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Chemotaxonomic Affinities of Eurasian Leafy Spurges (*Euphorbia* spp.) in Relation to a Biological Control Program

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Abstract

Leafy spurges (*Euphorbia* spp.; Euphorbiaceae), noxious weeds introduced into North America from Eurasia, possess laticifers containing diverse triterpenoid compounds. Latex exudate collected from Eurasian accessions was analyzed by gas chromatography for triterpenoids, chemicals which may influence the patterns of predation on spurge populations. Three different triterpenoid patterns, represented by qualitative differences for profile components, were detected among accessions indicating the presence of several distinctive chemotaxa among Eurasian spurge populations. Several Eurasian accessions possessed the triterpenoid profile of the chemotaxon, *E. esula*, indicating its presence in both Eurasia and North America. The other chemotypes did not correspond with those in North America. The presence of different chemical profiles among spurge populations and the reported obligate relationships of insects to spurges emphasize the necessity to chemotype each accession and its associated predator when collecting candidates for the biological control program so as to match the predator with the appropriate chemotype of the host.

Introduction

Leafy spurges (*Euphorbia* spp.; Euphorbiaceae), because of the highly toxic terpenoids in the latex, are serious weeds on grazing land and represent a health problem to livestock in North America. Latex causes severe dermatitis to mucus membranes of the digestive, respiratory tracts and sensory organs of animals. Spurges when eaten in large amounts can cause death (Dunn 1979). Conventional practices to control this weed have been unsuccessful, primarily because of the biology of leafy spurge, and a biological control program to manage this rapidly spreading weed has been proposed (Dunn 1985, Harris 1979).

The economic impact of leafy spurge to losses of pasture and illness or death of domesticated animals in North America is considerable, and increasing. For North Dakota alone in 1983 the annual loss in beef and hay production approximated $13 million (Messersmith and Lym 1983). Significant losses of similar or greater magnitude also occur in other livestock grazing states although no economic impact studies are available (Messersmith, pers. comm., 1988).

Euphorbia is unique in possessing the nonarticulated laticifer cell responsible for the synthesis and accumulation of diverse toxic terpenoids (Mahlberg and Sabharwal 1968, Mahlberg and Pleszczynska, 1983, Mahlberg *et al.* 1983). Initial analyses of North American accessions demonstrated the presence of several triterpenoid profiles among different accessions, emphasizing that several chemotypes may occur within the leafy spurge complex (Mahlberg *et al.* 1987). Triterpenoids are deterrents to insect feeding, and it has been suggested the inconsistent response of predators feeding on leafy spurges may reflect differences between plant populations (Harris 1984, Harris *et al.* 1985).

A biological control management program for leafy spurge requires identification of the plant taxon to associate it with on obligate predator. Traditional taxonomic studies of spurges describe numerous taxa (Radcliffe-Smith 1985). However, numerical analyses show these traditional morphological characters to be inadequate for delineating speciation (Harvey *et al.* 1988, Mahlberg *et al.* 1987). Spurges as adventive introductions into North America have unclear affinities with their Eurasian counterparts because the latter have not been studied. Thus it remains to be determined whether predators, as insects, obligate on Eurasian taxa will be effective in a biological control program in North America.

The purpose of this study was to initiate examination of Eurasian taxa of leafy spurges for the presence of triterpenoid profiles, and interpret them within the context of profiles reported from North American accessions (Mahlberg *et al.* 1987). It will be shown that Eurasian spurge populations differ in composition and can be grouped into chemotypes reflective of their triterpenoid profiles. Demonstration of triterpenoid differences and the known responses of predators to these compound emphasize the need to determine the triterpenoid profile of spurge accessions when collecting insects or other predators with the objective of matching predator to host in the biological control program.

