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# Leafy spurge taxonomy: A re-evaluation

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## Introduction

Leafy spurge is a very aggressive noxious perennial weed of great and increasing economic importance in rangelands of central and western North America (Morton, 1937). Chemical, cultural and biological strategies are being developed to control this difficult weed. It is important to both chemical and biological control that we properly identify the target weed. In biological control management it is important to correlate the target weed to the equivalent type in the plants' native range for identification of appropriate biological control agents (Harris, 1979).

Using current classification schemes, we have been unable to satisfactorily assign the plants we see at field sites to any one specific taxon, but rather we find that several taxa are present. 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 (Bakke, 1936, Dunn and Radcliffe-Smith, 1980; Groh, 1935; Moore, 1958; Radcliffe-Smith, 1985).

We are also interested in the potential of chemical constituents in the plants as taxonomic indicators. For biological control, the differences between host plants and non-host plants are likely to be chemically related rather than morphological and the efficiency of searches for biological control agents of North American spurge are likely to be low until these differences are identified (Harris, 1979).

The purpose of our study was 1) to quantify the within-plant, within clone, between-clone and between-site variability of leaf characteristics, 2) to quantify the within-plant, within-clone, between-clone and between-site variability of latex triterpenoid composition, 3) to determine if there were any logical groupings of accessions based on leaf and/or triterpenoid characteristics, and 4) to evaluate the taxonomic value of those characteristics. European reference specimens were included in the study to relate Montana specimens to European specimens. Four of the European specimens were root cuttings from the Austrian specimens used in the Ebke and McCarty (1983) study and verified as *E. esula* by Radcliffe-Smith for that work.

## Materials and methods

Multiple root cuttings from 27 Montana and 12 European accessions were established in the greenhouse in the summer of 1984. Leaves from flowering stems were collected in January and February, 1985. Every 15th leaf was removed from the plant and preserved between a self-adhesive acetate sheet and standard graph paper (a total of 1,776). This technique allowed the leaves to dry while preserving their shape and dimensions. From these leaves, the three longest from each plant were selected for measurement of length, width, length/width ratio, and area. Leaf shape, shape of the leaf apex and shape of the leaf base were also recorded.

**Latex triterpenoid content.** Latex exuded from severed leaves from each accession was collected in acetone-washed vials containing spectroscopic grade acetone. The acetone was then evaporated and the vials sealed until analysis. Gas chromatic analysis (GC) of the latex triterpenoids was done at Indiana University, using a Hewlett-Packard 5710A gas-liquid chromatograph.

Individual compounds (peaks) were identified by their retention time (adjusted to theinternal standard for each sample) and quantified on a Hewlett-Packard 3380A integrator with data expressed as area percent.

## Results and discussion

**Leaf morphological characteristics**. Overall leaf shape, shape of the leaf apex, shape of the leaf base, and position of the maximum leaf width are qualitative leaf charateristics that have been used as taxonomic characters in published classifications of leafy spurge (for example, see Radcliffe-Smith, 1981, 1985). Leaf shape and the position of maximum leaf width were well correlated. Shape of the leaf apex and shape of the leaf base were independent from leaf shape, position of greatest width and from each other.

Within a plant leaf morphological characteristics vary markedly. Sampling only the longest leaves reduces the variation, but does not eliminate it. Within-clone variation is also high; 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 contained marked variation.

Between-site variation in Montana is similar to the between-clone variation, with more than 60% of the sites having marked variation for position of maximum width and leaf apex shape.

**Leaf phenetic characteristics.** Leaf dimensions were as variable as leaf morphology. Statistical analysis for within-plant, within-clone, between-clone and between-site coefficients of variation for length, width, length/width ratio and area detected no significant differences.

Taxonomists increasingly use multivariate methods to guide the choices of characteristics for taxonomic evidence and for grouping specimens into taxa (Gaugh, 1982). Detrended correspondence analysis (Hill, 1979) of the data was employed to objectively determine groupings of replications amongst all accessions. Three clusters and one outlier were identified, but replications from individual clones occurred in two or even all three of the different clusters. This suggested that the groups were very 'artificial' rather than 'natural' groups and that the leaf characteristic that was the driving force for the selection of the groups (maximum width position) was not a valid characteristic for distinguishing taxa. Analysis of variance of data set based on these groupings showed no significant difference between groups ($P > 0.10$, df = 21), reflecting the large variation of the leaf characteristics.

