

HARNESSING PLANT BIOMASS FOR BIOFUELS AND BIOMATERIALS

Harnessing plant trichome biochemistry for the production of useful compounds

Anthony L. Schillmiller¹, Robert L. Last^{1,*} and Eran Pichersky²¹Departments of Biochemistry and Molecular Biology and Plant Biology, Michigan State University, East Lansing, MI 48824-1319, USA, and²Department of Molecular, Cellular and Developmental Biology, The University of Michigan, Ann Arbor, MI 48109-1048, USA

Received 8 October 2007; revised 3 December 2007; accepted 6 December 2007.

*For correspondence (fax +1 517 353 9334; e-mail lastr@msu.edu).

Summary

Plant trichomes come in a variety of shapes, sizes and cellular composition. Some types, commonly called glandular trichomes, produce large amounts of specialized (secondary) metabolites of diverse classes. Trichomes are implicated in a variety of adaptive processes, including defense against herbivores and microorganisms as well as in ion homeostasis. Because trichomes protrude from the epidermis and can often be easily separated from it and harvested, the mRNAs, proteins and small molecules that they contain are unusually accessible to analysis. This property makes them excellent experimental systems for identification of the enzymes and pathways responsible for the synthesis of the specialized metabolites found in these structures and sometimes elsewhere in the plant. We review the literature on the biochemistry of trichomes and consider the attributes that might make them highly useful targets for plant metabolic engineering.

Keywords: glandular secreting trichome, secondary metabolites, specialized metabolism, tomato, lamiaceae, biosynthetic pathways.

Introduction

Trichomes are small protrusions of epidermal origin on the surfaces of leaves and other organs of many plants. They range in size, shape, number of cells and morphology, as well as composition, from long cotton fibers, which are seed trichomes, to tiny bumps on the surface of a leaf. Trichomes are well known to humans, even those who do not normally think about plant morphology and biochemistry. This is because they give many plants their distinctive look and smell: anyone familiar with the smell released when you handle the leaf of a tomato plant, crush fresh or dried herbs such as basil, mint or thyme, or who notices the stickiness or fuzziness of plant leaves or stems has experienced the impact of trichomes on our everyday perception of plants.

Although trichome morphology and function appear to form a continuum, trichomes are normally divided into two general categories: non-glandular and glandular. The extensively studied *Arabidopsis* trichomes are single-celled, non-glandular types (Hulskamp, 2000; Hulskamp

and Kirik, 2000; Larkin *et al.*, 2003; Walker and Marks, 2000). Storage and secreting glandular trichomes range from small structures consisting of a few cells to large and elaborate structures with differentiated basal, stalk and apical secreting cells. It is common for multiple trichome types to co-exist on the same individual or even on the same organ (see Figure 1 for examples). Consistent with their diverse structures, trichomes store or release a wide variety of different substances, ranging from insect-attracting nectars or salts to highly toxic specialized (secondary) metabolites. This review focuses primarily on the biochemistry and chemistry of trichomes that produce specialized metabolites, and readers seeking more detailed information are directed to reviews that describe trichome morphological diversity (Werker, 2000), secretion mechanisms (Fahn, 2000) and transcriptional networks that regulate trichome development in model flowering plant species (Serna and Martin, 2006).

Figure 1. Examples of plant glands visualized by scanning electron microscopy.

(a) Peltate and capitate glands on the surface of a basil leaf. The peltate glands on the leaves are located at the base of a depression.

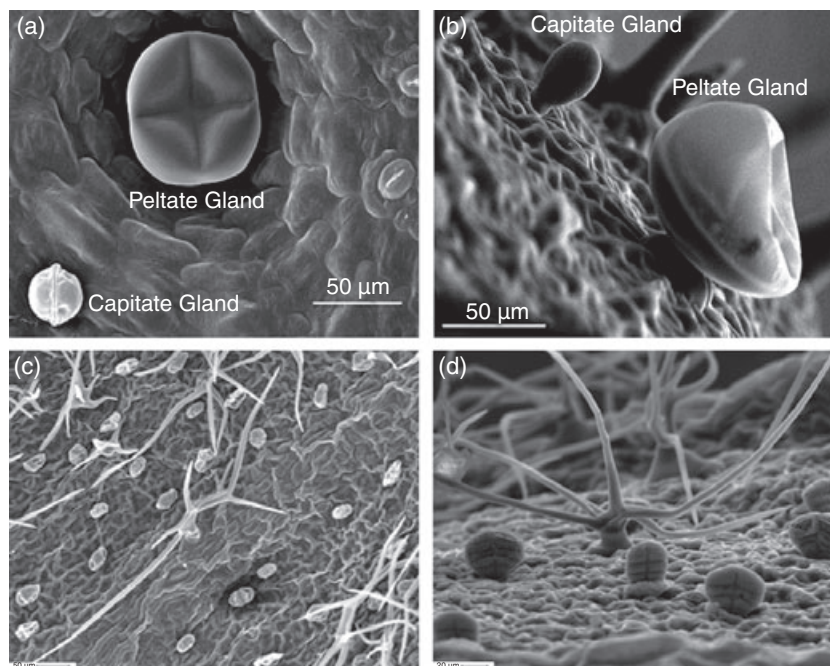
(b) Peltate and capitate glands on the surface of a basil sepal. On sepals, the peltate glands are not sunken. The cuticle above the gland cells is extended to contain the material secreted by the cells.

(c) Glands on the surface of a Russian tarragon leaf (*Artemisia dracunculus*), showing multicellular, oval-shaped glands that contain phenylpropenes, and multi-pronged trichomes.

(d) A higher-magnification side view of the Russian tarragon leaf glands.

(a, b) Reproduced from Gang *et al.*, 2001, © ASPB, with permission (American Society of Plant Biologists).

(c, d) Courtesy of Adam Schmidt (University of Michigan, USA).



One of the most remarkable features of trichomes is their capacity to synthesize, store and sometimes secrete large amounts and varied types of specialized metabolites (see Figure 2 for examples). These include various classes of terpenes (Gershenzon *et al.*, 1992; Hallahan, 2000; van der Hoeven *et al.*, 2000), as well as phenylpropanoid derivatives (Gang *et al.*, 2001), acyl sugars (Kroumova and Wagner, 2003; Li and Steffens, 2000), methylketones (Fridman *et al.*, 2005) and flavonoids (Voinir *et al.*, 1993). Many trichome-borne compounds have significant commercial value as pharmaceuticals, fragrances, food additives and natural pesticides (Duke *et al.*, 2000; Wagner, 1991; Wagner *et al.*, 2004). For this reason, the prospect of exploiting trichomes as 'chemical factories' to produce high-value plant products has recently caught the attention of plant biochemists and biotechnologists alike (Duke *et al.*, 2000; Mahmoud and Croteau, 2002; Wagner *et al.*, 2004). Because they are epidermal appendages, the contents of trichomes can be sampled relatively easily, facilitating analysis of small molecules, proteins and mRNAs (Fridman *et al.*, 2005; Gang *et al.*, 2001; Gershenzon *et al.*, 1992). This has permitted the identification of biosynthetic enzymes for a variety of pathways.

