 1 to 2 inches of ground level with a sickle bar mower June 30, 1982, 21 days prior to treatment with herbicides. The herbicide treatments were applied July 21, 1982, to a mature stand of leafy spurge 6-8 inches in height, with a 13-nozzle truck mounted sprayer using 23 gpa water carrier. Plots were 21.5 by 55 ft with one replication.

Shoot counts made May 19, 1983 and May 22, 1984 indicated that mowing prior to herbicide treatment may have potential for reduced rates of chemical for leafy spurge shoot control. The treatment of 1.0 lb ai/A of 2,4-D LV ester was as effective as 0.5 lb ai/A of picloram. However, more data is necessary to fully evaluate the value of mowing as a setup treatment for controlling leafy spurge.

Leafy spurge shoot control.

Treatment	Rate lb ai/A	Percent[2] Shoot Control	
		1983	1984
dicamba	1.0	32	36
picloram (K salt)	0.5	86	75
2,4-DLVE	1.0	91	85
Check	---		
shoots/ft²		*23.2*	*27.9*

[1]Plots mowed June 30, 1982 and treatments applied July 21, 1982.

[2]Shoot counts May 19, 1983 and May 22, 1984.

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Leafy spurge research update, 1984

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Three research projects were reported, as a portion of the research conducted on leafy spurge at Montana State University. It is important to understand the reproductive abilities of leafy spurge including the mechanisms that control regrowth from underground structures, the effect of root crown injury on regrowth, and seed dispersal by birds.

The first study examined the effect of glyphosate [N-(phosphoremethyl)-glycine] on the regulation of bud dormancy in leafy spurge. The objectives of this study were to measure the field responses of leafy spurge to glyphosate applied at several stages of plant growth, and to monitor the movement of ^{14}C-glyphosate in the root system of mature plants grown under field conditions. This information can be used to determine if a relationship between the pattern of glyphosate movement in leafy spurge and lateral bud release from dormancy exists.

Glyphosate was applied to leafy spurge in the field at sublethal and lethal rates. A proliferation of growth ("witches' broom") was observed on stems of leafy spurge plants that were treated with glyphosate the previous spring. Fall applications of glyphosate stimulated witches' broom growth and an increase in the number of stems/m ^{2} as a result of bud growth on the crown region of the root system.

An average of 74% of the total ^{14} C-glyphosate applied to an upper leaf was absorbed. There was increased absorption in plants that were senescing. There was a decrease in the amount of labelled glyphosate translocated out of the treated leaf as applications were made later in the season. The highest concentration of labelled glyphosate other than the treated leaf was in the root crown buds of plants that were senescing at the time of application (Table 1). Increased concentration of ^{14}C-glyphosate in the root crown buds of senescing plants may be directly related to the number of buds released from dormancy the following summer.

When leafy spurge is pulled, it generally breaks off below the thickened crown causing considerable damage and removing a large percentage of the buds from which regrowth normally occurs. Hand pulling experiments indicated that by pulling the leafy spurge plants in the bloom stage the regrowth vigor was significantly reduced for 2 years.

In June of 1983 an experiment was initiated to compare the effect of machine pulling of leafy spurge with mowing, an application of 0.56 kg ai/ha of picloram (4-amino-3,5,6-trichloropicolinic acid), an application of 2.24 kg ai/ha of 2,4-D amine (2,4-dichlorophenoxy acetic acid), and application of 2,4-D amine (1.12 kg ai/ha) to regrowth after pulling and mowing. Measurements taken on August 11, 1983 indicated that 2,4-D

applied alone in June provides better control than the other treatments (Table 2). None of the machine pulling, mowing, herbicides, or regrowth treatments significantly decrease the density of leafy spurge 1 year after application.

The best application of the pulling concept to leafy spurge control may be inoculation of the soil with pathogens. Injury to the root system can increase potential infection of plants by pathogens.

The third research project[1] was initiated to determine if mourning doves were disseminating leafy spurge seed. The gizzard and crop were collected from seven mourning doves during hunting season in an area infested with leafy spurge. No intact seeds were found in the gizzards and only one intact seed was found in the crop of one bird. The single seed was viable.

