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Taxonomic evaluation of leaf and latex variability of leafy spurge for Montana and European accessions[1]

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Abstract:

Leafy spurge (*Euphorbia* spp.), an aggressive noxious perennial weed of North America, is a complex group that has been designated as several different species, including *E. esula* L. (#[2] EPHES) and *E. virgata* Wald. & Kit. [*E. waldsteinii* (Sojak) Radcliffe-Smith]. Current classification keys are unsatisfactory in assigning plants in the field to specific taxa (or to a single taxon). These keys rely heavily upon morphological characteristics of leaves, but great variation in the leaves has been noted by us and previously reported by others. In this study we demonstrate qualitatively and quantitatively that the within-plant, within-clone, among-clone, and among-site variation in leaf morphology and triterpenoid content of the latex of leafy spurge is inherently high. Leaf characters were of little value in separating any of the accessions considered in our study. Latex triterpenoid profiles were useful in distinguishing *E. lucida* W. & K. × *salicifolia* Host. and *E. salicifolia* from European *E. esula, E. waldsteinii*, and *E. sequieriana* Neck. ssp. *seguieriana,* and all Montana accessions previously described from morphological studies. We concluded that Montana leafy spurge and the European *E. esula, E. waldsteinii*, and *E. sequieriana* belong to a single taxon: *Euphorbia esula* L.

Additional index words:

Triterpenes, chemotaxonomy, *Euphorbia esula*, *Euphorbia lucida*, *Euphorbia* lucida × *salicifolia*, *Euphorbia salicifolia*, *Euphorbia sequieriana*, *Euphorbia virgata*, *Euphorbia waldsteinii*, EPHES.

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[2] Letters following this # symbol are a WSSA-approved computer code from Composite List of Weeds. Weed Sci. 32, Suppl. 2. Available from WSSA, 309 West Clark Street, Champaign, IL 61820.

Introduction

Leafy spurges (*Euphorbia* spp., Euphorbiaceae) are a polymorphic group of poorly defined taxa that have been grouped together into the *esula* complex (6) or the *virgata* group (19). All are native to Eurasia, but some species are adventive in North America where they have become persistent noxious weeds of rangelands in the central and western regions (16, 22). Long-term chemical control has so far proven ineffective and/or too expensive (1, 13) and alternative management strategies, such as biological control, are being developed (10). Correct plant identification is prerequisite to the selection of appropriate biological control organisms for introduction and may influence the degree of success realized from introduced natural enemies (21).

Taxonomic treatments have regarded the complex to be composed of a single species (for example, 2) to several species (19). These classification schemes rely heavily on morphological characteristics of vegetative structures, particularly leaves. Great variability of these characteristics has been noted including within-site and within-plant variation (2, 5, 8, 14, 19). Using current identification keys, we have been unable to satisfactorily assign plants at field sites in North America to one specific taxon, but rather we find several apparent nominate taxa to be present at most sites. Many of the plants observed are assigned only with great difficulty because of the variability within the characters used in keys. Evaluation of the morphological variation within leafy spurges and comparison of North American and Eurasian specimens are necessary before the proper nomenclatural assignment of North American leafy spurges can be made.

The use of chemical constituents in plants as possible taxonomic indicators has become one area of focus in our research. For herbivores, the choice between host plants and non-host plants within the leafy spurge complex are more likely to be chemically rather than morphologically determined. As alluded to by Harris (10), the efficient search for biological control agents of leafy spurge cannot proceed until these differences are characterized.

The objectives of our study were to: a) quantify the within-plant, within-clone, among-clone, and among-site variability of leaf characters; b) quantify the within-plant, within-clone, among-clone, and among-site variability of latex triterpenoid composition; and c) evaluate the taxonomic value of these characters.

Materials and methods

Multiple root cuttings from 27 Montana (Table 1) and 15 European accessions (Table 2) were established in the greenhouse in the summer of 1984. The study was conducted in the greenhouse to enhance the comparison of genetically induced variation rather than that caused by the environment. Herbarium specimens of European accessions were identified by Dr. Radcliffe-Smith. Cuttings were planted in 18-cm pots in an equal volume mix of Bozeman silt loam, sand, and peat. Supplemental wide spectrum fluorescent lighting was used to maintain a 16-hour photoperiod. Daytime temperatures were 24 to 35º C

and night temperatures were 18 to 24º C. These plantings provided the source for all data collection.

Table 1. Montana leafy spurge accessions used in this study.