Biochemical pathway identification

Although it was proposed in the 19th century that plants produce specialized compounds to protect themselves from animals and insects (Stahl, 1888), interest in this area of chemical ecology declined in the early 20th century just as biochemistry was becoming an independent scientific area of investigation, and did not re-emerge until the late

1950s (Fraenkel, 1959). Well into the 1950s and even the 1960s, awareness of the ecological roles of what were called 'secondary' metabolites was not widespread, and the predominant view was that such chemicals were most likely waste products. Given the animal model of excretion of toxic or useless waste products, it is not surprising that trichomes, together with hydathodes, first caught the attention of the modern plant physiologists and biochemists as structures that function to remove such compounds from the plant (Uphof, 1962). For example, it was noted that the trichomes of some plants accumulate heavy metals, and that the leaf surfaces of some plants are covered by sticky material that appears to be secreted from the trichomes (reviewed by Wagner *et al.*, 2004). However, there was little interest in identifying specific compounds found in trichomes or elucidating their biosynthetic pathways, particularly as it was generally believed that such waste products were the result of random or indeterminate chemical reactions.

Systematic analysis of specialized metabolites in the trichome and elucidation of their biosynthetic pathways have enjoyed a re-awakening of interest in recent years for several reasons. Interest in these compounds was kindled by the discovery that some appear to play important roles in interaction of the plant with its biotic and abiotic environments. The demonstration of classes of compounds with significant pharmacological value or importance in the taste and odor of foods has also attracted the attention of researchers. Technical breakthroughs in analytical instrumentation have improved our ability to identify and quantify these diverse and sometimes structurally complex compounds (Last *et al.*, 2007). Finally, genomic and

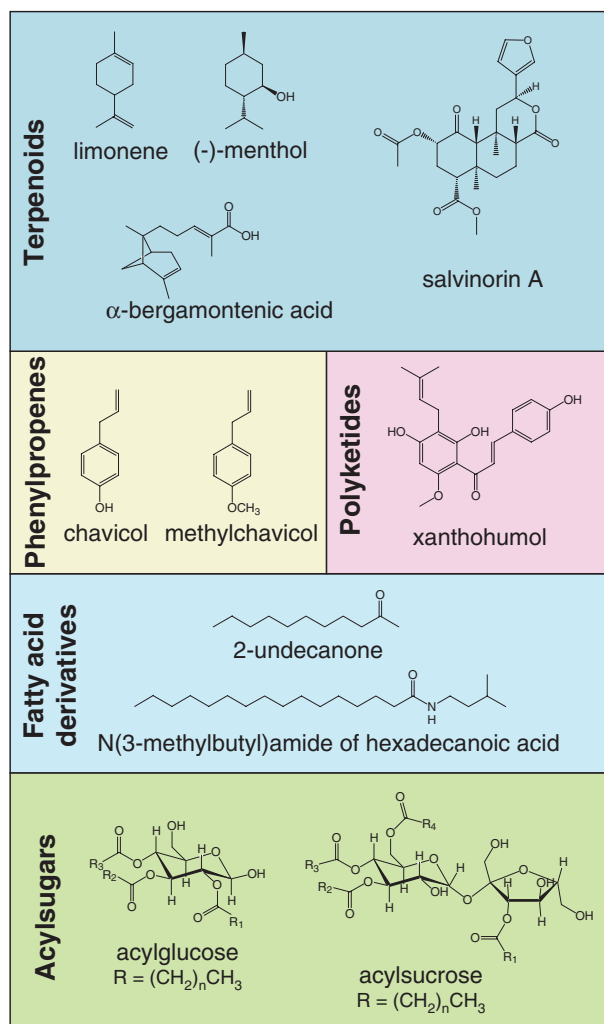


Figure 2. Examples of specialized metabolites synthesized by the trichomes of various plants.

bioinformatics approaches to identify plant genes, and molecular biology techniques to express plant genes in heterologous systems and test the proteins thus produced, have made the enzymes accessible to analysis (Fridman and Pichersky, 2005).

When considering the biosynthetic pathways for the specialized metabolites in trichomes, it is important to note that, in most cases, trichomes are not connected to the vascular system of the plant and instead are appendages extending from the epidermis. Moreover, the glandular parts of the trichomes are generally not strongly photosynthetic: many are pale green or completely non-green, and data mining of glandular trichome EST databases has indicated low levels of expression at best for genes encoding the components of the photosynthetic apparatus or enzymes of many basic biochemical pathways (e.g. Fridman *et al.*, 2005; Lange *et al.*, 2000). Analyses of such EST

databases have further shown that the trichomes operate as a closed biochemical system with a simple input and few highly active biochemical pathways of both primary metabolism (for generating energy and precursors) and specialized metabolism (for generating final products; Gang *et al.*, 2001). The primary metabolite that fulfils a dual role as the energy source and the starting material for building blocks is probably sucrose in most cases, as it can easily be imported into the trichome.

While amino acids are often required as the starting material for synthesis of specialized metabolites (e.g. phenylalanine for phenylpropanoids), it appears that import of amino acids into trichomes is minimal. In basil lines that accumulate phenylpropanoids, where the final product does not contain nitrogen, enzymes that recycle the amino group (e.g. amino transferases) are present in the trichomes (Gang *et al.*, 2001). As compounds that are produced in trichomes appear not to return to the rest of the plant, the lack of import of reduced nitrogen into the trichomes may be an evolutionary adaptation against losing this expensive resource, and may be the reason for the previously noted observation (Wagner, 1991) that, while the compounds found in trichomes represent all major classes of specialized metabolism found elsewhere in the plant, alkaloids are substantially under-represented.

In the sections below, the synthesis of some of the major compounds found in trichomes, grouped into specific classes, is discussed. It should be noted, however, that while trichomes are widespread in the plant kingdom, the majority of investigations into the biochemical composition and synthesis of specialized metabolites in trichomes have been carried out in plants belonging to two families, Solanaceae and Lamiaceae, whose species display an extensive diversity of trichome types and chemistry. In addition, while researchers have reported that compounds could be obtained by quick extraction of leaf or stem surfaces of plant species throughout the plant kingdom, direct proof that these compounds are present inside, or are derived from, trichomes (as opposed to the epidermal cell layer) is often not presented, and when such proof is present (e.g. by shearing off trichomes, isolating them and analyzing their content), the specific type of trichomes responsible for each compound is rarely determined. Therefore, while it is likely that many of these compounds are indeed derived from trichomes, information regarding surface-extractable compounds must be regarded with caution.

Terpenes

Terpenes constitute a coherent class of compounds that are all biosynthetically derived from the same basic C₅ building blocks [isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP)]. In addition to multiple isomers of the simple mono- (C₁₀), sesqui- (C₁₅) and di- (C₂₀) terpenes,

the terpene backbones are often further modified by oxidation reactions and conjugation to other moieties, and consequently the total number of plant-synthesized terpenes is estimated in the tens of thousands (see Figure 2 for examples). Terpenes are widespread in the plant kingdom and beyond, and therefore it is not surprising that the biochemical pathways leading to their biosynthesis have been highly studied. They are also present in the trichomes of many plants. The pioneering work of Croteau *et al.* (2005) on the biochemical pathway for synthesis of menthol via limonene in mint (*Mentha piperita*, Lamiaceae) peltate glands, using classical enzyme purification and EST database mining, resulted in identification of most of the enzymes of this pathway, including the heterodimeric geranyl diphosphate synthase (GPPS, an enzyme that uses IPP and DMAPP), the monoterpene synthase limonene cyclase, and additional modifying enzymes including cytochrome P450 mono-oxygenases and dehydrogenases (Croteau *et al.*, 2005).

