

TRICHOMES AND ROOT HAIRS: NATURAL PESTICIDE FACTORIES

Franck E. Dayan and Stephen O. Duke from the USDA-ARS's Natural Products Utilization Research Unit (National Center for Natural Products Research) at Oxford, Mississippi, USA, describe the natural pesticides produced by these specialized structures on the epidermis of aerial and rhizosphere plant parts

Introduction

In their continual struggle to overcome pests and competitors, plants have evolved biosynthetic pathways to produce a variety of natural pesticides. The pesticide industry has capitalized on the biological activity of these phytochemicals in their efforts to discover new pesticides, usually exploiting their structural backbones to develop commercial pest control agents (Dayan, 2002). While little effort has been made to understand how and in which organs, tissues, and cells plants manufacture these compounds, some of the most potent phytochemicals are made at the interface of the plant with its biotic environment – the plant epidermis. More specifically, specialized structures on the epidermis of aerial and rhizosphere plant parts (trichomes and root hairs, respectively) often produce relatively large amounts of natural pesticides (Duke *et al.*, 2000). This short review will discuss the production of natural pesticides by these amazing structures, as well as summarizing the practical significance of this information.

Trichome and root hair anatomy

Plant anatomists define 'trichomes' as any specialized cells derived from the epidermis. Thus, from a morphological standpoint, root hairs are classified as trichomes (Werker, 2000). Interestingly, recent advances in the field of molecular biology have corroborated their somatic similarity by demonstrating that root hairs are under some of the same genetic controls as leaf, stems and flower trichomes (*e.g.*, Kellogg, 2001). For purpose of simplicity, however, the terms trichomes and root hairs will be used for specialized epidermal cells found on shoots and roots, respectively.

Trichomes have been classified into several categories, based on their morphology and other criteria (Werker, 2000), but can also be broadly categorized as either glandular or non-glandular, depending on whether or not they produce secretory products. This review will focus on glandular trichomes, as only this type of trichome produces natural compounds with pesticidal activity. Glandular or secretory trichomes can be single celled or large, multicellular structures (Figure 1a, b). These specialized structures usually secrete bioactive secondary products into the space between the cell wall and the cuticle of cells at the trichome tip (Figure 1c). The bulbous appearance of glandular trichomes is due to the cuticle stretching outward over the cells as it engorges with secretory product. The localization