Methods and Materials

Plant Materials

Seeds designated as *Euphorbia esula* L., obtained from the National Botanic Garden, Dublin, Ireland and Natural History Museum, Paris, France, were grown to adult plants in an Indiana University greenhouse. Latex from the shoot apex was collected from three individuals of a taxon into spectroanalyzed grade acetone in acetone-washed vials, and portions of the plants accessioned as herbarium specimens. Plants are not killed during the sampling procedure and can be sampled repeatedly as desired. For Eurasian accessions, latex was collected into acetone in acetone-washed vials and sent to me for analyses. Plant and latex samples collected in Europe included 84HU006 (Debrecen, HU), 84HU008 (Derecske, HU), 84HU011 (Kisujszallas, HU), 84UO17 (Szolnok, HU), 84YU004 (Batrage, YU), 84YU008 (Negotina, YU), and 84AS002 (Alland, AS), and supplied by R. Nowierski, Montana State University. Plants are being maintained at Montana State University. Herbarium vouchers were prepared from these living materials.

These samples were compared with an *E. esula* accession 78AS001 obtained from Krems, AS (Ebke and McCarty 1983) and described as the chemotaxon for this taxon (Mahlberg *et al.* 1987).

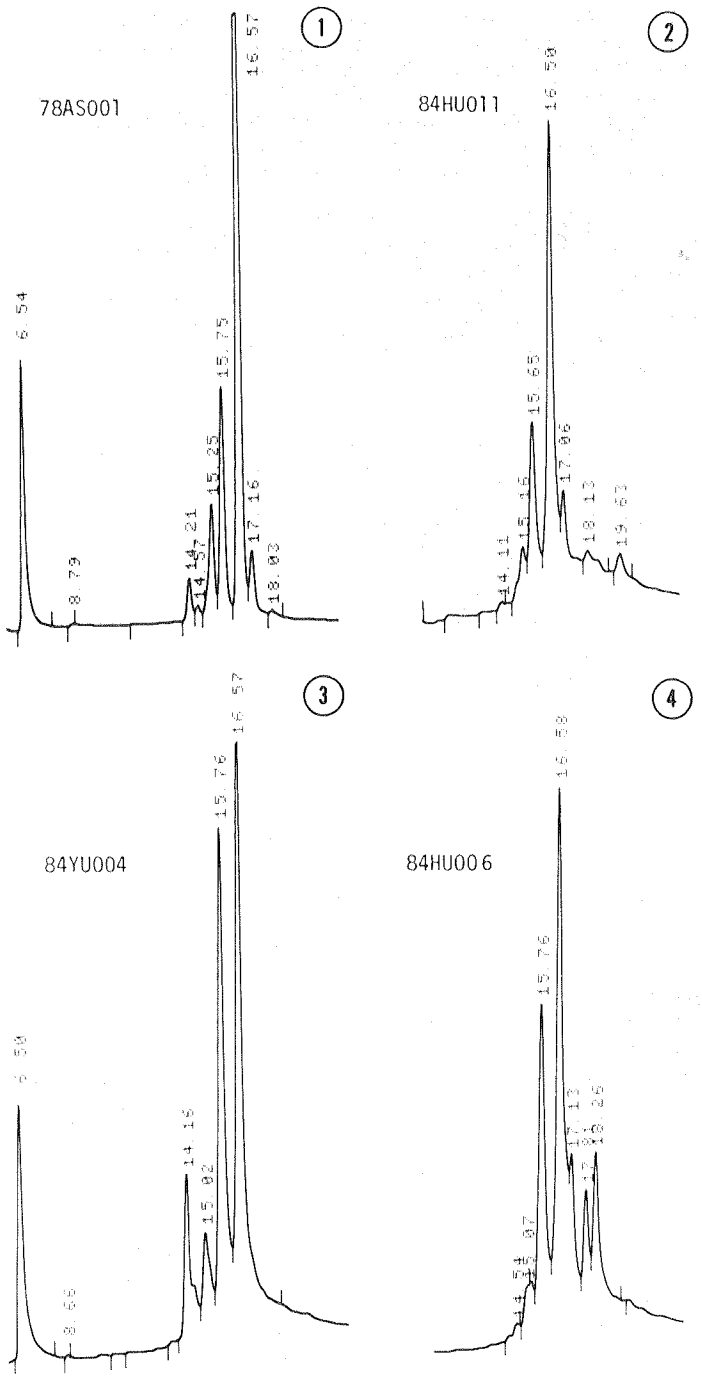
Chromatographic Analyses

Five to ten drops of exuded latex from severed shoot tips of a specimen were collected into spectral grade acetone. Triterpenoids in the acetone supernatant in all vials were decanted into fresh vials and centrifuged in a clinical centrifuge for 5 min to remove particulate matter in suspension. An aliquot was evaporated to dryness over nitrogen, and resuspended in 100 µl acetone containing 0.5 mg/ml 4-androsten-3,17-dione (androstenedione) as an internal standard (IS). One µl of each sample typically was injected into the chromatographic column.

Analyses were performed on a Hewlett-Packard 5710A gas-liquid chromatograph (GLC) equipped with a flame ionization detector and operated by programming from 240 to 290°C at 4° / min followed by a 16 min isothermal period. Nitrogen was the carrier gas (20 ml/min flow rate). Injection port temperature was 250°C; detector temperature was 350°C. Glass columns (2 mm ID x 2.43 m), treated with 5% dimethyldichlorosilane in toluene, were packed with 3% OV-1 on 100/120 mesh Supelcoport. Peaks of individual compounds were quantified on a Hewlett-Packard 3380A integrator with data expressed as area percent.

Results

