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Update on Plant-Insect Interactions

The Role of Cytochrome P450 Monooxygenases in Plant-Insect Interactions[1]

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Plants and herbivorous insects interact at the most fundamental of levels. With few exceptions, in the battle waged daily between these two groups of organisms, one serves as food source, the other as consumer. From a plant's perspective, success in this interaction is measured by its ability to defend itself from devastation by insect feeding. From an insect's perspective, success is measured by its ability to protect itself from a variety of toxic plant defense compounds, thereby allowing it to utilize plants as its sole food source. These interactions are multifaceted and dynamic. Many classes of insect repellents and toxic substances, including isoflavonoids, furanocoumarins, terpenoids, alkaloids, and cyanogenic glycosides, are synthesized in plants. The biosynthetic pathways leading to these allelochemicals are continually evolving to generate new secondary metabolites. A variety of defense mechanisms, including enzymatic detoxification systems, physiological tolerance, and behavioral avoidance, protect insects from the hazards of these compounds. These mechanisms continue to evolve as insects attempt to colonize new plant species.

P450s have a fundamental role on both sides of the plant-versus-insect equation. They define the spectrum of the defense compounds synthesized by plants and the plant toxins metabolized by insects. Although many other insect enzyme systems participate in the synthesis and degradation of plant allelochemicals, P450s occupy a position of unique importance in the evolution of interspecies adaptive strategies due to their extraordinary versatility. The multiplicity and diversity of their substrate recognition sites, as well as their transcriptional regulatory cascades, have allowed for tremendous biochemical flexibility in the metabolic profiles of individual organisms. This flexibility has in turn provided the necessary genetic variability required for reciprocal evolutionary responses between these trophically linked taxa.

Although their common name implies similarity in function, P450s actually include a highly diverse array of protein sequences possessing common catalytic chemistry but different metabolic capabilities. Classical microsomal P450s are heme-dependent, mixed-function oxidases that utilize NADPH and/or NADH to reductively cleave atmospheric oxygen to produce a functionalized organic product and a molecule of water. Several types of products can be formed as a result of the P450-mediated insertion of oxygen into substrates (Fig. 1A). In many cases, the products are hydroxylated derivatives of the substrate at one of its carbons; in other cases, P450s mediate hydroxylations at nitrogen and sulfur heteroatoms, dehalogenations, dealkylations, deaminations, and epoxidations. Even though no single P450 utilizes this entire array of oxygenation processes as part of its natural function, it is not uncommon for a single substrate to be metabolized at alternative positions by a group of P450s or for a single P450 to metabolize multiple substrates.

Although prominent in microsomal membranes, P450s are not exclusively restricted to this subcellular location. Mammalian mitochondria contain a small number of steroid biosynthetic P450s that utilize adrenodoxin and a NADPH-dependent adrenodoxin reductase for their electron transfer. This is in contrast to the "classical" microsomal enzymes, which utilize NADPH-dependent P450 reductase and/or Cyt b_5/Cyt b_5 reductase for electron transfer. Other P450s, which might be termed "nonclassical" in that they either do not utilize flavoproteins for dioxygen activation or they do not incorporate molecular oxygen into their substrates, exist in some organisms. Some of these P450s occur in microsomes, others do not.

Despite the variety of electron-transfer partners and subcellular locations, P450 polypeptides generally range between 45 and 62 kD. Classical and nonclassical P450s in their reduced state bind CO, forming a reduced P450:CO complex that has a spectral absorption maximum at 450 nm (Fig. 1B); this is the characteristic feature that has provided the basis for their common name. Nearly all classical P450s contain a conserved F--G-R-C-G sequence, sometimes termed the P450 signature motif, approximately 50 to 60 amino acids from their C termini. The Cys located within this sequence serves as the ligand for the ferriprotoporphy-

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Abbreviations: F3′H, flavonoid 3′-hydroxylase; F3′,5′H, flavonoid 3′,5′-hydroxylases; P450, Cyt P450 monooxygenase; *t*-CAH, *trans*-cinnamic acid hydroxylase.