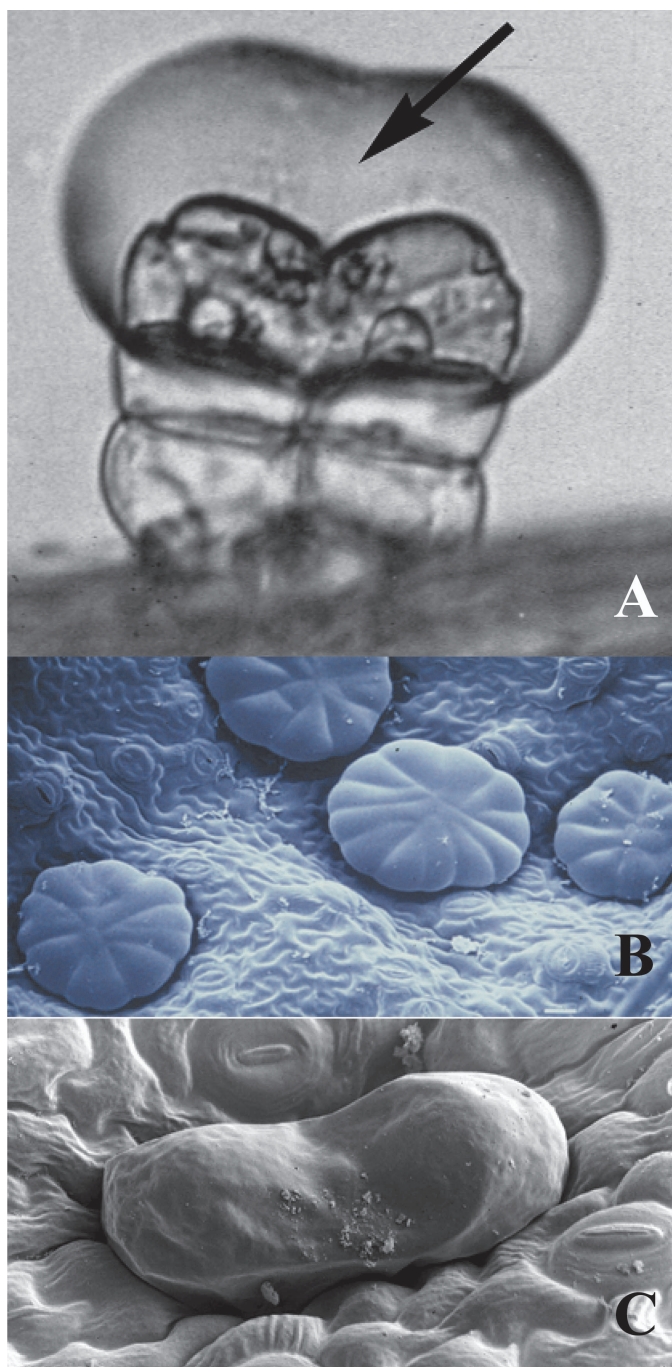


Figure 1. **A.** Light micrograph of multicellular *Artemisia annua* glandular trichome. Arrow denotes subcuticular space engorged with secretory products. **B & C.** Scanning electron micrographs of glandular trichomes of *Callicarpa americana* (**B**) and *A. annua* (**C**). Only the expanded cuticle is seen in these micrographs.

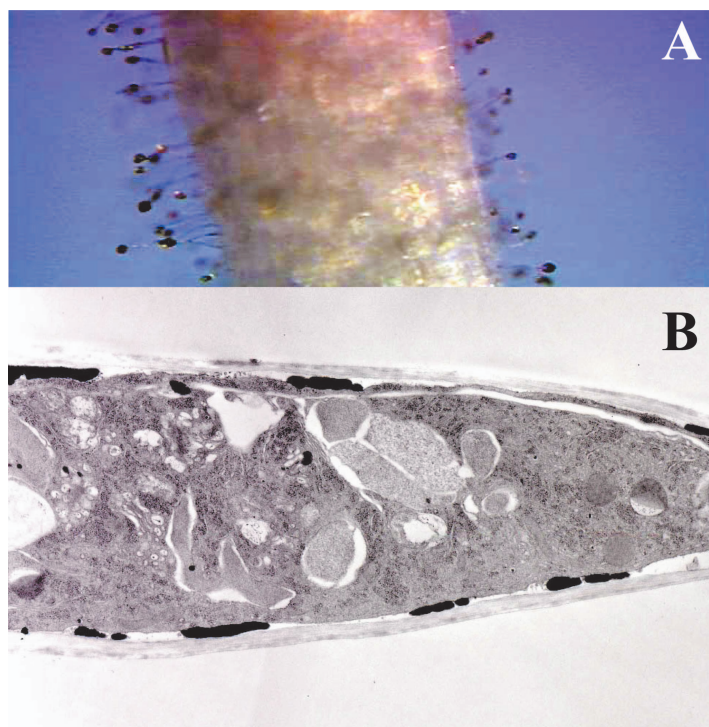


Figure 2. A. Roots of 6-day-old *Sorghum bicolor* showing oily droplets exuding from the root hairs. Sorgoleone and its analogues account for 95% of the exudate. B. Electron micrograph of *Sorghum bicolor* root hair showing numerous mitochondria and dense endoplasmic reticulum network underscoring the high level of physiological activity occurring in these cells.

of trichomes varies, with some species having trichomes on both sides of their leaves, while others have them on only one side. Some plants have trichomes on their green stems and flowers, while others do not.

Root hairs are structurally less diverse, as they all consist of protrusions of single epidermal cells. Root hairs of some species secrete secondary products in a similar fashion to glandular trichomes, the principle difference being that the material is not trapped between the cell wall and a cuticle. Root hairs of *Sorghum* spp. are an example of this phenomenon (Figure 2a). They exude oily droplets of secondary products that are up to 95% sorgoleone and its analogues (Czarnota *et al.*, 2003) (Figure 3). The secreting cells of root hairs and glandular trichomes have small or no vacuoles and are enriched in smooth endoplasmic reticulum (*e.g.*, Figure 2b), underscoring the high level of physiological activity associated with the production of these secondary metabolites.

Specialized pesticide factories

Glandular trichomes are recognized as sites of concentrated production of secondary products, many of which have strong biocidal activity. Relatively little is known about the biosynthetic

capabilities of root hairs, but recent work in our laboratory suggests that these modified epidermal cells can also produce bioactive natural products (Dayan *et al.*, 2003). Unfortunately, whether many known pesticidal natural products from plants are produced by trichomes or root hairs has not been determined. In a few carefully studied cases, glandular trichomes have been found to be the exclusive site of production of many bioactive phytochemicals. For example, many of the biologically active terpenoids of *Artemisia annua* L. are produced and stored solely in glandular trichomes (Duke *et al.*, 1994; Tellez *et al.*, 1999). Similar findings have been made with some of the compounds of cotton (reviewed in Duke *et al.*, 2000). Sorgoleone, the highly phytotoxic compound of *Sorghum* spp., is produced only by root hairs (unpublished data). Thus, these structures deserve more scrutiny in the quest for natural product-based pesticides.