The chemotype triterpenoid profile of *E. esula* (78AS001) obtained from Krems, Austria by Ebke and McCarty (1983) was used as a reference in this study (Fig. 1; Mahlberg *et al.* 1987). This accession has six peaks of 0.5% or more for its triterpenoid content (Table 1). The profile peaks in accessions described here were correlated with those present in this *E. esula* sample.



Figures 1-4: 1. Triterpenoid profile of chemotaxon *Euphorbia esula* 78AS001 detected in both North American and Eurasian spurge populations; 2. Triterpenoid profile comparable in composition to *E. esula* and included in that taxon; 3. Profile of accession differing qualitatively from *E. esula* but possessing two components in common. 4. Profile of accessions differing qualitatively and quantitatively from *E. esula* for most components. RT 6.54 and 6.50 are IS.

The profiles of *E. esula* seed stock from both Ireland and France closely resembled the *E. esula* chemotype from Austria (Fig. 2). These accessions contained six peaks and their similar retention times (RT) indicated the composition was identical for these plants. The quantitative ratio of peak contents were similar (Table 1). Some intra-population variation for contents among the three plant samples was indicated by the standard deviation for each peak; some inter-population variation among the several accessions also was evident. However, the differences between the accessions was relatively low.

Since the accessions have peaks in common with the *E. esula* chemotype, they possess the specific triterpenoids identified for 78AS001 (Table 1). These compounds include (from the left), euphol as peak 1, cycloartenol as peak 4 and 24-methylene cycloartenol as peak 5. Cycloartenol and its 24-methylene derivative, the most prominent component in the profile, represent 70-81% of the composition in these accessions. The similarity of the accessions from Ireland and France to 78AS001 support the interpretation that they represent the chemotype described as *E. esula.*

Table 1. Comparison of triterpenoid profiles from *Euphorbia esula* seed stock with chemotype profile of *E. esula* from Austria.

Peak No.	Retention time (min)[1]	Percent triterpenoids Austria[2]	Ireland	France
1	10.28	3	12 ± 1.4	4 ± 1.4
2	10.49	1	3 ± 0.3	3 ± 1.6
3	11.00	10	7 ± 1.3	8 ± 0.6
4	11.39	25	14 ± 1.6	13 ± 3.2
5	12.01	56	56 ± 4.5	67 ± 2.3
6	12.47	5	8 ± 2.3	5 ± 1.1

[1] Relative RT adjusted to IS 6.54 min for all chromatograms.
[2] Data for 78AS001 (Mahlberg *et al.* 1987).