Number	Location	Number	Location
82MT001	Gallatin County, MT	83MT015	Stillwater County, MT
82MT002	Gallatin County, MT	83MT016	Stillwater County, MT
82MT003	Gallatin County, MT	83MT020	Carter County, MT
82MT004	Gallatin County, MT	83MT022	Flathead County, MT
82MT005	Gallatin County, MT	84MT001	Roosevelt County, MT
82MT006	Gallatin County, MT	84MT002	Richland County, MT
83MT001	Gallatin County, MT	84MT003	Judith Basin County, MT
83MT002	Gallatin County, MT	84MT004	Custer County, MT
83MT004	Teton County, MT	84MT005	Gallatin County, MT
83MT006	Teton County, MT	84MT006	Gallatin County, MT
83MT012a	Fergus County, MT	84MT007	Gallatin County, MT
83MT012b	Fergus County, MT	84MT008	Sweetgrass County, MT
83MT014	Stillwater County, MT	84MT009	Cascade County, MT
		84MT021	Jefferson County, MT

Table 2. European accessions used in this study.

Number	Location	Taxon
78AS001a	Krems, Austria	*E. esula* L.
78AS001b	Krems, Austria	*E. esula* L.
78AS001c	Krems, Austria	*E. esula* L.
78AS00ld	Krems, Austria	*E. esula* L.
84AS002	Alland, Austria	*E. esula* L.
84HU006	Debrecen, Hungary	*E. waldsteinii* (Sojak) Radcliffe-Smith (*E. virgata* W. & K., non - Desf.)
84HU008	Derecske, Hungary	*E. waldsteinii* (Sojak) Radcliffe-Smith (*E. virgata* W. & K., non-Desf.)
84HU011	Kisujszallas, Hungary	*E. waldsteinii* (Sojak) Radcliffe-Smith (*E. virgata* W. & K., non -Desf.)
84HU017	Szolnok, Hungary	*E. waldsteinii* (Sojak) Radcliffe-Smith (*E. virgata* W. & K., non-Desf.)
84YU004	Batrage, Yugoslavia	*E. waldsteinii* (Sojak) Radcliffe-Smith (*E. virgata* W. & K., non-Desf.)
84YU007	Negotina, Yugoslavia	*E. waldsteinii* (Sojak) Radcliffe-Smith (*E. virgata* W. & K., non -Des .)
84YU008	Negotina, Yugoslavia	*E. waldsteinii* (Sojak) Radcliffe-Smith (*E. virgata* W. & K., non - Desf.)
84HU016	Szolnok, Hungary	*E. salicifolia* Host.
84HU003	Nyiregyhaza, Hungary	*E. lucida* W. & K. × *E. salicifolia* Host.
84HU014	Kecskemet, Hungary	*E. seguieriana* Neck. ssp. seguieriana

Leaf morphology

Leaves from flowering stems were collected in January and February 1985. Every 15th leaf was removed and preserved between a self-adhesive acetate sheet and standard graph paper. The leaves were allowed to dry while preserving their shape and dimensions. A total of 1776 leaves from 255 plants was collected. The three longest leaves from each plants subset were measured for length, width, and area and the length-width ratio was calculated. The position of maximum leaf width was classified as widest above the middle, at the middle, or below the middle of leaf length. Leaf shape was classified as oblanceolate, linear to narrow-oblong, oblong to elliptic-oblong, or oblong-lanceolate. Apex shape was classified as round, round-mucronulate, obtuse, obtuse-mucronulate, or acute. Base shape was classified as attenuate or abruptly acute.

Latex triterpenoid content

Gas chromatography of the latex triterpenoids was performed at Indiana University, Bloomington, IN. Exuded latex was collected from severed leaves at ≥ 5 drops/accession in acetone-washed vials containing spectroscopic grade acetone, evaporated, and sealed.

The dried contents in each vial were reconstituted with analytical grade acetone, filtered through acetone-washed (3×) Whatman #1 paper into fresh vials, and evaporated to dryness over nitrogen. The residue was resuspended in 1 ml acetone as a stock solution. For each sample, 300 L of stock solution was transferred to each of several fresh vials and evaporated over nitrogen to dryness. These residues were resuspended in acetone containing 0.5 mg/ml 4-androsten-3,17-dione (androstenedione) as an internal standard. Different quantities of acetone were employed (ranging from 0.05 to 0.5 ml) depending on the relative concentration of triterpenoids in the sample, so as to obtain quantitatively comparable chromatograms of all the samples. One L of each sample was injected onto the chromatographic column.