The peltate glands of basil (*Ocimum basilicum*, Lamiaceae), which contain only four cells as opposed to the eight cells in the mint peltate glands, synthesize a large number of monoterpenes and sesquiterpenes. Some cultivars have been bred to synthesize predominantly one terpene. Lemon basil, for example, synthesizes geraniol at a high rate in its peltate glands in a reaction that uses GPP as the substrate. This reaction is catalyzed by geraniol synthase, a member of the large family of enzymes to which all plant monoterpene, sesquiterpene and diterpene synthases belong (Bohlmann *et al.*, 1998; Iijima *et al.*, 2004b). Geraniol does not accumulate in the glands but instead is further oxidized to the aldehyde geranial, which undergoes keto–enol tautomerization to form neral (Iijima *et al.*, 2006). The mixture of geranial and neral is known as citral, and it imparts a lemony flavor to this basil variety.

Another Lamiaceae species, the sage *Salvia divinorum*, produces several diterpenes with hallucinogenic properties in its peltate trichomes, the most potent of which is salvinorin A (Siebert, 2004). Salvinorin A is a highly oxidized diterpene that is also methylated and acylated (see Figure 2). Its method of synthesis has not yet been elucidated.

Solanaceae trichomes are also known to express terpene synthases and produce a variety of terpenes (Guo and Wagner, 1995). In tobacco, the diterpenes cembratrieneols (CBTols) and cembratrienediols (CBTdiols), important defense compounds, are produced in the trichomes (Wang and Wagner, 2003; Wang *et al.*, 2001). CBTols are first synthesized from geranylgeranyl diphosphate (GGPP) in a reaction catalyzed by a diterpene synthase, and can then be modified to the CBTdiols in an oxidation reaction catalyzed by a cytochrome P450 enzyme. The genes encoding these two enzymes have been shown to be expressed in the trichomes (Wang *et al.*, 2001), and, at least in the case of the cytochrome P450, specifically in the

cell at the tip of a long trichome (Wang *et al.*, 2002). Presumably, the entire terpene pathway is active in this cell. Tomato species are also known to produce various terpenes in their glands. Li *et al.* (2004) and van Schie *et al.* (2007) showed that expression of the monoterpene-forming enzyme linalool synthase, which also occurs mostly in the glands, could be induced by treating the plant with methyl jasmonate.

The trichomes of *Artemisia annua* (Asteraceae) produce the compound artemisinin, which has recently received renewed attention as a drug to treat malaria (Chang *et al.*, 2007). Artemisinin is a sesquiterpene with five oxygens, including a peroxide group (Chang *et al.*, 2007). The sesquiterpene backbone is synthesized from the precursor farnesyl diphosphate (FPP) by the enzyme amorpha-4,11-diene synthase (Mercke *et al.*, 2000), and one of the enzymes responsible for the subsequent oxidation reactions has been identified (Teoh *et al.*, 2006), although none of the enzymes responsible for the downstream steps are known.

Phenylpropanoids, polyketides and mixed-type compounds

Phenylpropanoids are also commonly found in trichomes. For example, in some varieties of basil, the peltate glands not only make terpenes but also phenylpropanoids, mostly the phenylpropenes chavicol, eugenol, methylchavicol and methyleugenol (Gang *et al.*, 2001). These compounds are synthesized from monolignol alcohol precursors in a pathway that involves acetylation, reduction and methylation (Gang *et al.*, 2002; Koeduka *et al.*, 2006). Trichomes of *Phyllyrea latifolia* (Oleaceae) accumulate flavonoid glycosides (Tattini and Gucci, 1999), and those of *Orobancha ramosa* (Orobanchaceae) accumulate both (unspecified) phenylpropanoids and monoterpenes (Sacchetti *et al.*, 2003). Flavonoid aglycones are found on the surface of many plant species (Wollenweber, 1984), and are found in the peltate glands of peppermint (*Mentha × piperita*) leaves (Voirin *et al.*, 1993), apparently dissolved in the hydrophobic terpene matrix.

Other polyketides in addition to flavonoids are also found in the trichomes. Primin, a benzoquinone that causes dermatitis, is synthesized by a polyketide synthase in the trichomes of *Primula obconica* (Primulaceae) from hexanoyl CoA and three malonyl CoA units, giving the intermediate olivetolic acid, followed by decarboxylation, hydroxylation and methylation (Horper and Marner, 1996). In the family Cannabaceae, the trichomes of *Cannabis sativa* produce and accumulate the psychoactive tetrahydrocannabinol, which is synthesized through C10 prenylation of olivetolic acid, followed by a cyclization reaction and decarboxylation (Raharjo *et al.*, 2004). In hops (*Humulus lupulus*, Cannabaceae), which provide flavor to beer, the trichomes contain, in addition to terpenes, many

C5-prenylated polyketides, with the major compound being xanthohumol (Figure 2; Matousek *et al.*, 2002). Lower levels of more oxidized prenylated polyketides, the so-called 'bitter acids' (e.g. humulone), are also present and are important for the flavor (Fung *et al.*, 1997). Analysis of an EST database constructed from hops glands, followed by biochemical assays of candidate enzymes, have recently identified a methyltransferase involved in the synthesis of xanthohumol (Nagel *et al.*, 2008).

Fatty acid derivatives

Many specialized metabolites in trichomes are derived from fatty acids or have fatty acid moieties. As described above, the synthesis of olivetolic acid, which is one of the precursors in the synthesis of both primin and tetrahydrocannabinol, requires hexanoic acid as a precursor. The type VI trichomes of *Solanum habrochaites glabratum* (previously named *Lycopersicon hirsutum glabratum*) synthesize insecticidal C11, C13 and C15 methylketones by hydrolysis of the β -ketoacyl ACP intermediates of fatty acid biosynthesis, followed by decarboxylation (Fridman *et al.*, 2005). Both reactions are catalyzed by the same enzyme, methylketone synthase (MKS), which is related to other plant esterases (Fridman *et al.*, 2005). Analysis of a type VI-specific EST database showed that the genes encoding enzymes for fatty acid biosynthesis in the plastids are highly expressed, as is MKS (Fridman *et al.*, 2005).

In the type IV glands of the related species *Solanum pennellii* (previously *Lycopersicon pennellii*), as well as in many other Solanaceae species, including tobacco, *Datura* and *Petunia* spp., glucose and sucrose are acylated with 3–4 acyl groups of both straight and branched chains ranging in carbon number from 2 to 12 (Van Dam and Hare, 1998). The resulting polyester acyl sugars (Figure 2) are secreted from the glands, sometimes in relatively large quantities, and the exudate causes the plant surface to become sticky and provides a strong deterrent to insects. The first acylation reaction of glucose is catalyzed by UDP-glucose fatty acid:glycosyltransferase, and additional acylations of the sugar moiety appear to occur by a series of disproportionation reactions, the first of which is catalyzed by an enzyme that belongs to the serine carboxypeptidase family (Li and Steffens, 2000). The subsequent reactions have not been yet characterized. The acyl groups are derived from elongation of short straight and branched fatty acids, which themselves are derived from degradation of amino acids (Walters and Steffens, 1990). It appears that, in *Solanum* and *Datura* species, elongation occurs via the fatty acid biosynthetic pathway, thus adding two carbons to the chain per cycle, while in tobacco and petunia, elongation occurs via the α -ketoacid route that also operates in leucine biosynthesis as well as in the synthesis of glucosinolates (Kroumova and Wagner, 2003).

