



An education program, hydrocarbon extractions and allelopathy studies on leafy spurge (*Euphorbia esula* L.)
by Claire Louise Barreto

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE
in Agronomy
Montana State University
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Abstract:

Leafy spurge (*Euphorbia esula* L.) is an introduced perennial weed infesting one half million acres in Montana. Once established, leafy spurge is difficult to eradicate due to its deep extensive root system. Three approaches to the leafy spurge problem were utilized.

A statewide education program utilizing articles, newsletters and field tours was initiated in an effort to make more people aware of the leafy spurge problem. A ten part series of articles covering the history, biology, control and research of leafy spurge was sent to Montana newspapers. Twenty newspapers printed some or all of the articles. The articles resulted in requests from producers for additional information and copies of the complete series.

A quarterly leafy spurge newsletter was initiated in 1980. The newsletter included research results and updates from five universities and the Biological Control Laboratory in California. The newsletter was mailed to county agents, weed district supervisors, county commissioners, legislators, research personnel, chemical company representatives, farmers and ranchers. The mailing list for the newsletter numbered over 850 in October, 1981.

Eighteen field tours were conducted in 1980 and 1981. The tour sites utilized demonstration plots showing chemical control of leafy spurge using picloram (4-amino-3,5, 6-trichloropicolinic acid), dicamba (3,6-dichloro-o-anisic acid) and 2,4-D [(2,4-dichloro-phenoxy) acetic acid]. Displays and discussions covering statewide infestations, leafy spurge biology, biological control and new chemical application methods were included in the field tour. Two hundred and forty people attended the 1980 tours and over 300 attended the 1981 tours.

Leafy spurge was examined as a possible source of hydrocarbons. Twelve strains of leafy spurge were harvested three times or as many times as regrowth allowed during the 1980 growing season. A benzene:hexane mixture was used to extract hydrocarbons. No statistical differences were observed among strains for June, August and October harvests. Four ecotypes contained high amounts of hydrocarbon when harvested in July. Several ecotypes contained more than two percent hydrocarbons on a plant dry weight basis. Fertilizer did not improve hydrocarbon content.

Native and introduced grass species were grown in soil taken from two leafy spurge infestations to determine whether leafy spurge root exudates have allelopathic properties. There was no difference in the rate of emergence of the grass species in the infested and check soils. No differences were observed in the growth rate of the species grown in each soil. Four species showed weight differences between the Missoula infested and check soil one month after planting, however, there were no weight differences two months after planting. Plant dry weight of *Alopecurus arundinaceus* was lower in Missoula infested soil than in check soil two months after planting. Plant dry weight of *Bromus inermis* was lower when grown in Vaughn infested soil one month after planting.

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AN EDUCATION PROGRAM, HYDROCARBON EXTRACTIONS AND
ALLELOPATHY STUDIES ON LEAFY SPURGE (*Euphorbia esula* L.)

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CLAIRE LOUISE BARRETO

A thesis submitted in partial fulfillment
of the requirements for the degree

of

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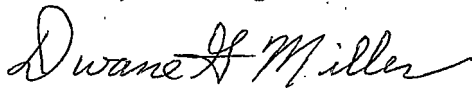
in

Agronomy

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Bozeman, Montana

March, 1982

ACKNOWLEDGMENTS

I would like to express my deep appreciation to my advisor, Peter Fay, for his ideas, enthusiasm and friendship; and for the challenges and responsibilities he gave me.

Sincere thanks are extended to Ronald Lockerman and Charles Rust for serving on my committee; to Greg Northcutt, the county agents, weed district personnel and ranchers for their involvement with the education program and to Dow and Velsicol Chemical companies for their chemical and financial support.

I am grateful for the weeds crew including Glenn Rohde, Bill Dyer, Bev Seder, Jeff Dickerson, Barb Landgraf and Tim Chicoine and many others who gave their time, friendship and advice.

Special gratitude is expressed to my family, Roger Veseth and Sally Metz who gave continued support and encouragement.

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ABSTRACT

Leafy spurge (*Euphorbia esula* L.) is an introduced perennial weed infesting one half million acres in Montana. Once established, leafy spurge is difficult to eradicate due to its deep extensive root system. Three approaches to the leafy spurge problem were utilized.

A statewide education program utilizing articles, newsletters and field tours was initiated in an effort to make more people aware of the leafy spurge problem. A ten part series of articles covering the history, biology, control and research of leafy spurge was sent to Montana newspapers. Twenty newspapers printed some or all of the articles. The articles resulted in requests from producers for additional information and copies of the complete series.

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Leafy spurge was examined as a possible source of hydrocarbons. Twelve strains of leafy spurge were harvested three times or as many times as regrowth allowed during the 1980 growing season. A benzene:hexane mixture was used to extract hydrocarbons. No statistical differences were observed among strains for June, August and October harvests. Four ecotypes contained high amounts of hydrocarbon when harvested in July. Several ecotypes contained more than two percent hydrocarbons on a plant dry weight basis. Fertilizer did not improve hydrocarbon content.

Native and introduced grass species were grown in soil taken from two leafy spurge infestations to determine whether leafy spurge root exudates have allelopathic properties. There was no difference in the rate of emergence of the grass species in the infested and check soils. No differences were observed in the growth rate of the species grown in each soil. Four species showed weight differences between the Missoula infested and check soil one month after planting, however, there were no weight differences two months after planting. Plant dry weight of *Alopecurus arundinaceus* was lower in Missoula infested soil than in check soil two months after planting. Plant dry weight of *Bromus inermis* was lower when grown in Vaughn infested soil one month after planting.

INTRODUCTION

Leafy spurge (*Euphorbia esula* L.) is a noxious perennial weed which probably originated in the Caucasus region of Russia (Croizat, 1945). It was first collected in the United States in Massachusetts in 1827 (Britton, 1921). It was found as far west as Michigan in 1881 (Dunn, 1979). A distribution map produced by Hanson and Rudd (1933) showed limited infestations of spurge as far south as Colorado, and the heaviest infestations in North Dakota, eastern South Dakota and Minnesota. An updated infestation map (Dunn, 1975) showed leafy spurge infestations were centered in Montana, North Dakota, South Dakota, and Wyoming. The weed had not spread southward since the Hanson and Rudd (1933) survey. Noble et al. (1979) reported that more than 2.3 million acres in the United States are currently infested with leafy spurge. By 1970 leafy spurge was present in every province in Canada except Newfoundland (Harris and Alex, 1971).

The spread of leafy spurge is attributed to its efficient reproduction by seed and vegetative buds (Bakke, 1936). Vegetative root buds are found as deep as ten feet below the soil surface and shoots are capable of emerging from as deep as three feet (Selleck et al., 1962). Buds from root tissue ten feet deep can produce shoots when brought to the soil surface (Raju et al., 1964). Bakke (1936) and Selleck et al. (1962) attribute the persistence of leafy spurge to its deep, extensive root system which accumulates a large carbohydrate reserve.

Reproduction by seed is an efficient process since a leafy spurge plant can produce several hundred seed per year (Muenscher, 1930). Seeds are normally produced in a three-lobed capsule. At maturity the seeds dehisce and can be projected as far as fifteen feet (Bakke, 1936).

Leafy spurge seeds are spread by hay, machinery and waterways. Animals aid seed dispersal (Selleck, 1962).

The extensive root system and vegetative buds make it difficult to eradicate leafy spurge by chemical, cultural or mechanical means (Dunn, 1979; McIntyre, 1972). Several chemicals are available to control topgrowth but none eradicate the plant. Biological control has been attempted with two insects, the spurge hawkmoth, *Hyles euphorbiae*, and a root mining moth, *Chamaesphecia tenthredin-informis*. Neither insect was successful. A root boring beetle, *Oberea erythrocephala*, is being evaluated in the field for its potential as a biological control agent on leafy spurge.

The following thesis examines three aspects of leafy spurge:

(1) In an effort to create an awareness of the leafy spurge problem and the possibilities for control, an education program was started in the state of Montana. The education program was carried out over a two year period and constitutes the major emphasis of the thesis work.

(2) The latex of certain members of the Euphorbiaceae family has been studied as a source of hydrocarbons to replace some petroleum products (Calvin, 1979b; Coffey and Halloran, 1979). Distinct ecotypes of leafy spurge exist (Barreto et al., 1980) so it is possible that high latex-bearing strains of spurge exist. Since leafy spurge is a perennial with excellent regrowth capacity, it might be possible to obtain several hydrocarbon harvests per season.

(3) There is evidence to suggest that some *Euphorbia* species exude toxic substances into soil that inhibit growth of nitrifying bacteria (Rice, 1974). Studies have shown that leafy spurge extracts will inhibit sensitive indicator plants (Steenhagen and Zimdahl, 1979;

LeTourneau et al., 1956). Leafy spurge is often found in areas which were formerly productive grassland. The third portion of the thesis was an investigation of the allelopathic potential of leafy spurge against native and introduced grass species.

PART I

A LEAFY SPURGE AWARENESS PROGRAM

CHAPTER ONE

LITERATURE REVIEW: AGRICULTURAL COMMUNICATION

Communication is the process of imparting or exchanging information (Bradfield, 1966). Kelsey (1963) further defined communication as "the process of transferring an idea, skill or aptitude from one person to another accurately and satisfactorily. Satisfaction is achieved when the communicator gets the attention, interest, decision and actions of the audience."

Mass communications are used in agriculture to aid in bridging the gap between farmers and research which enhances agricultural development (Myren, 1964; Bradfield, 1966). Mass communication techniques multiply contacts with farmers, help change habits of farmers, and transmit a *mood* of change (Myren, 1964). [The emphasis is the author's.]

Bradfield (1966) cautions that the communicator should know beforehand what type of change is desired in order to present the material correctly. How much the audience learns is affected by the confidence they have in the communicator and the communicator's attitude. The information transferred should be interesting to the people participating, understood by the audience and useful to those learning (Morgan, 1976; Mager, 1968).

Communication Studies

National and international organizations have recognized that communication is a necessary part of agriculture, and that methods of communication can be improved. In 1953 the Kellogg Foundation funded the National Project in Agricultural Communications "to study, stimulate and apply communication research and knowledge to the field of agricultural communications" (Michigan State University). The first interamerican research

symposium on the role of communications in agricultural development was held in Mexico City in 1962 (Myren, 1964). The symposium participants identified key research areas which identified guidelines for action programs.

Agricultural producers need an increasing amount of knowledge as agriculture technology becomes more advanced (Jensen et al., 1964). Training and education programs are necessary to keep producers current on new products and techniques (Bender et al., 1972). The Cooperative Extension Service has been an integral part of the search for new and innovative methods to reach the public with educational material since 1914 (Trent and Kinlaw, 1979). A major thrust of the Extension Service has involved adult education programs which offer a dissemination technique for presenting the newest farming practices and information. Adult education is uniquely beneficial since it can respond to the demands made by people involved with changing technologies (Jensen et al., 1964). The goals for an instructional program for adults need to be clearly defined at the onset so that there is a basis for selecting materials, content or instructional methods (Mager, 1962).

Communication Methods

Agricultural communication is accomplished through a five step diffusion process. Awareness of an idea initiates the process. Interest, evaluation, trial and adoption of the new idea complete the process. People are influenced to begin the process and make changes in proportion to the number of different teaching methods with which they come into contact. Contacts can be made via radio, newspaper, bulletins, meetings or demonstrations (Kelsey, 1963; Bender et al., 1972).

Operators of large commercial family farms have identified agricultural media including farm publications, newspapers, radio and television as important sources of infor-

mation (Brown and Collins, 1978). The audience and the response desired will determine the method of communication used (Bradfield, 1966).

Audio-Visual Communication

Radio first came to be of general interest in the 1920s. Programs were initially very general since one station covered a statewide area. Today, the radio approach can be more local since most towns have a radio station (Kelsey, 1963). Radio is an inexpensive means to reach a large number of people that would not be contacted otherwise (Dale, 1954; Kelsey, 1963).

Television altered the use of radio as a means of communication in agriculture (Kelsey, 1963). Radio is currently used as method of stimulating the listener to think and then follow through. Radio supports other methods of communication and has the advantage of being easily changed as conditions change (Bradfield, 1966).

Television is a tool of agricultural communication in the home and classroom. North Carolina agricultural educators first used television in 1966 to present 30 minute lessons to adult education classes (Boone, 1968). Teachers were available to prepare the class for the lesson, localize the information and help with individual questions. A similar program was used in Virginia with ten weekly lessons on farm management (Jewell and Oliver, 1968).

Agricultural extension agents in Ohio used a TV program "The Crop Game" for four years and gained a wide audience for teaching new farming practices (Koetz and Cole, 1978). The program covered areas relating to tillage, weed control, marketing, fertility and crop management. One-half of the estimated 10,000 viewers indicated changes in farming practices as a result of the program.

Written Communication

Whether the communication is through journal articles, bulletins, circulars or mimeographs, it must be clear and precise so the reader knows exactly what the writer is saying (Fuccillo, 1980). The communicator must understand the audience; readers may be intelligent but not be technically informed.

Written information comes in a variety of forms. Circulars, newspapers, flyers and bulletins can all work in separate ways to create interest, publicize events and dispense information (Bradfield, 1966; Myren, 1964). Magazines have an advantage in that they will often be saved for future reference (Myren, 1964). Newsletters rank high on the most-preferred list of written communications when they are well-written and pertinent to the problems of the receiver (Kelsey, 1963; Reisbeck, 1980; Brown and Collins, 1978).

Agricultural communication reaches out to other means of written information. The Public Health Service, Soil Conservation Society of America and the Environmental Protection Agency have successfully used comic books to portray poison control, pesticide safety and environmental improvement. The United States Department of Agriculture and the Wisconsin Extension Service have used cartoon series to teach soil fertility and food prevention (Trent and Kinlaw, 1979).

Many authors stress that the communicator should understand the audience (Fucillo, 1980; Kelsey, 1963; Bradfield, 1966; Morgan et al., 1976). Awa (1974) demonstrated that in using mass media to help the poor, the message sent was understood best by the sender and not by the poor. Joseph Pulitzer aptly stated the facts when he said, "Put it before them briefly so they will read it, clearly so they will understand it, forcibly so they will

appreciate it, picturesquely so they will remember it and above all, accurately so they will be guided by its light" (Kelsey, 1963).