Analyses of latex from all samples were performed on a gas-liquid chromatograph[3] equipped with a flame ionization detector and operated by programming from 240 to 310º C at 4º C/min. Nitrogen was used as the carrier-gas (20 ml/min flow rate). The injection port temperature was 250º C; the detector temperature was 350º C. Glass columns (2-mm i.d. by 2.43 m) were treated with 5% dimethyldichlorosilane in toluene (v/v) and packed with 3% OV-1 on 100/120 mesh Supelcoport. Individual compounds (peaks) were identified by their retention time (adjusted to the internal standard for each sample) and quantified on an integrator[4] with data expressed as area percent. Peaks present only as a shoulder on a larger peak or as a peak too small to be integrated by the analyzer were arbitrarily assigned a value of 0.05%. Peaks with retention times similar to contamination peaks in one of the five acetone blanks used as controls were eliminated from the analysis.

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[3] Hewlett-Packard model 5710A gas-liquid chromatograph

[4] Hewlett-Packard model 3380A integrator

Numerical analyses

Leaf and latex data were analyzed with Detrended Correspondence Analysis (DCA) (11) using various transformation techniques as discussed in the results. Latex triterpenoid composition of Montana accessions were also analyzed by analysis of variance.

Results and discussion

Leaf morphological characters

Leaf shape and position of maximum leaf width were strongly related (Figure 1). Oblanceolate leaves were widest above the middle, and leaves widest below the middle were oblong-lanceolate. Most leaves widest at the middle were narrow-oblong (82%) but varied from linear to elliptic-oblong. Shape of the leaf apex and the leaf base was independent of overall leaf shape, position of maximum width, and of each other.

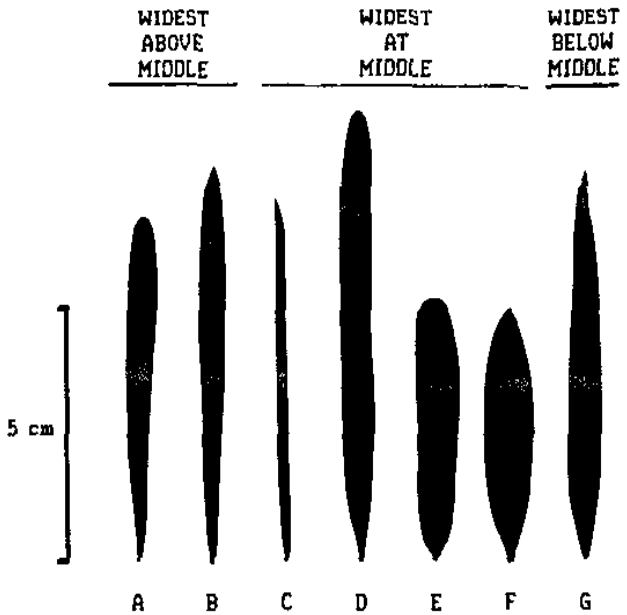

Figure 1. Range of variation in leaf morphology of individual leaves of leafy spurge.

Widest above the middle:	A. Oblanceolate, rounded apex
	B. Oblanceolate, acute apex
Widest at the middle:	C. Linear
	D. Narrowly-oblong
	E. Oblong
	F. Elliptic-oblong
Widest below the middle:	G. Oblong-lanceolate

Within a plant, leaf morphological characters vary markedly. Figure 2 illustrates typical within-plant variation of leaf morphology and dimensions. It is evident from Figure 2 that the developmental age of a leaf affects its shape and dimensions. By using only the longest leaves, we attempted to reduce within-plant variation and base the among-plant comparisons on leaves of the same developmental age.

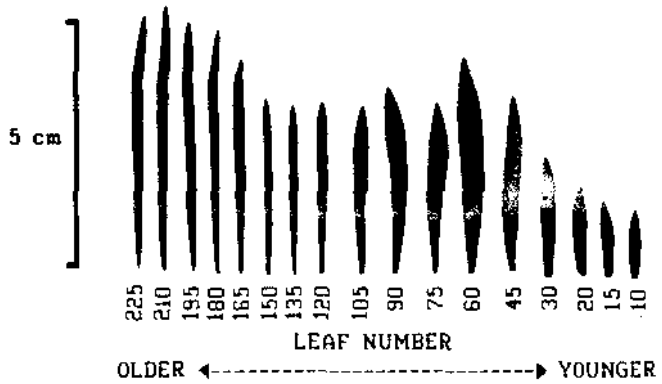

Figure 2. Typical within-plant variation of leaf morphology in leafy spurge (accession 82MT006). Leaf number refers to the chronological position of the leaf on the stem leaf 1 being the first leaf produced.

Within-clone variation was also high (Table 3); 80% of the clones sampled in Gallatin County showed marked variation for either position of maximum width or shape of the leaf apex. Leaf base shape was less variable, but 40% of the clones still exhibited marked variation.