Ranger *et al.* (2005) have reported that the stem trichomes of an alfalfa (*Medicago sativa*, Leguminosae) line that is resistant to potato leafhopper contain a series of *N*(3-methylbutyl)amides of saturated C14, C15, C16 and C18 fatty acids, and that these compounds may contribute to the observed resistance (see Figure 2 for an example). The enzymes responsible for the synthesis of these amides have not yet been described.

Alkaloids

As discussed above, alkaloids are under-represented among specialized metabolites found in glands, and there have been no reports on the *de novo* synthesis of alkaloids in glands. However, tobacco species are known to contain the toxins nicotine and related alkaloids. Nicotine itself is synthesized in the roots and is mobilized to all aerial parts of the plant, including trichomes, upon herbivory (Zador and Jones, 1986). While nicotine predominates in roots, leaves and trichomes contain higher levels of nornicotine (demethylated nicotine) than of nicotine. Neither nicotine nor nornicotine are appreciably secreted from the plant. However, the trichomes produce a stress-induced enzymatic activity that acylates nornicotine with C10–C14 straight-chain fatty acids and C10–C14 straight-chain β -hydroxy fatty acids. These hydrophobic *N*-acyl nornicotines are then secreted from the gland to coat the leaf surface (Laue *et al.*, 2000; Zador and Jones, 1986).

Trichomes without high levels of specialized metabolites

While trichomes of many species have been found to synthesize high levels of one or a few specialized metabolites, it is likely that many others do not. For example, Aziz *et al.* (2005) reported that there are only low levels of specialized metabolites, mostly phenylpropanoids and fatty acids, in the trichomes of the alfalfa line that is resistant to the potato leafhopper (but see Ranger *et al.*, 2005; discussed above). These glands are also unusually photosynthetically active, and analysis of an EST database prepared from RNA extracted from both the gland and stalk cells indicated that transcripts of genes involved in photosynthesis are highly represented. In contrast, genes involved in metabolism are expressed in these trichomes at similar frequency to that seen in other parts of the plant (Aziz *et al.*, 2005). In the trichome ('stinging hair') of the stinging nettle (*Urtica thunbergiana*, Urticaceae), a needle-shaped structure with a pointed, secreting cell at the tip, it was recently shown that, previous reports notwithstanding, the pain-causing compounds are neither the alkaloids serotonin and histamines nor formic acid, which are present at very low concentrations, but oxalic and tartaric acids, the latter being the most abundant (Fu *et al.*, 2006).

It is possible that many types of trichomes that appear non-glandular will nevertheless be found to have active metabolism upon further examination. For example, *Arabidopsis* trichomes were serendipitously discovered to accumulate sinapyl esters (Sinlapadech *et al.*, 2007), and the gene encoding the enzyme responsible for the formation of methylsalicylate was found to be highly expressed in these trichomes (Chen *et al.*, 2003). The lack of prior chemical or ecological knowledge suggesting the presence of active compounds in a given type of trichome in a given species, however, is a hindrance for such a system to become a focus of investigation.

Gland-specific biosynthetic reactions and the evolution of new specialized metabolites

Many of the biosynthetic pathways operating in trichomes are also present elsewhere in the plant. However, often one or a few unique enzymes operate in the trichome to further modify the end product of the 'standard' pathway. For example, some of the highly functionalized prenylated flavonoids are found uniquely in the trichomes of hops; the prenylation makes the flavonoids more hydrophobic and most likely allows them to be secreted. Presumably, the hop genome evolved genes encoding specific prenyltransferases that are expressed only in the trichome, although currently there is no published information about such genes and enzymes. Another example is the synthesis of methylketones, which relies on a single new enzyme, MKS, to intercept the β -ketoacyl ACP intermediates of the fatty acid biosynthetic pathway and convert them to the final methylketone products. The gene encoding MKS is expressed only in type VI trichomes, which also show high levels of expression of genes encoding fatty acid biosynthesis (Fridman *et al.*, 2005). It is intriguing that the fatty acid biosynthetic pathway also contributes to synthesis of the medium-chain fatty acids that are used to synthesize abundant acyl sugars in type IV secreting trichomes of another wild tomato species, *S. pennellii*. In this case, expression of an evolutionarily novel serine carboxypeptidase-like acyltransferase provides the biosynthetic potential to produce these unusual polyesters (Li and Steffens, 2000). This observation suggests that robust fatty acid biosynthesis, or at least the potential to easily increase the flux of this pathway, in the glands of Solanaceae species is an ancestral trait, and the availability of high levels of fatty acids and their precursors provided substrates for further metabolic evolution.

Interplay among competing pathways

Because the biosynthetic capacity of the trichomes is limited by the amount and type of the carbon source imported into them, it is not surprising that, even when a

given type of trichome is capable of synthesizing different classes of compounds, the total output is limited. For example, the peltate glands of various cultivars of basil synthesize either predominantly terpenes, predominantly phenylpropenes or a mixture of both, and the output of one class of compounds is inversely correlated with the levels of the other (Iijima *et al.*, 2004a). Similarly, the type VI glands of *S. habrochaites glabratum* synthesize high amounts of methylketones and low amounts of terpenes, while the glands of some other *S. habrochaites* accessions contain high levels of sesquiterpene acids and no methylketones (Fridman *et al.*, 2005). These differences are controlled at the transcriptional level, although the components of the regulatory mechanism have not yet been identified.

Trichome defense proteins

Although the majority of work aimed at understanding trichome function has focused on the specialized metabolites produced within the trichome, more recent studies have demonstrated that proteins that do not function in traditional biosynthetic pathways are produced by trichomes in significant amounts and contribute to trichome function. Proteinase inhibitor proteins, which interfere with herbivore physiology by inhibiting the digestive proteinases of insects and animals, are known to accumulate in many plant tissues, either constitutively (for example in flowers) or inducibly upon wounding or insect feeding (e.g. in leaves). Given the ability of trichomes to act as a first line of defense on the surface of tissues consumed by herbivores, identification of a constitutively expressed *proteinase inhibitor 2b* (*SaPIN2b*) gene in *S. americanum* (nightshade) trichomes further supports a role for trichomes as more than simply physical barriers in plant defense (Liu *et al.*, 2006). Surprisingly, ectopic overexpression of *SaPIN2b* in transgenic tobacco caused an increase in the density of trichomes and promoted branching, but the mechanism by which this occurs is not known. Analysis of trichome density on transgenic plants with reduced levels of *SaPIN2b* should provide further insight into this observed phenotype.

