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Biological Weed Control: The Plant-Insect Interaction

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Charles Darwin (1859) said, "The preservation of favorable variations and the rejection of injurious variations, I call natural selection." Selection is defined as the survival of those few individuals that have successfully outlasted the "struggle for existence." In every generation evolution mixes the genetic make-up of the few survivors, creating new genotypes, which are then tested in the next generation. The ability of a weed to improve fitness by protecting itself from phytophagous organisms amplifies its weediness. A natural enemy's ability to overcome these defenses is fundamental to classical biological control practices. Some insects, plant pathogens and other organisms within the area of origin of targeted weeds co-evolve with the plant, developing mechanisms (in some cases quite complex) to overwhelm defense strategies. This relationship may result in the phytophagous organism becoming dependent upon the weed for food and shelter. Inter-relationships among weeds and their predators, coupled with the unique nature of the weed, have facilitated the selection of biological control agents. In the example of the rangeland weed, leafy spurge (*Euphorbia esula*), a varying array of biologically active chemicals have evolved within the plant, typical of the Euphorbiaceae. Most terpenoids are contained in the milky latex; however, some are present in the epicuticular leaf waxes. Further, these terpenoids vary by species and probably by biotype. A successful introduction can be assessed by the host-specificity of the introduced agent(s) and the impact of the biological control agent on the targeted weed as the agent's population increases.

Introduction

Ernst Mayr, in his book *Towards a New Philosophy of Biology* (1988), stated

> "more than 99.9 percent of all species that ever existed on earth have become extinct. This includes even such temporarily so-flourishing groups as the trilobites, ammonites, and dinosaurs. Here it does not matter what factor was responsible for the extinction: competition, a pathogen, a climatic catastrophe, or an asteroid's impact. In each case selection had been unable to find, among the available variants, an appropriate answer to the new situation."

Mayr (1988) sensed that "Darwin apparently took it for granted that there was always sufficient variability in natural populations to satisfy the demands of natural selection. Many geneticists tended to disagree. De Vries, Bateson, and other Mendelians did not actually deny the existence of variation, but they thought that ordinary continuous variation was evolutionarily irrelevant and that evolution proceeded via occasional more or less drastic mutations. This was contradicted by the work of Chetverikov (1926) and his school and by the ecological geneticists (Dobzhansky 1937; Ford 1964), who showed that abundant natural variation was always available. But, said Muller, and this opinion was widely adopted, this variation simply consists of deleterious recessives that are eliminated as quickly as they become homozygous. Finally, in 1966 Darwin seemed to be vindicated when Lewontin and Harris demonstrated the enormous variability of enzyme genes as revealed by electrophoresis. But that by no means ended the argument, because Kimura and others presented evidence

that much of this variation was neutral, that is, not suitable as material for natural selection."

Methods have evolved within the plant kingdom for increasing fitness. Techniques for increasing reproductive potential include profuse seeding, hairy plant parts, thick fruit exocarp and thick bark such as seen on *Quercus suber* L. (Fagaceae), the cork tree of commerce. Natural selection in various plant families has also resulted in the production of a variety of organic chemical compounds occurring within plant tissue. Macedo and Langenheim (1989) found that insect feeding was affected by tropical Leguminosae that had variable amounts of sesquiterpenes in the leaves. Insect herbivory of leaves was found to vary both within trees and between trees and was correlated with sesquiterpene composition.

Selecting agents for the biological control of a weed involves the search for organisms that have evolved mechanisms to overcome the particular traits that a plant species has evolved to protect itself against predation. The more successful a weed is in a new environment, the more likely that its competitive advantages include methods to lessen predation. The family Euphorbiaceae contains some of the most potent tumor-promoting agents, called diterpenes or esters of phorbol (Evans 1986). The family Euphorbiaceae has long been noted for toxicological effects on animals and man, including skin and mucus membrane inflammation, conjunctivitis in the eyes, production of scouring in animals and purgative actions in man. Plant derivatives have been used as fish poisons, ingredients in arrow poisons and have been used as drugs in both traditional and alternative medicine (Evans 1986). Nonarticulated, multinucleated lactifer cells in leafy spurge extend the entire length of the plant from the root tip to the shoot tip. These lactifer cells produce a latex containing toxic diterpene and triterpene esters (Mahlberg *et al.* 1987). One weedy member of the family, leafy spurge, *Euphorbia esula,* L., produces various terpenes that probably serve as a means of defense against predation.