Among-site variation in Montana was similar to the among-clone variation, with 63 and 75% of the sites having marked variation for position of maximum width and leaf apex shape, respectively. Leaf base shape was again the least variable, with only 6% of the sites showing variability. All European accessions were widest above the middle, had an attenuate leaf base, and were oblanceolate or narrow-oblanceolate.

Table 3. Leaf morphological characteristics of leafy spurge accessions from Montana and Europe (percent of the leaves sampled ± standard deviation).

		Position of maximum width			Shape of leaf apex				Shape of leaf base	
Sample number	No. reps[a]	Above middle	At middle	Below middle	Round	Round Mucro-nulate	Obtuse or acute	Obtuse mucronulate	Cuneate attenuate	Abrupt acute
		(%)								
Gallatin County accessions:										
82MT001	18	77 ± 38	17 ± 35	6 ± 17	39 ± 33	55 ± 38	6 ±13	0 ± 0	80 ± 38	20 ± 38
83MT001	16	100 ± 0	0 ± 0	0 ± 0	65 ± 38	35 ± 38	0 ± 0	0 ± 0	100 ± 0	0 ± 0
83MT002	13	3 ± 9	54 ± 37	43 ± 37	0 ± 0	13 ± 29	23 ± 34	64 ± 40	18 ± 38	82 ± 38
82MT002	9	96 ± 11	4 ± 11	0 ± 0	0 ± 0	0 ± 0	31 ± 43	69 ± 43	100 ± 0	0 ± 0
82MT003	28	98 ± 9	2 ± 9	0 ± 0	31 ± 38	2 ± 9	2 ± 9	65 ± 37	94 ± 16	6 ± 16
82MT004	17	100 ± 0	0 ± 0	0 ± 0	0 ± 0	100 ± 0	0 ± 0	0 ± 0	100 ± 0	0 ± 0
82MT005	15	86 ± 31	5 ± 18	9 ± 27	40 ± 48	9 ± 27	35 ± 44	16 ± 33	93 ± 19	7 ± 19
82MT006	43	97 ± 15	2 ± 10	1 ± 5	0 ± 0	29 ± 43	71 ± 43	0 ± 0	100 ± 0	0 ± 0
84MT006	19	14 ± 28	16 ± 30	70 ± 41	0 ± 0	0 ± 0	100 ± 0	0 ± 0	100 ± 0	0 ± 0
84MT007	16	94 ± 18	6 ± 18	0 ± 0	2 ± 8	77 ± 38	21 ± 36	0 ± 0	100 ± 0	0 ± 0
Other Montana accessions:										
83MT020	12	47 ± 41	31 ± 36	22 ± 33	0 ± 0	0 ± 0	100 ± 0	0 ± 0	100 ± 0	0 ± 0
84MT004	10	75 ± 32	15 ± 32	10 ± 18	46 ± 39	7 ± 14	7 ± 14	40 ± 41	100 ± 0	0 ± 0
83MT004	3	78 ± 19	11 ± 19	11 ± 19	78 t 19	22 ± 19	0 ± 0	0 ± 0	100 ± 0	0 ± 0
83MT006	3	100 ± 0	0 ± 0	0 ± 0	33 ± 34	11 ± 19	11± 19	45 ± 20	100 ± 0	0 ± 0
82MT002	3	11 ± 19	22 ± 19	67 ± 19	0 ± 0	0 ± 0	0 ± 0	100 ± 0	100 ± 0	0 ± 0
84MT001	3	78 ± 19	22 ± 19	0 ± 0	100 ± 0	0 ± 0	0 ± 0	0 ± 0	100 ± 0	0 ± 0
83MT012a	13	80 ± 32	20 ± 12	0 ± 0	13 ± 32	3± 9	46 ± 44	38 ± 43	92 ± 28	8 ± 28
83MT012b	22	64 ± 40	27 ± 34	9 ± 15	14 ± 27	41 ± 42	41 ± 46	4 ± 21	100 ± 0	0 ± 0
83MT014	15	100 ± 0	0 ± 0	0 ± 0	7 ± 26	53 ± 47	31 ± 41	9 ± 27	100 ± 0	0 ± 0
83MT015	3	100 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	100 ± 0	100 ± 0	0 ± 0
83MT016	3	89 ± 19	0 ± 0	11 ± 19	0 ± 0	0 ±0	11 ± 19	89 ± 19	100 ± 0	0 ± 0
84MT008	3	89 ± 19	11 ± 19	0 ± 0	33 ± 34	11 ±19	11 ± 19	45 ± 20	100 ± 0	0 ± 0
83MT003	3	100 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	11 ± 19	89 ± 19	100 ± 0	0 ± 0
84MT021	10	78 ± 19	11 ± 19	11 ± 19	0 ± 0	0 ±0	7 ±14	93 ± 14	100 ± 0	0 ± 0
84MT009	10	100 ± 0	0 ± 0	0 ± 0	90 ± 16	10 ±16	0 ± 0	0 ± 0	100 ± 0	0 ± 0
83MT022	3	100 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ±0	44 ± 20	56 ±20	100 ± 0	0 ± 0
European accessions:										
84HU006	3	100 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ±0	11 ±19	89± 19	100 ± 0	0 ± 0
84HU008	3	100 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ±0	78 ±19	22± 19	100 ± 0	0 ± 0
84HU011	3	100 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	22 ±39	78 ±39	100 ± 0	0 ± 0
84HU014	3	100 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	11 ±19	89 ± 19	100 ± 0	0 ± 0
84HU017	3	100 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	33 + 0	67 ± 0	100 ± 0	0 ± 0
84YU008	3	100 ± 0	0 ± 0	0 ± 0	0 ± 0	100 ± 0	0 ± 0	0 ± 0	100 ± 0	0 ± 0
84HU003	3	100 ± 0	0 ± 0	0 ± 0	100 ± 0	0 ± 0	0 ± 0	0 ± 0	100 ± 0	0 ± 0
84HU016	3	100 ± 0	0 ± 0	0 ± 0	100 ± 0	0 ± 0	0 ± 0	0 ± 0	100 ± 0	0 ± 0
84AS002	3	100 ± 0	0± 0	0± 0	100 ± 0	0 ± 0	0 ± 0	0 ± 0	100 ± 0	0 ± 0