In another experiment 150 grams of 81% viable seeds were fed to 10 doves in captivity. The fecal matter was collected and all intact seeds (including all species) were separated out. One intact seed was found which was viable.

Currently germination tests are being conducted on leafy spurge seeds found in mourning dove nests. Nine of the 13 nests collected contained leafy spurge seeds and 54 to 9 seeds were found in each nest.

Table 1. The concentration of ^{14}C-glyphosate (expressed as DPM's per gram of oven dried tissue) in root crown buds of leafy spurge 120 hours after application at three stages of growth.[a]

Herbicide treatment date	Growth stage of leafy spurge at application	DPM's per gram of oven dried tissue 120 hours after application[b]
6-2-83	Pre-bloom	18,800 a
7-25-83	Full bloom	22,660 a
9-5-83	Senescent	79,750 b

[a] Data are averages from four replications.
[b] Means within a column followed by the same letter are not significantly different at the 5% level using the LSD test.

[1] This project is in cooperation with David Blockstein at the James Ford Bell Museum of Natural History, University of Minnesota.

Table 2. The effect of machine pulling, mowing, herbicide treatments, and combination treatments on leafy spurge dry weight biomass, dry weight biomass of perennial grasses, and cattle use the same season as application.

			Data Collected on 8-11-83		
			Dry Weight Biomass		
	Application			Perennial	Cow Feces
Treatment	Rate	Date	Leafy spurge	Grass	Per Plot
Bourquin Puller	---	6-29-83	656 abc	731 ab	1.7 ab
Mow	---	6-29-83	1140 bcd	333 a	2.7 ab
Bourquin Puller + 2,4-D Amine	---	6-29-83			
	1.12	7-21-83	183 a	828 ab	3.7 b
Mow + 2,4-D Amine	---	6-29-83			
	1.12	7-21-83	333 ab	366 a	4.3 b
2,4-D Amine	2.24	6-29-83	161 a	1140 b	5.0 b
Picloram	0.56	6-29-83	1624 d	871 a	0.7 a
Control	---	---	1527 cd	1226 b	2.0 ab

[a] Means within a column followed by the same letter are not significantly different at the 0.05 level using the LSD test.

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Leafy spurge control in North Dakota - 1984

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Economical control of leafy spurge continues to be a major goal of the leafy spurge program in North Dakota. An experiment to evaluate cost effective long-term leafy spurge management was established at four sites in North Dakota in 1980. The predominate grasses were bluegrass (*Poa* spp.) with occasional crested wheatgrass, smooth brome, big bluestem or other native grasses. All sites were established in early June except one site which was established in September 1980. The herbicides applied in 1980 (Year 1) included 2,4-D ((2,4-dichlorophenoxy)acetic acid), dicamba (3,6-dichloro-*o*-anisic acid) and picloram (4-amino-3,5,6-trichloropicolinic acid). The treatments were applied using a tractor mounted sprayer delivering 8.5 gpa water at 35 psi. The plots were 15 by 150 feet and treatments were replicated twice at each site in a randomized complete block design. In June 1981 (Year 2) each plot was divided into six 7.5 by 50 feet subplots for retreatments of 2,4-D, picloram, dicamba or no retreatment. Retreatments were applied again in June 1982 and 1983 (Years 3 and 4). Forage yields were obtained from each plot by harvesting a 3 by 25 foot section with a flail mower in July 1981 and a 4 by 15 foot section with a rotary mower in July 1982 and 1983. Sub-samples were taken by hand along each harvested strip so that leafy spurge and forage weight could be separated. The samples were oven dried and are reported with 12% moisture content. Economic return was estimated by converting forage production to hay sold for $48.00/T minus the cost of the herbicide and estimated application cost, i.e. 2,4-D = $2.17/lb ai, picloram 2S = $40.00/lb ai, dicamba = $10.30/lb ai, broadcast application = $2.50/A and roller or wick application = $4.10/A.