Over 100 yrs ago leafy spurge was accidentally introduced to North America. The plant's apparent protection mechanisms have reduced herbivory to a few insect species, including common rangeland grasshoppers. For this reason, insects have been imported from the Old World into the United States and Canada for its control. Key to the successful use of these organisms is host-specificity. In the case of leafy spurge, host-specificity is ameliorated by the specific toxic chemicals that are found in the plant. Gary Manners and David Davis (1984) studied epicuticular wax constituents of leafy spurge leaves in an effort to separate genotypes and later (1987) examined the characterization of esulone C and chemotaxonomy of jatrophane diterpenes in leafy spurge. Mahlberg *et al.* (1987) considered laticifers in the classification of leafy spurge. The chemical distinctiveness of individual genotypes was verified. Many of these chemical constituents are toxic to other plants and animals. The chemical constituents of leafy spurge regulate the plant's ability to protect against predation and attack by micro-organisms. The chemical variation in this target weed affects the degree of establishment and the level of control achieved by the biological control agent(s).

Methods and Materials

A native flea beetle was collected from leafy spurge in Richland Co., Montana. The species was identified by Richard White of the USDA/ARS Systematic Entomology Laboratory as *Glyptina cerina* LeConte (Coleoptera: Chrysomelidae). *G. cerina* used in laboratory testing originated from a colony maintained on potted leafy spurge in an environmental chamber at 27°C day and 20°C night temp. with a 16:8 L:D (light:dark) photoperiod. Two species of flea beetle, *Aphthona nigriscutis* Foudr. and *A. flava* Guill. LeConte (Coleoptera: Chrysomelidae), were obtained from colonies in the United States. These species originated in Europe and were imported under federal permit for the biological control of leafy spurge. Adults were maintained on leafy spurge plants in the same environmental chamber used for *G. cerina*.

A gas chromatographic method was used for the detection of terpenoids in the latex of leafy spurge. Forty µl samples of latex were extracted from each test plant using a micro-

capillary tube. The samples were transferred to 1 ml of GC grade acetone and centrifuged for 15 mins at 12,300 rpm. Approximately 0.5 ml of the supernatant was pipeted into an acetone-washed crimped-sealed autosampler vial. Four µl were injected into a Hewlett-Packard 5890 gas-liquid chromatograph[1] (GLC) equipped with a flame ionization detector and a split/splitless injector system. This GLC was programmed with an oven temperature from 65 to 150°C at 5/min. Helium was used as the carrier gas. The injection port temperature was 275°C; the detector temperature was 300°C. The glass capillary column (0.314 mm ID X 30 m) used was a J & W Scientific SE-30. Individual compounds were quantified on a Hewlett-Packard 3380A integrator with data on detected peaks expressed as percentage area.

Adult *A. nigriscutis* were collected at Spruce Woods, Manitoba, Canada and imported into the United States under federal permit. One thousand insects (>90%) were released at each leafy spurge study site in early July 1990. In June 1991 sites were evaluated and the degree of establishment measured by counting the number of *A. nigriscutis* collected in 5 sweeps of a 38 cm dia. insect net used over the area of the initial release. These data were used as an indicator of the suitability of the site for insect reproduction and increase.

Insect feeding preferences for leafy spurge from the different study sites was measured in the laboratory under conditions designed to limit results due to factors other than differences in plant chemistry. Leafy spurge plants were established in 1989 and 1990 in the greenhouse at the U.S. Department of Agriculture, Agricultural Research Service (USDA, ARS) facility in Sidney, Montana, from various sites in Montana and North Dakota. The accessions were held under similar conditions in greenhouse soil (80% sand, 3-5% organic matter & 15-18% silty clay) before testing. Leaves were removed from the upper portion of test plants and placed on white 9 cm dia. filter paper. Leaves were distinguished by colored/self-adhesive dots placed on the proximal end. The filter paper was placed in 9 x 2 cm plastic Petri dishes, with each Petri dish as an experimental unit. Leaves from test plants were tested by providing insects in the Petri dish with a choice between leaves from plants obtained from different leafy spurge infestation sites. Each test was replicated 10 times or more. Two adult beetles (unsexed) were placed in each Petri dish. The dishes were held in a closed plastic container to maintain high humidity. The container was kept in an environmental chamber at 30 ± 1°C temp. with a 14:10 L:D photoperiod. The amount of feeding damage was estimated daily for 3 d on a scale of 0 (no feeding) to 5 (leaf eaten).