In addition to proteinase inhibitors, trichomes of many *Solanum* species accumulate significant levels of polyphenol oxidase (Yu *et al.*, 1992). Members of this family of enzymes oxidize phenolics to highly reactive quinone compounds, and studies of polyphenol oxidase have led to many proposed functions, including defense against insects and pathogens as well as potential roles in the biosynthesis of phenolic specialized metabolites (reviewed by Mayer, 2006). In tomato, mRNAs for individual isoforms of the polyphenol oxidase gene family have been shown to be expressed in specific trichome types and not in others (Thipyapong *et al.*, 1997).

Proteins and peptides known to be involved in responses to toxins and heavy metal stress were also shown to be present at high levels in trichomes. In *Arabidopsis*, the concentration of glutathione in trichome cells was reported to be two to three times greater than in pavement or trichome basement cells (Gutierrez-Alcala *et al.*, 2000). In addition to the function of glutathione in detoxification of xenobiotics and as an antioxidant, it is a precursor for phytochelatin, which functions in the sequestration of heavy metals in the vacuole. High glutathione concentrations may allow trichomes to play a role in relieving heavy metal stress in plants (Küpper *et al.*, 2000; Salt *et al.*, 1995; Sarret *et al.*, 2002). In tobacco, trichomes have been shown to secrete metal (Cd, Zn) substituted calcium crystals (Choi *et al.*, 2001; Sarret *et al.*, 2006) when grown in medium supplemented with toxic levels of these metals. It is still not clear how the metals are accumulated in the trichomes and whether phytochelatins play a role in excretion of metal-substituted calcium crystals by the trichomes. There are also reports of high expression of metallothionein-like genes in trichomes of *Vicia faba* and *Arabidopsis* (Foley and Singh, 1994; Guo *et al.*, 2003). Metallothioneins are low-molecular-weight metal ion-binding proteins originally described in animals, and were recently shown to play a role in metal tolerance in plants (Zimeri *et al.*, 2005).

In addition to secreting small molecules (for example acyl sugars), recent evidence indicates that trichomes of tobacco actively secrete water-soluble low-molecular-weight glycoproteins, known as phylloplanins, to the leaf surface (Kroumova *et al.*, 2007; Shepherd *et al.*, 2005). These studies demonstrated that phylloplanins can inhibit spore germination and the onset of blue-mold disease caused by the oomycete pathogen *Peronospora tabacina*; tobacco plants with reduced levels of phylloplanin mRNA and protein are more susceptible to pathogen infection. These small proteins deposited at the leaf surface thus represent an early line of defense against microbial invaders. Pathogen spore germination assays using water-soluble leaf proteins from other plants showed that leaf surface-localized antimicrobial proteins are not restricted to tobacco (Kroumova *et al.*, 2007). However, it is not yet known whether the leaf surface proteins on other plants are homologous to the tobacco phylloplanins. Other peptides, including pathogenesis-related proteins, have been shown to be secreted to the leaf surface of barley (*Hordeum vulgare*) and tobacco (*Nicotiana tabacum*) by guttation of fluid from hydathodes, a mechanism that does not involve trichomes (Grunwald *et al.*, 2003; Komarnytsky *et al.*, 2000). Although secretion of small molecules to the leaf surface from trichomes is thought to occur either through cuticular pores or simply following rupture of the cuticle, the exact mechanism for secretion of larger peptides and proteins from trichomes is not known (Fahn, 2000).

Global analysis of mRNA and proteins expressed in trichomes

Although our understanding of trichome-localized metabolites has greatly increased, identification of the genes and enzymes controlling specialized metabolism in trichomes has proceeded at a much slower pace. EST sequencing of trichome-specific cDNA libraries from various plants has yielded the majority of genes known to function in trichome metabolic pathways (e.g. Aziz *et al.*, 2005; Fridman *et al.*, 2005; Gang *et al.*, 2001; Lange *et al.*, 2000; Teoh *et al.*, 2006). Aziz *et al.* (2005) reported a microarray experiment comparing transcripts from isolated trichomes to those from stems with the trichomes removed as another way of identifying genes that are preferentially expressed in trichomes. Obviously, this approach is only applicable when using plant species for which microarrays are available. However, reductions in sequencing costs, and, more significantly, new sequencing technologies such as single molecule sequencing by synthesis (Margulies *et al.*, 2005), will allow larger-scale genomics projects with the promise of increased identification of genes controlling specialized metabolism.

Likewise, advances in proteomics technologies provide another avenue for identifying candidate enzymes. Amme *et al.* (2005) reported the use of two-dimensional gel electrophoresis to separate proteins from tobacco trichomes, and identified several stress-related proteins including a superoxide dismutase and glutathione peroxidase. Because of the relatively small number of available tobacco genomic sequences, the authors used *de novo* sequencing of peptides for most protein identifications, rather than using the un-interpreted probability-based matching algorithms (e.g. Mascot, <http://www.matrixscience.com>) that are suitable for organisms with larger sequence databases. For organisms with a full genomic sequence or for which a large collection of EST sequences is available, higher-throughput shotgun proteomics experiments are possible. Wienkoop *et al.* (2004) reported the use of a nano-LC/MS/MS based method for profiling *Arabidopsis* trichome proteins. This method allowed the use of smaller tissue samples and had reduced bias against certain protein classes compared with two-dimensional SDS-PAGE. Of the 63 proteins identified, several were involved in sulfur metabolism, consistent with the previously reported finding of high glutathione levels in *Arabidopsis* trichomes (Gutierrez-Alcala *et al.*, 2000). However, due to the lack of DNA sequence information for many organisms with interesting trichome chemistry, the problem of identifying trichome proteins from proteomic mass spectra remains a serious one. Improvements in cost-effective DNA sequencing should allow researchers to combine DNA sequencing and proteomics experiments when using organisms with few available genomics resources.

Turning trichomes into factories

Many of the characteristics associated with glandular trichomes have generated interest in using them to engineer the production of large amounts of specialized chemicals or proteins. One appealing feature of trichomes is that they are dispensable structures; trichome-deficient mutants of *Arabidopsis* and other plants grow normally under controlled environment conditions, and in general there is little evidence that compounds made in trichomes are ever re-absorbed into the rest of the plant. This suggests that modifying the original chemistry of the trichomes and engineering them to make and accumulate novel small molecules or proteins will not have deleterious effects on the plant, even if the engineered product would have phytotoxic effects if accumulated in other cell types. In addition, trichomes appear to be programmed to single-mindedly produce large amounts of specific small molecules or proteins, a precondition for the production of useful quantities of novel products. Examples of coordinated regulation of genes in trichome biosynthetic pathways suggest that successful engineering of metabolism will require the use of appropriate promoters to drive the expression of pathway-modifying enzymes (Gang *et al.*, 2001; McConkey *et al.*, 2000). Unlike other structures that are specialized for high-level production of specialized metabolites (for example, laticifers and resin ducts), trichomes are accessible to purification and analysis. In addition, there is an increasing understanding of the genetic and physiological regulatory networks that specify trichome differentiation and patterning (Hulskamp, 2000; Larkin *et al.*, 2003; Li *et al.*, 2004; Serna and Martin, 2006). Taken together, trichomes offer a unique opportunity to understand and engineer the development of structures that produce and sequester specialized metabolites, and the pathways leading to them.