Group Meetings

Field demonstration or result demonstration sites for new agricultural practices and products can be effective communication tools (Cunningham and Simeral, 1977). The site should be in an area where the practices demonstrated offer a practical solution to a common problem (Kelsey, 1963). In some areas the farmers participate in observing and recording data and the programs continue year after year (Witt, 1968). Part of the success of farm tours or demonstration sites depends on planning ahead, a good location, a respected cooperator and media coverage before the tour or field day (Cunningham and Simeral, 1977; Bradfield, 1966).

Group meetings can be in the form of conferences, workshops, seminars, lectures, formal classes or open discussion. The amount of information transferred will depend on the communicator, the type of information and the audience (Morgan, 1976; Kelsey, 1963).

Communication transfer, written or oral, can be reinforced and clarified by visual aids (Bradfield, 1966). These may include drawings or photographs with written material or real objects and models at meetings or demonstrations.

One of the newest agricultural communication tools is the computer (Harrison, 1974). Currently, Montana, North and South Dakota, Wyoming, Nebraska and Minnesota are making computer programs available through county agents to help farmers make com-

plex management decisions (Kresge, 1981). Clarity, reliability and accessibility are important to the program users (Harrison, 1974).

Communication Survey

Brown and Collins (1978) surveyed farmers in the U.S. to determine the educational needs of large scale family farms and current sources of information. Suggestions from the survey ranged from returning to the old techniques of personal visits from county agents to experimenting with the latest electronic and computer devices.

The survey indicated that the source for agricultural information depended on the type of information being sought. Radio was the most popular source for market information, farm magazines provided new product information and universities and extension services were listed as the most important source for information on product technology.

Brown and Collins (1978) pointed out that agribusinessmen felt that the Extension Service and agribusiness firms should combine efforts to reach a larger audience with greater efficiency.

In closing, the survey indicated that innovative programs need to be developed that will meet the needs of young farmers.

CHAPTER TWO

LEAFY SPURGE ARTICLES

Abstract

A ten part series of newspaper articles covering the history, biology, control and research of leafy spurge was written and sent to Montana daily and weekly newspapers in 1980. The series was printed either partially or in their entirety in 20 newspapers. After the articles began, requests from producers were received for additional leafy spurge information, missed articles and copies of the complete series. In 1981, the articles were sent out again to newspapers that agreed to reprint them and were made available to other states and agencies for their use.

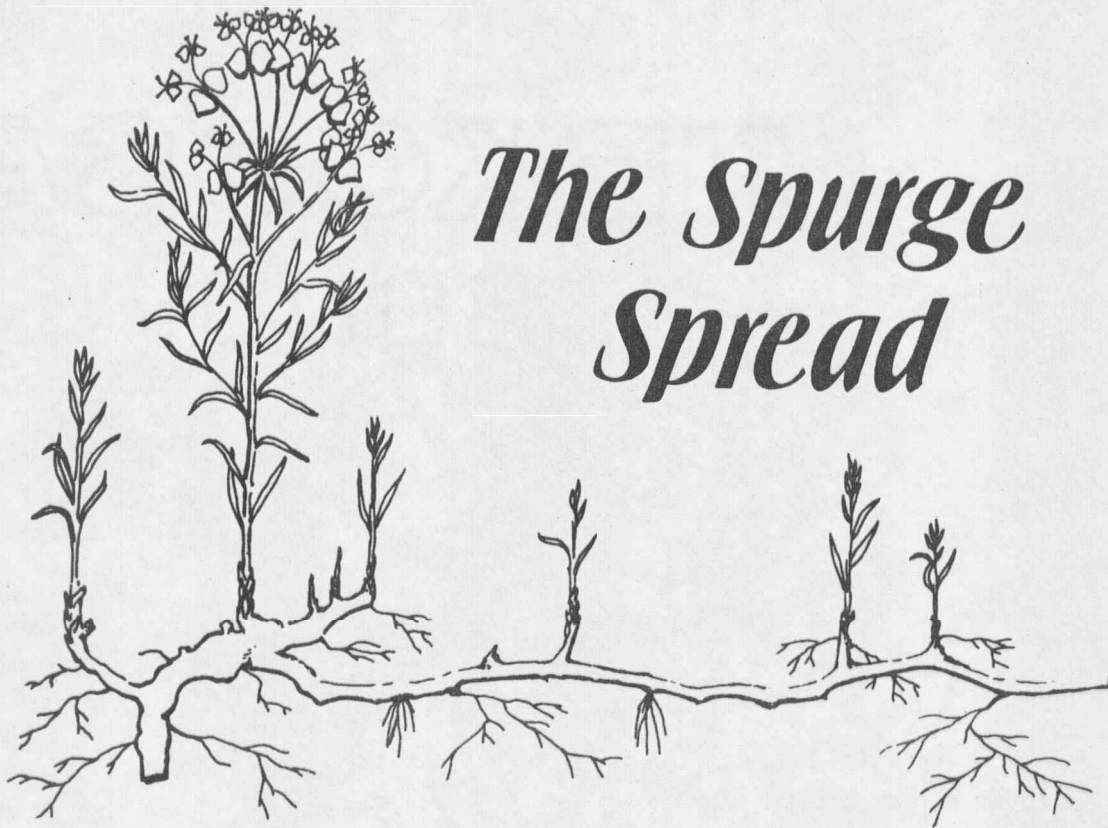
Introduction

Newspapers have a wide audience and few households go without at least one. When attempting to reach a large population, information can be easily disseminated by the use of newspapers. In an agricultural state like Montana, sections of newspapers are written specifically for farmers and ranchers. Problems pertinent to a local area can be addressed.

Materials and Methods

A ten part series of articles on leafy spurge was written in early 1980. The series included the history of leafy spurge, seed production, flowering, roots, biological, chemical and cultural control, economic impact of the weed, leafy spurge research and taxonomy of the plant.

An appropriate logo (Figure 1) was selected for accompanying the articles to aid in their identification and continuity. The logo was drawn and lettered by the Montana State



The Spurge Spread

Figure 1. Logo for leafy spurge newspaper articles.

University Art Services. Photographic plates of the logo were made in sufficient quantity to send one to each newspaper in Montana.

Preceding each article was a brief description of the leafy spurge problem and the number of Montana acres involved. Concluding each article was a listing of the writer's name and address for those people interested in obtaining additional leafy spurge information or for those interested in being placed on the leafy spurge newsletter mailing list.

The articles were sent out on a bi-weekly basis to 82 daily and weekly papers in Montana. The initial article was accompanied by an introductory letter informing the newspaper editor of the leafy spurge problem in the state and requesting his assistance by printing the articles. All articles were sent to the newspaper through the Montana Agricultural Experiment Station News and Publication Office.

One year after the original articles were printed, the complete series was rewritten and sent to Montana papers that agreed to print them. The articles were also made available to other states and agencies by use of an announcement to that effect in the April 1981 issue of the Leafy Spurge Newsletter.

Results and Discussion

The logo was widely used by the newspapers printing the articles.¹

Twenty-one of the 82 newspapers printed the series either partially or in its entirety (Table 1). Of those newspapers carrying the series, the large majority were in areas of the state where leafy spurge is a problem.

Various responses were received from readers of the articles. Several requests were made for additional information on control of leafy spurge or for the complete series. The articles contributed approximately 30 names to the leafy spurge newsletter mailing list.

¹ Appendix A.

Table 1. Newspapers printing some or all of the leafy spurge articles

| Newspaper | Town |
|--------------------------------|---------------------------|
| <i>Miles City Star</i> | Miles City, MT |
| <i>Winnett Times</i> | Winnett, MT |
| <i>Judith Basin Press</i> | Stanford, MT |
| <i>Ravalli Republic</i> | Hamilton, MT |
| <i>The Madisonian</i> | Virginia City, MT |
| <i>Silver Star Post</i> | Deer Lodge, MT |
| <i>Glacier Reporter</i> | Browning, MT |
| <i>River Press</i> | Fort Benton, MT |
| <i>Laurel Outlook</i> | Laurel, MT |
| <i>Tribune Examiner</i> | Dillon, MT |
| <i>Times Clarion</i> | Harlowton, MT |
| <i>Meagher County News</i> | White Sulphur Springs, MT |
| <i>Phillips County News</i> | Malta, MT |
| <i>Glasgow Courier</i> | Glasgow, MT |
| <i>Big Timber Pioneer</i> | Big Timber, MT |
| <i>Montana Farmer Stockman</i> | Statewide |
| <i>Fairfield Times</i> | Fairfield, MT |
| <i>Roundup Record Tribune</i> | Roundup, MT |
| <i>Ranger Review</i> | Glendive, MT |
| <i>Forsyth Independent</i> | Forsyth, MT |
| <i>Prairie Star</i> | Statewide |

One editor whose publication did not print the series stated that the articles needed to be written in more specific local terms. Other editors selected certain articles they felt were more important or that fit into the layout of their newspaper.

Several county agents and weed district supervisors requested an article series. They felt their county newspapers would be more likely to print an article if it was presented to them from someone within the county.

The newspaper articles provided the background on leafy spurge and were the first step in the awareness program.

CHAPTER THREE

LEAFY SPURGE NEWSLETTER

Abstract

A leafy spurge newsletter was initiated on a quarterly basis in April, 1980. The newsletter included leafy spurge research results and updates from five universities, progress reports on biological control of leafy spurge and summaries of leafy spurge conferences. Legislative action concerning leafy spurge was included in the newsletter. The newsletter was mailed to county agents, weed district supervisors, county commissioners, legislators, research personnel, chemical company representatives, farmers and ranchers. The mailing list for the newsletter numbered over 850 in October, 1981.

Introduction

Agricultural newsletters are a convenient means for communication between university agronomists and producers. The reader is given a brief summary of new findings, remedies for problems, and upcoming events. A number of specific problems such as crop varieties, weed control or harvesting can be addressed at a given season of the year.

Newsletters are usually printed several times a year so information can be updated as needed. The information can reach a large number of people in a short period of time.

Newsletters are not meant to replace research reports or farm magazines but rather to present a summary of available information.

Materials and Methods

The first issue of the leafy spurge newsletter was printed in April 1980. A letterhead was designed by Montana State University Art Services (Figure 2). The picture from the

letterhead was used as part of the return address on the backside of the second page of the newsletter (Figure 3).

Information contacts were developed at each of four universities other than Montana State University. Research personnel working with leafy spurge at North Dakota State University, South Dakota State University, University of Nebraska and University of Wyoming were contacted for details regarding current research programs on leafy spurge research results. Personnel from the United States Department of Agriculture Biological Control Laboratory in Albany, California, were contacted for updates on new insect releases for biological control of leafy spurge. United States Forest Service personnel in Montana and South Dakota were contacted for leafy spurge information.

All university, state and federal agency contacts were notified one month prior to the printing deadline to submit information or research results for the newsletter. Contacts who had not submitted articles by the deadline were telephoned in order to solicit late submissions.

News items received were rewritten in one or two paragraphs. The items were compiled by state and put together in newsletter form. The newsletter was typed on plain white paper and was reprinted on letterhead sheets by multilith process.

The leafy spurge newsletter mailing list was initially developed from a list of persons attending two leafy spurge symposia held in 1979. County agents, weed district supervisors, county commissioners and legislators were added to the mailing list. Names of persons attending the 1980 and 1981 leafy spurge tours were placed on the newsletter mailing list. The entire mailing list was placed on computer cards for printing address labels.

All newsletters with the except of those mailed overseas were mailed bulk postage.



Leafy Spurge News

PLANT & SOIL SCIENCE DEPARTMENT
MONTANA STATE UNIVERSITY
BOZEMAN, MONTANA 59717

Figure 2. Letterhead for leafy spurge newsletter.

PLANT & SOIL SCIENCE DEPARTMENT
MONTANA STATE UNIVERSITY
BOZEMAN, MONTANA 59717

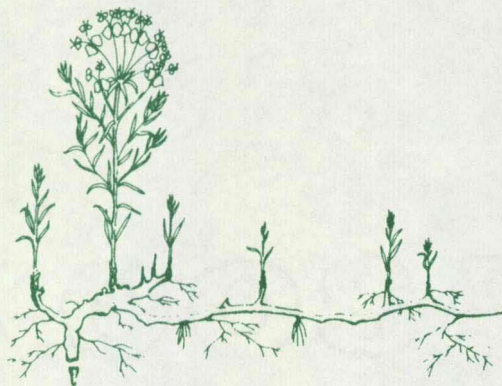


Figure 3. Return address for leafy spurge newsletter.

The leafy spurge newsletter was printed on a quarterly basis beginning in April, 1980.

Results and Discussion

The newsletter² was received favorably. This was indicated not only by comments from persons receiving the newsletter but also by continual additions to the mailing list. The April, 1980 issue was mailed to 240 people in five states. The October 1981 issue was mailed to 850 people in 14 states and four foreign countries.

The newsletter reported new leafy spurge research projects ranging from sheep feeding trials at Montana State University to herbicide root kills at University of Wyoming. Weed legislation involving leafy spurge in Montana and North Dakota resulted in articles in the newsletter.

The newsletter serves a purpose in providing an update on research to scientists at other universities. Farmers, ranchers, county agents, legislators and weed district personnel in the state of Montana are becoming informed about leafy spurge research being conducted at Montana State University and are aware that research is an on-going process.

The farmer/rancher population reached by the newsletter is limited to Montana. Researchers at the contributing universities were made aware that they were welcome to submit names for the mailing list. Very few names were received. In May 1982 the newsletter will be headquartered at one of the other cooperating universities. It is hoped that this move will help add more names of the ranchers in that state to the mailing list.

² Appendix B.

CHAPTER FOUR

LEAFY SPURGE FIELD TOURS

Abstract

Leafy spurge field tours were held at eight locations in 1980 and ten locations in 1981. The majority of the tour sites had demonstration plots showing chemical control of leafy spurge using picloram (4-amino-3,5,6-trichloropicolinic acid), dicamba (3,6-dichloro-o-anisic acid) and 2,4-D[(2,4-dichloro-phenoxy) acetic acid]. Displays and discussions covering the statewide infestations, leafy spurge biology, biological control and new chemical application methods were included in the field tour. Two hundred and forty people attended the 1980 tours and over 300 attended the 1981 tours.

Introduction

Field tours in agricultural communities offer an on-the-site link between research and the producer. The topic of the field tour is based on the needs and interests of those in the area (Kelsey, 1963).

Producers attend tours to gain knowledge about their problem. It is the responsibility of tour personnel to satisfy that need and learn from the producer's experiences.

The tours must be carefully planned. The objective of the tour must be evaluated and sufficient pre-planning conducted to meet the objective (Morgan, 1976). Cooperating farmers and county agents must be contacted well in advance to set up the location and plot sites. Immediately prior to the tour, publicity announcements need to be made, using as many different news media as possible (Cunningham and Simeral, 1977; Bradfield, 1966).