A second detrended correspondence analysis using only leaf phenetic characteristics (i.e. leaf dimension data) was employed to determine which dimension characteristics were most important and to see if any groups were evident from only dimension data. The analysis could not distinguish any meaningful groupings of replicates and strongly indicates a continuum of leaf dimensions (The first factor endpoints were determined by leaf width and leaf area, but only 6% of the variance in the data was accounted for in the analysis).

**Triterpenoid content.** Analysis of variance of within-plant composition, within-clone composition, between-clone composition at a single site and between-sites in Montana were all non-significant ($P > 0.85$) and suggested strong similarity.

The triterpenoid composition was also analyzed with detrended correspondence analysis using three different forms of the data: i) original percent area data, ii) downweighting of the rare peaks (see Gaugh, 1982; Hill, 1979), and iii) data transformed to presence/absence form. The total variance accounted for by the first factors were only 11.5%, 10.4% and 21.5% for the original data form, rare downweighting and presence/abscence form, respectively. All three analyses produced similar results; no clear groupings of triterpenoid profiles and one outlier. Although some clustering of samples occurs, samples from the same clone and even from the same plant appear in more than one cluster. The reliability of any clusters is also doubtful with the low eigenvalues associated with the analyses, therefore the clusters do not represent any definable taxonomic entities. The results support the conclusion that the inherent variability of the plants is high even for these chemical components.

**Classification.** Sample data has four components: redundancy, relationships, outliers and noise (Gaugh, 1982). In our study, replication within a plant (several leaves or multiple latex samples from the same plant) and replication within a clone (an assumed genetic individual) represent redundancy. When a data set includes diverse samples, clear sample relationships will emerge despite any imperfections in the individual samples if the difference in the between-sample variation exceeds the within sample variation (Gaugh, 1982). Among relationships we see are the similarity of different clones and different sites for leaf morphology, leaf dimensions and triterpenoid profiles. Our study identified one well defined outlier that is clearly not in the '*esula* complex'.

The fourth component of sample data is noise. Noise is composed of both sampling error and inherent variability. By growing the plants in a uniform environment (i.e., the greenhouse) we have eliminated any environmental variation present in field collected specimens (i.e., herbarium collections and field studies). This type of variability falls into the sampling error category since it does not represent genotypic variability that is the basis for a natural classification. The inclusion of large replicate samples also reduces the potential for sampling error (Snedecor and Cochran, 1980). Multivariate analysis is a good test for homogeneity and discontinuous versus continuous variation (Goodall, 1954), and the orthoganality of the method maximizes the variance that is accounted for in the original data set (Gaugh, 1982). Detrended correspondence analysis demonstrated the presence of continuous variation in leaf dimension and in latex composition. The

European samples included in the study to represent the European leafy spurge complex were indistinguishable from the Montana plants.

Our study indicates that the common leaf characteristics used as taxanomic indicators, both singly and in combination, are highly variable and produce very artificial groupings of questionable value for classifying Montana and European accessions. Others have also noted the variation in leafy spurge (Ebke and McCarty, 1983; Bakke, 1936; Groh, 1935; Moore, 1958). Latex triterpenoid composition did not enhance the ability to detect meaningful clusters of samples.

There is little agreement in the literature on the proper taxon that this diverse group of plants belongs to. *E. esula* L. was preferred by Wheeler (1939) and Moore (1958), but Hanson and Rudd (1933), Groh (1935), Bakke (1936), and Morton (1937) preferred *E. virgata* Wald. & Kit. while Radcliffe-Smith (1980, 1985) has asserted that several taxa are present in this polymorphic complex. However, Croizat (1945, 1947) proposed *E. podperae* Croiz. for the complex, as it includes *E. esula*, *E. virgata*, and *E. intercedens*. The chronological history for the name is complicated and has, been reviewed by Richardson (1968) and Galitz (1980).

All the accessions in our study must be considered as one taxonomic unit based on the characteristics that we used, which included those leaf characteristics used in previously published classification schemes as well as triterpenoid profiles. In addition, the samples included in this study that represent the *E. esula-virgata* complex from Europe, and identified as being *E. esua* L., are indistinguishable from the Montana samples.

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