[a]Number of replications (plants) from which the three largest leaves were measured.

Table 4. Phenetic characteristics of leaves of leafy spurge accessions from Montana and Europe.

Sample number	No. reps[a]	Mean length	Min length	Max length	Mean width	Min width	Max width	Length/width	Area
		(mm)	(mm)	(mm)	(mm)	(mm)	(mm)		(cm^2)
Gallatin County accessions:									
82MT001	18	54.2 ± 12.6	36.3	86.0	6.4 t 1.0	4.7	9.0	8.8 ± 1.3	3.1 ± 1.2
83MT001	16	61.8 ± 8.0	36.1	77.0	6.6 ± 0.9	4.1	8.6	9.6 ± 1.1	3.4 ± .8
83MT002	13	68.1 ± 10.8	51.2	85.2	9.0 ± 1.9	5.3	11.5	8.0 ± 1.7	5.2 ± 1.6
82MT002	9	69.1 ± 9.5	47.5	85.2	5.8 ± 1.4	3.3	8.6	11.3 ± 2.9	3.8 ± .8
82MT003	28	44.8 ± 7.9	26,8	63.1	9.5 ± 1.7	5.7	13.9	4.8 ± .6	3.6 ± 1.0
82MT004	17	54.8 ± 10.0	36.1	75.4	5.1 ± 1.4	2.5	7.8	11.1 ± 1.8	2.6 ± 1.0
82MT005	15	40.6 ± 16.7	21.3	77.8	5.5 ± 1.7	2.6	9.4	7.6 ± 1.8	2.2 ± 1.4
82MT006	43	48.5 ± 10.7	18.0	70.4	5.2 ± 1.8	1.9	9.2	10.4 ± 3.9	2.3 ± .8
84MT006	19	57.3 ± 12.7	29.7	79.8	4.4 ± 1.5	1.7	8.2	14.4 ±3.5	2.5 ± .9
84MT007	16	47.5 ± 9.0	34.3	67.8	5.6 ± 0.9	4.0	8.5	9.3 ± 2.2	2.6 ± .8
Other Montana accessions:									
83MT020	12	44.2 ± 5.0	31.5	53.3	3.9 ± 0.8	2.5	5.7	12.2 ± 1.8	1.8 ± .4
84MT004	10	52.4 ± 12.6	29.4	62.6	5.1 ± 2.6	2.6	8.2	10.7 ± 2.3	2.1 ± .8
83MT004	3	51.3 ± 6.3	44.1	53.1	5.4 ± 0.5	4.6	6.3	9.7 ± 1.6	2.0 ± .8
83MT006	3	56.2 ± 8.0	44.1	62.6	6.1 ± 1.7	4.7	8.5	9.6 ± 1.5	2.0 ± .9
82MT002	3	41.4 ± 5.1	32.4	48.8	3.8 ± 0.5	2.7	4.1	10.8 ± 2.2	2.3 ± 1.0
84MT001	3	37.2 ± 4.5	26.8	45.6	4.8 ± 0.6	3.6	5.7	7.9 ± 2.2	1.7 ± .7
83MT012a	13	49.4 ± 9.3	29.5	70.1	4.4 ± 1.5	2.0	7.0	13.0 ± 5.0	2.3 ± 1.0
83MT012b	22	49.9 ± 9.1	20.9	74.2	6.1 ± 0.9	3.7	8.2	9.3 ± 1.7	2.8 ± .7
83MT014	15	62.1 ± 12.3	41.0	84.8	7.4 ± 1.9	3.7	11.5	9.1 ± 2.4	4.4 ± 1.7
83MT015	3	44.4 ± 4.8	33.0	52.1	4.4 ± 1.4	3.3	4.9	10.3 ± 2.3	2.3 ± .8
83MT016	3	36.2 ± 3.1	30.6	41.7	4.1 ± 1.1	3.6	4.6	8.9 ± 1.7	2.4 ± .8
84MT008	3	47.5 ± 8.6	39.6	51.9	4.6 ± 1.3	2.9	5.3	10.6 ± 1.9	2.3 ± .9
83MT003	3	37.3 ± 5.2	31.8	42.3	4.2 ± 1.2	3.5	4.3	9.3 ± 1.1	2.1 ± .8
84MT021	10	38.8 ± 9.1	29.9	46.4	4.0 ± 1.2	3.1	4.6	9.9 ± 1.6	2.2 ± 1.0
84MT009	10	39.3 ± 7.6	30.0	49.3	4.3 ± 1.4	3.2	4.8	9.3 ± 1.8	2.1 ± .9
83MT022	3	35.5 ± 3.3	30.1	38.1	3.2 ± 0.6	2.7	3.8	11.3 ± 21	2.4 ± .9
European accessions:									