Field tours offer an opportunity for farmers and ranchers to come together and discuss common problems. The tour becomes a social gathering for producers in an area. Refreshments may be beneficial in these situations to allow an informal intermingling of producers and university personnel (Morgan, 1976).

Materials and Methods

Field tours. In October, 1979, demonstration plots showing chemical control of leafy spurge were put out in Judith Basin, Cascade and Missoula counties in Montana for field tours in June, 1980. In September, 1980, plots were put out in Park, Phillips, Powder River, Fergus and Deer Lodge counties for use as tour sites in June, 1981. Site selection was based on county agent or county weed district supervisor recommendations. Only picloram and dicamba were applied in 1979. In 1980, picloram, dicamba and 2,4-D were applied (Table 2). Tour sites where the writer did not apply treatments were in areas treated by Extension or Weed District personnel (Table 3).

Displays were constructed to show statewide leafy spurge infestations, the biology of the leafy spurge plant, biological control and demonstration models of the rope wick and roller applicators. The displays were constructed in a manner so they could be easily set up and taken down in the field.

Bumper stickers were designed and printed by Colorworld of Montana, Bozeman, Montana (Figure 4). These were disseminated at the tours as an awareness tool.

Publicity for the tours was coordinated by Greg Northcutt, editor for the Montana Agricultural Experiment Station. Radio announcements were taped and sent to the radio

Table 2. Chemicals applied for leafy spurge control at eight demonstration locations

| County | Herbicide | Formulation | Rates of application kg/ha |
|--------------|-----------|-------------|-------------------------------|
| Judith Basin | picloram | 5G | 2.24 |
| | dicamba | 5G | 8.96 |
| Cascade | picloram | 2E | .56, 1.12, 2.24, 3.36 |
| | dicamba | 4E | 4.48, 6.72, 8.96 |
| Missoula | picloram | 2E | .56, 1.12, 2.24, 3.36 |
| | dicamba | 4E | 4.48, 6.72, 8.96 |
| Park | picloram | 2E | .56, 1.12, 2.24 |
| | picloram | 2G | 1.12, 2.24 |
| | dicamba | 4E | 4.48, 6.72 |
| | dicamba | 5G | 4.48, 6.72 |
| | 2,4-D | 4E | 2.24 |
| Powder River | picloram | 2E | 1.12, 2.24 |
| | picloram | 2G | 1.12, 2.24 |
| | dicamba | 4E | 4.48, 6.72 |
| | dicamba | 5G | 4.48, 6.72 |
| | 2,4-D | 4E | 2.24 |
| Fergus | picloram | 2E | 1.12, 2.24 |
| | picloram | 2G | 1.12, 2.24 |
| | dicamba | 4E | 4.48, 6.72 |
| | dicamba | 5G | 4.48, 6.72 |
| | 2,4-D | 4E | 2.24 |
| Phillips | picloram | 2E | 1.12, 2.24 |
| | picloram | 2G | 1.12, 2.24 |
| | dicamba | 4E | 4.48, 6.72 |
| | dicamba | 5G | 4.48, 6.72 |
| | 2,4-D | 4E | 2.24 |
| Deer Lodge | picloram | 2E | .56, 1.12, 2.24 |
| | picloram | 2G | 1.12, 2.24 |
| | dicamba | 4E | 4.48, 6.72 |
| | dicamba | 5G | 4.48, 6.72 |
| | 2,4-D | 4E | 2.24 |

Table 3. Herbicides were applied at 10 tour locations by Cooperative Extension or weed district personnel

| County | Herbicide Demonstration Location | Applicator |
|-------------|----------------------------------|--|
| Carter | Fix Farm Ekalaka, MT | M. Jackson MSU Ext. Weed Spec. |
| Fallon | Rugg Ranch Plevna, MT | M. Jackson Ext. Weed Spec. |
| Yellowstone | Morledge Ranch Billings, MT | M. Jackson Ext. Weed Spec. |
| Blaine | Bob Sharples Chinook, MT | Blaine Co. Weed District |
| Hill | Robert Davey Havre, MT | Gregg Carlson Hill Co. Ext. Agent |
| Lake | Several | Ranch owners |
| Silver Bow | Several | Silver Bow Co. Weed District |
| Sheridan | Don Hedges Plentywood, MT | Sheridan Co. Weed District |
| Roosevelt | Berry Bros. Bainville, MT | Roger Ashley Roosevelt Co. Ext. Agent |
| Stillwater | Several | Stillwater Co. Weed District |

station nearest the tour site several weeks before the tour. Newspaper releases regarding the tours were sent to the county agents for distribution.

Hot dogs, beer and pop were available at each tour to provide refreshments and act as an icebreaker.

Funding for the travel expenses and refreshments was obtained from Dow Chemical Company in 1980 and Dow and Velsicol Chemical companies in 1981.



Figure 4. Bumper sticker utilized in leafy spurge field tours.
(Reduced from original 7.6 cm by 38.1 cm.)

Table 4. Attendance at 1980 and 1981 leafy spurge field tours

| Year | County | Number of persons in attendance |
|-------|--------------|---------------------------------|
| 1980 | Carter | 6 |
| | Fallon | 6 |
| | Yellowstone | 15 |
| | Judith Basin | 50 |
| | Hill | 70 |
| | Cascade | 33 |
| | Blaine | 20 |
| | Missoula | 40 |
| 1981 | Lake | 25 |
| | Park | 25 |
| | Silver Bow | 30 |
| | Hill | 70 |
| | Phillips | 27 |
| | Sheridan | 20 |
| | Roosevelt | 53 |
| | Stillwater | 18 |
| | Powder River | 14 |
| | Judith Basin | 400 |
| total | | 922 |

In addition to the funding, Dow and Velsicol Chemical companies donated herbicides which were given away as door prizes at the tours.

Slide-tape sets. As a follow-up for the field tours, leafy spurge slide-tape sets were made available at a minimum price to county agents and vocational agriculture instructors. A narrative on the biology and control of leafy spurge was recorded on cassette tape and reproduced in quantity by the Modern Languages Department, Montana State University. The tape accompanied twenty-one slides showing leafy spurge plant stages, root systems

and various means of controlling the weed. A Riker mount containing a leafy spurge plant and seeds was also made available at minimum cost.

Results and Discussion

Field tours. The 1980 tours were completed in June, 1980. The 1981 tours were held in June, 1981. There were additional requests for tours in 1981 from county agents and county weed district personnel. Fergus and Deer Lodge county tours were cancelled because of poor weather conditions. The author set up and manned a leafy spurge booth at the Deer Lodge county fair in place of a field tour.

The tours were enthusiastically received by the county agents and producers. Attendance exceeded original expectations (Table 4). Tours were held in both 1980 and 1981 in Judith Basin and Hill counties due to county agent requests. The 1981 Judith Basin leafy spurge tour was part of a statewide Weed Fair.

Picloram applied at a rate of 1.12 kg/ha or 2.24 kg/ha provided the best control of leafy spurge one year after application with the least amount of grass injury (Table 5). Because of the cost of the 2.24 kg/ha treatment³, many ranchers decreased the rate of picloram applied to .56 kg/ha, added 2,4-D and treated every year. When examining the control by the various chemicals, it was stressed that the roots were not killed and retreatments would be necessary.

The producers were a valuable source of information. They suggested numerous practices which were working for them. Several ranchers at the tour in Stillwater County had obtained excellent topgrowth control of leafy spurge by grazing sheep. The pastures

³ Chemical cost of 2.24 kg/ha of picloram is currently \$90.00.

Table 5. Percent topgrowth control of *Euphorbia esula* in six tour locations. 0 = no control; 100 = complete control

| Chemical | Rate kg/ha | Formulation | County | | | | | |
|----------|---------------|-------------|---------|-----------------|----------|------|-----------------|----------|
| | | | 1980 | | | 1981 | | |
| | | | Cascade | Judith Basin | Missoula | Park | Powder River | Phillips |
| picloram | .56 | 2EC | 60* | | 62 | 75 | | |
| picloram | 1.12 | 2EC | 90 | | 91 | 95 | 95 | 100 |
| picloram | 2.24 | 2EC | 100* | 100* | 97 | 98 | 99 | 100 |
| picloram | 3.36 | 2EC | 100* | | 99 | | | |
| picloram | .56 | 2G | | | 10 | | | |
| picloram | 1.12 | 2G | | | 45 | 85 | 85 | 95 |
| picloram | 2.24 | 2G | | | 85 | 93 | 90 | 95 |
| picloram | 3.36 | 2G | | | 95 | | | |
| dicamba | 4.48 | 4EC | 70* | | 75 | 85 | 20 | 90 |
| dicamba | 6.72 | 4EC | 80* | | 80 | 94 | 70 | 85 |
| dicamba | 8.96 | 4EC | 90* | 100* | 88 | | | |
| dicamba | 4.48 | 5G | | | 10 | 98 | 60 | 50 |
| dicamba | 6.72 | 5G | | | 50 | 98 | 70 | 60 |
| dicamba | 8.96 | 5G | | | 70 | | | |
| 2,4-D | 2.24 | 4EC | | | | 20 | 0 | 0 |

*Grass injury greater than 50%.

were in good condition, however, the ranchers noted that spurge would return if sheep grazing was terminated.

A common statement made by ranchers was that they would not object to the price of picloram if one application would control the weed. Since retreatments are necessary for leafy spurge control, most ranchers feel they cannot afford to use picloram on grazing land because of its low rate of annual return.

Many ranchers commented that a use should be found for the weed since it grows so well in Montana. Ranchers whose land adjoined state land said it would take cooperative

efforts from the ranchers, the state and railroads before the problem could be brought under control.

Some older ranchers stated that leafy spurge was first introduced to their area by threshers and hay from infested areas.

The leafy spurge tours were helpful in that some people who did not recognize the plant before the tour left with an awareness of the weed and the problems it can cause once it becomes established. Frequently, ranchers who would not identify leafy spurge before the tour took plants home so they could alert their neighbors to the identity and threat of leafy spurge.

The tours demonstrated side-by-side comparisons of chemical control, gave a review of leafy spurge research and offered encouragement that universities are studying the weed and control methods.

Slide-tape sets. Twenty-two leafy spurge slide tape sets and 28 Riker mounts of the weed were sent to county agents and vocational agricultural instructors. Although materials were ordered by some county agents who had hosted leafy spurge tours in 1980 or 1981, many were requested by county agents or instructors in whose county a tour was not held.

The slides, tapes and plant mounts allowed the information to reach a large number of people around the state with a minimum amount of time, travel and money. The educational materials provided a continuity of the awareness program even when Montana State University personnel were not available to present a program.

CHAPTER FIVE

SUMMARY

Leafy spurge infests over one half million acres in Montana and continues to spread. The weed is most easily controlled when infestations are small. An education program was initiated in Montana in 1980 to aid in identification of and knowledge about the weed.

Newspaper articles, leafy spurge newsletters, summer tours and slide sets were utilized to provide needed information on a troublesome weed problem. The education program created a greater awareness of leafy spurge and the possibilities for control. The articles, tours and slide sets reached a large number of people and delivered basic information about the weed and the leafy spurge newsletter is continuing to update that information. Legislative action on leafy spurge has occurred as a result of the increased awareness of the problem.

The education and awareness program is not completed. Many people still need to become informed about the weed. The ideas and background material have been disseminated and local county agents and weed district personnel can continue the program. Areas utilized as demonstration plots can be retreated and used again or county personnel can set up their own treated areas. Local weed authorities can draw on information provided in the education program to improve or add to their existing weed programs.

PART II

HYDROCARBON EXTRACTIONS

CHAPTER SIX

LITERATURE REVIEW: PLANT PRODUCTION OF HYDROCARBONS

Historical Research

The extraction of natural rubber from the rubber tree (*Hevea brasiliensis*) is a familiar form of hydrocarbon extraction. During the nineteenth century rubber was tapped from scattered trees in the Amazon jungle. From 1910 until 1940 most natural rubber came from rubber plantations in Malaysia (Calvin, 1977).

The blockades against Germany during World War I stimulated unsuccessful attempts to synthesize rubber (Polhamus, 1967). United States, British and German scientists successfully developed an economical synthetic rubber from petroleum during World War II (Calvin, 1977). By the end of World War II natural rubber could no longer compete economically with synthetic rubber.

As a result of the German rubber shortage during World War I, Thomas Edison examined thousands of plants that could be grown in the United States as a domestic source of rubber (Polhamus, 1967). Edison reported that plants in the genus *Euphorbia* contained high amounts of latex.

Buehrer and Benson (1945) reported the results of numerous studies that examined rubber content of native American plants. They restricted their investigation to plants indigenous to the southwestern United States. Minshall (1957) used a similar procedure to study the rubber content of plants found in Canada.

Current Research

The oil shortages of the past decade prompted scientists to examine plants not only for natural rubber content but also for other petroleum-like products (Calvin, 1974, 1976,

1977, 1979a, 1979b; Coffey and Halloran, 1979; Gartside, 1977; Nielsen et al., 1977; Sachs et al., 1981). Nishimura et al. (1977) point out that carbohydrates from sugar cane are converted to alcohols and then to olefins. Olefins are routinely produced at present by cracking crude petroleum products. Approximately ten percent of fossil hydrocarbons are cracked down to ethylene and used for the production of synthetic fibers and plastics. Calvin (1979b) feels that short-chained plant hydrocarbons could be used instead of fossil fuel for fiber and plastic production.

Several authors (Buchanan et al., 1978a, 1978b; Buchanan, 1978; Calvin, 1977; Gartside, 1975) suggest that agricultural production of hydrocarbons may be compatible with a need for increased food and fiber production if the entire plant could be harvested for hydrocarbons, fiber, protein and carbohydrates.

Potential Sources of Plant Material

Over 2,000 plant species produce hydrocarbons (Calvin, 1976). Plants in the Euphorbiaceae and Asclepiadaceae families contain hydrocarbons similar to those found in crude oil (Coffey and Halloran, 1979; Buchanan et al., 1978a). It is presently economically feasible to extract plant-derived hydrocarbons if they constitute two percent or more of the plant's dry weight (Buchanan et al., 1978a; Polhamus, 1967; Buehrer and Benson, 1945; Minshall, 1957). Coffey and Halloran (1979) stated that *Euphorbia tirucalli*, *E. lactea* and *E. lathyris* may have potential for hydrocarbon production.