Picloram applied at 1.0 or 2.0 lb/A in 1980 gave only 20 and 40% leafy spurge control, respectively, in June 1984, but increased to 87 and 89% if retreated annually with picloram at 0.25 lb/A (Table 1). Annual forage production was increased compared to the control, but due to the high initial cost of picloram at 1.0 or 2.0 lb/A, plus annual retreatments, the net return after four years ranged from -$29 to -$120/A. Picloram applied at 1.0 lb/A without a retreatment in 1981-1983 resulted in a net return of $12/A, but would require a retreatment in 1984 to maintain satisfactory control. Annual application of picloram at 0.25 lb/A or picloram plus 2,4-D at 0.25 + 1.0 lb/A resulted in 52 and 67% leafy spurge control, respectively, after three applications, the highest forage production of all treatments at 6380 and 6768 lb/A, respectively, and a net return of $43 and $45/A, respectively. Annual application of 2,4-D at 2.0 and 1.0 lb/A resulted in a return of $44/A, but only 17% leafy spurge control.

Previous research by North Dakota State University has shown that picloram at 0.25 to 0.5 lb/A applied annually will give satisfactory leafy spurge control after 3 to 5 years. An experiment was begun in 1981 to establish the number of annual applications of picloram and picloram plus 2,4-D treatments needed to provide 90 to 100% control of leafy spurge, to investigate synergism between picloram and 2,4-D at various concentrations, and to evaluate picloram residue in three soil types following annual application.

The experiment was established on 25 August 1981 at Dickinson, 1 September 1981 at Sheldon and on 11 June 1982 at Valley City. All treatments were applied annually except 2,4-D alone which was applied biannually (both spring and fall). Picloram treatments were applied in late August 1981 and in June of 1982 and 1983. Thus the Dickinson and Sheldon sites have received three picloram and picloram plus 2,4-D treatments and five 2,4-D treatments, while the Valley City site has received two and four treatments, respectively. The plots were 10 by 30 feet and each treatment was replicated four times in a randomized complete block design at all sites. Evaluations were based on percent stand reduction as compared to the control.

A soil bioassay was conducted to determine the herbicide residue from annual broadcast applications of picloram at 0.25, 0.375 and 0.5 lb/A. Three soil samples per plot to a 4 inch depth were taken to form a composite sample in June and August of each year. Sunflower height, fresh weight and dry weight in a greenhouse bioassay were used to determine picloram residues. The soil at Dickinson was a loamy fine sand with pH 7.2 and 0.6% organic matter, at Sheldon was a silty clay loam with pH 5.8 and 3.4% organic matter, and at Valley City was loam with pH 6.0 and 3.3% organic matter.

Picloram at 0.25, 0.375 and 0.5 lb/A provided 42, 61 and 75% leafy spurge control, respectively, after three treatments when averaged across the Dickinson and Sheldon locations in August 1983 (Table 2). Control in August 1983 was not increased when compared to the August 1982 evaluations. 2,4-D alone provided between 19 and 30% control of leafy spurge after biannual applications for three years.

Leafy spurge control increased when 2,4-D, regardless of rate, was applied with picloram at 0.25 lb/A and when 2,4-D at 1.5 lb/A was applied with picloram at 0.375 lb/A (Table 2). Leafy spurge control with picloram at 0.25 or 0.375 increased from 42 and 61%, respectively, to 71 and 81%, respectively, when 2,4-D at 1.5 lb/A was applied with picloram. Picloram at 0.5 lb/A plus 2,4-D provided 78 to 85% leafy spurge control and was similar to picloram at 0.5 lb/A alone at 75%. The greatest synergism of 2,4-D and picloram seems to be with 2,4-D rates of 1.5 lb/A or less and picloram at 0.375 lb/A or less. In general, leafy spurge control was lower at Valley City than at Dickinson or Sheldon after two years. Also at Valley City, the addition of 2,4-D to picloram tended to increase leafy spurge control compared to picloram alone and control in August 1983 was similar to or slightly higher than control in August 1982.

Control decreased 10 to 20% in June of 1984 for all treatments just prior to retreatment (Table 2). Previous research at North Dakota State University has shown that after leafy spurge control reaches approximately 70% or more, control is maintained for a much longer period than when below 70%. Several of the treatments are near 70% control after three years and it is expected leafy spurge control will reach 80 to 90% with

many of the picloram and picloram plus 2,4-D treatments and remain at that level after the fourth treatment.