The benefit associated with the location and cellular compartmentalization of the production of these bioactive compounds is at least two-fold. First, the pesticidal compounds are produced in the epidermis, the external layer most exposed to biotic challenges. Second, the biological activity of these natural products may also be harmful to the

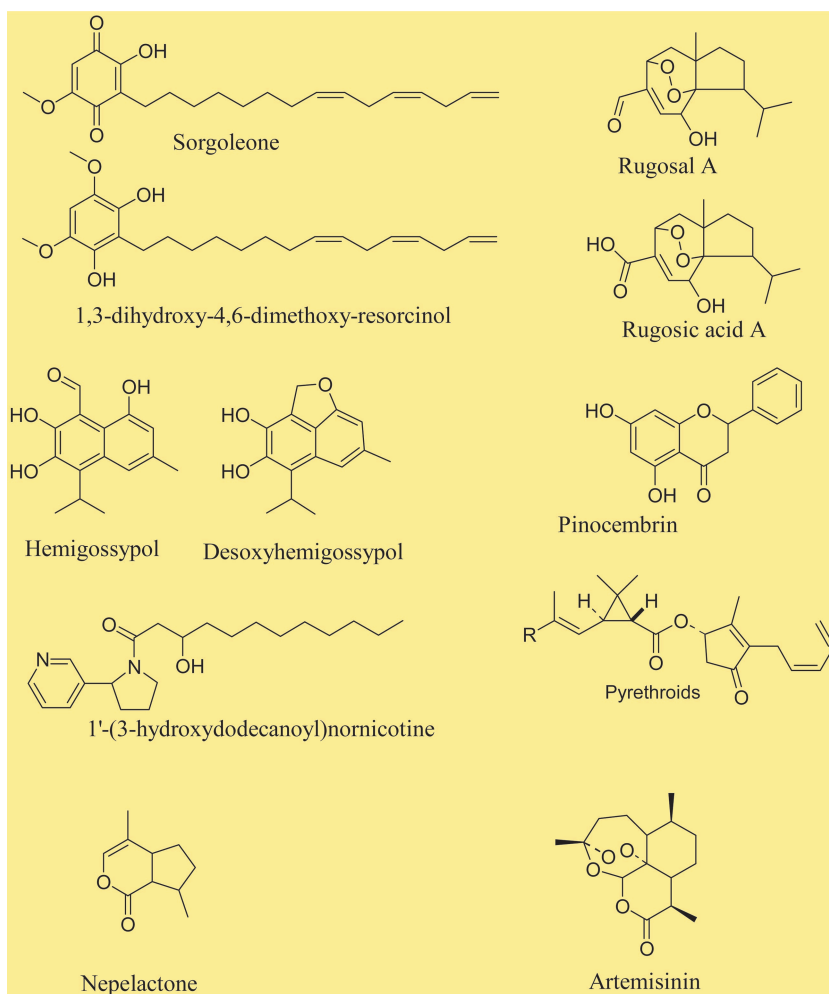


Figure 3. Chemical structures of natural pesticidal compounds produced by glandular trichomes or root hairs.

plant itself, thus requiring isolation from the rest of the plant. For example, artemisinin is as phytotoxic to the producing species, *Artemisia annua*, as to other species, but its compartmentalization apparently prevents autotoxicity (Duke *et al.*, 2000).

Antifungal agents

Plants produce numerous antifungal natural products that protect them from pathogenic fungal infection. Many of these compounds are synthesized in glands and are sometimes exuded or distributed over the leaf surfaces after rupture of the glandular cuticle. Such compounds often act as first line of defense against fungal spores that land on the surface of leaves. Numerous antifungal sesquiterpenes are produced in glandular trichomes. For example, hemigossypol and desoxyhemigossypol are produced by cotton, and rugosal A and rugosic acid are produced by *Rosa rugosa* (Figure 3). Plants compartmentalize the biosynthesis of other antifungal chemical classes in glandular trichomes, such as the flavanone pinocembrin produced by cottonwood (*Populus deltoides*) (Figure 3). The amount of antifungal compounds present in glands can increase in response to fungal infection, suggesting that plants can allocate more of their energy to synthesize compounds involved in plant defense when exposed to biotic stresses (Shain and Miller, 1982; Talley *et al.*, 2002).