While these characteristics suggest that trichomes are good targets for metabolic engineering, the unusual structures and biochemistry of trichomes could create barriers to their utility. The observation that most trichomes are not photosynthetic suggests that limited amounts of sugars and biosynthetic intermediates are available to biosynthetic pathways in the trichome. Depending on the reason for modifying trichome metabolism, the fact that some trichomes secrete the chemicals they produce rather than store them may complicate efforts to recover engineered metabolites. Another major concern arises from the knowledge that these structures are so strongly oriented to making large amounts of a few specific products. If relatively few biosynthetic pathways operate in each type of trichome, engineering novel specialized metabolic pathways in a given type could be thwarted by a lack of availability of biosynthetic precursors. It is likely that it would be more difficult to engineer novel biosynthetic pathways than to introduce variations in an existing pathway. However, a recently

published example that involved engineering the conversion of tyrosine to melanin in cotton fibers (which are highly modified trichomes) suggests that lack of primary metabolic precursors is not always an insurmountable problem (Xu *et al.*, 2007). Unleashing the bioengineering potential of trichomes will require a deeper understanding of the biosynthetic pathways operating in these fascinating chemical factories.

Acknowledgements

Work on trichome biology in the Last laboratory is funded by NSF grants DBI-0604336 and DBI-0619489 and that in the Pichersky laboratory is funded by grant DBI-0604336 and by National Research Initiative of the USDA Cooperative State Research, Education and Extension Service grant 2004-35318-14874.

References

- Amme, S., Rutten, T., Melzer, M., Sonsmann, G., Vissers, J.P., Schlesier, B. and Mock, H.P. (2005) A proteome approach defines protective functions of tobacco leaf trichomes. *Proteomics*, **5**, 2508–2518.
- Aziz, N., Paiva, N.L., May, G.D. and Dixon, R.A. (2005) Transcriptome analysis of alfalfa glandular trichomes. *Planta*, **221**, 28–38.
- Bohlmann, J., Meyer-Gauen, G. and Croteau, R. (1998) Plant terpenoid synthases: molecular biology and phylogenetic analysis. *Proc. Natl Acad. Sci. USA*, **95**, 4126–4133.
- Chang, M.C., Eachus, R.A., Trieu, W., Ro, D.K. and Keasling, J.D. (2007) Engineering *Escherichia coli* for production of functionalized terpenoids using plant P450s. *Nat. Chem. Biol.* **3**, 274–277.
- Chen, F., D'Auria, J.C., Tholl, D., Ross, J.R., Gershenzon, J., Noel, J.P. and Pichersky, E. (2003) An *Arabidopsis thaliana* gene for methylsalicylate biosynthesis, identified by a biochemical genomics approach, has a role in defense. *Plant J.* **36**, 577–588.
- Choi, Y.E., Harada, E., Wada, M., Tsuboi, H., Morita, Y., Kusano, T. and Sano, H. (2001) Detoxification of cadmium in tobacco plants: formation and active excretion of crystals containing cadmium and calcium through trichomes. *Planta*, **213**, 45–50.
- Croteau, R.B., Davis, E.M., Ringer, K.L. and Wildung, M.R. (2005) (-)-Menthol biosynthesis and molecular genetics. *Naturwissenschaften*, **92**, 562–577.
- Duke, S.O., Canel, C., Rimando, A.M., Tellez, M.R., Duke, M.V. and Paul, R.N. (2000) Current and potential exploitation of plant glandular trichome productivity. *Adv. Bot. Res.* **31**, 121–151.
- Fahn, A. (2000) Structure and function of secretory cells. In *Plant Trichomes* (Hallahan, D.L. and Gray, J.C., eds). New York: Academic Press, pp. 37–75.
- Foley, R.C. and Singh, K.B. (1994) Isolation of a *Vicia faba* metallothionein-like gene: expression in foliar trichomes. *Plant Mol. Biol.* **26**, 435–444.
- Fraenkel, G.S. (1959) The raison d'être of secondary plant substances; these odd chemicals arose as a means of protecting plants from insects and now guide insects to food. *Science*, **129**, 1466–1470.
- Fridman, E. and Pichersky, E. (2005) Metabolomics, genomics, proteomics, and the identification of enzymes and their substrates and products. *Curr. Opin. Plant Biol.* **8**, 242–248.
- Fridman, E., Wang, J., Iijima, Y., Froehlich, J.E., Gang, D.R., Ohlrogge, J. and Pichersky, E. (2005) Metabolic, genomic, and biochemical analyses of glandular trichomes from the wild