[1]Mention of trade names are included for the benefit of the reader and does not constitute endorsement by the USDA.

Results and Discussion

G. cerina were found commonly on leafy spurge in Montana and North Dakota. This genus was recorded in the United States from the Euphorbiaceae, but not from leafy spurge (Horn 1889). *G. cerina* either moved from another Euphorbiaceae producing latex with a similar chemical composition or they are not native to North America. The most recent revision of the genus by Horn (1889) lists 7 species in North America. *G. cerina* is reported to occur in California and Arizona. *G. cerina* has been reared on leafy spurge in an environmental chamber and in a greenhouse in Sidney, Montana for over 2 yrs. Adult flea beetles are 2–2.25 mm long and feed on the apical leaves of leafy spurge. Caged adults will feed gregariously, giving leaves a lacy appearance. Larvae feed in the soil on leafy spurge roots. Leaf removal by *G. cerina* was less than by the 2 *Aphthona* species, reflecting the smaller size of adult *G. cerina* (less than half the size of *A. flava* or *A. nigriscutis*).

It was found that particular leafy spurge sites where *A. nigriscutis* was released produce many insects within 1 yr, while other sites with similar parameters produce few insects. Fig. 1 shows the selection of leafy spurge leaves from 3 sites by *G. cerina*. These data were replicated with *A. flava* (Fig. 2).

These data were analyzed on a day-by-day basis using the chi^2 statistic. The degree of significance for biotype selection was at $p=<0.0001$. GLC chromatograms of latex extracted from the test plants show discrete differences in terpenoid.content (Fig. 3). These

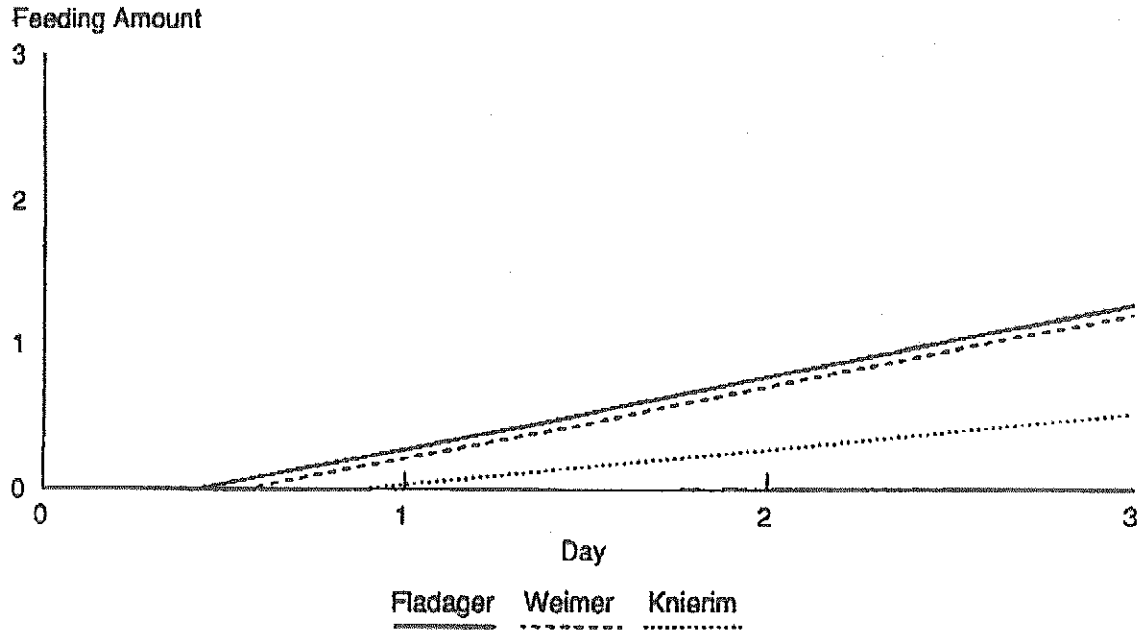

Figure 1. Selection of leafy spurge biotypes from three sites in Montana by *Glyptina cerina*. Feeding amount: 0= no feeding; 1= some feeding; 2= 1/4 leaf eaten; and 3= 1/2 leaf eaten.