Insecticidal agents

Plants produce numerous insecticidal secondary metabolites. The effectiveness of these compounds in natural settings has led to the development of commercial chemical insecticide classes based on their structural features. For example, the insecticidal properties of powders made from *Chrysanthemum* spp. flower head was known for centuries, and the discovery of the bioactive pyrethrin components (Figure 3) of this powder led to the successful development of numerous pyrethroid insecticides (*e.g.*, cypermethrin, permethrin, cyfluthrin). There is good evidence that *Chrysanthemum* spp. produce pyrethrins in specialized glands on the leaf and flower surfaces or within the epidermis (Zito *et al.*, 1983). Pyrethroid insecticides cause prolonged opening of sodium-ion channels in nerve membranes of insects, resulting in a deadly interruption of the nervous signal.

Tobacco leaves have high levels of nicotine, an alkaloid with insecticidal properties. Nicotine acts as an acetylcholine receptor agonist and is highly toxic to insects and mammals. The search for insect-specific analogs, such as nithiazin, imidacloprid, nitenpyram and acetamiprid, led to the discovery of neonicotinoid analogs that specifically bind to insect acetylcholine receptors. While most of the nicotine (Figure 3) is produced in the roots and translocated to the leaves, sometimes accumulating in trichomes, some nicotine derivatives are produced solely in glandular trichomes on the surface of leaves (Zador and Jones, 1986). Nicotine levels are increased in response to insect attack (Ohnmeiss *et al.*, 1997), indicating that glandular trichome biosynthesis of these compounds can be manipulated.

Many monoterpenes such as limonene have strong insecticidal activity and most of these are produced in glands on the surface of leaves (Colby *et al.*, 1993). Another group of monoterpenes, the nepetalactones, are produced by catmints or catnips (*Nepeta* spp.) (Figure 3). In addition to being cat attractants, these compounds are insect repellents (Hallahan, 2000). In some cases, glandular trichomes produce volatile compounds (*e.g.*, methylhexanol) in response to insect feeding (Kandra and Wagner, 1998). It is postulated that these chemical signals are used to attract other insects that either feed on or deposit their eggs on the insect causing the damage.

Herbicidal agents

Many of the compounds found in glandular trichomes are phytotoxic. For example, artemisinin, an anti-malarial sesquiterpene lactone from *Artemisia annua*, is quite phytotoxic to several plant species, including *A. annua* (Figure 3). At least one pesticide company considered using artemisinin as a herbicide lead. Its molecular target site is unknown. This compound and a number of other phytotoxic terpenoids are produced solely in the glandular trichome of *A. annua*. Glandular trichomes may be lucrative sources of natural phytotoxins from plants, as potent phytotoxins must be sequestered from the cytoplasm in places such as the subcuticular space of glandular trichomes or excreted from the cell, as found with excretion of phytotoxins by roots and root hairs. Some known phytotoxic phytochemicals may be localized in glandular trichomes, but few studies have identified the tissue or cellular locations of these compounds.

Even less is known of root hair-generated phytotoxins. Few allelopathy studies have examined root-generated phytotoxins, and even fewer have identified the cells of the root that produce such compounds. An exception is the well-studied sorgoleone, a root hair-produced allelochemical from *Sorghum* spp. This compound and several of its analogues have at least three molecular target sites, photosystem II, *p*-hydroxyphenylpyruvate dioxygenase, and a target in mitochondrial respiration. Based on dose-response studies, the first two sites appear to be the most important. Evolution of resistance to such a compound with multiple target sites would have to involve metabolic inactivation.

Using biotechnology to enhance pest control functions of trichomes and root hairs

The past decade has seen the introduction of plants made insect- and pathogen-resistant through the use of transgenes. However, this approach has not been used to manipulate trichome- or root hair-produced compounds to improve pest resistance. Certain laboratories are involved in transgenic manipulation of valuable compounds found in glandular trichomes (*e.g.*, McCaskill and Croteau, 1999; Mahmoud and Croteau, 2002). Improved pest resistance could be an indirect effect of these efforts. Our laboratory is in the early stages of manipulating sorgoleone production in root hairs of crops by molecular genetics approaches, with the intent

of reducing or eliminating the need for synthetic herbicides (Duke *et al.*, 2002).