Picloram soil residue did not accumulate from annual applications regardless of location (Table 3). Picloram residue ranged from 0 to 0.18 ppm in August following June application, but generally was undetectable the following spring. Thus, the general increase in leafy spurge control was due to a gradual stand reduction and not to an increased picloram level that would prevent reestablishment.

A third aspect of the leafy spurge control program has been to evaluate various plant growth regulators (PGR) in combination with herbicides for leafy spurge control. Although many PGR's have been evaluated, only dikegulac sodium (tradename Atrinal by Maag Agrochemicals, Vero Beach, Florida) was found to be synergistic with 2,4-D and picloram for leafy spurge control. Dikegulac sodium causes temporary inhibition of plant growth, reduction or elimination of flowering and promotion of axillary plant growth. Dikegulac sodium activity on leafy spurge decreases as the plant matures. The purpose of these experiments was to evaluate the synergism of dikegulac sodium with picloram or 2,4-D in the field both as a tank mix and split application.

The experiments were established at Lisbon, ND in a mused quarry with a heavy infestation of leafy spurge. The first two experiments were established on 26 May 1982 when the leafy spurge was in the yellow bract growth stage and before true flower initiation. The weather was partly cloudy, 76º F and 67% relative humidity with a soil temperature of 76° and 65º F at 1 and 4 inches, respectively. The plots were 10 by 30 feet, and treatments were replicated four times in a randomized conplete block design. The treatments were applied in 8.5 gpa, at 35 psi. Evaluations were based on percent stand reduction as compared to the control.

In the first experiment dikegulac sodium at 0.5, 1.0 and 2.0 lb/A was applied alone and tank-mixed with picloram at 1.0 or 2.0 lb/A and 2,4-D at 2.0 lb/A. One month after application leafy spurge plants treated with dikegulac sodium alone were stunted, with many axillary branches and most flowers had been aborted. In general, the number of axillary branches increased as the dikegulac sodium rate increased. By the end of the growing season plants treated with dikegulac sodium at 2 lb/A still had many axillary branches, but plants treated at the lower rates had resumed normal growth. Leafy spurge control was increased when picloram at 1.0 lb/A was applied with dikegulac sodium (Table 4). Leafy spurge control was 27% 24 months following application of picloram at 1.0 lb/A alone, but was 64, 68 and 76% when tank-mixed with 0.5, 1.0 and 2.0 lb/A, respectively, of dikegulac sodium. Dikegulac sodium tank-mixed with picloram at 2.0 lb/A tended to increase leafy spurge control compared to herbicide applied alone. Leafy spurge control with 2,4-D was not affected by dikegulac sodium.

In the second experiment dikegulac sodium was applied as a tank mix or split treatment with picloram and 2,4-D. Dikegulac sodium alone at 0.5 and 1.0 lb/A was applied on 26 May 1983. Picloram or 2,4-D at 1.0 lb/A were applied on 30 June 1983, as a split treatment alone or as a tank mix treatment with dikegulac sodium. The weather was clear with 76º F, 69% relative humidity and a soil temperature of 80° and 76º F at 1 and 4 inches, respectively. The leafy spurge was in the true flower growth stage and beginning seed set. Dikegulac sodium had no observable effect on leafy spurge growth when ap-

plied later in the growing season. However, leafy spurge control with picloram at 1.0 lb/A increased slightly when dikegulac sodium was used as a pretreatment or a tank mix compared to picloram applied alone (Table 5). Leafy spurge control with 2,4-D was not affected by dikegulac sodium.

The third experiment was similar to the second experiment with dikegulac sodium alone applied on 7 September 1982 and 2,4-D or picloram applied on 4 October 1982 either alone for the split treatments or tank-mixed with dikegulac sodium. On 7 September the sky was partly cloudy with 78º F and 80% relative humidity, the soil was dry and leaf spurge was under moisture stress. On 4 October the temperature was 57º F with 45% relative humidity and the leafy spurge was red and yellow with slight frost damage. Dikegulac sodium alone did not affect leafy spurge growth or control with picloram and 2,4-D when applied as a fall treatment to mature plants (Table 6).

Dikegulac sodium was very active on leafy spurge early in the growing season before flower initiation, as indicated by increased axillary branching, flower abortion and stem shortening, but it shows little effect on more mature plants. Leafy spurge control increased when dikegulac sodium at 0.5 to 2.0 lb/A was applied with picloram at 1.0 and 2.0 lb/A compared to picloram alone.