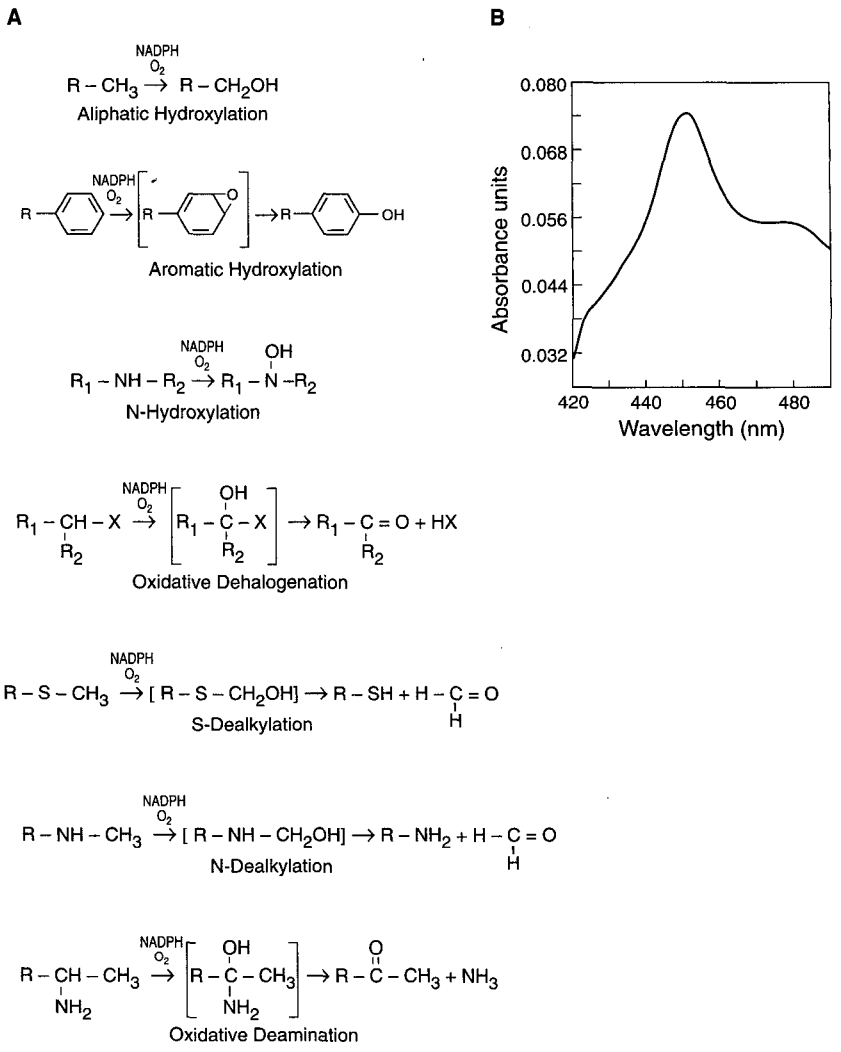

Figure 1. General P450 characteristics. A, Examples of monooxygenase activities mediated by different P450 enzymes. B, CO difference spectrum of the reduced form of P450 ($P450_{red}$) generated by subtracting the absorption spectra of $P450_{red}$ obtained in the absence of CO from that obtained in the presence of CO.

rin IX (heme) prosthetic group present in the catalytic site. Some nonclassical P450s retain only the C-G of this motif.

Reflecting a wide diversity of reactive sites and a high degree of amino acid variation, P450s are encoded by a highly divergent gene superfamily containing more than 450 Cyt P450 (CYP) sequences distributed among 65 gene families (Nelson et al., 1993, 1996). (These sequences can be accessed via the Internet address http://drnelson.utmem.edu/nelsonhomepage.html.) Structural studies conducted primarily with vertebrate P450s have suggested that the extensive divergence in catalytic site, as well as noncatalytic residues, accounts for the high degree of primary structure variation existing in this P450 superfamily and the diverse array of substrates synthesized and catabolized by these proteins. Gene copy number estimates based on DNA Southern blot analyses suggest that the P450 gene superfamily contains between 60 and 250 genes per vertebrate genome (Nelson et al., 1993, 1996). These estimates provide only a slight indication of the repetition frequency of P450 genes in plants and insects, which are continually evolving biosynthetic P450s for the production of new defense toxins as well as catabolic P450s for the elimination of these toxins.

PLANT P450s MEDIATING THE SYNTHESIS OF ALLELOCHEMICALS

P450s exist at many points in the biosynthetic pathways for plant allelochemicals, which are naturally occurring compounds that serve variously as insect toxins, repellents, or attractants, depending on the compound and the insect exam. Because metabolic assays have been developed for relatively few plant P450s, the following discussion serves to highlight the array of allelochemical biosynthetic steps/pathways known or postulated to be P450-mediated, the modes of action of these allelochemicals, and the few allelochemical biosynthetic P450s that have actually been cloned and characterized. Additional biochemical and molecular information on these biosynthetic and other detoxicative plant P450s are available in reviews by Bolwell et al. (1994) and Schuler (1996).

Many of the most prominent allelochemicals, including coumarins, furanocoumarins, and isoflavonoids, are products of the phenylpropanoid biosynthetic pathway. *t*-CAH, the first P450 in this pathway, catalyzes the hydroxylation of *trans*-cinnamic acid to *para*-coumaric acid, which is subsequently activated to its CoA thioester and funneled into

one of the branched pathways leading to the production of plant defense compounds (furanocoumarins, flavonoids, or isoflavonoids), cell-wall constituents (lignin), or pigments (flavonoids) (Dixon and Paiva, 1995; Werck-Reichhart, 1995). Because it is central to all of these branched pathways and because of its high degree of amino acid conservation relative to other P450s, *t*-CAH was one of the first plant P450s purified, biochemically characterized, and cloned. By current counts, *t*-CAH cDNAs have been characterized from seven plant species and share a higher degree of amino acid identity (>86%) than has been observed for any other group of plant P450 sequences.

In the furanocoumarin biosynthetic pathway that branches from the core phenylpropanoid pathway, linear furanocoumarins are synthesized from umbelliferone, their coumarin precursor, by the attachment of a furan ring to the 6 and 7 positions of the coumarin nucleus (Fig. 2A) (Berenbaum and Zangerl, 1996). Marmesin synthase and psoralen synthase are P450s leading to the production of psoralen; psoralen 5-monooxygenase and psoralen 8-monooxgenase are P450s that derivatize psoralen to form bergapten and xanthotoxin, respectively, two of the most common linear furanocoumarins occurring in umbelliferous plants (e.g. parsley and parsnip). These furanocoumarins are highly toxic to insects and a wide variety of other organisms that ingest them, because when photoactivated they are capable of cross-linking DNA strands and modifying proteins (Berenbaum, 1991).