Conclusion

In contrast to the large body of knowledge associated with numerous bioactive natural products produced by plants, strikingly little information has been published on the biosynthetic potential of trichomes and root hairs. As demonstrated by the few examples mentioned in this review, these amazing structures have an unusual ability to produce highly active compounds. The full potential of these natural chemical factories remains to be exploited.

References

- Colby, S. M.; Alonso, W. R.; Katahira, E. J.; McGarvey, D. J.; Croteau, R. (1993) 4S-Limonene synthase from the oil glands of spearmint (*Mentha spicata*). cDNA isolation, characterization, and bacterial expression of the catalytically active monoterpene cyclase. *Journal of Biological Chemistry*, **268**, 23016–23024.
- Czarnota, M. A.; Rimando, A. M.; Weston, L. A. (2003) Evaluation of root exudates of seven sorghum (*Sorghum* spp.) accessions. *Journal of Chemical Ecology* (in press).
- Dayan, F. E. (2002) Natural pesticides. Pages 521–525 in D. Pimentel, ed. *Encyclopedia of Pest Management*. Marcel Dekker, New York.
- Dayan, F. E.; Kagan, I. A., and Rimando, A. M. (2003) Elucidation of the biosynthetic pathway of the allelochemical sorgoleone using retrobiosynthetic NMR analysis. *Journal of Biological Chemistry* (in press).
- Duke, M. V.; Paul, R. N.; Elsohly, H. N.; Sturtz, G.; Duke, S. O. (1994) Localization of artemisinin and artemisitene in foliar tissues of glanded and glandless biotypes of *Artemisia annua*. *International Journal of Plant Science*, **155**, 365–373.
- Duke, S. O.; Canel, C.; Rimando, A. M.; Tellez, M. R.; Duke, M. V.; Paul, R. N. (2000) Current and potential exploitation of plant glandular trichome productivity. *Advances in Botanical Research*, **31**, 121–151.
- Duke, S. O.; Rimando, A. M.; Baerson, S. R.; Scheffler, B. E.; Ota, E.; Belz, R. G. (2002) Strategies for the use of natural products for weed management. *Journal of Pesticide Science*, **27**, 298–306.
- Hallahan, D. L. (2000) Monoterpenoid biosynthesis in glandular trichomes of Labiate plants. *Advances in Botanical Research*, **31**, 77–120.
- Kandra, L.; Wagner, G. J. (1998) Pathway for the biosynthesis of 4-methyl-1-hexanol volatilized from petal tissue of *Nicotiana sylvestris*. *Phytochemistry*, **49**, 1599–1604.
- Kellogg, E. A. (2001) Root hairs, trichomes and the evolution of genes. *Trends in Plant Science*, **6**, 550–552.
- Mahmoud, S. S.; Croteau, R. B. (2002) Strategies for transgenic manipulation of monoterpene biosynthesis in plants. *Trends in Plant Science*, **7**, 366–373.
- McCaskill, D.; Croteau, R. (1999) Strategies for bioengineering the development and metabolism of glandular tissues in plants. *Nature Biotechnology*, **17**, 31–36.
- Ohnmeiss, T. E.; McCloud, E. S.; Lynds, G. Y.; Baldwin, I. T. (1997) Within-plant relationships among wounding, jasmonic acid, and nicotine: implications for defense in *Nicotiana sylvestris*. *New Phytologist*, **137**, 441–452.
- Shain, L.; Miller, J. B. (1982) Pinocembrin: an antifungal compound secreted by leaf glands of eastern cottonwood. *Phytopathology*, **72**, 877–880.
- Talley, S. M.; Coley, P. D.; Kursar, T. A. (2002) Antifungal leaf-surface metabolites correlate with fungal abundance in Sagebrush populations. *Journal of Chemical Ecology*, **28**, 2141–2168.
- Tellez, M. R.; Canel, C.; Rimando, A. M.; Duke, S. O. (1999) Differential accumulation of isoprenoids in glanded and glandless *Artemisia annua* L. *Phytochemistry*, **52**, 1035–1040.
- Werker, E. (2000) Trichome diversity and development. *Advances in Botanical Research*, **31**, 1–35.
- Zador, E.; Jones, D. (1986) The biosynthesis of a novel nicotine alkaloid in the trichomes of *Nicotiana stocktonii*. *Plant Physiology*, **82**, 479–484.
- Zito, S. W.; Zieg, R. G.; Staba, E. J. (1983) Distribution of pyrethrins in oil glands and leaf tissue of *Chrysanthemum cinerariaefolium*. *Planta Medica*, **47**, 205–207.