A major emphasis of the leafy spurge control program in the future is to investigate the proper timing of picloram applications to leafy spurge for maximum control. Parameters under consideration are plant growth stage, air temperature, relative humidity and soil nutrients. Also, picloram translocation and loss from the leafy spurge root system is being studied.

Table 1. Cost comparisons of various leafy spurge control regimes from selected long-term treatments 1980-84.

1980 Treatment	Rate	1981-83 Retreatment	Rate	% L.S. Control June 1984	1980-83 Total Forage Yield	Total Value As Hay	Cost	Net Return
	(lb/A)		(lb/A)		(lb/A)	($/A)	($/A)	($/A)
2,4-D	2.0	2,4-D	1.0	17	5715	137	19	+44
Pic 2S	1.0	---	---	20	5355	128	42	+12
Pic 2S	2.0	---	---	40	4228	102	82	-54
---	---	Pic+2,4-D	0.25+1.0	67	6768	162	43	+45
---	---	Picloram	0.25	52	6380	153	36	+43
---	---	Dicamba	2.0	65	4592	110	68	-32
Pic 2S	1.0	Pic+2,4-D	0.25+1.0	88	6451	155	110	-29
Pic 2S	1.0	Picloram	0.25	87	4139	99	103	-78
Pic 2S	2.0	Picloram	0.25	89	5088	122	168	-120
Control	---	---	---	0	3083	74	0	0

Table 2. Leafy spurge control from annual picloram or picloram plus 2,4-D treatments and biannual 2,4-D treatments at three locations in North Dakota.

		Site/Evaluation Date								
		Sheldon		Dickinson		Valley City		Mean		
		August		August		August		August		June
Herbicide	Rate	1982	1983	1982	1983	1982	1983	1982	1983[a]	1984[a]
	(lb/A)	---------- (% Control) ----------								
Picloram	0.25	49	48	48	37	68	25	49	42	33
Picloram	0.375	79	77	56	49	78	63	66	61	58
Picloram	0.5	75	80	74	70	81	48	74	75	68
2,4-D bian	1.0	22	35	30	27	5	14	27	30	24
2,4-D bian	1.5	15	33	20	9	14	29	18	19	24
2,4-D bian	2.0	20	54	9	11	37	28	14	30	31
Pic+2,4-D	0.25+1.0	54	76	69	62	41	33	63	68	59
Pic+2,4-D	0.25+1.5	58	91	61	56	50	54	60	71	60
Pic+2,4-D	0.25+2.0	78	83	49	45	49	49	61	61	57
Pic+2,4-D	0.375+1.0	78	87	64	65	67	68	70	74	65
Pic+2,4-D	0.375+1.5	74	84	67	78	61	65	70	81	67
Pic+2,4-D	0.375+2.0	81	87	69	39	64	56	74	60	68
Pic+2,4-D	0.5+1.0	77	89	79	83	61	59	78	85	74
Pic+2,4-D	0.5+1.5	58	78	65	84	82	68	62	84	72
Pic+2,4-D	0.5+2.0	75	76	80	81	87	71	78	78	64
LSD(0.05)		26	22	19	24	30	28	18	18	21

[a] Experiment at Valley City began in June 1982 and is not included in August 1983 or June 1984 means.

Table 3. Picloram soil residue following annual applications in 1981, 1982, and 1983 at Dickinson and Sheldon, and in 1982 and 1983 at Valley City, North Dakota.

		Location/Evaluation Date									
		Dickinson			Sheldon				Valley City		
		1982	1983		1982		1983		1982	1983	
Treat-ment	Rate (lb/A)	Aug	June[a]	Aug	June[a]	Aug	June[a]	Aug	Aug	June[a]	Aug
		(PPM)									
Picloram	0.25	0	0	0	0	0.10	0	0	0	0	0.10
Picloram	0.375	0	0	0.01	0	0.12	0	0	0	0	0.08
Picloram	0.5	0	0.01	0.08	0	0.18	0	0.03	0	0	0.09

[a]Soil samples were obtained immediately before the annual picloram treatment was applied.