Several species of *Euphorbia* were planted for hydrocarbon production in southern California (Calvin, 1979b). A Japanese firm planted *E. tirucalli* and estimated that five to ten barrels of oil¹ could be produced per acre per year. Calvin (1979b) estimated that *E.*

¹ Oil refers to fats, fatty acids, resins, terpenes and hydrocarbons.

lathyris could produce eight to twelve percent of the plant dry weight in oil which would yield ten to twenty barrels of oil per acre per year.

Selection and genetic improvement may improve the yield of hydrocarbons (Buchanan et al., 1978a; Calvin, 1979b; Coffey and Halloran, 1979). Strains of *Parthenium argentatum* contain different amounts of latex hydrocarbons (Coffey and Halloran, 1979). Economic pressures can influence agronomy and plant breeding. Rubber yields increased from an average of 224 kg/ha in 1945 to over 2240 kg/ha by 1965 (Calvin, 1977). The chemical composition of plant hydrocarbons could also be manipulated. Coffey and Halloran (1979) found distinct qualitative differences in hydrocarbons among *Euphorbia* species.

Agronomic and edaphic factors influence hydrocarbon content. Hydrocarbon levels of *Parthenium argentatum* were regulated by annual rainfall, growth temperature and time of harvest (Coffey and Halloran, 1979). Alternatively, Buehrer and Benson (1945) feel that climate and soil conditions are of lesser importance in determining rubber content than plant genus and age of the plant at the time of analysis.

Buchanan et al. (1978b) evaluated 100 plant species from 13 families and 60 genera for hydrocarbons, fiber, protein and carbohydrates. Several species in the family Labiatae contained more than one percent rubber. Several Compositae, *Cacalia atriplicifolia*, *Solidago graminifolia* and *S. rigida* contain significant amounts of crude rubber. These findings were supported by Edison (Polhamus, 1967). *Euphorbia dentata* and *E. heterophylla* contain high oil percentage.

Buchanan et al. (1978) found oils and hydrocarbons distributed throughout the plant. Variations in whole plant oils may be due to growing conditions, soil fertility, disease and insect infestations.

Euphorbia lathyris is a potential source of hydrocarbons (Coffey and Halloran, 1979; Calvin, 1979b). Sachs et al. (1981) studied irrigation and fertilization effects on hydrocarbon yield. Although their irrigated plots produced more dry matter than non-irrigated plots, the percentage of rosin was greater in non-irrigated plants because of a greater proportion of leaf to stem dry weight. Little or no response was noted to additional nitrogen.

In this same study, problems were encountered with seedling emergence and crop establishment, pathogens, and plant drying. Early expectations were that *Euphorbia lathyris* could be established and grown without irrigation. This is unlikely. According to Sachs et al. (1981), the economic potential of *E. lathyris* for hydrocarbon production is low. Genetic improvement may be difficult since no differences were observed in hydrocarbon content among the germplasm tested.

Extraction Procedures

The determination of hydrocarbon content in most plants is usually carried out by an acetone extraction to remove resins, fat, saponifiable oils, sugars, coloring matter and essential oils. This is followed by a benzene extraction that removes the hydrocarbon substance (Buehrer and Benson, 1945; Buchanan, 1978a; Buchanan, 1978; Minshall, 1957; Polhamus, 1967).

A survey of the methods used for hydrocarbon extraction found that sample size varied from two grams (Buehrer and Benson, 1945) to greater than 500 grams (Buchanan

et al., 1978). Plant samples were air dried and coarsely ground before storing. Samples were finely ground prior to the extraction (Buchanan et al., 1978).

Buehrer and Benson (1945) recommend air drying plant material out of direct sunlight since ultraviolet light tends to destroy the rubber substance. Drying the plant material in a vacuum oven also resulted in partial decomposition of the rubber substance.

Extraction periods range from six (Minshall, 1957) to 24 hours using a Soxhlet apparatus (Buchanan et al., 1978a). The extraction time, sample size and particle size of the materials may affect the final results of an extraction (Buehrer and Benson, 1945).

CHAPTER SEVEN

HYDROCARBON CONTENT OF LEAFY SPURGE

Abstract

Twelve strains of leafy spurge were harvested three times or as many times as regrowth allowed during the 1980 growing season. The topgrowth was dried, ground and extracted with acetone and benzene:hexane to determine hydrocarbon content. The extracted hydrocarbons were expressed as a percent of plant dry weight.

No statistical differences were observed among strains for June, August and October harvests. Four ecotypes contained higher amounts of hydrocarbon than the rest when harvested in July. Several ecotypes contained more than two percent hydrocarbons on a plant dry weight basis. Additional research is needed to completely assess the potential of leafy spurge as a source of hydrocarbons.

Introduction

Hydrocarbons and seed oils have the potential to supply chemicals similar to those presently derived from crude petroleum oil. Green plants utilize solar energy to produce a wide variety of products that are competitive with synthetic petrochemicals. Collectively referred to as hydrocarbons, they include isoprene polymers, waxes and terpenoids.

Leafy spurge (*Euphorbia esula*) is a well-established perennial plant in the north-central United States and Canada. Since no diseases or insects attack leafy spurge this would not pose a threat to production in North America. The plant does not need to be established each year, has excellent regrowth potential after cutting, and contains hydrocarbons. The following study was conducted to measure the hydrocarbon content of

several ecotypes of leafy spurge and to measure the potential of the plant for hydrocarbon production.

Materials and Methods

An existing collection of *Euphorbia esula* from twelve locations was used as the source of plant material in this study. Ecotypes were identified according to collection location: Lethbridge, Saskatchewan, Colorado, Wyoming, Idaho, South Dakota, North Dakota I, North Dakota II, Missoula MT, Moccasin MT, Bozeman MT and Antelope MT. Plants were established in 17.8 cm diameter by 90 cm long metal cans which were placed in concrete pipe sunk into the ground on 1.2 m centers. The plants were established in 1962 at the Agricultural Experiment Station, Bozeman. The plants were placed on a randomized complete block design. There were three replications.

Harvest dates. Plants were harvested June 18, July 15 or August 1, 1980, at three stages of plant growth: bloom, seed set and post seed dispersal, respectively. Regrowth was harvested six weeks after the initial harvest. Plants were air dried and ground with a Wiley mill to pass through a 20-mesh screen. The samples were finely ground and oven dried at 30°C immediately before extraction.

A 85 ml Soxhlet apparatus equipped with 90 by 33 mm Schleicher and Schuell extraction thimbles was used in the extraction process. Each sample was weighed, extracted for 24 hours with acetone, dried, reweighed and extracted for 24 hours with a 2:3 (V:V) benzene:hexane mixture. Samples were dried and weighed after the benzene:hexane extraction. The difference in weight before and after the benzene extraction was taken to be the weight of the hydrocarbons.

Effect of fertilizer. The effect of nitrogen fertilizer on hydrocarbon content was measured on three ecotypes. Ammonium nitrate fertilizer (67 kg/ha) was surface applied July 5, 1980. Plants were harvested July 15 or August and October (two harvests) and analyzed for hydrocarbons as described above.

Results and Discussion

All ecotypes were not represented in the first harvest because of drying problems encountered with harvested material. The ecotypes did not produce regrowth at the same rate and all ecotypes were not harvested at all cuttings.

Harvest dates. The June and August harvests showed no statistical differences in hydrocarbon content among ecotypes (Tables 6 and 8).

The July harvest of Wyoming, Missoula and Bozeman ecotypes contained higher levels of hydrocarbons (Table 7). Each ecotype had one replication with an exceptionally high hydrocarbon content which may account for the difference. Ecotypes from Lethbridge, Saskatchewan and North Dakota I produced statistically less hydrocarbon than ecotypes from Missoula, Wyoming or Bozeman.

Only ecotypes from Lethbridge and Wyoming had sufficient regrowth to harvest in October (Table 9). Those plants had been previously harvested in July. Hydrocarbon content of those ecotypes was statistically different but neither reached the two percent level (Table 9).

Four ecotypes produced greater amounts of hydrocarbon with a first cutting in July than with a first cutting in June. Only the Saskatchewan ecotype produced a higher percentage of hydrocarbon in June (Table 10).

Table 6. Percent hydrocarbon in *Euphorbia esula* ecotypes harvested June 18, 1980.

| Ecotype | % hydrocarbon |
|--------------|---------------|
| Saskatchewan | 1.97 |
| Colorado | .12 |
| Idaho | .14 |
| South Dakota | .47 |
| Antelope | .17 |
| LSD (.05) | 2.02 |

Table 7. Percent hydrocarbon in *Euphorbia esula* ecotypes harvested July 15, 1980.

| Ecotype | % hydrocarbon |
|-----------------|---------------|
| Lethbridge | .40 |
| Saskatchewan | .60 |
| Colorado | 1.12 |
| Wyoming | 2.27 |
| Idaho | 1.56 |
| South Dakota | .73 |
| North Dakota I | .38 |
| North Dakota II | .88 |
| Missoula | 2.18 |
| Moccasin | .73 |
| Bozeman | 1.96 |
| Antelope | 1.24 |
| LSD (.05) | 1.58 |

Table 8. Percent hydrocarbon in *Euphorbia esula* ecotypes harvested August 1, 1980.

| Ecotype | % hydrocarbon |
|-----------------|---------------|
| Lethbridge | 1.22 |
| Wyoming | .42 |
| North Dakota I | .24 |
| North Dakota II | .60 |
| Moccasin | 1.36 |
| LSD (.05) | 1.20 |

Table 9. Percent hydrocarbon in *Euphorbia esula* ecotypes harvested in October, 1980.

| Ecotype | % hydrocarbon |
|------------|---------------|
| Lethbridge | 1.10 |
| Wyoming | .15 |
| LSD (.05) | .75 |

Table 10. Percent hydrocarbon in *Euphorbia esula* ecotypes harvested in June and July, 1980, for the first time.

| Ecotype | Month of harvest | % hydrocarbon |
|--------------|------------------|---------------|
| Saskatchewan | June | 1.97 |
| Saskatchewan | July | .40 |
| Colorado | June | .12 |
| Colorado | July | 1.12 |
| Idaho | June | .14 |
| Idaho | July | 1.56 |
| South Dakota | June | .47 |
| South Dakota | July | .73 |
| Antelope | June | .17 |
| Antelope | July | 1.24 |
| LSD (.05) | | 1.71 |

Effects of fertilizer. No statistical differences were measured between hydrocarbon content of fertilized and unfertilized ecotypes (Table 11).

Several problems are inherent in this study. The plants had been in the cans for 18 years. *Euphorbia esula* roots are extensive in a field situation; the confined roots of the experimental plants may have hindered the regrowth abilities of these plants. Some plants demonstrated vigorous regrowth while others produced only one or two shoots after cutting.

Euphorbia esula seed capsules can project seed as far as four meters. Since the cans were placed on 1.2 m centers, seed from one ecotype could have easily contaminated an

Table 11. Percent hydrocarbons in fertilized and non-fertilized ecotypes of *Euphorbia esula* harvested in August, 1980.

| Ecotype | Fertilizer added | % hydrocarbons |
|------------|------------------|----------------|
| Lethbridge | no | 2.65 |
| Lethbridge | yes | 1.33 |
| Wyoming | no | 3.21 |
| Wyoming | yes | .42 |
| Moccasin | no | .89 |
| Moccasin | yes | 1.36 |
| LSD (.05) | | 2.85 |

adjoining can. Contamination may explain the variation between replications.

The sample size used in the extraction can markedly change the outcome. Some ecotypes produced less than two grams of dried plant material. Small samples did not grind well and had larger particles than ten gram samples. Samples with large particles consistently gave higher hydrocarbon percentages. Buehrer and Benson (1945) note that if the acetone extraction is incomplete, results from the benzene extraction will be high. Resins and benzene-soluble compounds normally extracted by acetone remain in the sample and are extracted by the benzene, giving excessive estimates for hydrocarbon content. This is evident in the Saskatchewan ecotype at the June harvest, the Missoula ecotype at the July harvest and the unfertilized Lethbridge ecotype at the August harvest where the dried samples in one replication were 1.7 g, 1.2 g and 0.26 g, respectively.

Results from extraction of plant material from the unfertilized ecotype from Wyoming indicate it contained 3.21 percent hydrocarbons, considerably higher than the .41 percent plant dry weight reported by Minshall (1957) for leafy spurge. This number should be accepted cautiously because of a large variation between replications.

Ecotypes of leafy spurge cannot be recommended as a potential source of hydrocarbons. Results obtained in this study were unreliable due to the inconsistency of the replications and the uncertain identification of plant material.

CHAPTER EIGHT

SUMMARY

Euphorbia species may have potential as sources of hydrocarbons which could replace crude petroleum products in the production of synthetic fibers and plastics. Some of the *Euphorbia* species being studied are annuals and problems have been encountered in establishing the plants. *Euphorbia esula* is a perennial and is already established on millions of acres in northcentral United States and Canada. In this study, twelve ecotypes of *E. esula* were harvested and hydrocarbons were extracted in an effort to find ecotypes which contained at least two percent of the plant dry weight as hydrocarbons. None of the twelve ecotypes contained high levels of hydrocarbon.

Several ecotypes appeared to have at least two percent hydrocarbons but the data were not sufficiently consistent to recommend *Euphorbia esula* as a possible hydrocarbon source. Additional data needs to be collected from properly identified ecotypes.

Euphorbia esula was originally examined partially because of its regrowth abilities. Extensive regrowth was not observed in this study possibly because of physical root inhibition. Studies are needed of *E. esula* regrowth in a field situation where root growth is not inhibited.

The use of a limited amount of fertilizer did not appear to change the hydrocarbon content of the plant. Heavier applications of fertilizer and irrigation may improve the hydrocarbon content of *Euphorbia esula*.

Before *Euphorbia esula* becomes a potential source of hydrocarbons, increased hydrocarbon yields must be realized. Agronomic research in the areas of plant breeding and fertility may aid in increasing the hydrocarbon content.

PART III

ALLELOPATHIC ACTIVITY OF *Euphorbia esula* L.

CHAPTER NINE

LITERATURE REVIEW: ALLELOPATHY

Introduction

Allelopathy is defined by Muller (1969) to be "the release by one plant of a chemical compound into the environment which inhibits the growth of another plant." Rice (1974) further defines allelopathy to be "any direct or indirect harmful effects by one plant, including microorganisms, on another through the production of chemical compounds released into the environment." Allelopathy and competition are two forms of interference which is defined to be all forms of reactions by one plant that are deleterious to another (Muller, 1969). Whittaker (1970) states that allelopathy is "not a peculiarity of a few plants but rather a widespread and normal, though inconspicuous, phenomenon of natural communities."