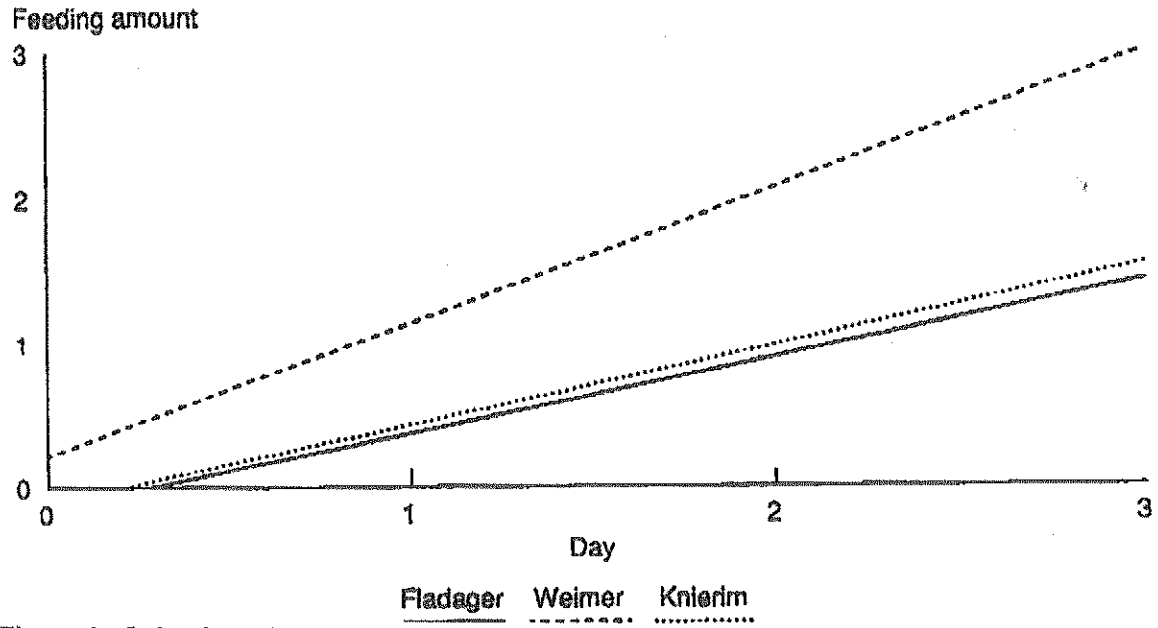

Figure 2. Selection of leafy spurge biotypes from three sites in Montana by *Aphthona flava*. Feeding amount: 0= no feeding; 1= some feeding; 2= 1/4 leaf eaten; and 3= 1/2 leaf eaten.