Table 4. Leafy spurge control with 2,4-D or picloram applied alone or with dikegulac sodium on 26 May 1982 near Lisbon, ND.

Treatment	Rate	Control		
		June 83	August 83	June 84
	(lb/A)	(%)		
Dikegulac sodium + picloram	0.5 + 1.0	92	70	64
Dikegulac sodium + picloram	0.5 + 2.0	100	90	68
Dikegulac sodium + picloram	1.0 + 1.0	91	60	56
Dikegulac sodium + picloram	1.0 + 2.0	100	83	87
Dikegulac sodium + picloram	2.0 + 1.0	96	68	76
Dikegulac sodium + picloram	2.0 + 2.0	99	94	90
Dikegulac sodium + 2,4-D	0.5 + 2.0	15	3	0
Dikegulac sodium + 2,4-D	1.0 + 2.0	15	3	4
Dikegulac sodium + 2,4-D	2.0 + 2.0	2	0	9
Dikegulac sodium	0.5	1	0	0
Dikegulac sodium	1.0	0	0	0
Dikegulac sodium	2.0	2	0	0
Picloram	1.0	90	19	27
Picloram	2.0	96	98	72
2,4-D	2.0	12	0	0
LSD (0.05)		13	15	21

Table 5. Leafy spurge control with 2,4-D or picloram applied with dikegulac sodium as a pretreatment or tank mix on 26 May and 30 June 1982, respectively, in Lisbon, ND.

Treatment	Rate	Control	
		1 June1983	22 August 1982
	(lb/A)	(%)	
Dikegulac sodium	0.5	0	0
Dikegulac sodium	1.0	7	0
Picloram	1.0	90	9
2,4-D	1.0	14	0
Dikegulac sodium+picloram (split)	0.5+1.0	94	19
Dikegulac sodium+picloram (split)	1.0+1.0	92	16
Dikegulac sodium+picloram (tank mix)	0.5+1.0	95	18
Dikegulac sodium+picloram (tank mix)	1.0+1.0	82	9
Dikegulac sodium+2,4-D (split)	0.5+1.0	4	0
Dikegulac sodium+2,4-D (split)	1.0+1.0	4	0
Dikegulac sodium+2,4-D (tank mix)	0.5+1.0	1	0
Dikegulac sodium+2,4-D (tank mix)	1.0+1.0	9	0
LSD (0.05)		14	10

Table 6. Leafy spurge control with 2,4-D or picloram applied with dikegulac sodium as a pretreatment or tank mix on 7 September and 4 October 1982, respectively, in Lisbon, ND.

Treatment	Rate	Control	
		1 June1983	22 August 1983
	(lb/A)	(%)	
Dikegulac sodium+picloram (tank mix)	0.5+1.0	72	1
Dikegulac sodium+picloram (tank mix)	1.0+1.0	52	4
Dikegulac sodium+picloram (split)	0.5+1.0	47	0
Dikegulac sodium+picloram (split)	1.0+1.0	64	8
Dikegulac sodium+2,4-D (tank mix)	0.5+2.0	2	0
Dikegulac soduim+2,4-D (tank mix)	1.0+2.0	2	0
2,4-D	2.0	4	0
Picloram	1.0	57	8
LSD (0.05)		20	3

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Chemotaxonomy discussion group

Two topics were discussed during our meeting resulting in the following recommendations:

I. Terminology related to specimen collection.

a. The group recommends that everyone use the term, *accession* or *accession number* to designate each collection. This term is preferred to others as biotype or ecotype, etc. because it does not convey any inferences about the plant or where it was collected.

   We anticipate at a future time that accessions will be designated as taxa or other hierarchal categories when such information becomes available.

II. Collection identification

a. The group recommends that a uniform numbering system be established for each plant collection. The numbers should include:

Year	Site	Accession Number
4 digits	2 letters	3 digits

b. We recommend that a standardized data collecting form be used. This form would contain: the accession number, date, collector/address, collection site, habitat, soil, and other pertinent data.

   The procedural aspects and data accumulation could follow the format of APHIS (used for the Exotic Weed Survey).

c. The Group recommends that there be a central source or center for processing information; the center would provide the accession number.

d. We recommend that one individual, in a state or province, be responsible (perhaps assigned the responsibility) for coordinating the collection of the information (data forms) and forwarding them to the Center.

e. We recommend that we obtain the support of the GPC-14 to implement this system.