In order to establish that allelopathy exists, two conditions are important (Muller, 1971). It must first be determined that the plant contains an inhibitory compound, and that there is a mechanism by which the phytotoxin can be released from the plant, transported in the environment and accumulated in the area of the inhibited plant. Second, the relative weight of all pertinent physical factors must be determined in order to rule out competition.

Muller (1971) cites Plenck for the first suggestion of chemical interactions between plants in 1795. Börner (1960) credits DeCandolle (1832) with the original theory of plants releasing toxic substances.

"Soil sickness" was sometimes used to describe soil when it was no longer productive (Woods, 1960). Woods (1960) states Pantanelli (1926) attributed soil sickness to be

the result of root excretions preventing the development of soil microorganisms. Schreiner and Sullivan (1909) demonstrated unfavorable soil conditions may be caused by organic substances arising through crop growth.

A publication by Molisch (1937) initiated a series of studies concerning plant interactions (Börner, 1960). Since that time, allelopathy has been shown to alter the structure, function and diversity of plant communities (Muller, 1969; Whittaker, 1970; del Moral and Cates, 1971). Phenomena previously considered to be due to competition for light, moisture or nutrients may have to be reconsidered with a recognition of possible allelopathic effects (Muller, 1971).

Occurrence of Allelopathy

Allelopathic effects are widespread in both agricultural and wild species (Whittaker and Feeney, 1971). Crop plants from the genera *Avena*, *Triticum*, *Secale*, *Hordeum*, *Bromus* and *Brassica* are known to release toxic substances from roots (Putnam and Duke, 1974).

By using a split root technique Buchholtz (1971) found that *Agropyron repens* influenced the accumulation and utilization of mineral nutrients by corn (*Zea mays*) by impairing the adsorptive capacity of corn roots. Gabor and Veatch (1981) isolated a phytotoxin from *A. repens* rhizomes that inhibited seedling growth of corn. Ahlgren and Aamodt (1939) determined that adjoining roots may have an effect on the development and activity of other roots.

In natural communities allelopathy may regulate the density and distribution of plant species (Putnam and Duke, 1974), the vegetation pattern or plant succession (Whittaker and Feeney, 1971; Einhellig and Rasmussen, 1973; Rice, 1974).

Rice (1971) studied the four stages in old field succession and the plant interactions that determine why each stage replaced the other. He found that several first stage plants produced inhibitory compounds which rapidly eliminated the major portion of first stage plants but did not inhibit stage two plants. He also determined that pioneer species which require very little nitrogen produce inhibitors of the nitrogen-fixing and nitrifying bacteria and thereby slow the rate of succession.

Whittaker (1971) feels that invading plant species can influence the rate of plant succession by the use of allelochemicals. Bonner (1950) noted that *Encelia farinosa* and *Artemisia absinthium* showed true inhibition under field conditions. This may indicate that association or non-association of different species may be the result of specific compounds being released.

Newman and Rovira (1975) obtained strong correlations when they compared the field vegetation patterns with laboratory results. They concluded that auto-inhibitory exudates are a key process in the control of species diversity in grassland.

Mechanisms of Allelopathy

Germination inhibitors are found in nearly all plant parts (Evanari, 1949). Release routes for allelochemicals include rainwash and fog drop from leaf surfaces, volatilization from leaves, excretion or exudation from roots and decay of above and/or below-ground plant parts (Whittaker and Feeney, 1971).

Allelopathic chemicals have been collected after washing leaves of *Artemisia absinthium* (Bode, 1939 from Bonner, 1950; Bennett and Bonner, 1953). Other allelopathic compounds were found in litter of the same plant. Bell and Muller (1973) leached water soluble toxins from dead stalks and leaves of *Brassica nigra*.

Toxic substances are produced by excretions from roots or underground stems (Bonner, 1946; Bonner, 1950; Putnam and Duke, 1974) or from decaying plant residues (Bode, 1939 from Bonner, 1950; Benedict, 1941).

Einhellig and Rasmussen (1973) postulated that many allelochemicals are water soluble which would facilitate their release into the environment from fresh leaves. Woods (1960) cautions against the use of aqueous plant extracts because many of the toxins in such a solution would not be present under normal growing conditions.

Allelopathic effects have been observed from fresh and dead tissue. Dilutions of aqueous extracts from the fresh leaf tissue of *Rumex crispus* inhibited *Amaranthus retroflexus*, grain sorghum (*Sorghum vulgare*) and field corn (Einhellig and Rasmussen, 1973). Undiluted root extracts of three perennials, *Cynodon dactylon*, *Cyperus rotundus* and *Sorghum halepense* reduced root length in barley (*Hordeum vulgare*), *Brassica nigra* and wheat (*Triticum aestivum*) (Friedman and Horowitz, 1970).

Friedman and Horowitz (1970) stated that perennial plants may be toxic during decay because they may cause temporary inhibition of growth in nearby plants. They found that leaves or rhizomes of *Sorghum halepense* decaying in soil inhibited germination and seedling development of weed species planted in the soil. Volatile and water soluble toxins from *Eucalyptus camaldulensis* litter were found to inhibit growth in the lab and greenhouse. This was thought to be the reason for a lack of annual herbs around the plot when litter accumulated in the field (del Moral and Muller, 1970).

Other perennials have displayed allelopathic effects. Hanawalt (1971) found inverse correlations between the amount of plant litter and weed seedling number in an area dominated by *Arctostaphylos glauca* and *A. glandulosa* var. *zacaensis*. Environmental factors

were ruled out as a cause for differential growth. Fresh leaf litter was more toxic than partially decayed litter. Toxins were no longer present nine weeks after soil was taken from the field to the greenhouse.

Rice (1974) noted that deCandolle (1832) first observed that thistles injure oats in the field. DeCandolle also saw flax injured by euphorbe (*Euphorbia* spp.) and *Scabosia* spp. Rye plants (*Lolium* spp.) injured wheat. Reports of allelopathy in these plants have been supported by later work. Stachon and Zimdahl (1980) found that the addition of Canada thistle (*Cirsium arvense*) litter, ground roots and ground foliage to the soil reduced growth of *Amaranthus retroflexus* and *Setaria viridis*. Rice (1974) stated that allelochemicals from *E. supina* may inhibit growth of pioneer weeds and speed their elimination from the first stage of plant succession in abandoned fields. *Euphorbia corollata* also inhibits first stage succession plants.

Frank and Dechoetz (1980) found that phytotoxins from dwarf spikerush (*Eleocharis coloradoensis*) reduced growth of American pondweed (*Potamogeton nodosus*) and sago pondweed (*P. pectinatus*).

Annual plants have been shown to have allelopathic effects. Decomposing plants of timothy (*Phleum pratense*), corn (*Zea mays*), rye (*Secale cereale*) and tobacco (*Nicotiana tabacum*) produced substances that inhibited respiration, germination and growth of tobacco seedlings (Patrick and Koch, 1958).

Allelopathic Compounds

Rovira (1971) identified a wide range of compounds that were exuded from intact roots including sugars, amino acids, peptides, enzymes, organic acids, nucleotides, and

fungal stimulants. Root exudation is affected by plant species, plant age, temperature, light, plant nutrients and soil moisture.

Rovira (1965) as cited by Foy (1971) lists four references which present major evidence for root exudation of organic molecules:

1. Knudson (1920) provided conclusive evidence that peas and maize release specific organic substances from roots.

2. Lyon and Wilson (1921) measured organic nitrogen released from major roots under sterile conditions.

3. Craner (1922) detected phosphatides from roots of both seedlings and mature plants.

4. O'Brien and Prentice (1930) demonstrated biological specificity of plant exudates.

Numerous toxic substances have been found in intact plants. Hydrolyzable tannins were isolated from extracts of *Euphorbia supina* after it was found to affect nitrifying bacteria (Rice, 1969). Cineole and camphor were found in toxic amounts in the air above *Salvia leucophylla* and *Artemesia californica*. The characteristic bare zones around these shrubs was shown to be a result of these terpenes which are bound by the soil (Muller, 1971). Muller (1971) demonstrated that volatile terpenes from *Salvia mellifera* and *Lepechinia calycina* inhibited growth of numerous other plant species.

Many substances are released from the roots into the soil, often as a result of death and decay (Whittaker, 1970). Chemicals responsible for allelopathic effects are often secondary plant substances such as phenols, terpenes, and alkaloids.

Jugalone (5-hydroxy-1,4-naphthoquinone) occurs as hydroxy jugalone in all plant parts of black walnut (*Juglans nigra*). After release from the leaves and dead tissue it is

converted to its toxic form (Davis, 1928; Whittaker, 1970). The inhibition of germination and growth as a result of allelochemical release by *Encelia farinosa* is partially attributed to 3-acetyl-6-methoxy benzaldehyde (Gray and Bonner, 1948). Went (1970) found trans-cinnamic acid to be the main inhibitor utilized by guayule (*Parthenium argentatum*). *Euphorbia corollata* was shown to produce gallic and tannic acids (Rice, 1974).

Börner (1971) found phlorizone in high concentrations in established apple (*Malus* spp.) plantings, however, the concentration was not high enough to cause toxic reactions.

Patterson (1981) examined ten compounds in the laboratory and found that t-cinnamic, caffeic, p-coumaric, ferulic, gallic, vanillic and 5-sulfosalicylic acids reduced plant growth. Uncertainty exists as to whether the concentration of these allelopathic compounds in the field ever reached the levels used in laboratory work. Long-term effects in the field may differ radically from short-term laboratory effects.

Plants have devised protective systems to avoid internal autotoxicity. Protective mechanisms include the synthesis and accumulation of inactive polymers such as tannins, the secretion of inactive compounds which become toxic after release such as jugalone, cellular segregation such as the storage of calcium oxalate crystals, separation in the cell by compartmentalization of the inactive toxin and the activating enzyme (Whittaker, 1971). Seeds of *Eucalyptus camaldulensis* are not sensitive to the toxins produced by the parent plant (del Moral and Muller, 1970).

Factors Affecting Allelopathy

Toxins can be leached in the soil. Rice (1969) cites Blum and Rice (1969) as having discovered that tannic acid from the leaves of *Rhus copallina* leached 75 cm into the soil profile. Small amounts of tannic acid reduced nodulation of bean (*Phaseolus vulgaris*)

plants. Bonner (1950) found that *Encelia farinosa* leaves may retain their toxic properties for a year or more after falling and the toxin may be leached into the soil.

Soil texture affects toxin concentration. Del Moral and Muller (1970) found that toxins from *Eucalyptus camaldealensis* affected the surrounding grassland only after being adsorbed by the soil colloids since the toxins failed to inhibit other plant species grown in sand. Allelopathic compounds can interact with soil and be selectively adsorbed (del Moral and Muller, 1970).

Autotoxicity exists between guayule roots if plant roots become pot-bound (Bonner, 1950). Benedict (1941) demonstrated dead bromegrass (*Bromus* spp.) roots inhibited growth of bromegrass plants. Putnam and Duke (1974) discovered that cucumber (*Cucumis sativus*) accessions and cultivars differ greatly in their ability to control weed growth.

Bonner (1971) suggests the definition of allelopathy be expanded to include the influence of the products of soil microorganisms since they can convert inactive compounds secreted from plant residues into phytotoxic substances.

Börner (1971) studied the effects of root secretions of weeds among cultivated plants in water culture.

Stachon and Zimdahl (1980) found that ethanolic extracts of Canada thistle (*Cirsium arvense*) produced toxic effects on test species but dried plant residues had no effect. Some studies show microbes are responsible for an increase or decrease in allelopathic effects (Bonner, 1950). Patrick and Koch (1958) determined that initial microbial breakdown of plant tissue produced a toxin and later aided in decomposition of the plant tissue.

Allelopathic Activity of *Euphorbia esula*

Allelopathic extracts of *Euphorbia esula* have been demonstrated. LeTourneau and Heggeness (1957) used an aqueous extract of *E. esula* foliage to inhibit root growth of *Triticum aestivum*. The same extract caused shortening and discoloration of 'Alaska' pea (*Pisum sativum*) roots. On a dry weight basis, fresh extracts were found to be more inhibitory than extracts of dried ground material. *Euphorbia esula* leaf extracts were more inhibitory than stem extracts (LeTourneau et al., 1956; Selleck, 1972). Extracts from young spurge plants were more inhibitory than extracts from older plants (LeTourneau et al., 1956).

Selleck (1972) found that leaf and stem extracts from leafy spurge inhibited germination of wheat, *Bromus inermis*, *Brassica kaber* and *Agropyron cristatum*. Root extracts depressed germination and coleoptile elongation of all the species listed above except wheat. Extracts of soil in close association with leafy spurge roots inhibited radicle elongation of wheat and *Bromus inermis*.

Steenhagen and Zimdahl (1979) demonstrated that dried, ground leafy spurge added to the soil would inhibit growth of tomato (*Lycopersicon esculentum*) and crabgrass (*Digitaria sanguinalis*). Their results suggest that toxin levels in soil may be too low to inhibit growth of competing species when the leafy spurge litter is removed.

Newman and Rovira (1975) stated that toxic compounds are found in many plants but cautioned that laboratory results are not always relevant to the field situation.

Indicator species are sometimes chosen for convenience rather than ecological significance. Del Moral and Cates (1971) agree that subsequent work with potential allelo-

pathic species should utilize native species which are routinely influenced by the suspected allelochemic plant.

CHAPTER TEN

ALLELOPATHIC SOIL RESIDUES OF LEAFY SPURGE

Abstract

Soil was collected from two leafy spurge infested areas near Missoula and Vaughn, Montana. Soil from a nearby non-infested area served as the control. Twelve grass species were grown in the soils in the greenhouse to determine if plant growth was influenced by allelopathic compounds.

There was no difference in the rate of emergence of the grass species in the infested and check soils. In addition, there was no difference in growth rate of the species grown in each soil.

Four species showed weight differences when grown in the infested and check soil from Missoula one month after planting; however, there were no weight differences two months after planting. Plant dry weight of *Alopecurus arundinaceus* was lower in Missoula infested soil than in check soil two months after planting. Plant dry weight of *Bromus inermis* was lower when grown in Vaughn infested soil one month after planting.

Introduction

Allelopathy may regulate the distribution of plant species or plant succession (Einhellig and Rasmussen, 1973; Putnam and Duke, 1974; Rice, 1974). The following research was conducted to determine if leafy spurge utilizes allelopathy as a major strategic device since there is evidence to suggest that related species (*Euphorbia supina* and *E. corollata*) inhibit pioneer weeds in the first stage of plant succession in abandoned fields (Rice, 1974).