In the flavonoid biosynthetic branch of the phenylpropanoid pathway in sweet orange (*Citrus sinensis*), a F3'H converts naringenin, the isomerized form of tetrahydroxychalcone, to eriodictyol, dihydrokaempferol to dihydroquercitin, and kaempferol to quercetin (Doostdar et al., 1995). The hydroxylated eriodictyol serves as a growth retardant for corn earworm (*Heliocoverpa zea*), whereas naringenin does not (Elliger et al., 1980). At later steps in the synthesis of flavonoid pigments, P450-mediated hydroxylations define the range of anthocyanins in flowers serving as insect attractants. In the best-characterized example of this type of P450-mediated reaction, variation in the proportion of delphinidin and cyanidin derivatives depends solely on the presence/absence of F3'H and F3',5'H (Forkmann, 1991; Holton and Cornish, 1995). When both P450 activities are present, as in petunia (*Petunia hybrida*), dou-

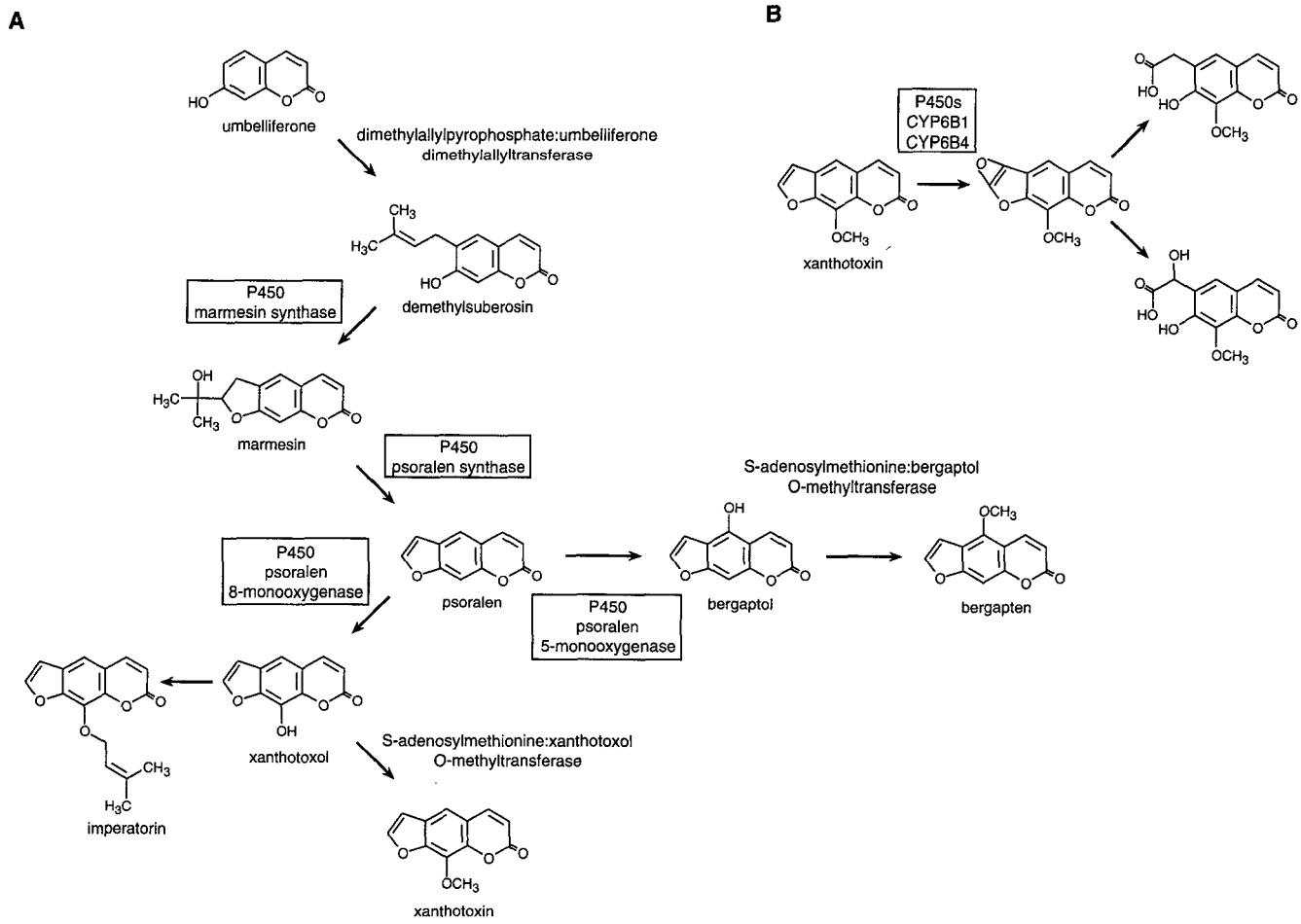

Figure 2. Furanocoumarin biosynthesis and degradation. A, The biosynthetic pathway for linear furanocoumarins occurring in most umbelliferous plants (e.g. parsley and parsnip) (Berenbaum and Zangerl, 1996). P450s known to exist in this pathway are boxed. B, P450 detoxification of the linear furanocoumarin xanthotoxin in *Papilio polyxenes* (black swallowtail) is postulated to occur via an epoxidation on the furan ring and subsequent hydroxylation reactions (Bull et al., 1984).

