Reprinted with permission from: GPC-14 Annual Report: Leafy Spurge Control in the Great Plains. 1982. pp. 108-112.

Published by: Great Plains Agricultural Council.

Research report:
Leafy spurge cooperative research project

Participating laboratories and individuals:

USDA-ARS Western Regional Research Center
Albany, California - Dr. Gary Manners, Dr. Kenneth Stevens

USDA-ARS Metabolism and Radiation Research Laboratory
Fargo, North Dakota - Dr. Dave Davis

USDA-ARS University of Nebraska
Department of Agronomy
Lincoln, Nebraska - Dr. Kelvin McCarthy

North Dakota State University
Department of Botany
Fargo, North Dakota - Dr. Don Galitz

Introduction

In July 1982, a cooperative research program involving personnel at USDA-ARS laboratories in Albany, California; Fargo, North Dakota; Lincoln, Nebraska and the Botany Department of North Dakota State University was initiated with the purpose of examining the chemistry of leafy spurge in three main areas: 1) chemical constituents of leafy spurge, 2) chemical constituents of plants allelopathic to leafy spurge and 3) chemical taxonomic classification of leafy spurge biotypes. This report will define the scope of this program and the research results obtained to date.

I. Chemical constituents of leafy spurge

Purpose of investigation - An investigation of the chemical composition of *Euphorbia esula* was initiated to obtain basic chemical information about leafy spurge relative to: 1) the allelopathy of the plant to preferred range plant species, 2) the resistance of the plant to effective control by herbicides, 3) the toxicity of the plant to livestock and the potential of the plant as a chemical source.

Scope of the investigation - The initial exploration of plant chemicals of leafy spurge is directed toward, 1) the isolation and characterization of phytotoxic (allelopathic) chemicals from the plant and 2) the characterization of leaf wax constituents of leafy spurge.

A. Research progress

1. Allelopathic chemicals of leafy spurge

A water extract of defatted plant material (46 lb) was obtained. A radish seed bioassay of the water extract confirmed bioactivity. The aqueous extract was concentrated and liquid/liquid extracted to obtain a biologically active ethyl acetate extract (150 g).

Further manipulation of the extract produced a bioactive fraction which upon crystallization yielded Kaempherol-3-glucuronide (13.1 g) and p-Coumaric (0.1 g). An accompanying fraction yielded Kaempherol (0.12 g) and the coumarin, scopoletin (0.04 g).

The isolated compounds are common plant constituents and Kaempherol-3-glucuronide has been previously described to occur in *E. esula*, albeit in yield 100 times less than obtained from our plant material. At 125 ppm the glucuronide produces a 13% reduction in the root growth of lettuce seeds thereby suggesting some participation in the phytotoxic activity of leafy spurge.

Several other flavonoid and phenolic constituents have been isolated from the ethyl acetate extract. These compounds will be identified and biologically assessed leafy spurge root material will also be extracted and examined.

2. Leaf Wax Constituents of Leafy Spurge

Photo-electron micrographs of leafy spurge leaves reveal the occurrence of crystalline wax materials which are most effectively removed by chloroform dipping. The chloroform leaf dip material has been solvent partitioned and chromatographically

examined. The most prominent wax constituent (50-60% of total wax) has been characterized as n-hexacosonal which has been previously reported in *E. esula*. The characterization of several other leaf wax constituents is underway. A comparison of leaf wax constituents among leafy wax biotypes will be conducted and the leaf wax composition relative to growing season timing will be pursued. Cell cultures and callus tissue cultures of several *E. esula* biotypes are available at MRRL for further primary level chemical studies. An examination of leafy spurge leaf volatiles in cooperation with Dr. Robert Flath, WRRC is planned.

II. Chemical constituents of plants allelopathic to leafy spurge

Purpose of the investigation - Plants observed or reported to be allelopathic to leafy spurge will be chemically examined with the purpose of establishing the chemical source of phytotoxicity and its specific identity. The characterization of these allelochemicals will provide basic chemical information with potential use in the development of specific herbicides for leafy spurge.

Scope of the investigation - The initial investigation is limited to the only plant presently reported to be allelopathic to leafy spurge - *Antennaria microphylla*. The investigation will include the detailed chemical examination of this plant as guided by biological assay.

A. Research Progress

1. Allelopathic chemicals of *Antennaria microphylla.*

Whole plant material has been sequentially extracted and the extracts have been biologically assessed utilizing a lettuce seed bioassay. A fraction from one of the extracts has produced a 50% reduction of lettuce seed root growth at 150 ppm with complete arresting of seed germination at 500 ppm. This fraction is presently being evaluated in tissue cultures of *E. esula* maintained at NDSU and MRRL.

Further fractionation of this active mixture is underway. A "target organism" tissue culture bioassay technique is being developed. Further field observations of allelopathy to leafy spurge are planned pursuant to the collection of other plant materials phytotoxic to *E. esula.*

III. Chemical taxonomic classification of leafy spurge biotypes

Purpose of investigation – It appears as if several species or biotypes of leafy spurge are dominant in North America which has hampered the importation of predator insects from Europe which are species specific. Usual taxonomic classification methods have met with little success. A chemical taxonomic classification is thus proposed

Scope of the investigation - We have proposed to look at the primary metabolites of leafy spurge, i.e., the proteins and enzymes and thus avoid difficulties often associated with secondary plant metabolites. Comparative studies of the genetic material (protein) of each leafy spurge biotype should for the most part eliminate environmental factors which often change both the quality and quantity of secondary plant metabolites. Both seed and leaf protein will be investigated.

Research Progress

Gel electrophoresis (SDS-PAGE) of seeds from several leafy spurge biotypes (supplied by Dr. Melvin McCarty) has shown differences between the seeds. These differences may be due to genetic differences, however this point remains to be confirmed.