In addition, suspected allelochemicals from leafy spurge inhibited root growth of wheat, *Bromus inermis*, *Brassica kaber* and *Agropyron cristatum* (LeTourneau and Heggeness, 1957; Selleck, 1972). Steenhagen and Zimdahl (1979) inhibited growth of tomato and *Digitaria sanguinalis* by addition of leafy spurge residues to soil.

Several authors (del Moral and Cates 1971; Newman and Rovira, 1965) have emphasized the following points: (1) plants may contain toxic substances, however, these substances are not always utilized in the field situation, and (2) studies related to allelopathy need to utilize native species which are found in association with the suspected allelopathic plant.

The following study used twelve native and introduced grass species commonly found in areas infested with leafy spurge.

Materials and Methods

Soil was excavated under dense stands of leafy spurge in October, 1979, at Missoula and Vaughn, Montana. An equal quantity of soil was collected from an uninfested area within 30 m of the infested soil at each location. Soil was sieved to remove debris and plant material and stored in plastic bags at -4°C to await greenhouse testing. Soil pH, organic matter, phosphorus, potassium, salt hazard and texture were determined (Table 12).

Seed of 12 grass species was obtained from the Bridger Plant Center, Bridger, Montana (Table 13).

Plant height comparisons. Soil from infested and uninfested sites in Missoula as well as greenhouse potting soil (peat:sand:Huffine silt loam, 1:1:3 v/v/v) was placed in 3.8 cm diameter by 21 cm long containers. Four seeds of *Agropyron spicatum*, *A. trachy-*

Table 12. Soil properties of greenhouse, Missoula infested and Missoula check soils.

| Soil | pH | Organic matter % | P ppm | K ppm | Salt hazard | Soil texture |
|-------------------|-----|---------------------|----------|----------|----------------|-----------------|
| Greenhouse | 7.6 | 5.6 | 392 | 724 | 1.0 | loam |
| Missoula infested | 7.1 | 5.8 | 95 | 785 | 0.4 | loam |
| Missoula check | 7.1 | 5.0 | 104 | 587 | 0.7 | loam |

Table 13. Twelve grass species grown in infested and uninfested soils from Missoula and Vaughn, Montana.

| Genus and species | Common name |
|--------------------------------|----------------------------|
| Native species | |
| <i>Agropyron smithii</i> | western wheatgrass |
| <i>A. trachycaulum</i> | slender wheatgrass |
| <i>A. spicatum</i> | bluebunch wheatgrass |
| <i>A. dasystachum</i> | thickspike wheatgrass |
| <i>Calomvilfa longifolia</i> | prairie sandreed |
| <i>Stipa viridula</i> | green needlegrass |
| <i>Elymus cinereus</i> | basin wildrye |
| Introduced species | |
| <i>Agropyron cristatum</i> | Fairway crested wheatgrass |
| <i>Elymus junceus</i> | Russian wildrye |
| <i>Elymus angustus</i> | 'Altai' wildrye |
| <i>Alopecurus arundinaceus</i> | creeping foxtail |
| <i>Bromus inermis</i> | smooth brome |

caulum, *A. smithii*, *Elymus junceus*, *E. angustus*, *E. cinereus*, *Alopecurus arundinaceus* or *Bromus inermis* were planted in each container. After planting the soil surface in each container was covered with 0.5 cm sand to reduce evaporation. There were three replications per treatment.

The containers were placed in a germination chamber set at an alternating 15-25°C temperature. After 10 days they were placed in a growth chamber set to operate at a constant 22°C with 12 hours of light (22 Klux) per 24 hour period. Each container received 10 ml water every other day. Plant height was measured every other day for one month.

Plant weight comparisons. Soil from infested and uninfested sites from Missoula and Vaughn was placed in 13.5 cm by 21.5 cm by 9.0 cm deep flats. Twenty seeds of a species were planted 0.7 cm deep in rows 13 cm long. There were 4 species planted in each flat. There were three replications per treatment. The flats were maintained in the greenhouse without supplemental light with a temperature range of 18 to 25°C. Flats were watered as needed.

The number of emerged seedlings was counted daily and the speed of emergence was calculated. One month after planting half of the plants in each flat were cut at the soil surface, oven dried for three days at 38°C and weighed. Two months after planting the remaining plants were cut, dried and weighed.

Results and Discussion

Plant height comparison. There were plant height differences for four plant species in two soils on one or more days. *Agropyron smithii* and *Elymus cinereus* produced more growth in greenhouse soil than in the check or infested soils from Missoula (Figures 5 and

6). The greenhouse soil was slightly different in some soil properties which may explain differences in plant growth. *Agropyron trachycaulum* showed differences in growth on three dates among the soils tested (Figure 7). *Elymus junceus* was taller when grown in infested soil on three days (Figure 8). There were no differences in the growth curves of the other species tested.¹

Two differences were noted in growth between soils using regression analysis (Table 14). Two species had significant differences between slopes of the regression lines. The regression line of *Agropyron spicatum* in the check soil had less of a slope than the infested or greenhouse soils (Figure 9). The regression line of *Elymus junceus* in greenhouse soil had a steeper slope than either of the other two soils (Figure 10). All other species showed similar regression lines for the three soils.²

Similar slopes of the regression lines indicate no differences in rate of growth among the three soils. Dry weight accumulation for each species was the same in all soils. This would imply that no growth inhibitors were present in the infested soil to slow the growth rate.

The mean plant height of *Elymus cinereus* and *Elymus angustus* was lower when grown in uninfested soil compared to the infested or greenhouse soils (Table 15).

Plant weight comparison. Speed of emergence was determined by the following equation: (Wiesner, 1981).

$$\frac{\text{no. of plants emerged}}{\text{day 1}} + \frac{\text{no. of plants emerged}}{\text{day 2}} + \dots + \frac{\text{no. of plants emerged}}{\text{day 5}} = \text{speed of emergence}$$

¹ Appendix C.

² Appendix D.

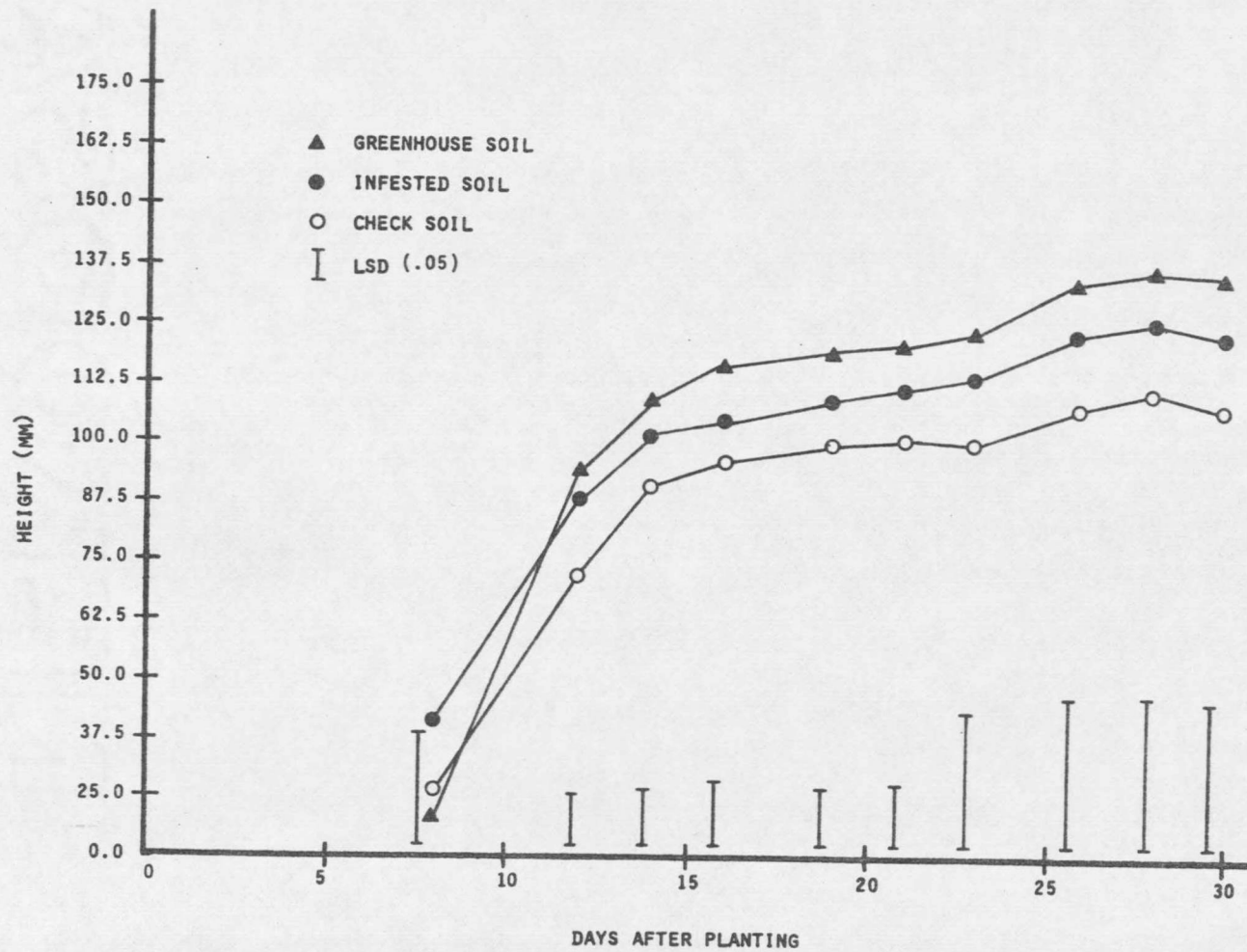


Figure 5. Growth curve of *Agropyron smithii* in three soils.

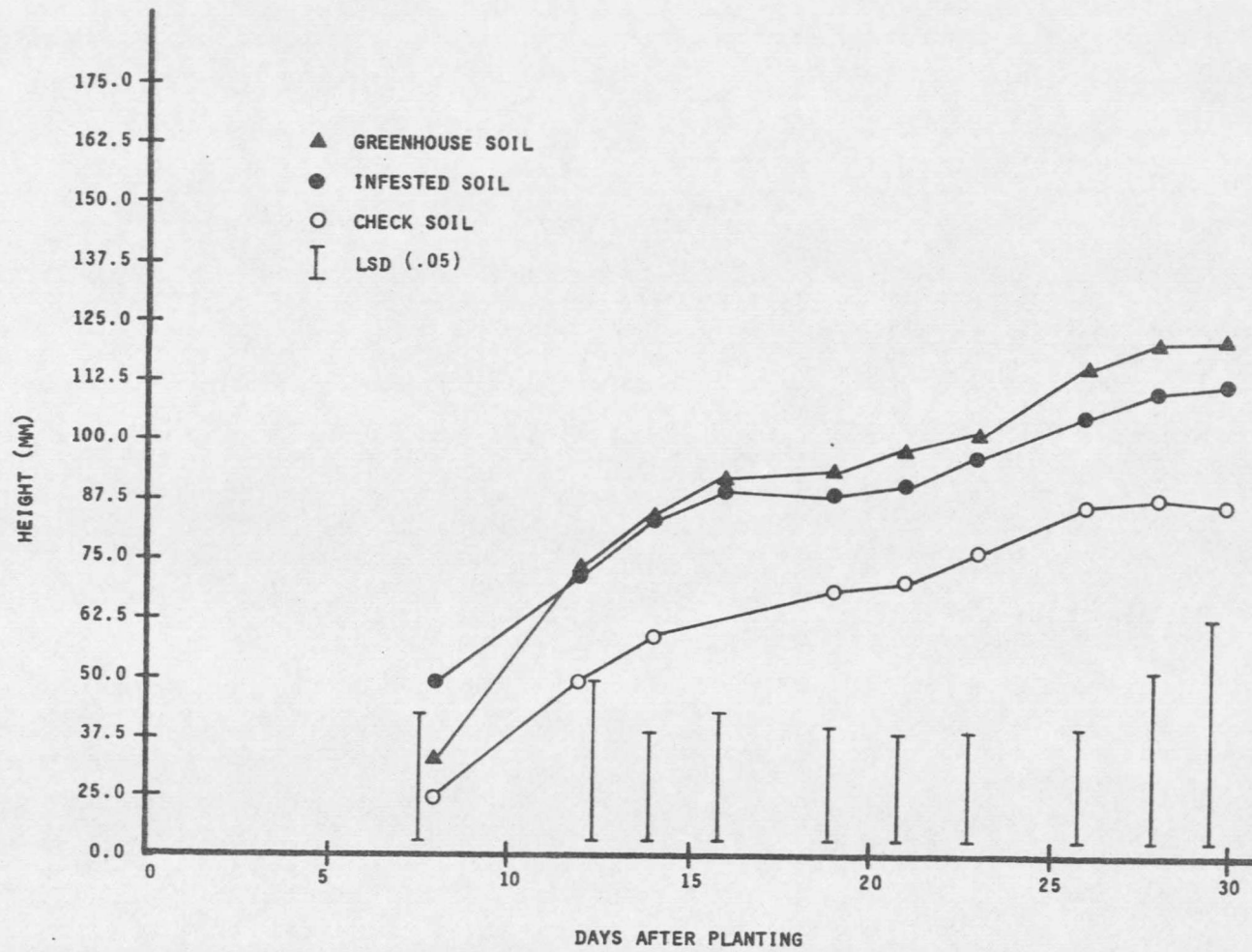


Figure 6. Growth curve of *Elymus cinereus* in three soils.