bly hydroxylated delphinidin derivatives predominate; when only the F3'H activity is present, singly hydroxylated cyanidin derivatives predominate. The importance of F3',5'H in these conversions was definitively demonstrated by complementation of delphinidin-deficient petunia mutants with cDNAs encoding two different F3',5'H proteins (Holton et al., 1993).

P450s mediate the synthesis of allelochemicals in other pathways unrelated to phenylpropanoids. The large class of natural products called terpenoids includes many plant defense compounds (monoterpenes and sesquiterpenes), as well as accessory pigments (carotenoids) and hormones (GAs and ABA) (McGarvey and Croteau, 1995). P450-mediated hydroxylations alter the structure of monoterpene derivatives, often in a species-specific manner, to produce a variety of toxic terpenoids. One of the best-characterized examples in this regard is the hydroxylation of limonene at the C-3, C-6, or C-7 positions in peppermint (*Mentha piperita*), spearmint (*Mentha spicata*), and perilla (*Perilla frutescens*) (Karp et al., 1990). The exclusive nature of these hydroxylations generates the notable fragrances in these mint species, which serve as attractants for some insects and repellents for others. Examples of other monoterpenes hydroxylated by P450s include sabinene (Karp et al., 1987), camphor (Funk and Croteau, 1993), and abietin (Funk and Croteau, 1994).

Alkaloids represent the largest (>10,000 structures) and one of the most structurally diverse groups of substances that serve as plant defense agents. Many of these compounds are biologically active in *Homo sapiens* (hence their importance in pharmaceuticals) and act as feeding deterrents for insects at concentrations above 1% (dry weight) (Wink, 1993; Kutchan, 1995). The largest subclass of alkaloids, isoquinoline and benzylisoquinoline alkaloids, are rich in these biologically active constituents. Simple isoquinoline structures are synthesized in cacti of the Sonoran Desert of North America from their tetrahydroisoquinoline precursor in a species-specific manner. Saguaro (*Carnegiea gigantea*) and cardon (*Pachycereus pringlei*) cacti contain low levels (1% dry weight) of the monomeric isoquinoline alkaloids, e.g. carnegine and gigantine (Fig. 3A). Carnegine and gigantine are nontoxic to desert *Drosophila* species, including resident species that live in the saguaro cactus and nonresident species that live in other cacti species, and are highly toxic to nondesert species (e.g. *Drosophila melanogaster*) (Fogleman et al., 1982). Senita (*Lophocereus schottii*) cactus contains high levels (3–15% dry weight) of the more complex isoquinoline alkaloid, lophocerine, and its trimers, pilocereine and piloceredine (Fig. 3A). Pilocereine, but not the monomeric lophocereine, has a demonstrated toxicity for all *Drosophila* species except those that live in or in close proximity to senita cactus.

The dimeric bisbenzylisoquinoline alkaloids such as berbamunine (Fig. 3B), which is synthesized in barberry (*Berberis stolonifera*), act as cytotoxic and hypotensive agents. Another in this subclass, tubocurarine (an arrow poison), which is synthesized in *Chondrodendron tomentosum* and is a component of tube-curare, blocks neuromuscular function (Wink, 1993; Kutchan, 1995). The barberry P450, designated berbamunine synthase (Fig. 3B), catalyzes the stereoselective intermolecular coupling of oxygen in one molecule of *N*-methylcoclaurine to the carbon in an enantiomeric molecule of *N*-methylcoclaurine (Kutchan, 1995). The resulting dimeric bisbenzylisoquinolone alkaloid generated in this nonclassical P450 reaction forms without the incorporation of molecular oxygen into its product alkaloids. The recent cloning of this cDNA (Kraus and Kutchan, 1995) has demonstrated that berbamunine synthase lacks at least one of the amino acids thought to be essential for the formation of the oxygen-binding pocket and for oxygen activation, as might be expected of an enzyme that does not utilize molecular oxygen in its reaction cycle. The F--G-R-C-G of the heme-binding domain, however, is conserved. Benzophenanthridine alkaloids represent a subclass of benzylisoquinoline alkaloids that accumulate in response to fungal elicitors and wounding, and act variously as antimicrobial, antifungal, and cytotoxic agents, as well as hallucinogens. Macarpine, one of the most highly oxidized alkaloids in this class, is synthesized by an elaborate pathway in *Eschscholtzia californica* (California poppy) and *Papaver somniferum* (opium poppy) that contains at least six P450s (Kammerer et al., 1994, and previous refs. therein), none of which have been cloned.