84HU006	3	33.1 ± 5.6	25.8	39.4	3.3 ± 0.7	2.8	3.8	10.2 ± 2.4	2.2 ± 1.1
84HU008	3	33.6 ± 8.3	25.1	39.9	3.4 ± 0.6	2.8	3.9	9.7 ± 1.6	2.2 ± 1.2
84HU011	3	44.0 ± 9.1	31.3	47.5	3.3 ± 0.6	3.0	3.9	13.5 ± 2.5	2.1 ± 1.0
841IU014	3	35.2 ± 8.8	23.8	40.7	3.6 ± 0.6	3.1	4.0	9.9 ± 1.7	1.9 ± .8
84HU017	3	44.1 ± 9.1	32.3	50.3	3.8 ± 0.8	2.8	4.2	11.2 ± 1.9	1.9 ± .8
84YU008	3	44.4 ± 7.7	37.5	49.0	4.3 ± 0.6	4.0	4.9	10.4 ± 2.1	1.8 ± .8
84HU003	3	38.1 ± 7.4	31.6	47.5	4.5 ± 0.5	3.9	5.4	8.7 ± 2.0	2.1 ± .9
84HU016	3	42.1 ± 7.8	35.5	50.1	6.5 ± 0.7	5.6	7.0	6.7 ± 1.6	2.1 ± 1.0
84AS002	3	40.0 ± 8.7	36.6	49.4	6.7 ± 0.6	6.2	7.3	6.2 ± 1.5	2.2 ± 1.1

[a] Number of replications (plants) from which the three longest leaves were measured.

Leaf phenetic characteristics

Leaf dimensions were also highly variable (Table 4). Detrended correspondence analyses were employed to objectively determine groupings of replications amongst all accessions (11). In a first level analysis (normalized leaf morphology and dimension data), the first factor represents a morphological gradient, with the end-points determined solely by position of the maximum width. This resulted in three data clusters: 1) widest above the middle, 2) widest at the middle, and 3) widest below the middle. Factor 1 accounted for 56.5% of the variance in the data. Replications from individual clones occurred in two or even all three of the different clusters. Thus the groups were very "artificial", and maximum width position was not a useful characteristic for distinguishing taxa. The second factor was related to leaf dimension, with end points determined by leaf width, area, and length/width ratio and accounting for only 1% of the variance in the data.

A second DCA using leaf dimension data was employed to determine which characteristics were most important and to see if any groups were evident from dimension data only. The first factor end points were determined by leaf width and leaf area but accounted for only 6% of the variance in the data. The second factor accounted for less than 1% of the variance. As predicted by the low eigenvalues, the analysis did not distinguish any meaningful groupings of replicates and strongly reflected a continuum of leaf dimensions (Figure 3).

