*Reprinted with permission from: Proceedings of the 1984 Leafy Spurge Annual Meeting. Dickinson, ND. June 27-28, 1984. pp. 4-6.* 

Published by: Great Plains Agricultural Council. Leafy Spurge Symposium.

## The chemotaxonomy program on leafy spurge in Fargo, ND and the confusion regarding numbering of plant collections

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Chemical constituents of leafy spurge have been used as criteria in attempts to determine taxanomic relationships between North American and European plants grown under identical environments. In 1983, Manners reported at the Leafy Spurge Symposium in Sundance, Wyoming, on the possible use of epicuticular wax constitutents to distinguish different leafy spurge biotypes (accessions, collections, etc.) collected from North America and Europe. From his results, it was concluded that the North American biotypes could be grouped together as having similar wax compositions, but slight differences were found in the wax composition of *Euphorbia esula* collected from near Krems, Austria (Manners and Davis, in press; Phytochemistry). The biotypes selected were from a wide geographical location, and of quite variable leaf and shoot morphology. However, only four North American and one Austrian biotype were compared; consequently definitive relationships were not established.

More recently, Davis, Galitz, Manners, Pleszczynska and Mahlberg (Submitted to American Journal of Botany) studied the shoot latex triterpenoids from these same biotypes and from several other North American biotypes in an effort to corroborate and extend these studies. This work will be presented in detail by Dr. Mahlberg in this symposium, and at the meetings of the Botanical Society of America. A gas chromatography method of fairly low resolution was used. The results indicated that the relationships between the various spurges tested appear to be more complex than those proposed by Manners and Davis in their wax study. At least three different groupings of leafy spurge appeared to be possible from that analysis. These differences between the two studies need to be resolved, and correlated with cytological observations underway by several of the people in this symposium. Also, preliminary high resolution gas chromatographic analyses of these same latex constituents lead us to conclude that the relationships are more complex than hoped.

A single analysis of root latex and shoot latex from one plant by Dr. Manners (USDA, Berkeley, California) resulted in different triterpenoid profiles (gas chromatog-raphy). If true, this result contradicts the concept that the laticifer in leafy spurge is a single continuous cell with a uniform distribution of triterpenoids throughout the plant. This needs to be investigated further, if the triterpenoids are to be considered for chemotax-

onomic classifications. Perhaps other constituents of the laticifer might be used, as well as the triterpenoids.

Dr. Manners is presently looking for chemical constituents of leafy spurge that can be used as taxonomic tools and/or allelopathic agents. He has found at least one compound of interest extracted from roots of flowering leafy spurge collected in the sandhills of North Dakota in May, 1983. He is characterizing that compound, and will be testing it as the possible irritant factor on the skins of cattle. He has also indicated that the compound appears to be in a class of compounds reported in the literature obtained only from three members of the Euphorbiaceae (two in Japan). This compound may be a potential taxonomic marker, and the European spurges should be checked for its presence or absence. He is following this up, and will extract roots from non-flowering plants as well as shoots from flowering and non-flowering material to determine whether it is organ specific and transient. It exists at a concentration of  $2x10^{-4}$ % which is quite high. He may report on these results next year.

In summary, it appears that a great deal more work needs to be done to determine whether the laticifer contents or other chemical constituents can be used to separate taxonomic relationships amongst the various collections of leafy spurge. Dr. Mahlberg will discuss this at a greater length, in this symposium.

In Fargo, tissue cultures of several biotypes have been established, and significant differences in the characteristics of the cultures have been observed. One biotype has been regenerated, another appears to be amenable to regeneration, but five others have shown little evidence of being capable of regeneration. Dr. Galitz (North Dakota State University) has compared some of these cultures in their response to the herbicide dicamba, and Dr. Frear (USDA, Fargo, ND) has compared their abilities (and those of intact plants of the same biotype) to metabolize dicamba. A striking difference was found in the metabolism of dicamba by one biotype or selection. Dr. Schaeffer (Montana State University) made an assessment to determine whether the cell cultures might be a good source of material to study the karyotypes of these materials. He has indicated that the cultures do <u>not</u> appear to be useful for his work, for technical reasons.

A problem that should be addressed by this group in this meeting is in the reporting of information obtained from different leafy spurge collections. A concensus of opinion by this group hopefully will eliminate confusion in the literature. First, what should these collections of leafy spurge be called? We have used the term <u>biotype</u> for our own convenience. The advisability of using the term <u>accession</u> was discussed by some in this group at a meeting in Spokane, Washington this past winter. The term <u>collection</u> has been used by Bruckhart at Frederick, Maryland. No matter what term we use in our own research, it would be most useful to be consistent in published articles.

Secondly, a consistent and useful numbering system would be helpful, especially when material is exchanged between locations, as is being done frequently. At Fargo, the material collected was simply numbered consecutively as it was collected in the field or obtained from Dr. McCarty (USDA, Lincoln, Nebraska). Last November, Ebke and McCarty published their results on the taxonomy of their collection based on leaf characteristics, using their numbering system; again, theirs was a numberical system with *E. esula* from Austria being numbered 1-4, and the remaining numbers were consecutive

according to the order of collection. Their numbers include different species of spurge as well as different variations of leafy spurge. This numbering system works well for an individual location, or for one or two publications, but can cause complications later when material is moved from one location to another. In McCarty's case, his nursery has been moved out of Lincoln, with duplicate root stock being taken to Bozeman, Montana and mailed to Fargo, North Dakota. Whose numbering system do you then use? McCarty's or your own?

Several people in this meeting have already been contacted regarding the numbering system. And, of course, several solutions have been proposed. One such solution was to retain numbers 1-100 for McCarty's original collection, 101-200 for the collection at Fargo, 201-300 for a collection in Montana, etc. for other states.

Dr. Messersmith suggests using a two letter zip code (e.g. ND01, ND02, etc.) according to the state, province, or country followed by a numerical sequence of collection. Pros and cons of this system were discussed by Messersmith. Some confusion might arise. For example, would MN be Minnesota or Manitoba? Eileen Sutker and Dr. Bruckhardt (USDA, Frederick, Maryland) are using such a zipcode. They use BC for British Columbia, and Eileen suggests CM for Manitoba (Canada Manitoba). Again, possible confusion arises, since they also use the first letter to designate species other than *Euphorbia*, e.g. CMT = *Cyparissas* from Montana. They use IC to refer to *Euphorbia esula* from Italy, Campito (the town from which the plant was collected). Eileen also recommends using letters rather than just numbers for the pertinent and practical reason that numbers often get lost on pot tags in the greenhouse, Letters seem to be easier to retain and see when you are working with them.

If Dr. Galitz collects plants in North Dakota, and Dr. Lym does also, who's numbers should be used, and how is the information communicated quickly enough to be useful and avoid unnecesary problems? These appear to be minor points, but they are a nuisance when you want to get on with a research program and write up results without being bothered by a lot of interruptions and complication of details. All of the above points merit serious discussion here because many of the people involved in leafy spurge research in the U.S. and Canada are here, and it probably affects us all.