Reprinted from: 1985 Leafy Spurge Symposium. Bozeman, MT. July 17-18, 1985. pp. 16-18.

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

Triterpenoids in latex: Their synthesis and possible role in *Euphorbia*

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Introduction

The topic that I want to discuss today is related to the latex of the *Euphorbia* plant, including *Euphorbia esula* L., the spurge. In our last meeting I emphasized that the latex is produced in a specialized cell termed the laticifer cell. This cell is initiated in the embryo and then progressively grows throughout the plant as a single-cell type to form a long coenocytic cell. It is the longest of biological cell types (1). The unusual feature about this cell in the genus *Euphorbia* and some related genera is that it undergoes a specialized synthetic activity for production of various toxic triterpenoids, the accumulation of rubber, and also the accumulation of starch within laticifer plastids.

Last year I emphasized that the triterpenoids from the latex of 10 different populations analyzed by gas liquid chromotography separated these populations into three distinctive groups based on the profile of their triterpenoids (2). Specific profiles of triterpenoids have been recorded for individual taxa examined in other studies in my laboratory (3). From these results I have postulated that the triterpenoid profile represents a fingerprint for a taxon (4). A question posed by the occurrence of stable and distinctive profiles within a given population or taxon relates to where these triterpenoids are produced in the plant. We undertook studies to examine the sites of synthesis of these compounds in latex fractions using labeled acetate and malvalonate of the squalene pathway (5). The purpose of this study is to interrelate triterpenoid synthesis with organellar components of the cell and project a hypothesis of the function of the laticifer and its unusual contents.

Results and discussion

Latex, upon fractionation, is separated into three distinctive fractions. An upper fraction, which is the so-called triterpene particle fraction including rubber content, a large, clear serum middle fraction, and a small bottom fraction which includes the plastids and membranes of tubular form. Similar fractions are obtained from latex of all examined *Euphorbia*. This material is very difficult to handle because as soon as you begin manipulating the rubber fraction of exuded latex, it begins to coagulate. This phenomenon can be minimized by collecting the latex exudate directly into phosphate buffer, passing it

through a sephadex column, and eluting the particles from the column with water. With this procedure one can isolate the triterpene-rubber particles as specific particles (5).

Each triterpene particle from the upper fraction, when examined by transmission electron microscopy, consists of an electron dense body surrounded by a membrane. These particles are capable of incorporating both mevalonate and acetate to synthesize triterpenes. The serum fraction contains no morphological details when examined by electron microscopy. It does not incorporate labeled acetate or mevalonate (5).

In the bottom fraction, we find starch grain-containing plastids as well as abundant tubular membranes associated with the plastids. This fraction was most active in triterpenoid synthesis and incorporated both acetate and mevalonate for this activity (5). Structural details of the membranes show small electron dense masses between the membranes. These masses resemble closely the larger rubber particles of the upper triterpene-containing fraction; and it appears that these small bodies, which typically appear as spherical bodies along these membranes, may be pinched off to form membrane-bound particles in the cytoplasm. In this way, the plastic membrane fraction is associated with the synthesis of triterpenoids and the formation of rubber particles. These particles upon release from the membranes contributes to the upper triterpenoid-rubber fraction. It is unclear, at present, how these particles become enlarged after their release from the tubular membranes.

The lipophilic rubber particles physically represent the prominent component of the upper fraction. Since the triterpenoids accumulate to high concentrations, as high as 40% dry weight of latex, the rubber particles compartmentalize these compounds and remove them from the metabolic stream. Thus, the rubber particles function to store these generally toxic compounds. This interpretation is supported from studies on other latex bearing plants where, if triterpenoids are absent, the rubber is absent. Thus, the evolution of rubber may correlate with the evolution of the triterpenoid pathway in the laticifer.

The function of the laticifer contents is interpreted to relate to protection against predation. Phytophagous insects and larger animals foraging upon the plant would receive amounts of latex exudate containing high concentrations of triterpenoids. The qualitative and qualitative differences for triterpenoids among different taxa is interpreted to be a result of coevolutionary pressures between the plants and on obligate insects. An obvious example would be reflected by a qualitative alteration of the triterpenoid composition representing speciation (chemotype alteration) and resulting in a plant now unpalatable to the obligate predator. However, insect evolution can be expected to result in a form able to forage upon the evolved species with its altered triterpenoid composition. Repetition of this coevolutionary scenario provides an explanation of the origins of *Euphorbia* species with diverse triterpenoid components and the obligate or selective feeding habits of insects associated with the genus.

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