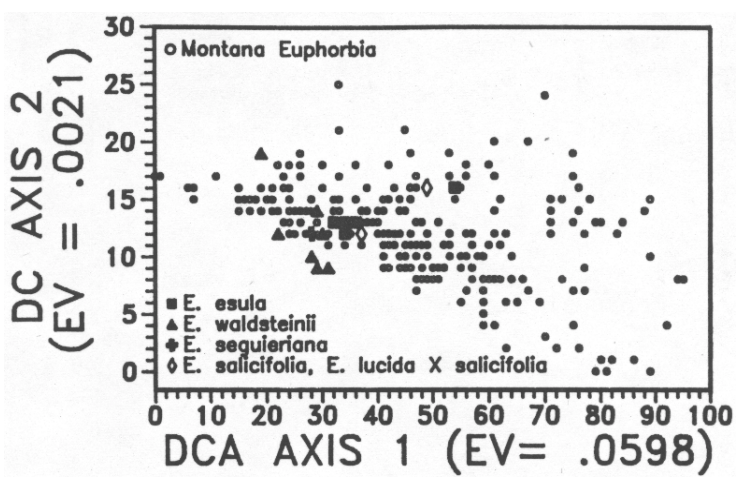

Figure 3. Detrended correspondence analysis of leafy spurge leaf morphology (313 Montana and 27 European plants). EV = eigenvalue (percent of the variance in the data accounted for by that axis).

Triterpenoid content

Twenty-three peaks were identified from chromatograms: retention times are presented in Table 5. Three compounds have been chemically identified: peak 10, euphol; peak 14, cycloartenol; and peak 16, 24-methylene cycloartenol. Compounds from the remaining peaks have not been specifically identified, but these compounds extracted as typical triterpenoids had retention times similar to known triterpenoids and hence were considered as such (12).

Table 5. Retention times of triterpenoid peaks (compounds) identified by gas chromatography of leafy spurge latex.

Peak	Retention time	Peak	Retention time
	—— (min) ——		—— (min) ——
1	13.69- 13.88	14[b]	15.66 - 15.68
2	12.79- 12.89	16[c]	16.49 - 16.63
3	11.58 - 11.64	17	17.04 - 17.08
4	11.29- 11.39	18	17.64 - 17.98
5	10.69 - 10.73	19	18.06 - 18.16
6	10.30- 10.38	20	18.40 - 18.51
7	10.08 - 10.19	22	19.43 - 19.76
8	8.92	23	19.77 - 20.00
10[a]	14.06 - 14.13	24	13.11 - 13.32
11	14.42- 14.58	25	12.01 - 12.41
12	14.74- 15.05	26	11.08
13	15.14- 15.18		

[a] Euphol.
[b] Cycloartenol.
[c] 24-methylene cycloartenol.

Analysis of variance of within-plant, within-clone, and among-clone composition at a single site (Gallatin County, MT, accessions), and among sites in Montana were all non-significant (P>0.85) and suggested strong similarity.

Triterpenoid composition was also analyzed with DCA to elucidate relationships among accessions. Analysis of untransformed data accounted for 39% of the variation in the data. Three groupings of accessions were identified (Figure 4): 1) European *E. lucida × salicifolia,* 2) European *E. salicifolia*, and 3) European *E. esula, E. waldsteinii*, and *E. sequieriana*, and all Montana accessions. The end points for this axis represent the presence and absence of peaks unique or nearly unique to *E. lucida × salicifolia* and *E. salicifolia*. Peaks 3, 4, 6, and 8 were present only in these two taxa and peaks 1 and 2 were present only in minute amounts in three other samples, compared to *E. lucida × salicifolia* and *E. salicifolia*. The second axis accounted for only 5.2% of the variation in the data.

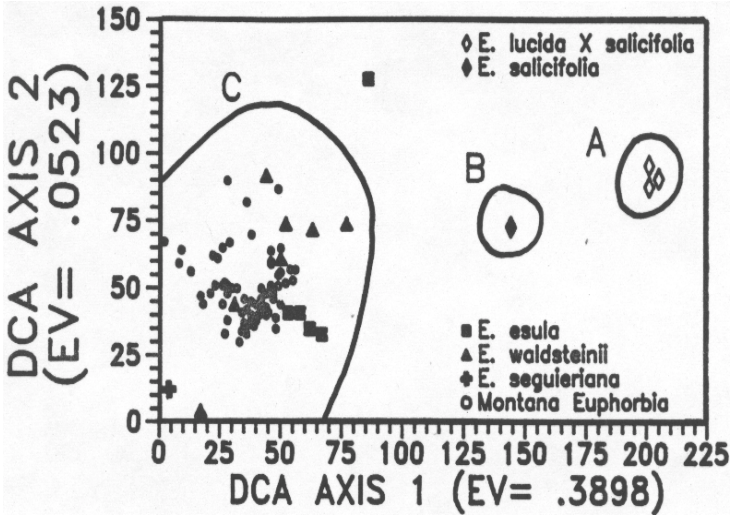

Figure 4. Detrended correspondence analysis of leafy spurge latex triterpenoid composition (71 Montana and 13 European plants). EV = eigenvalue (percent of the variance in the data accounted for by that axis).