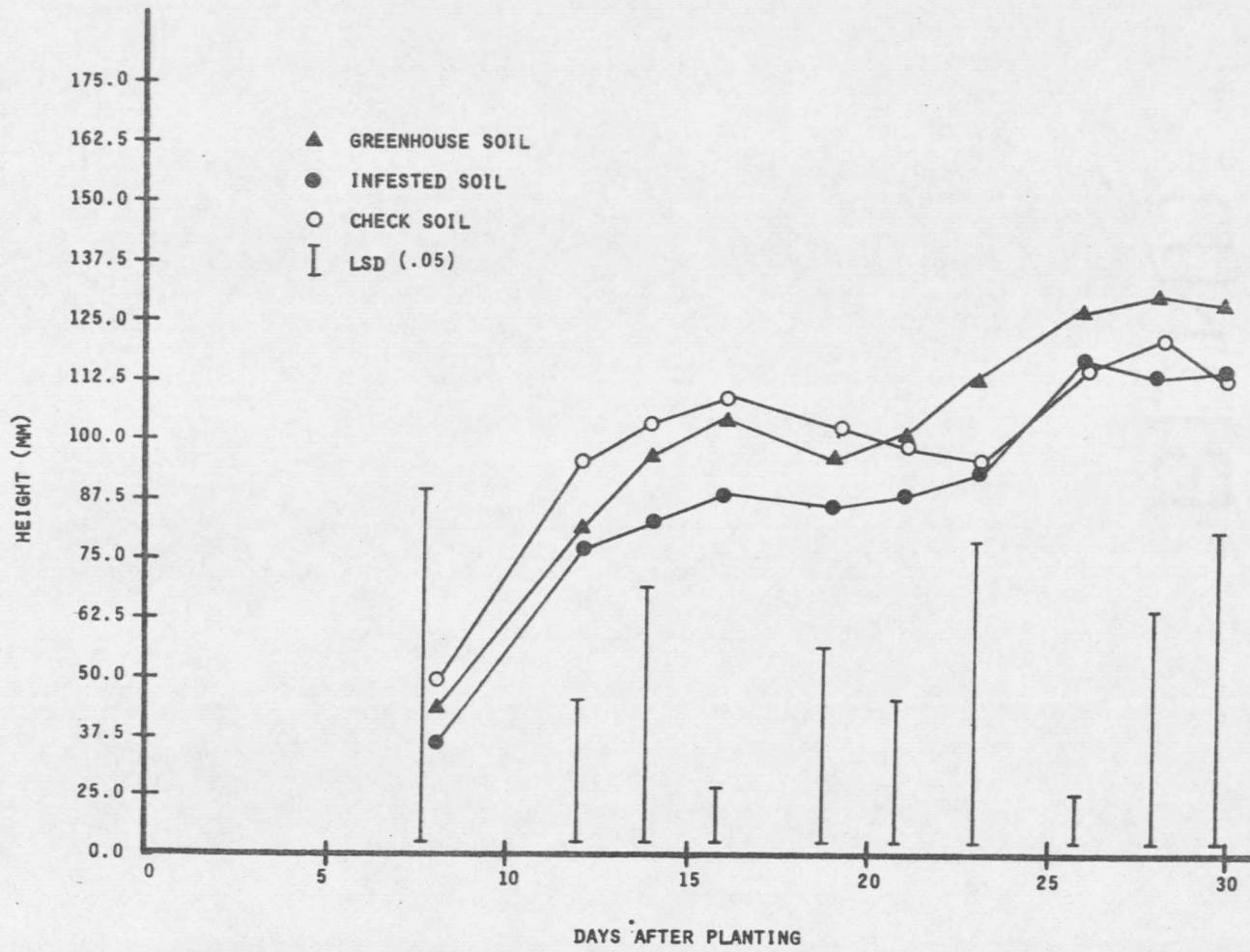


Figure 7. Growth curve of *Agropyron trachycaulum* in three soils.

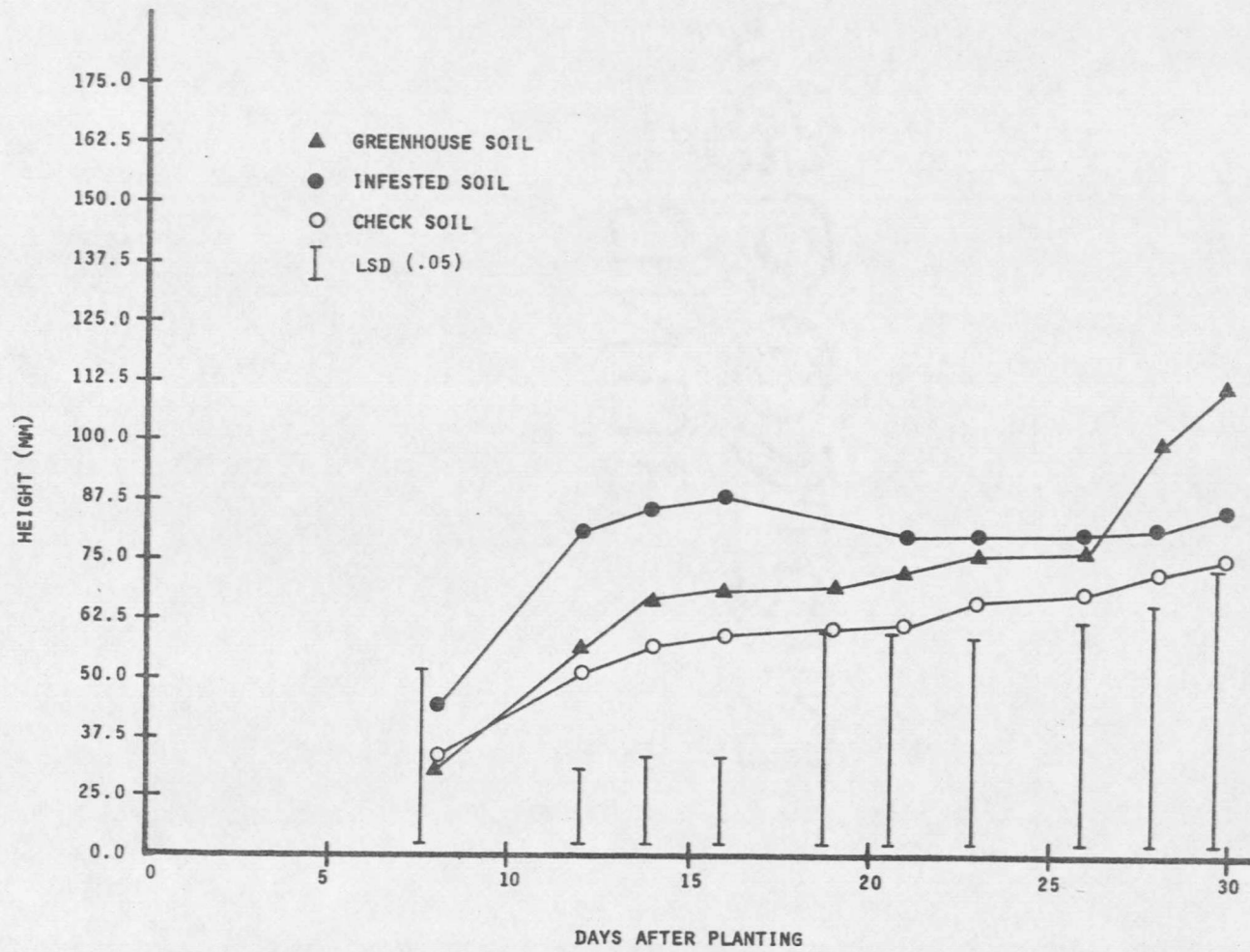


Figure 8. Growth curve of *Elymus junceus* in three soils.

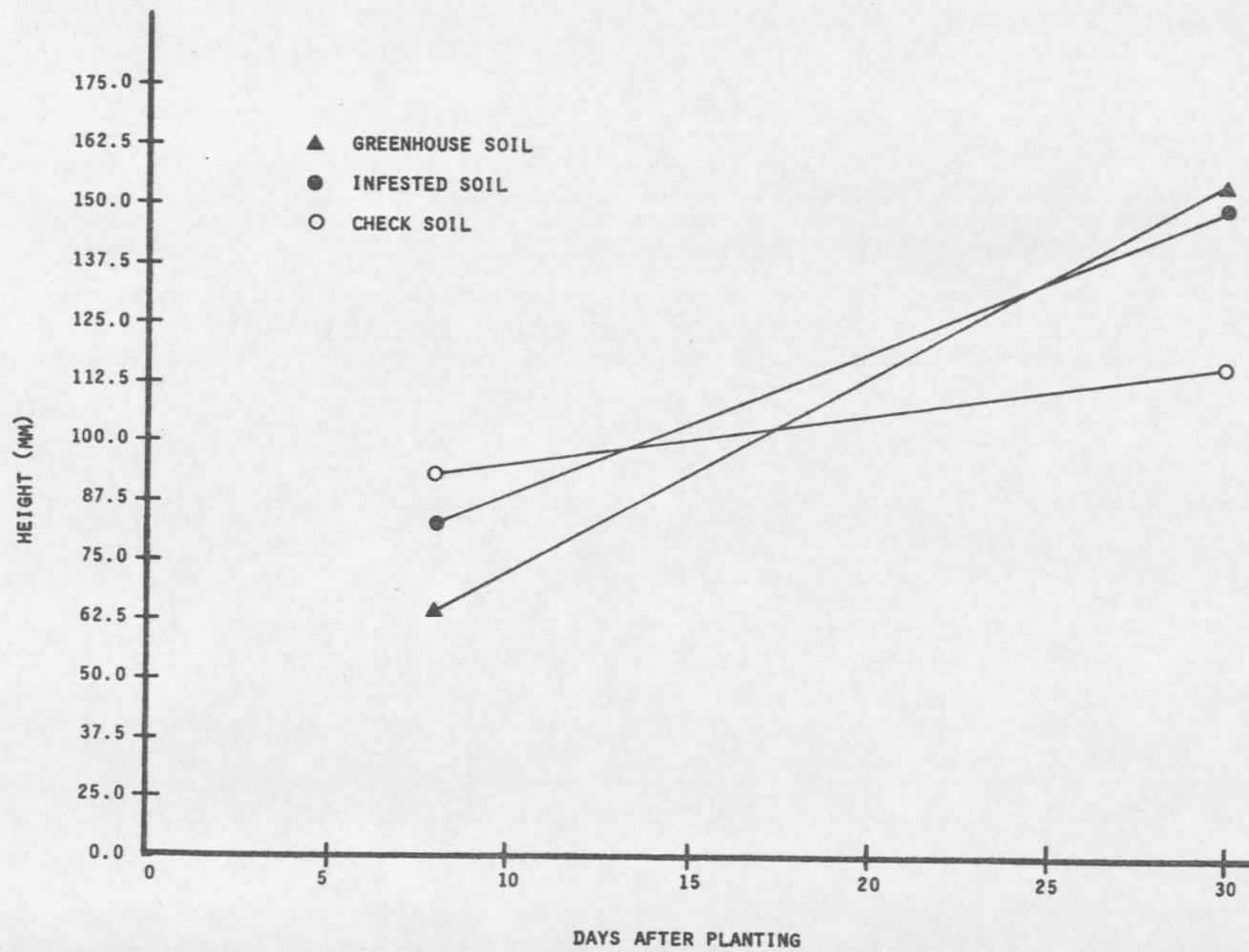


Figure 9. Regression lines for growth of *Agropyron spicatum* in three soils.

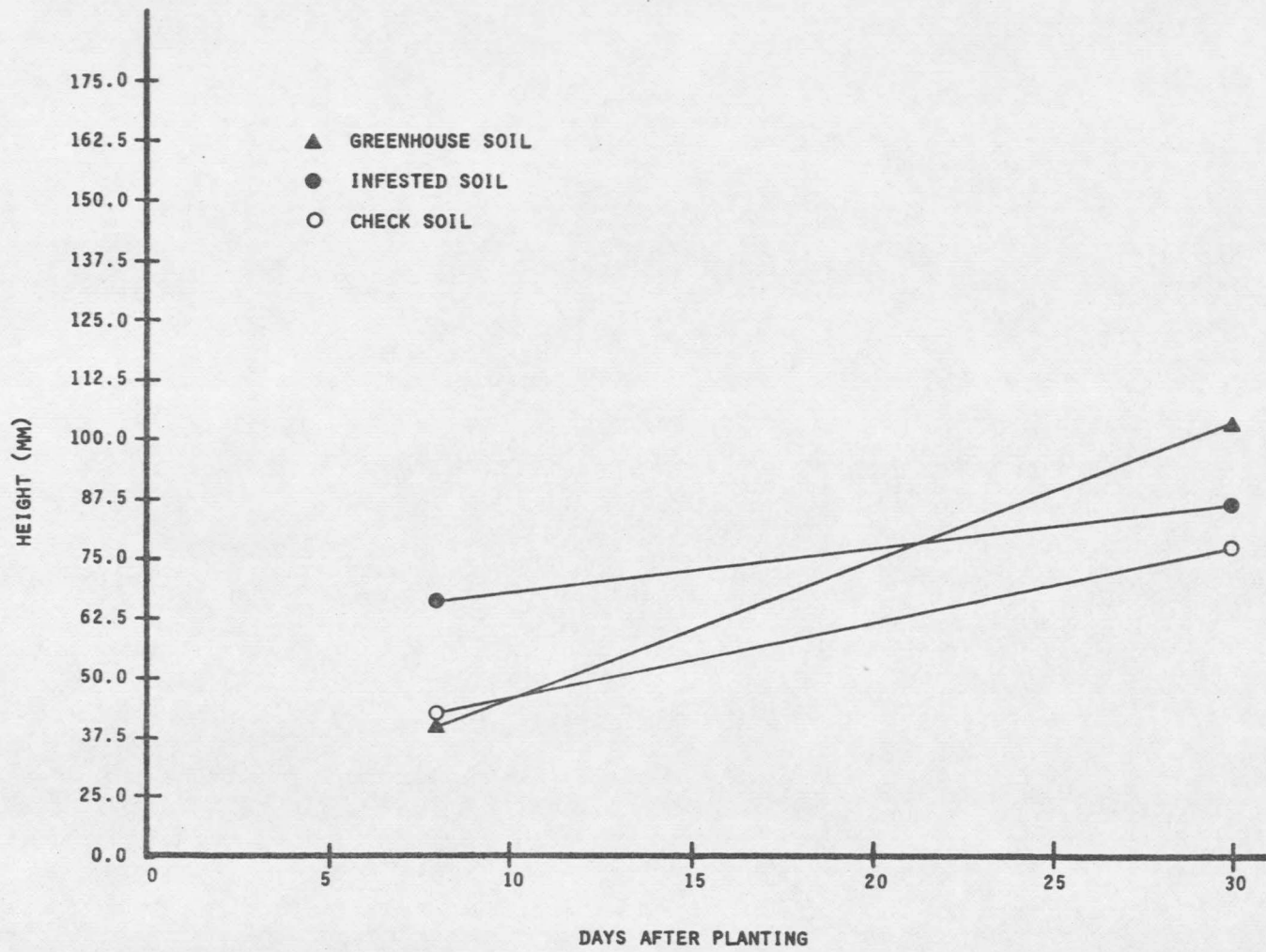


Figure 10. Regression lines for growth of *Elymus junceus* in three soils.