Monoterpene indole alkaloids, another large subclass of alkaloids, contain an array of antimitotic agents (vinblastine), cytotoxins (quinine), and muscle toxins (strychnine), which are known to serve as insect deterrents and toxins (Wink, 1993; Kutchan, 1995). Monoterpene iridoids, such as nepetalactone, which is synthesized in catnip (*Nepeta racemosa*), are closely related to insect sex pheromones (nepetalactols). One of the first steps in the synthesis of monoterpene indole alkaloids in periwinkle (*Catharanthus roseus*) or iridoid alkaloids in catnip is the conversion of geraniol and nerol (monoterpene alcohols) to their 10-hydroxy derivatives by a P450 designated geraniol/nerol 10-hydroxylase (Fig. 3C) (Meijer et al., 1993). The array of hydroxylations that occur at later points in the synthesis of these indole alkaloids represent logical candidates for P450-mediated reactions, but none of these reactivities has been specifically defined.

The tropane and nicotine alkaloids produced in solanaceous plants are naturally occurring insecticides and feeding deterrents derived from a common *N*-methyl-Δ^1-pyrrolinium cation precursor by pathways not known to contain P450s (Kutchan, 1995). Even so, they are covered here as examples of allelochemicals that are detoxified by P450-mediated reactions in insects feeding on the Solanaceae. Nicotine (Fig. 3D), the predominant alkaloid in tobacco (*Nicotiana*) roots, inhibits nervous-system function by binding to the acetylcholine receptor. As a result, growth of larvae such as the tobacco hornworm (*Manduca sexta*) is severely inhibited (Baldwin, 1988). Scopolamine and atropine, tropane alkaloids in *Hyoscyamus niger* (henbane) and *Atropa belladonna* (deadly nightshade), resemble endogenous neurotransmitters. By binding to the muscarinic receptor, scopolamine and atropine inhibit neuromuscular function, effectively paralyzing insects that ingest them in large concentrations.

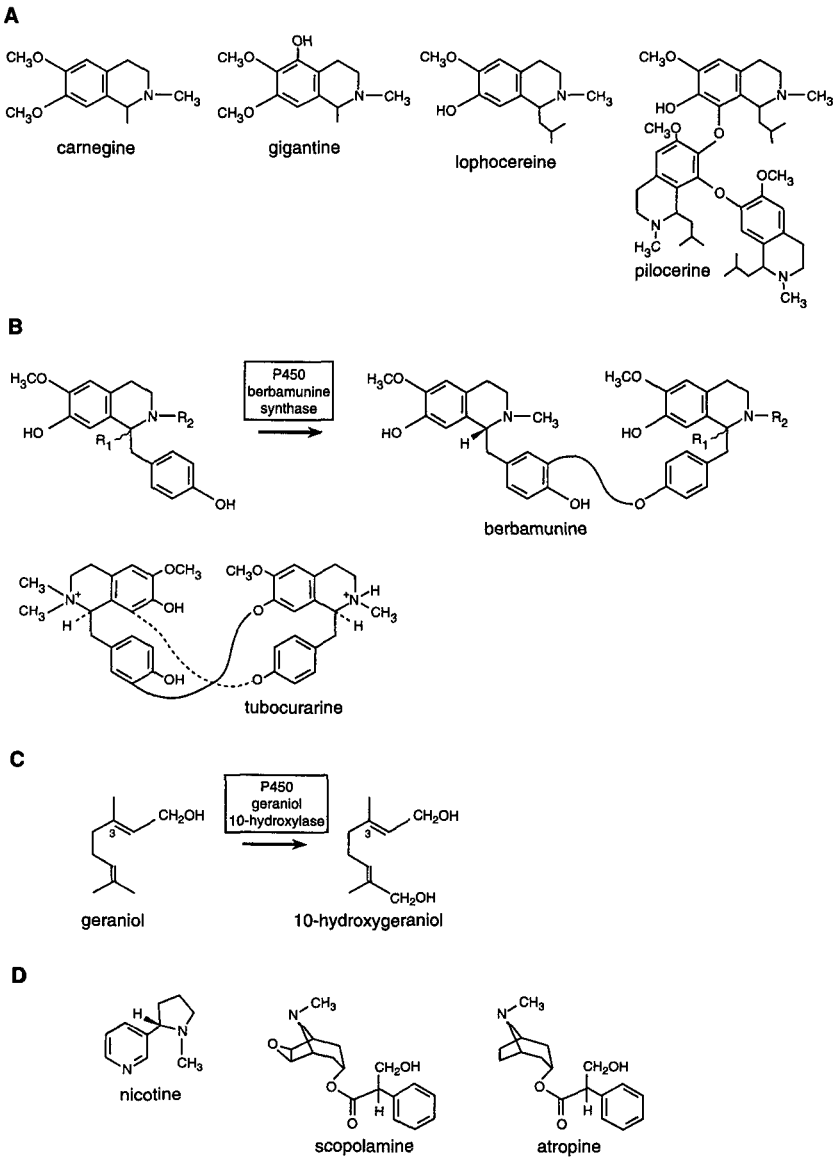

Figure 3. Alkaloid subclasses. A, Isoquinoline alkaloids; B, bisbenzylisoquinoline alkaloids; C, monoterpene indole alkaloids; D, nicotine and tropane alkaloids.