- tomato species *Lycopersicon hirsutum* identify a key enzyme in the biosynthesis of methylketones. *Plant Cell*, **17**, 1252–1267.
- Fu, H.Y., Chen, S.J., Chen, R.F., Ding, W.H., Kuo-Huang, L.L. and Huang, R.N.** (2006) Identification of oxalic acid and tartaric acid as major persistent pain-inducing toxins in the stinging hairs of the nettle, *Urtica thunbergiana*. *Ann. Bot.* **98**, 57–65.
- Fung, S.Y., Zuurbier, K.W.M., Paniego, N.B., Scheffer, J.J.C. and Verpoorte, R.** (1997) Conversion of deoxyhumulone into the hop alpha-acid humulone. *Phytochemistry*, **44**, 1047–1053.
- Gang, D.R., Wang, J., Dudareva, N., Nam, K.H., Simon, J.E., Lewinsohn, E. and Pichersky, E.** (2001) An investigation of the storage and biosynthesis of phenylpropenes in sweet basil. *Plant Physiol.* **125**, 539–555.
- Gang, D.R., Lavid, N., Zubieta, C., Chen, F., Beuerle, T., Lewinsohn, E., Noel, J.P. and Pichersky, E.** (2002) Characterization of phenylpropene O-methyltransferases from sweet basil: facile change of substrate specificity and convergent evolution within a plant O-methyltransferase family. *Plant Cell*, **14**, 505–519.
- Gershenzon, J., McCaskill, D., Rajanarivony, J.I., Mihaliak, C., Karp, F. and Croteau, R.** (1992) Isolation of secretory cells from plant glandular trichomes and their use in biosynthetic studies of monoterpenes and other gland products. *Anal. Biochem.* **200**, 130–138.
- Grunwald, I., Rupprecht, I., Schuster, G. and Klopstschek, K.** (2003) Identification of guttation fluid proteins: the presence of pathogenesis-related proteins in non-infected barley plants. *Physiol. Plant.* **119**, 192–202.
- Guo, Z. and Wagner, G.J.** (1995) Biosynthesis of labdenediol and sclareol in cell-free extracts from trichomes of *Nicotiana glutinosa*. *Planta*, **197**, 627–632.
- Guo, W.-J., Bundithya, W. and Goldsbrough, P.B.** (2003) Characterization of the Arabidopsis metallothionein gene family: tissue-specific expression and induction during senescence and in response to copper. *New Phytol.* **159**, 369–381.
- Gutierrez-Alcala, G., Gotor, C., Meyer, A.J., Fricker, M., Vega, J.M. and Romero, L.C.** (2000) Glutathione biosynthesis in Arabidopsis trichome cells. *Proc. Natl Acad. Sci. USA*, **97**, 11108–11113.
- Hallahan, D.L.** (2000) Monoterpene biosynthesis in glandular. In *Plant Trichomes* (Hallahan, D.L. and Gray, J.C., eds). New York: Academic Press, pp. 77–120.
- van der Hoeven, R.S., Monforte, A.J., Breeden, D., Tanksley, S.D. and Steffens, J.C.** (2000) Genetic control and evolution of sesquiterpene biosynthesis in *Lycopersicon esculentum* and *L. hirsutum*. *Plant Cell*, **12**, 2283–2294.
- Horper, W. and Marner, F.J.** (1996) Biosynthesis of primin and miconidin and its derivatives. *Phytochemistry*, **41**, 451–456.
- Hulskamp, M.** (2000) Cell morphogenesis: how plants split hairs. *Curr. Biol.* **10**, R308–R310.
- Hulskamp, M. and Kirik, V.** (2000) Trichome differentiation and morphogenesis in Arabidopsis. In *Plant Trichomes* (Hallahan, D.L. and Gray, J.C., eds). New York: Academic Press, pp. 237–260.
- Iijima, Y., Davidovich-Rikanati, R., Fridman, E., Gang, D.R., Bar, E., Lewinsohn, E. and Pichersky, E.** (2004a) The biochemical and molecular basis for the divergent patterns in the biosynthesis of terpenes and phenylpropenes in the peltate glands of three cultivars of basil. *Plant Physiol.* **136**, 3724–3736.
- Iijima, Y., Gang, D.R., Fridman, E., Lewinsohn, E. and Pichersky, E.** (2004b) Characterization of geraniol synthase from the peltate glands of sweet basil. *Plant Physiol.* **134**, 370–379.
- Iijima, Y., Wang, G., Fridman, E. and Pichersky, E.** (2006) Analysis of the enzymatic formation of citral in the glands of sweet basil. *Arch. Biochem. Biophys.* **448**, 141–149.
- Koeduka, T., Fridman, E., Gang, D.R. et al.** (2006) Eugenol and isoeugenol, characteristic aromatic constituents of spices, are biosynthesized via reduction of a coniferyl alcohol ester. *Proc. Natl Acad. Sci. USA*, **103**, 10128–10133.
- Komarnytsky, S., Borisjuk, N.V., Borisjuk, L.G., Alam, M.Z. and Raskin, I.** (2000) Production of recombinant proteins in tobacco guttation fluid. *Plant Physiol.* **124**, 927–934.
- Kroumova, A.B. and Wagner, G.J.** (2003) Different elongation pathways in the biosynthesis of acyl groups of trichome exudate sugar esters from various solanaceous plants. *Planta*, **216**, 1013–1021.
- Kroumova, A.B., Shepherd, R.W. and Wagner, G.J.** (2007) Impacts of T-phylloplanin gene knockdown and of Helianthus and Datura phylloplanins on *Peronospora tabacina* spore germination and disease potential. *Plant Physiol.* **144**, 1843–1851.
- Küpper, H., Lombi, E., Zhao, F.-J. and McGrath, S.P.** (2000) Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri*. *Planta*, **212**, 75–84.
- Lange, B.M., Wildung, M.R., Stauber, E.J., Sanchez, C., Pouchnik, D. and Croteau, R.** (2000) Probing essential oil biosynthesis and secretion by functional evaluation of expressed sequence tags from mint glandular trichomes. *Proc. Natl Acad. Sci. USA*, **97**, 2934–2939.
- Larkin, J.C., Brown, M.L. and Schiefelbein, J.** (2003) How do cells know what they want to be when they grow up? Lessons from epidermal patterning in Arabidopsis. *Annu. Rev. Plant Biol.* **54**, 403–430.
- Last, R.L., Jones, A.D. and Shachar-Hill, Y.** (2007) Towards the plant metabolome and beyond. *Nat. Rev. Mol. Cell. Biol.* **8**, 167–174.
- Laue, G., Preston, C.A. and Baldwin, I.T.** (2000) Fast track to the trichome: induction of N-acyl nornicotines precedes nicotine induction in *Nicotiana repanda*. *Planta*, **210**, 510–514.
- Li, A.X. and Steffens, J.C.** (2000) An acyltransferase catalyzing the formation of diacylglycerol is a serine carboxypeptidase-like protein. *Proc. Natl Acad. Sci. USA*, **97**, 6902–6907.
- Li, L., Zhao, Y., McCaig, B.C., Wingerd, B.A., Wang, J., Whalon, M.E., Pichersky, E. and Howe, G.A.** (2004) The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. *Plant Cell*, **16**, 126–143.
- Liu, J., Xia, K.F., Zhu, J.C., Deng, Y.G., Huang, X.L., Hu, B.L., Xu, X. and Xu, Z.F.** (2006) The nightshade proteinase inhibitor IIb gene is constitutively expressed in glandular trichomes. *Plant Cell Physiol.* **47**, 1274–1284.
- Mahmoud, S.S. and Croteau, R.B.** (2002) Strategies for transgenic manipulation of monoterpene biosynthesis in plants. *Trends Plant Sci.* **7**, 366–373.
- Margulies, M., Egholm, M., Altman, W.E. et al.** (2005) Genome sequencing in microfabricated high-density picolitre reactors. *Nature*, **437**, 376.
- Matousek, J., Novak, P., Patzak, J., Briza, J. and Krofta, K.** (2002) Analysis of true chalcone synthase from *Humulus lupulus* L. and biotechnology aspects of medicinal hops. *Rostlinna Vyroba*, **48**, 7–14.
- Mayer, A.M.** (2006) Polyphenol oxidases in plants and fungi: going places? A review. *Phytochemistry*, **67**, 2318–2331.
- McConkey, M.E., Gershenzon, J. and Croteau, R.B.** (2000) Developmental regulation of monoterpene biosynthesis in the glandular trichomes of peppermint. *Plant Physiol.* **122**, 215–223.
- Mercke, P., Bengtsson, M., Bouwmeester, H.J., Posthumus, M.A. and Brodelius, P.E.** (2000) Molecular cloning, expression, and characterization of amorpho-4,11-diene synthase, a key enzyme