Table 14. Test for equality of slopes of three soil regression lines for each grass species.

| Species | Test result p-value |
|--------------------------------|------------------------|
| <i>Agropyron spicatum</i> | .008 ** |
| <i>A. trachycaulum</i> | .181 NS |
| <i>A. smithii</i> | .887 NS |
| <i>Elymus junceus</i> | .011 * |
| <i>E. cinereus</i> | .258 NS |
| <i>E. angustus</i> | .923 NS |
| <i>Bromus inermis</i> | .083 NS |
| <i>Alopecurus arundinaceus</i> | .096 NS |

* = .05 significance

** = .01 significance

NS = not significant

Table 15. Mean height for each grass species after one month of growth in the Missoula check, Missoula infested and greenhouse soils.

| Soil | Mean height (mm) ^a | | | | | | | |
|------------|-------------------------------|-------|-------|-------|--------|-------|-------|---------|
| | Grass species ^b | | | | | | | |
| | Agsm | Alar | Agtr | Elju | Agsp | Brin | Elci | Elan |
| Greenhouse | 92.7a | 38.1a | 89.8a | 60.9a | 98.9a | 60.1a | 82.3a | 134.8a |
| Check | 78.4a | 29.3a | 87.8a | 48.3a | 92.5a | 80.8a | 50.6b | 78.7b |
| Infested | 92.3a | 32.3a | 76.7a | 64.5a | 104.9a | 66.2a | 81.6a | 92.9a,b |

^a Means within each column followed by the same letter are not statistically different at the 5% level as determined by LSD test.

^b Agsm = *Agropyron spicatum*
 Alar = *Alopecurus arundinaceus*
 Agtr = *Agropyron trachycaulum*
 Elju = *Elymus junceus*

Agsp = *Agropyron spicatum*
 Brin = *Bromus inermis*
 Elci = *Elymus cinereus*
 Elan = *Elymus angustus*

No grass species showed differences in the speed of emergence between the Missoula check and infested soils (Table 16).

Table 16. Speed of emergence index number of twelve grass species grown in Missoula check and infested soils.

| Soil | Grass species ^a | | | | | | | | | | | |
|--------------|----------------------------|------|------|------|------|------|------|------|------|-------|------|------|
| | Calo | Agsm | Agcr | Stvi | Elci | Elan | Alar | Agda | Agsp | Brin | Elju | Agtr |
| Check | .91 | 5.35 | 4.88 | 1.79 | 4.75 | 7.69 | 7.57 | 8.92 | 8.94 | 10.30 | 4.57 | 7.52 |
| Infested | 2.37 | 5.84 | 2.20 | 1.82 | 6.61 | 5.83 | 6.30 | 8.62 | 8.01 | 9.69 | 5.57 | 6.99 |
| LSD (.05) | 1.77 | 3.00 | 3.12 | 1.16 | 4.27 | 4.81 | 3.36 | 4.39 | 1.81 | 2.31 | 8.62 | 5.15 |

^aCalo = *Calomovilfa longifolia*
 Agsm = *Agropyron smithii*
 Agcr = *Agropyron cristatum*
 Stvi = *Stipa viridula*
 Elci = *Elymus cinereus*
 Elan = *Elymus angustus*

Alar = *Alopecurus arundinaceus*
 Agda = *Agropyron dasystachum*
 Agsp = *Agropyron spicatum*
 Brin = *Bromus inermis*
 Elju = *Elymus junceus*
 Agtr = *Agropyron trachycaulum*

There were plant weight differences one month after planting for four species planted in soil from Missoula (Table 17). *Calomovilfa longifolia*, *Agropyron spicatum*, *Elymus junceus* and *A. trachycaulum* produced more plant tissue when grown in uninfested soils. Two months after planting there was no longer a weight difference among the four species in the two soils. *Alopecurus arundinaceus* had a lower plant weight in infested soil two months after planting.

Only *Bromus inermis* produced less growth in the infested soil from Vaughn one month after planting (Table 18). No significant differences in plant weight were detected at the time of the second cutting.

The plant weight of *Alopecurus arundinaceus* was three-fold higher two months after planting when grown in uninfested soil from Vaughn. Although not statistically dif-

Table 17. Weight of grass species grown in Missoula check and infested soil one and two months after planting.

| Soil | Grass species ^a | | | | | | | | | | | |
|------------|----------------------------|-------|--------|-------|--------|-------|-------|--------|-------|--------|-------|--------|
| | Calo | Agsm | Agcr | Stvi | Elci | Elan | Alar | Agda | Agsp | Brin | Elju | Agtr |
| | -----mg/plt----- | | | | | | | | | | | |
| One Month | | | | | | | | | | | | |
| Check | 18.0 | 31.3 | 39.3 | 12.0 | 22.7 | 31.7 | 6.0 | 28.0 | 28.7 | 26.7 | 16.0 | 44.0 |
| Infested | 5.7 | 21.7 | 27.7 | 7.3 | 16.0 | 26.7 | 3.3 | 24.0 | 23.0 | 18.7 | 11.7 | 18.0 |
| LSD 5% | 7.69 | 13.45 | 13.45 | 5.08 | 21.06 | 16.06 | 2.91 | 22.89 | .73 | 9.99 | 1.92 | 15.46 |
| Two Months | | | | | | | | | | | | |
| Check | 25.7 | 117.0 | 204.3 | 48.0 | 75.0 | 88.3 | 31.0 | 128.7 | 121.7 | 142.7 | 45.7 | 213.7 |
| Infested | 26.7 | 95.0 | 230.0 | 55.7 | 61.0 | 78.0 | 9.7 | 99.7 | 108.7 | 105.3 | 32.3 | 111.0 |
| LSD 5% | 89.26 | 50.73 | 164.69 | 50.69 | 124.81 | 81.35 | 18.97 | 223.59 | 99.65 | 186.60 | 16.54 | 114.23 |

^aCalo = *Calamovilfa longifolia*
 Agsm = *Agropyron smithii*
 Agcr = *Agropyron cristatum*
 Stvi = *Stipa viridula*
 Elci = *Elymus cinereus*
 Elan = *Elymus angustus*

Alar = *Alopecurus arundinaceus*
 Agda = *Agropyron dasystachum*
 Agsp = *Agropyron spicatum*
 Brin = *Bromus inermis*
 Elju = *Elymus junceus*
 Agtr = *Agropyron trachycaulum*

Table 18. Weight of grass species grown in Vaughn check and infested soil one and two months after planting.

| Soil | Grass species ^a | | | | | | | | | | | |
|-------------------|----------------------------|-------|--------|-------|-------|-------|--------|-------|-------|-------|-------|--------|
| | Calo | Agsm | Agcr | Stvi | Elci | Elan | Alar | Agda | Agsp | Brin | Elju | Agtr |
| -----mg/plt----- | | | | | | | | | | | | |
| One Month | | | | | | | | | | | | |
| Check | 3.7 | 35.3 | 23.7 | 11.7 | 21.0 | 20.7 | 1.7 | 28.3 | 29.0 | 14.0 | 6.3 | 24.0 |
| Infested | 8.0 | 21.0 | 13.7 | 7.3 | 11.3 | 13.3 | 4.0 | 24.7 | 16.3 | 11.3 | 8.7 | 20.0 |
| LSD 5% | 11.20 | 61.68 | 30.22 | 13.68 | 22.27 | 25.38 | 3.79 | 13.68 | 13.68 | 1.43 | 5.17 | 9.94 ♂ |
| Two Months | | | | | | | | | | | | |
| Check | 34.3 | 113.3 | 127.0 | 39.3 | 70.3 | 54.7 | 73.3 | 99.3 | 82.0 | 72.3 | 24.3 | 117.0 |
| Infested | 35.3 | 99.0 | 36.3 | 38.0 | 46.0 | 60.3 | 24.3 | 53.7 | 63.0 | 145. | 18.7 | 104.0 |
| LSD 5% | 24.47 | 98.64 | 147.51 | 77.23 | 28.25 | 11.20 | 169.97 | 62.08 | 94.96 | 269.7 | 10.34 | 131.95 |

^aCalo = *Calamovilfa longifolia*

Agsm = *Agropyron smithii*

Agcr = *Agropyron cristatum*

Stvi = *Stipa viridula*

Elci = *Elymus cinereus*

Elan = *Elymus angustus*

Alar = *Alopecurus arundinaceus*

Agda = *Agropyron dasystachum*

Agsp = *Agropyron spicatum*

Brin = *Bromus inermis*

Elju = *Elymus junceus*

Agtr = *Agropyron trachycaulum*

ferent, this trend does correlate with the differences observed when the same plant was grown in soil from Missoula.

Confirmation trials were established with three of the four grasses which showed weight differences one month after planting when grown in soil from Missoula (Table 19). There were no differences in plant weights at either time of cutting in the confirmation trial.

Table 19. Weight of three grass species grown for a second time in Missoula soil one month and two months after planting.

| Soil | Grass species ^a | | |
|------------|----------------------------|-------|------|
| | Agsp | Agtr | Elju |
| | -----mg/plt----- | | |
| One Month | | | |
| Check | 35.0 | 38.0 | 13.0 |
| Infested | 30.0 | 34.0 | 13.5 |
| LSD (.05) | 162.2 | 101.7 | 57.2 |
| Two Months | | | |
| Check | 69.3 | 56.3 | 59.0 |
| Infested | 72.7 | 78.5 | 43.5 |
| LSD (.05) | 15.6 | 33.7 | 22.8 |

^a Agsp = *Agropyron spicatum*, Agtr = *Agropyron trachycaulum*, Elju = *Elymus junceus*.

The weight differences in *Alopecurus arundinaceus* between the infested and uninfested soils from Missoula were not evident until the time of the second cutting. If the observed differences were caused by an allelochemical in soil, the toxin must not be readily broken down after eight weeks in a greenhouse experiment. No other differences appeared among the other species two months after planting. It is unlikely that the observed difference in plant growth was caused by a toxin released by leafy spurge since the growth

of *Alopecurus arundinaceus* was not reduced when the plant was grown in soil from Vaughn. The growth reduction of this plant in infested soil from Missoula must have been a result of an unknown variable.

Selleck (1974) observed allelopathic responses from soil extracts by using soil directly associated with leafy spurge roots. The soil from Missoula and Vaughn was sieved to remove plant debris. Since growth of four species was reduced in infested soil at the first cutting, a toxin may have been present initially, but without continuous release from leafy spurge roots the allelopathic effects were not maintained. Under field conditions root and foliar debris would be present in soil or on the soil surface. Steenhagen and Zimdahl (1979) observed inhibition of tomato and *Digitaria sanguinalis* only when leafy spurge litter was incorporated in the soil.

The time of year of soil collection may have affected the results. If leafy spurge produces an allelopathic toxin it may not have been present in sufficient quantity in October to produce growth inhibition of the grass species tested.

CHAPTER ELEVEN

SUMMARY

Leafy spurge infests grazing land and will become the dominate vegetation over time. Several *Euphorbia* species exhibit allelopathic effects on plant species in old field succession. This study examined possible allelopathic soil residues from leafy spurge and their effect on grass species.

Soil was excavated from a dense stand of leafy spurge and from an uninfested area several meters away. Twelve grass species were grown in each soil. Plant height and dry matter accumulation were measured.

Several grass species showed decreased growth when grown in leafy spurge infested soil from one location. The effect on growth was not consistent between infested soils from two locations. Confirmation trials did not support the initial data. If leafy spurge produces allelopathic substances they do not remain in the soil at effective concentrations when plant debris is removed.

Results from this study indicate that soil from a dense stand of leafy spurge does not contain toxins in sufficient quantities to inhibit growth of native or introduced grass species when grown under greenhouse and growth chamber conditions.

Further studies in a controlled situation are needed. Native and introduced grass species should be observed when grown in association with intact leafy spurge roots. Growth of these species should also be monitored when natural litter is present. These studies may indicate which species would be useful in reseeding areas where leafy spurge is controlled.

SUMMARY

Leafy spurge is a serious weed problem in Montana, infesting over one half million acres. A three part program developed an education program on leafy spurge, analyzed the hydrocarbon of the weed and examined the allelopathic soil residues of leafy spurge.

The education program utilized newspaper articles, a leafy spurge newsletter, summer field tours and slide sets to increase awareness and knowledge of leafy spurge. The articles, summer tours and slide sets provided background information and the newsletters continue to update the information. Many people are still unaware of leafy spurge, the threat it presents and control methods. County agents and local weed personnel need to continue the program through utilization of the chemical control demonstration sites and incorporation of the educational materials into their existing programs.

Annual *Euphorbia* species have been examined as possible sources of hydrocarbons. In this study, 12 ecotypes of a perennial species, *Euphorbia esula*, were grown and harvested. Samples were harvested on three dates during the growing season and extracted for hydrocarbons. No ecotypes produced exceptionally high levels of hydrocarbons.

Leafy spurge regrowth was a problem. Extensive regrowth after cutting was expected but did not occur possibly due to restricted root growth. Low rates of fertilizer did not increase hydrocarbon content. Effects of heavier applications of fertilizer and irrigation on hydrocarbon content need to be researched. Additional research in plant breeding and soil fertility is needed to determine if leafy spurge has potential as a source of hydrocarbons.

Infestations of leafy spurge tend to dominate vegetation on grazing land. The effect of possible allelopathic soil residues from leafy spurge on grass species was studied.

Soil was excavated from a dense stand of leafy spurge and from an uninfested area several meters away. Native and introduced grass species were grown in the soils. Plant height and dry matter accumulation was measured.

Several grass species showed decreased plant dry matter when grown in infested soil. This effect was inconsistent between infested soils from two locations. The results from confirmation trials did not agree with results from the original experiment.

Results from this study indicate that soil from a dense stand of leafy spurge does not contain toxins in sufficient quantities to inhibit growth of certain grass species when grown under greenhouse or growth chamber conditions.

Leafy spurge research needs to be continued. Mechanisms of root bud activity need to be understood in order to control plant growth. Less expensive means of control must be found before leafy spurge acreage will decrease. Even though research has not developed a perfect control, an education program on leafy spurge should not be de-emphasized. People need to be aware of the weed, why it spreads, and controls presently available. While research is being continued, education of the public about leafy spurge is essential.

A wide array of personnel and media are available to disseminate information. Television, radio, slides, newspapers and special publications can be utilized by teachers, county agents, vocational agriculture instructors and weed district personnel to disperse information. Unless people are aware of leafy spurge, its problems and ongoing research, they will not initiate a control program.

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APPENDICES