Several accessions recently collected in Hungary, Yugoslavia and Austria also possessed the *E. esula* profile (Fig. 3). These accessions, 84YU008, 84HU011, 84HU017 and 84AS002, qualitatively contained the major components characterizing the *E. esula* chemotype (Table 2). There were quantitative differences for several of the components, represented by a given peak, between the accessions. In all four collections, however, the peaks at RT 16.29 and 17.53 composed 77-88% of the triterpenoid composition. The RT for these two peaks corresponded to those for cycloartenol and 24 methylene cycloartenol in accessions 78AS001, although it will be necessary to establish identity by mass spectrometry. The four accessions were placed together into Group 1 to facilitate comparison with other accessions.

Accession 84YU004 was found to have a distinctive profile (Fig. 4). Only four major triterpenoid peaks were detected in this profile, although shoulders indicative of additional peaks were evident near the baseline but not recognized by the integrator. This profile differed quantitatively and qualitatively from these in Group 1. Two peaks, ET 14.35 and 15.63, corresponded with peaks in the *E. esula* chemotype. The two remaining peaks, RT 15.09 and 16.57, represented new peaks composing 55% of the total triterpenoid content. The profile also included compounds not detected in the *E. esula* chemotype.

Two accessions, 84HU006 and 84HU008, also contained a very distinctive profile, one more complex in composition than detected in *E. esula* (Fig. 4). Seven peaks were evident in this profile (Table 2). Six of these peaks did not correspond with peaks in the *E. esula* chemotype of Group 1. Two of the peaks, RT 15.09 and 11.57, corresponded with components in the

profile of Group 2, whereas four of the peaks represented new compounds not evident among other accessions. This Eurasian profile was placed into Group 3.

Table 2. Percent triterpenoids in profiles of Eurasian spurge accessions.[1]

Peak Number	Retention time (min)	Group 1 84HU 011	84HU 017	84YU 008	84AS 002	Group 2 84YU 004	Group 3 84HU 006	84HU 008
1	14.15	-	-	-	-	-	-	1
2	14.35	1	1	1	3	11	t	t
3	15.09	-	-	-	-	8	4	7
4	15.63	9	7	11	12	34	-	-
5	15.71	-	-	-	-	-	22	20
6	16.29	22	19	18	33	-	-	-
7	16.57	-	-	-	-	47	44	39
8	17.09	-	-	-	-	-	10	10
9	17.53	55	56	70	51	-	-	-
10	17.69	-	-	-	-	-	7	4
11	18.42	6	12	-	-	-	13	19
12	20.20	3	2	-	1	-	-	-
13	23.10	4	3	-	2	-	-	-

[1] t = trace; - = no compound detected.

These nine accessions of Eurasian leafy spurge including those grown from seed separated into three groups. Group 1 represented samples closely resembling the *E. esula* chemotype (Tables 1 & 2). The groups were separated on the basis of their profile composition: accessions possessing similar components were grouped together, while those accessions differing in composition from each other were placed into separate groups.

RT values among Group 2 and 3 accessions indicate the presence of new triterpenoids in addition to those identified in *E. esula* (Table 2). Analyses show these accessions to contain in aggregate more than ten prominent compounds which are yet to be identified by mass spectrometry. The detectable quantitative differences for individual peaks or peak ratios within a taxon are not yet clearly understood. While these differences may reflect genetic changes in biosynthetic steps in the triterpenoid pathway, the mechanisms of their origin remain to be determined.

