Chemical Defense of a Rare Mint Plant

by Thomas Eisner, *et al.*

Analyses of leaf extracts of *Dicerandra frutescens*, a highly aromatic mint plant from central Florida listed as an endangered species, revealed presence of twelve closely related monoterpenes. The principal of these, (+)-trans-pulegol, is a new natural product, the synthesis of which is described. The terpenes are produced in glandular capsules that release their contents upon injury of the leaf. Data from bioassays with ants and cockroaches indicate that the terpenes serve for defense against insects. *Dicerandra* does, however, have a leaf-eating enemy, the caterpillar of a pyralid moth, *Pyrausta panopealis.* The discovery of a new natural product from an endangered species raises questions about the chemical implications of species extinction.

Introduction

Dicerandra frutescens, or scrub balm, is a rare mint plant (family Labiatae) (Huck 1987; Kral 1982) restricted in distribution to probably no more than a few hundred acres of a single county (Highlands County) of central Florida. A low, multi-branched herb, it is an endemic of the sandy scrub, a chief habitat of the region. It was only discovered in 1962, and is now listed as an endangered species.

Like many mint plants, *D. frutescens* is highly aromatic. Manually macerated leaves emit an intense terpenoid odor, which may be apparent also from meters downwind when a person walks through a patch of the plant or brushes across its branches by hand. Close examination of the plant revealed it to be remarkably free of insect-inflicted injury. This suggested that the odorants might be anti-insectan and therefore worth characterizing chemically, particularly given the endangered status of the plant. Chemical work done so far on plants of the genus *Dicerandra* must be considered tentative (Huck et al. 1989). We here deal with the glandular source and chemical nature of the insect repellents of *D. frutescens,* and present notes on a exceptional pyralid caterpillar (*Pyrausta panopealis*) that is undeterred by the chemicals and feeds on the plant.

Biological aspects of the study, including observations of the caterpillar, were carried out mostly at the Archbold Biological Station in Lake Placid, where *D. frutescens* occurs in patches. All leaves used for experimental purposes (chemical extraction, morphological work, and bioassays) stemmed from plants from the site.

Robin B. Huck, from *Florida Wild Flowers and Roadside Plants*

Scrub Balm

Indirect visualization of the glandular capsules of the plant was effected by pressing fresh leaves on thin-layer chromatography plates soaked in Tollen reagent. The technique, which provides a quick general means for revelation of plant glandular structures, has been described (Eisner et al. 1987).

In preparation for scanning electron microscopy, leaves were frozen by quick immersion in liquid Freon 22* (jacketed by liquid nitrogen) and immediately freeze-dried. To provide cross sectional views of the leaves, some were broken transversely while immersed in Freon.

Leaves were clipped in the field, or from potted plants transported to our Cornell greenhouses, and directly transferred to CH2Cl2 for extraction (at -20 C).

Caterpillars of *Pyrausta panopealis* (family Pyralidae) were field collected on the plant over a period of days in late October 1989. They live singly, in loose silken enclosures that they construct on branches of the plant, binding together numbers of leaves, including some upon which they feed. They are quickly mobile, moving back and forth with great agility when disturbed, and leaving the enclosure only when forced to do so by persistent prodding. When grasped with forceps, they writhe and anoint themselves and their immediate surroundings with regurgitated fluid. Scanning electron microscopic examination of leaves that were in the process of being fed upon by the larvae showed the glandular capsules at the margins of chewed portions of the leaf to be in various stages of rupture, indicating that they themselves, as well as in all likelihood their contents, were being ingested.

A number of larvae, which pupated within silken enclosures, gave rise to adults, providing specimens for definitive identification.

Mint plants are a rich source of "essential oils", terpenoid mixtures that account for their aroma, and for which they are often cultivated to provide chemicals or chemical blends for the scent and flavor industries. Essential oils are doubtless the basis of defense in these plants, and the specific terpenoid mixture that we isolated from *D. frutescens* is probably no exception. ...Neither [ants] nor [cockroaches] are actual enemies of plants. However, their demonstrated response to the

Dicerandra terpenes may be indicative of a more general insectan sensitivity, encompassing actual herbivores as well. The fact that *Pyrausta* was virtually the only herbivorous insect noted to be feeding on *D. frutescens*, attests to the high degree of invulnerability of this plant in nature. Interesting in this connection is that pennyroyal oil, with its rich pulegone content, has long been used as a flea repellent by humans.

While the demonstration that the chemical defenses of a plant are embodied in its epidermal glandular capsules is in itself not novel, two points brought out by our data are worth noting. First, the *Dicerandra* capsules appear to be hermetic enclosures. Exposure of the leaves to freeze-drying involved confinement at high vacuum for 12 to 20 hours. The fact that the capsules retained their volatile contents under these conditions signifies that their walls must be literally impervious to outward diffusion of the terpenes. In the normal plant, evaporative loss of defensive material must thus be held to a minimum.

The second point concerns the demonstration that leaves of *Dicerandra* proved repellent to ants only after transection. This strongly implies that it is through capsule rupture that repellency is initiated. While it is common knowledge that the scent of a mint plant is revealed to the human nose through leaf maceration, we know of no other direct demonstration that the insect repellency of the leaf is itself also thus activated and therefore linked to the scent. Clearly, the capsules are designed to externalize their defensive "message" when the plant is attacked. While insects may indeed be *Dicerandra*'s major potential enemies, other herbivores, including perhaps mollusks and vertebrates, may also find the plant unpalatable.

Plant defenses are diverse and varied, and differ in their adaptive merits and shortcomings. Laticiferous plants, for instance, which store latex under pressure in canals aligned with the venational system of the plant, derive protection from the latex that oozes from sites of vein injury. But the system is imperfect in that latex outflow invariably restricts the potential for further effluence in tissue distal to the initial cut. Insect specialists that feed on laticiferous plants exploit this shortcoming by cutting veins at intended feeding sites, thereby inducing localized latex depletion, and then feeding distal to the cuts.

So-called "inducible" defenses of plants are also sometimes circumventable. In some plants the induction involves direct translocation of deterrent chemicals to the site of an attack, a response that some insects are able to counter by chewing an encircling trench into a leaf prior to initiation of feeding at the center of the trench.

The defense of *Dicerandra* is subject to no such counterploys. Neither vein-cutting nor trenching can affect a defense based on permanently deployed, densely spaced glandular capsules. For *Dicerandra*, the chief encumbrance may be the cost of its defense. To maintain a chemical "mining" of the entire integumental surface doubtless requires investment of substantial metabolic energy.

Pyrausta evidently copes with *Dicerandra* by virtue of insensitivity to the terpenes of the plant. It apparently ingests these, indicating that it might itself use the chemicals defensively as part of the oral effluent it emits when disturbed. If so, it would not be unique in employing such a strategy. Regurgitative use of dietary resins for defense has been documented for sawfly larvae and a caterpillar. Although *P. panopealis* had not previously been reported from *D. frutescens,* it is known to feed on other mint plants in nature. The apparent tolerance of terpenes by this insect is in itself of interest, given the known toxicity of compounds such as pulegone to other caterpillars.

A final point concerns the implication of finding a novel compound in an endangered plant. How many plants, one wonders, are now threatened with extinction worldwide as a consequence of habitat destruction, and how many unknown chemicals of potential interest are in danger of vanishing forever as a result?

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