Cyanogenic glycosides, which are broken down to toxic cyanide by β-glucosidases and hydroxy nitrile lyases sequestered in plants, also serve as feeding deterrents for insect and vertebrate herbivores. Dhurrin, a cyanogenic glycoside in sorghum (*Sorghum bicolor*), is synthesized via a multifunctional $P450_{TYR}$ that catalyzes two initial hydroxylations on Tyr, generating *N,N*-dihydroxytyrosine. Sequential dehydration and decarboxylation reactions on this labile structure, probably proceeding nonenzymatically, generate *p*-hydroxyphenylacetaldehyde oxime, which is subsequently converted to *p*-hydroxymandelonitrile by a second multifunctional $P450_{OX}$ (Fig. 4) (Halkier and Moller, 1991; Koch et al., 1995). Sequence analysis of the cloned $P450_{TYR}$ cDNA indicates that it maintains the F--G-R-C of the heme-binding site, but replaces the final Gly in this conserved P450 motif with an Ala (Koch et al., 1995). This is also an atypical P450 in that substitutions exist at all three of the positions essential for formation of the oxygen-binding pocket.

In addition to these toxic allelochemicals, there are secondary metabolites in plants that function as specific inhibitors of the P450s present in insects. These compounds, some of which are lignans, enhance the efficacy of natural and synthetic insecticides by blocking the insect's ability to modify toxic agents. As a consequence, allelochemicals or insecticides are toxic at lower doses. Myristicin in nutmeg (*Myristica fragrans*), sesamin in sesame seed (*Sesamum indicum*), and safrole in sassafras (*Sassafras officinale*) are familiar lignans with the common methylene dioxyphenyl core structure necessary for P450 inhibition. Paradoxically, it is thought that P450s are involved in the synthesis of these P450 inhibitors, but, again, none of these P450 activities have been definitively identified.

Because of the potential for devastation by insects and due to the development of allelochemical resistances in insects, plants generally do not rely on just one chemical defense. The most successful plant species typically synthesize a wide array of moderately toxic defense com-

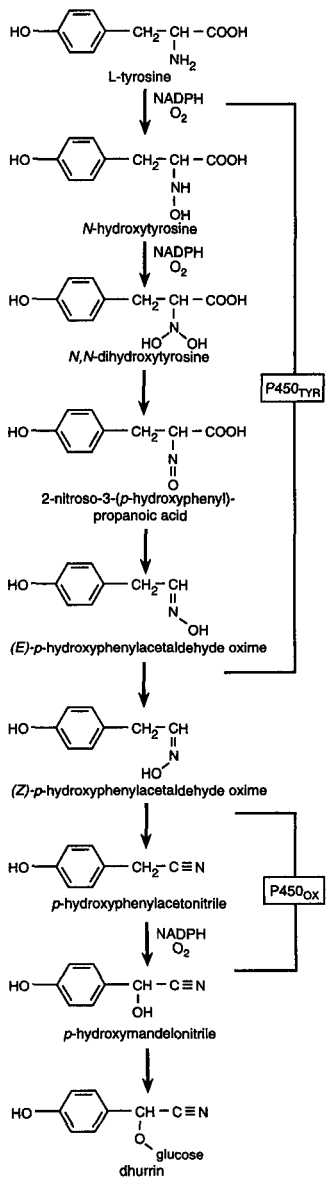

Figure 4. Biosynthetic pathway for the cyanogenic glucoside dhurrin in sorghum (adapted from Koch et al. [1995]). Reactions catalyzed by the multifunctional $P450_{TYR}$ and $P450_{OX}$ in this biosynthetic pathway are boxed. $P450_{TYR}$ catalyzes two initial NADPH-dependent steps leading to the production of *N,N*-dihydroxytyrosine, which is subsequently dehydrated and decarboxylated by processes that are probably nonenzymatic. $P450_{OX}$ catalyzes the two-step conversion of *p*-hydroxyphenylacetaldehyde oxime to *p*-hydroxymandelonitrile.

pounds or a small number of highly toxic substances (Berenbaum, 1995).

INSECT P450s MEDIATING THE DETOXIFICATION OF PLANT ALLELOCHEMICALS

Insect herbivores are classified into two groups: oligophagous species (specialists) feed on one or a small number of plant species and, thus, encounter a limited range of allelochemicals; polyphagous species (generalists) feed on a wide range of plant species, potentially encountering an array of toxic substances. Allelochemical detoxification systems, which in both groups of insects include P450s, glutathione *S*-transferases, and esterases, are typically concentrated in the insect's midgut, allowing for rapid elimination of ingested toxic substances, and/or the fat body, facilitating the detoxification of contaminants penetrating cuticular or tracheal structures.

From a historical perspective, insect P450s have only recently been defined at the biochemical and molecular level, despite the enormous importance of these proteins in the metabolism of endogenous substances (e.g. pheromones and hormones) and toxic foreign substances (e.g. allelochemicals and synthetic insecticides). Although the range of plant allelochemicals metabolized by insect P450s includes the furanocoumarins, alkaloids, lignans, nicotine, and pyrethrins described above, only two groups of insect P450s specifically involved in allelochemical metabolism have been identified to date. Only one of these groups has been cloned and definitively identified by overexpression in a heterologous protein expression system.