Discussion

The analysis of triterpenoid profiles of leafy spurges was undertaken because of its potential value in the biological control program. The toxicity of triterpenoids has been demonstrated in other plants (Reed *et al.* 1983). The irritating and toxic qualities of spurge latex to grazing livestock is well established (Watson 1985). Several authors have suggested that the inconsistent response of predatory insects on leafy spurges may reflect differences between spurge populations (Harris 1984, Harris *et al.* 1985). These differences in specificity or the obligate relationship between host and predator may reflect a response to differences in chemistry of spurges. The abundance of these toxic compounds in *Euphorbia*, up to 45 DW in latex, and their efficient delivery by the laticifer cell to a predator upon injury of the plant indicate a protective role for triterpenoids against foragers (Spilatro and Mahlberg 1986).

Analyses of latex triterpenoids in spurges show the presence of several distinctive profiles among Eurasian accessions. These observations parallel the detection of several profiles

among different spurge populations in North America (Mahlberg *et al.* 1987). Importantly the profile of *E. esula* occurs in populations in both North American and Eurasian accessions. Thus it is possible to identify this chemotaxon from widely separated geographical areas. The presence of this chemotaxon on both continents supports an interpretation that *E. esula* represents one of the early adventive introductions into North America (Dunn 1985). Recognition of the *E. esula* chemotaxon in North America indicates it has remained unchanged since its introduction. Its distinctive qualitative character makes it possible to recognize this taxon regardless of origin.

The detection of qualitatively different triterpenoid profiles among accessions emphasized the occurrence of chemical differences among Eurasian spurge populations. These accessions could be separated by their characteristic triterpenoid profile, whereas they could not be separated on their morphological characters (Harvey *et al.* 1988). Studies have shown the triterpenoid profiles for other *Euphorbia* taxa to be a fingerprint for the species and stable under diverse ecological and physiological conditions (Mahlberg *et al.* 1983, Mahlberg *et al.* 1985). It is suggested therefore that the detection of different profiles among Eurasian spurges is significant and provides the basis for recognizing different chemotaxa. As noted we find only one profile in common between spurges on the two continents. The other profiles do not interrelate; at this time sample size remains small for purposes of comparing different profiles. Thus this study should be expanded to include analyses of other Eurasian accessions to determine the number of chemotypes within these populations, and subsequently to study the interrelationship with spurges in North America.

It is important to chemotype both host and predator when collecting candidates for the biological control program. Several phytophagous insects have been reported to be obligate on specific taxa of *Euphorbia* (Harris 1979). The relationships between these insects and the triterpenoid profile of the host are yet to be determined. Chemical diversity in the host may reflect selection pressure against foraging. The composition of the chemical profile may be evolving at a more rapid rate than morphological characters, a phenomenon we find for leafy spurge. Similarly, physiological adaptation of an insect for predation on hosts undergoing chemical diversification may be closely linked in a coevolutionary relationship.

In initial observations on establishment of predators on spurges in North America, *Hyles euphorbiae* (L.) (Lepidoptera: Sphingidae) (leafy spurge hawkmoth) has become well established on spurge in the vicinity of Bozeman, Montana; *Obera erythrocephala* (Schrank) (Coleoptera: Cerambycidae) (stem boring beetle) has shown marginal establishment on these same population (Harvey and Nowierski, pers. comm.). These spurge populations possess the triterpenoid profile of the *E. esula* chemotaxon (Harvey *et al.* 1988, Mahlberg *et al.* 1987). Although we know these insects will forage on spurge possessing this profile, there is no record for the spurge chemotype from which insects were obtained. It is pertinent now to examine the response of these insects on spurges possessing other chemotypes to develop an understanding of the mechanism influencing the predator-host relationships on leafy spurges.

Analyses of spurge triterpenoid profiles can contribute to the biological control management program for this weed in important ways:

(1) utilize the chemotyping procedure to determine physiological and chemotaxonomic interrelationships of Eurasian and North American spurges;

(2) chemotype host and insect or other predator to correlate host-predator specificity and determine effectiveness of a predator on spurges with different chemotypes; and

(3) chemotype wild spurge populations in North America to determine, in advance of predator release, which spurge populations are most susceptible to predation by a given predator.

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