- of artemisinin biosynthesis in *Artemisia annua* L. *Arch. Biochem. Biophys.* **381**, 173–180.
- Nagel, J., Culley, L.K., Lu, Y., Liu, E., Matthews, P.D., Stevens, J.F. and Page, J.E.** (2008) EST analysis of hop glandular trichomes identifies an *O*-methyltransferase that catalyzes the biosynthesis of xanthohumol. *Plant Cell*, doi: 10.1105/tpc.107.055178.
- Raharjo, T.J., Chang, W.T., Choi, Y.H., Peltenburg-Looman, A.M.G. and Verpoorte, R.** (2004) Olivetol as product of a polyketide synthase in *Cannabis sativa* L. *Plant Sci.* **166**, 381–385.
- Ranger, C.M., Winter, R.E.K., Rottinghaus, G.E., Backus, E.A. and Johnson, D.W.** (2005) Mass spectral characterization of fatty acid amides from alfalfa trichomes and their deterrence against the potato leafhopper. *Phytochemistry*, **66**, 529–541.
- Sacchetti, G., Ballero, M., Serafini, M., Muzzoli, M., Tosi, B. and Poli, F.** (2003) Morphological and histochemical investigation on glandular trichomes of *Orobancha ramosa* subsp. *nana* (Orobanchaceae). *Phyton*, **43**, 207–214.
- Salt, D.E., Prince, R.C., Pickering, I.J. and Raskin, I.** (1995) Mechanisms of cadmium mobility and accumulation in Indian mustard. *Plant Physiol.* **109**, 1427–1433.
- Sarret, G., Saumitou-Laprade, P., Bert, V., Proux, O., Hazemann, J.-L., Traverse, A., Marcus, M.A. and Manceau, A.** (2002) Forms of zinc accumulated in the hyperaccumulator *Arabidopsis halleri*. *Plant Physiol.* **130**, 1815–1826.
- Sarret, G., Harada, E., Choi, Y.E., Isaure, M.P., Geoffroy, N., Fakra, S., Marcus, M.A., Birschwilks, M., Clemens, S. and Manceau, A.** (2006) Trichomes of tobacco excrete zinc as zinc-substituted calcium carbonate and other zinc-containing compounds. *Plant Physiol.* **141**, 1021–1034.
- van Schie, C.C., Haring, M.A. and Schuurink, R.C.** (2007) Tomato linalool synthase is induced in trichomes by jasmonic acid. *Plant Mol. Biol.* **64**, 251–263.
- Serna, L. and Martin, C.** (2006) Trichomes: different regulatory networks lead to convergent structures. *Trends Plant Sci.* **11**, 274–280.
- Shepherd, R.W., Bass, W.T., Houtz, R.L. and Wagner, G.J.** (2005) Phylloplanins of tobacco are defensive proteins deployed on aerial surfaces by short glandular trichomes. *Plant Cell*, **17**, 1851–1861.
- Siebert, D.J.** (2004) Localization of salvinorin A and related compounds in glandular trichomes of the psychoactive sage, *Salvia divinorum*. *Ann. Bot.* **93**, 763–771.
- Sinlapadech, T., Stout, J., Ruegger, M.O., Deak, M. and Chapple, C.** (2007) The hyper-fluorescent trichome phenotype of the *brt1* mutant of *Arabidopsis* is the result of a defect in a sinapic acid-UDPG glucosyltransferase. *Plant J.* **49**, 655–668.
- Stahl, E.** (1888) Pflanzen und Schnecken. Biologische Studie über die Schutzmittel der Pflanzen gegen Schneckenfraß. *Jenaer Z. Naturwiss.* **22**, 557–684.
- Tattini, M. and Gucci, R.** (1999) Ionic relations of *Phillyrea latifolia* L. plants during NaCl stress and relief from stress. *Can. J. Bot.* **77**, 969–975.
- Teoh, K.H., Polichuk, D.R., Reed, D.W., Nowak, G. and Covello, P.S.** (2006) *Artemisia annua* L. (Asteraceae) trichome-specific cDNAs reveal CYP71AV1, a cytochrome P450 with a key role in the biosynthesis of the antimalarial sesquiterpene lactone artemisinin. *FEBS Lett.* **580**, 1411–1416.
- Thipyapong, P., Joel, D.M. and Steffens, J.C.** (1997) Differential expression and turnover of the tomato polyphenol oxidase gene family during vegetative and reproductive development. *Plant Physiol.* **113**, 707–718.
- Uphof, J. and Hummel, K.** (1962) *Plant Hairs: Encyclopedia of Plant Anatomy IV*, Berlin: Borntraeger.
- Van Dam, N.M. and Hare, J.D.** (1998) Biological activity of *Datura wrightii* glandular trichome exudate against *Manduca sexta* larvae. *J. Chem. Ecol.* **24**, 1529–1549.
- Voirin, B., Bayet, C. and Couloso, M.** (1993) Demonstration that flavone aglycones accumulate in the peltate glands of *Mentha × piperita* leaves. *Phytochemistry*, **34**, 85–87.
- Wagner, G.J.** (1991) Secreting glandular trichomes: more than just hairs. *Plant Physiol.* **96**, 675–679.
- Wagner, G.J., Wang, E. and Shepherd, R.W.** (2004) New approaches for studying and exploiting an old protuberance, the plant trichome. *Ann. Bot.* **93**, 3–11.
- Walker, A.R. and Marks, M.D.** (2000) Trichome initiation in *Arabidopsis*. In *Plant Trichomes* (Hallahan, D.L. and Gray, J.C., eds). New York: Academic Press, pp. 219–236.
- Walters, D.S. and Steffens, J.C.** (1990) Branched chain amino acid metabolism in the biosynthesis of *Lycopersicon pennellii* glucose esters. *Plant Physiol.* **93**, 1544–1551.
- Wang, E. and Wagner, G.J.** (2003) Elucidation of the functions of genes central to diterpene metabolism in tobacco trichomes using posttranscriptional gene silencing. *Planta*, **216**, 686–691.
- Wang, E., Wang, R., DeParasis, J., Loughrin, J.H., Gan, S. and Wagner, G.J.** (2001) Suppression of a P450 hydroxylase gene in plant trichome glands enhances natural-product-based aphid resistance. *Nat. Biotechnol.* **19**, 371–374.
- Wang, E., Gan, S. and Wagner, G.J.** (2002) Isolation and characterization of the CYP71D16 trichome-specific promoter from *Nicotiana tabacum* L. *J. Exp. Bot.* **53**, 1891–1897.
- Werker, E.** (2000) Trichome diversity and development. In *Plant Trichomes* (Hallahan, D.L. and Gray, J.C., eds). New York: Academic Press, pp. 1–35.
- Wienkoop, S., Zoeller, D., Ebert, B., Simon-Rosin, U., Fisahn, J., Glinski, M. and Weckwerth, W.** (2004) Cell-specific protein profiling in *Arabidopsis thaliana* trichomes: identification of trichome-located proteins involved in sulfur metabolism and detoxification. *Phytochemistry*, **65**, 1641–1649.
- Wollenweber, E.** (1984) The systematic implication of flavonoids secreted by plants. In *Biology and Chemistry of Plant Trichomes* (Rodriguez, E., Healey, P.L. and Mehta, I., eds). New York: Plenum Press, pp. 53–69.
- Xu, X., Wu, M., Zhao, Q., Li, R., Chen, J., Ao, G. and Yu, J.** (2007) Designing and transgenic expression of melanin gene in tobacco trichome and cotton fiber. *Plant Biol.* **9**, 41–48.
- Yu, H., Kowalski, S.P. and Steffens, J.C.** (1992) Comparison of polyphenol oxidase expression in glandular trichomes of *Solanum* and *Lycopersicon* species. *Plant Physiol.* **100**, 1885–1890.
- Zador, E. and Jones, D.** (1986) The biosynthesis of a novel nicotine alkaloid in the trichomes of *Nicotiana stocktonii*. *Plant Physiol.* **82**, 479–484.
- Zimeri, A.M., Dhankher, O.P., McCaig, B. and Meagher, R.B.** (2005) The plant MT1 metallothioneins are stabilized by binding cadmiums and are required for cadmium tolerance and accumulation. *Plant Mol. Biol.* **58**, 839–855.