Analysis of the P450 activities present in caterpillar larvae from a series of *Papilio* (swallowtail) species provided the first evidence that host-plant utilization patterns within a genus are associated with the evolution of microsomal P450s capable of detoxifying plant allelochemicals, as well as P450 transcriptional regulatory cascades capable of responding to these host-plant toxins. Specialist species within this genus, such as *Papilio polyxenes* (black swallowtail), and generalist species, such as *Papilio glaucus* (tiger swallowtail), detoxify linear (xanthotoxin and bergapten) (Fig. 2A) and angular (angelicin and sphondin) furanocoumarins in their midguts significantly more efficiently than confamilial species outside of this genus (Cohen et al., 1992). Detoxification of xanthotoxin appears to occur by an initial P450-mediated epoxidation of the furan ring and, sometimes, by subsequent P450-mediated hydroxylations on the coumarin nucleus (Fig. 2B) (Bull et al., 1984). The highly charged, hydroxylated products resulting from these reactions are readily eliminated from the insect, thereby reducing further exposure to these toxins. cDNA cloning and baculovirus-mediated expression of the P450s responsible for these metabolic processes demonstrated that *P. polyxenes* and *P. glaucus* larvae express related P450s (CYP6B1 and CYP6B4, respectively; 63% amino acid identity) with specific reactivities that reflect to some degree the range of furanocoumarins present in the host plants for each species (Fig. 5) (Ma et al., 1994; Hung et al., 1997).

In the insect, exposure to these plant allelochemicals significantly induces expression of the P450s responsible for furanocoumarin detoxification. Furanocoumarin regulation of this P450 detoxification system occurs at a transcriptional level. *CYP6B1* and *CYP6B4* transcripts are highly induced in response to the presence of xanthotoxin and some other furanocoumarins in the insect's diet (Cohen et al., 1992; Hung et al., 1995, 1997). Expression of *P. polyxenes CYP6B1* promoter:CAT fusion constructs in Sf9 insect cell cultures has now identified a xanthotoxin-response element between −136 and −119 nucleotides (relative to the RNA initiation site) needed for transcriptional

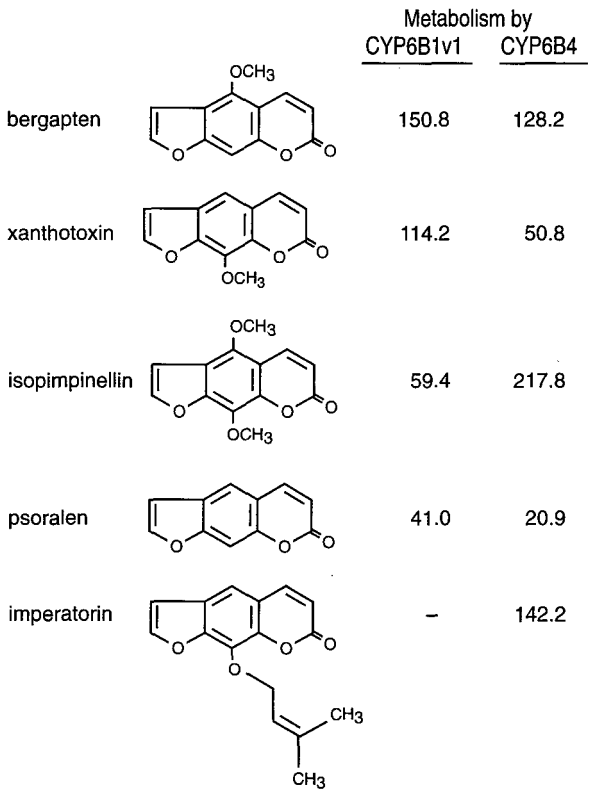

Figure 5. Substrate specificities of CYP6B1 from *P. polyxenes* and CYP6B4 from *P. glaucus*. *CYP6B1* and *CYP6B4* cDNAs cloned from *P. polyxenes* and *P. glaucus*, respectively, were expressed independently in lepidopteran cell lines using baculovirus vectors. The rates of linear furanocoumarin metabolism were defined in in vitro metabolism assays by HPLC analysis of the rates of substrate disappearance (Ma et al., 1994; Hung et al., 1997). All activities are reported as pmol min^{-1} mg^{-1} protein in total cell lysate.

induction of this promoter by xanthotoxin (Prapaipong et al., 1994, unpublished data). A region similar but not identical to the *P. polyxenes CYP6B1* xanthotoxin-response element is present in the xanthotoxin-inducible *CYP6B4* promoter of *P. glaucus* (Hung et al., 1996). These data indicate that insect P450 genes involved in furanocoumarin detoxification have been derived from a common ancestral gene, and that despite the evolutionary pressures dictating diversification of the host-plant ranges of these insects, promoter features pertinent to allelochemical regulation have been conserved.

In the second instance of allelochemical resistance, it has now become clear that P450s define the host-plant range of desert *Drosophila* species. The four *Drosophila* species that breed in isoquinoline alkaloid-rich saguaro and senita cacti metabolize carnegine and gigantine (Fig. 3A) more efficiently than *Drosophila* species not typically exposed to these alkaloids (e.g. *D. melanogaster*) (Frank and Fogleman, 1992). Although the products of these P450-mediated reactions are not yet known, it is now evident that the basal activities of the P450s responsible for alkaloid metabolism are induced in response to the presence of cactus alkaloids in the diet of *Drosophila* larvae and adults (Frank and Fogleman, 1992; Danielson et al., 1994). Full-length cDNA clones encoding several isoquinoline alkaloid-inducible P450s have been obtained, but their substrate specificities have not yet been defined (J.C. Fogleman, personal communication).