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Role of biocontrol agents in the management of weeds[1]

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Discussion centered around the need for more and more effective biocontrol agents. Various ranchers pointed out the high cost of chemical control, the necessity for repeated applications, the sometimes inability of chemicals to provide complete control and the possible detrimental effect of long term chemical use on the land, as reasons. As expressed by Dwane Woolworth, "we must take a stewardship of the land; we have an obligation to hand down the land to the next generation in better condition than we received it."

Dr. Harold Alley correctly pointed out that had chemicals been applied to small patches of leafy spurge as they appeared, we would not now be faced with the necessity of attempting to control leafy spurge on over a million acres. He noted that it has been possible with judicious use of chemicals to contain the 40,000 acre infestation in Wyoming. While in individual instances the cost of control of spurge can exceed the original cost of the land, by extrapolation the cost per acre would only be $7.50 if one assumes that uncontrolled the 40,000 acres would in six years have expanded to one million. As he correctly points out, we cannot wait for biocontrol agents.

However, since we are now faced with a major leafy spurge range expansion, it behooves us to consider other ways to control this weed. Biocontrol is certainly one promising path. In this regard, the primary concern expressed by the group was that biocontrol agents were not becoming available as fast as everyone would like. As Dr. Warren Shaw (USDA) correctly pointed out there is an established protocol for the release of bioagents which is consistent with the concept of safe release so as not to endanger plants of economic importance.

Several people expressed the concern that maybe we are putting a little too much emphasis on the potential conflict of the natural enemies with native plants, and thus perhaps unnecessarily reducing the list of promising natural enemies and retarding the rate at which new control agents are released. Dr. Nowierski pointed out that the displacement and elimination of native plants by the weed itself and herbicide impact on native plants are also important issues that need to be considered.

[1] Some pertinent comments have also been added from the general discussions throughout the symposium.

Discussion proceeded on what levels of risk should be taken as it relates to the use of biocontrol agents. How much economic loss are we prepared to take while we wait for bioagents to become effective? Concern was also expressed that potential bioagents, once released, would not only control the weed, but would wipe out related native plants which might be of marginal economic importance, have aesthetic value, or have long term potential as a genetic pool. As was correctly pointed out by Dr. Nowierski, one of the basic premises of the use of bioagents is that a natural enemy never completely eliminates its host. Thus one sees the reduction of the host followed by a population crash of the bioagent and then a subsequent regeneration of the host with another population explosion of the bioagents, i.e., a cyclical phenomenon. It was pointed out that a program of education was needed to propagate this idea, and reduce concerns for the environment expressed by many people. Dr. Nowierski pointed out that the level of risk one can afford for natural enemies attacking native plants or other desirable flora should probably be based on the economic damage caused by the weed, weighed against any beneficial attributes the weed may possess (such as providing nectar and pollen to honey bees) and the number of native and/or economically important plants in potential conflict. More risk (that a natural enemy may attack a native plant or other desirable flora) may have to be tolerated for a severely damaging weed on marginal economic land, where conventional control is too expensive or impossible to implement.

Because of the genetic plasticity of leafy spurge, there is obviously no single answer to the problem. As was correctly pointed out by several individuals, a multiple (Integrated Pest Management) approach is a necessity consisting of chemical, cultural, biological and mechanical control. In this regard, it is obviously necessary that we know our enemy, thus the emphasis on taxonomy and cytogenetics of the spurge complex. It was pointed out that the best approach was multiple stress on the weed.

Concern was then expressed, that where leafy spurge had been controlled, secondary problems could arise. Mr. Stephenson pointed out that flooding and erosion along the Heart River would ensue if leafy spurge were controlled there. In other locations, other weeds have invaded the areas left clear by dead spurge plants. As was pointed out by Lavigne, the solution might be to utilize a grass seeding program to restabilize the environment.

Whatever, the direction taken for the control of leafy spurge, one thing is certain – as pointed out by Mr. Lentsch, ranchers cannot afford to pay the high costs of chemical control for the next 20 years in light of the reduced consumption of beef and concurrent low beef prices.