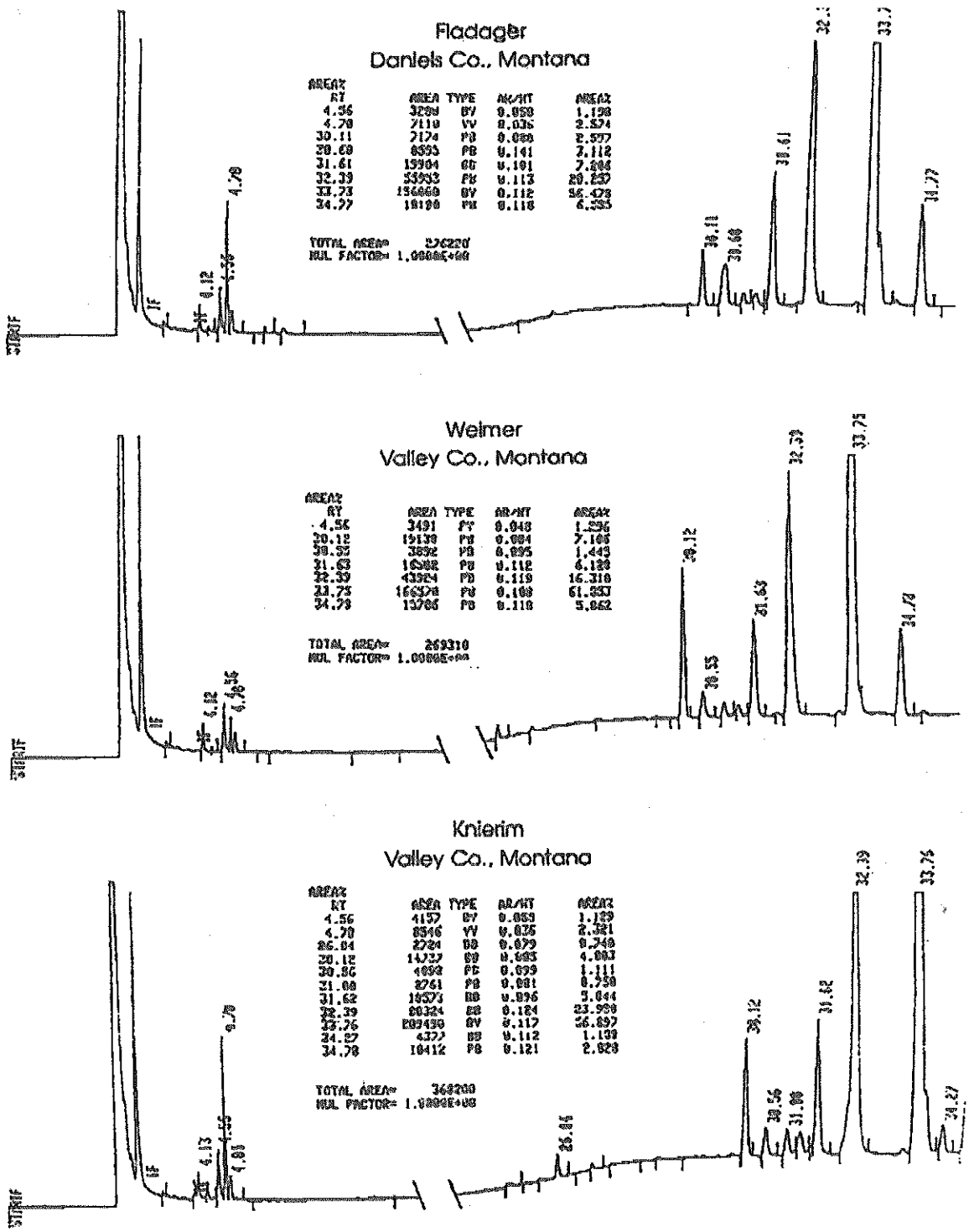

Figure 3. GLC chromatographs of latex from leafy spurge biotypes from three sites in Montana.

data show that the insects are responding to the chemical content of the plant. Quite possibly, leafy spurge terpenoids affects the establishment and insect population at any given site. Variation between plant terpene concentration and chemical composition should affect herbivory. In another study of insect-plant interaction, Macedo and Langenheim (1989) found that insect feeding was affected by tropical Leguminosae that had variable amounts of sesquiterpenes in the leaves. Insect herbivory of leaves was found to vary both within trees and between trees and was correlated with sesquiterpene composition.

I believe that the spurge plant chemistry may be an important factor in establishing new insect populations. While I feel that the above analysis is essentially correct, I am not forgetting that physical parameters of a site such as soil type can also be limiting. However, I feel that the abiotic factors have a range in which the insect is unrestricted.

The above discussion is based upon the idea of obtaining a rapidly expanding insect population for weed control and does not consider genetic selection over time. There is an ever-evolving system in which the insect population will adapt to leafy spurge plants that are not being attacked. This condition should be seen when large numbers of insects increase on specific plant biotypes. Thus, at first we may see some patchy control, but large area control should be expected to occur over time. In the northern rangelands of the United states and across the border, into Canada, millions of acres of once productive land is now dominated by leafy spurge. Active biological control of even small areas in the 1990s will give hope to the agricultural community in the 2 nations. Large scale biological control will probably not occur in this century but signs of its potential will be present as biological control begins to exert its force.

My data show that both a native flea beetle and 2 closely related flea beetles originally from Europe feed preferentially on specific biotypes of leafy spurge. This feeding can be correlated with the terpenes found in the leaves. As additional data is collected from research sites in the states of Montana and North Dakota, I believe that leafy spurge chemistry will be found to be a selective factor on the mortality rate, *i. e.* survival rate, of introduced insects for biological control of leafy spurge. Other factors, such as soil type and soil moisture are important but may not exert as strong a selective pressure within an area as the plant itself.

In Europe, there are 105 native *Euphorbia* species in the subgenus *Esula*, the group to which leafy spurge belongs. In North America, there are only 21 native species in the subgenus *Esula* (Pemberton 1985). This disparity in numbers provides the framework for biological control, namely, the importation from Europe of biotic agents which have evolved the ability to overcome leafy spurge's chemical defenses. In many cases, the evolved specialization has resulted in host-specificity; feeding limited to only one, or a few closely related species.

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