Transformation of the data to presence/absence form (qualitative rather than quantitative) yielded nearly identical results. The first axis accounted for 43.9% of the variation, with the same selection of end points as in the untransformed analysis. The second axis only accounted for 8.9% of the variation. Similarity between the two analyses indicated that the relative quantity of a triterpenoid present carried little more information than the presence or absence of that triterpenoid.

To explore the effect of the presence of rare peaks in the data, the rare peaks were downweighted (11) and DCA was performed. The first axis only accounted for 20.5% and the second axis for 3.9% of the variation. With the data transformed to presence/absence and the rare peaks downweighted, the results were nearly identical (19.7 and 5.4% for the first and second axes, respectively). The end points for the first axis were the same as for the analyses without downweighting. In all these analyses, three groups were identified as described above. Eliminating data for peaks present in quantities of less than 0.5% resulted in the first axis accounting for only 6.1% of the variation, indicating that the presence of the less common triterpenoids is critical in identifying differences among accessions.

To further examine the large group of unresolved accessions, the *E. lucida* × *salicifolia* and *E. salicifolia* were omitted from the data and a DCA was performed. Only 13.7% of the data variation was explained by the first axis and 5.5% by the second axis. One outlier was identified, but the reliability of any clusters is doubtful with the low eigenvalues associated with the analyses. The results support the conclusion that the accessions in the large cluster (C) in Figure 2 had a high inherent variability in triterpenoid composition.

Classification

Latex triterpenoid analysis identified two well-defined sets of outliers in the leafy spurge accessions we have sampled: *E. lucida* × *salicifolia* and *E. salicifolia*. Multivariate analysis techniques (DCA) demonstrated the presence of continuous variation in leaf dimension and latex composition in the large clustering of accessions (nonoutliers). The European *E. esula, E. waldsteinii*, and *E. seguieriana* accessions included in the study were indistinguishable from Montana accessions (Figure 4).

Leaf characteristics have been widely used as taxonomic characters in published classifications of leafy spurge (17, 18, 19). Leaf shape (including shape of the apex and base, and position of maximum width) and dimensions are often used as criteria for separation at the species level. For example, Prokhanov (17) used leaf apex shape as the sole criterion for separating *E. virgata* Waldst. et Kit. from *E. boissieriana* (Woron.) Prokh. The classification key presented by Dunn and Radcliffe-Smith (5) and Radcliffe-Smith (19) and used by Ebke and McCarty (6) has 19 couplets of which seven use leaf shape, four use position of the maximum width, five use leaf apex shape, and three use leaf base shape in various combinations to help determine to which taxa a given specimen or population should be assigned. We have demonstrated in this study that these characteristics, both singly and in combination, are highly variable and produce very artificial groupings of questionable value for classifying accessions. Even though Ebke and McCarty (6) placed their accessions in the classification structure proposed by Radcliffe-Smith (18), they state, "The nursery material represented a large degree of variability which could reflect a normal field situation". Variability was seen from season to season as well as within a season. Some variability was noted in plants within the same tube (two plants from the same site per tube) as well. Bakke (2) noted that the leafy spurge he was working with was highly variable, even on the same plant. Groh (8) and Moore (14) also noted the same variability.

The accessions analyzed in this study can be separated into three major groups. Two groups are represented by the outliers *E. lucida* × *salicifolia* and *E. salicifolia*. The remaining accessions, including phenetically identified taxa collected in Europe and Montana, represent the third group of apparently diverse plants. Leaf characteristics and latex triterpenoid profiles were not useful in illucidating any differences in this third group. It is possible that further study of this group will show it to represent a single polytypic species.

There is little agreement in the literature on the proper name for this diverse group of plants. *Euphorbia esula* L. was preferred by Wheeler (23) and Moore (14) but *E. virgata* Wald & Kit. was preferred by Hanson and Rudd (9), Groh (8), Bakke (2), and Morton (15). Radcliffe-Smith (18, 19) has asserted that several taxa are present in this polymorphic complex. Croizat (3, 4) proposed *E. podperae* Croiz. for the complex including *E. esula*, *E. virgata*, and *E. intercedens*. The nomenclatural history of this group is complicated and has been reviewed by Richardson (20), Galitz (7), and Mahlberg *et al*. (I 2).

Because all the Montana accessions fall together with *E. esula*, *E. waldsteinii*, and *E. seguieriana*, we accept the oldest name available, *Euphorbia esula* L., for these populations.

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