In this P450-focused discussion, it is important to note that different insect species respond to the presence of insecticidal allelochemicals in their diets in different ways; only some of the detoxification strategies are based on P450 (Brattsten, 1979). For example, constant exposure to nicotine alkaloids in their *Nicotiana* host plants has caused the tobacco wireworm (*Conoderus vespertinus*) and larvae of the cigarette beetle (*Lasioderma serricorne*) to evolve P450-mediated detoxification systems for converting nicotine into nontoxic cotinine (Self et al., 1964). In contrast, the tobacco hornworm (*Manduca sexta*) and cabbage looper (*Trichoplusia ni*) have evolved excretion systems that eliminate nicotine many times faster than other insects due to a specialized alkaloid transport system in their Malpighian tubules (Maddrell and Gardiner, 1976). Green peach aphids (*Myzus persicae*) simply avoid ingesting nicotine by feeding on phloem tissue and avoiding the toxic, nicotine-containing xylem (Guthrie et al., 1962).

Other insect defense strategies against plant allelochemicals do not involve P450 detoxification systems at all. Cyanogenic glycosides are either sequestered in specialized organs or metabolized by *b*-cyanoalanine synthase, which converts cyanide to the less toxic Asn (Seigler, 1991). In other insects, the cyanide target site is modified or absent so that cyanide cannot bind (Berenbaum, 1986). Glutathione *S*-transferases, which mediate conjugations to the tripeptide glutathione, and esterases, which catalyze ester hydrolysis, represent other significant insect defenses against exogenous toxins (Brattsten, 1992).

In addition to these defenses against naturally occurring allelochemicals, insecticide-resistant strains have evolved the ability to detoxify synthetic organic insecticides and botanicals via P450s. Examples of such metabolism include the housefly (*Musca domestica*) CYP6A1 protein that epoxidizes the cyclodiene insecticides aldrin and heptachlor (Andersen et al., 1994), and the CYP6D1 protein that hydroxylates the pyrethroid insecticides deltamethrin and permethrin on their aromatic rings and also metabolizes organophosphate insecticides to some extent (Wheelock and Scott, 1992).

Because many organic insecticides resemble plant allelochemicals or are, in the case of the pyrethroids, derived from them, it remains possible that the P450s responsible for insecticide detoxification are related to the P450s responsible for allelochemical metabolism. Few insect P450s have been expressed in one of the heterologous systems (baculovirus, yeast, or *Escherichia coli*) that can be used to define a P450's substrate specificity in the absence of other P450 activities, as in microsomes. Thus, several alternatives exist for the evolutionary relationship between allelochemical- and insecticide-metabolizing P450s. The first alternative is that particular P450s may, in response to selective agrochemical pressure, have evolved the ability to detoxify insecticides in addition to their normal array of

allelochemicals. In this scenario, the acquisition of insecticide resistance would be predicted to have little effect on insect viability and fitness. A second alternative is that P450s have evolved the ability to detoxify insecticides to the detriment of their normal catabolic functions. In this latter scenario, acquisition of insecticide resistance would be predicted to negatively affect insect viability.

P450 REGULATION

One of the most fascinating aspects of the plant and insect P450s involved in these interspecies interactions is their level of reciprocal regulation. In plants, the levels of allelochemical biosynthetic P450s adjust rapidly to the presence of antagonists such as insect and vertebrate herbivores in the environment. In insects, the levels of allelochemical-detoxifying P450s acclimate to the presence of plant allelochemicals in the insect's environment. This regulatory interface between plant and insect P450s is especially evident in the case of xanthotoxin and related linear furanocoumarins. Wounding and insect feeding induce plant P450 activities required in the core and furanocoumarin branch of the phenylpropanoid pathway (Fig. 2A) significantly above that normally found in nonwounded tissue. As a consequence, furanocoumarin levels are induced 3- to 4-fold in damaged plants compared with the basal level present in undamaged plants (Zangerl, 1990). Accumulation of these furanocoumarins in the plant, in turn, induces expression of the insect P450s that detoxify xanthotoxin and bergapten (Fig. 2B) (Hung et al., 1995, 1997). There is a clear advantage to maintaining transcriptional regulatory control on both sides of this allelochemical equation: plants invest energy in producing large quantities of furanocoumarins only when the potential for insect damage is high, and insects invest energy in expressing P450s for furanocoumarin detoxification only when exposed to these toxins.

FUTURE PROSPECTS

Many plant and insect P450s involved in these plant-insect interactions remain to be cloned. The new PCR-based technologies available for cloning P450 cDNAs (Schuler, 1996) provide exciting opportunities for identifying plant P450s involved in the synthesis of other defense compounds, as well as insect P450s involved in their detoxification. These technologies will circumvent the problems most frequently encountered in biochemically purifying P450s from plant and insect systems (i.e. low P450 content, susceptibility to degradation, etc.) and allow for the direct isolation of cDNAs with encoded substrate specificities that can be defined in heterologous systems. There are now several examples of plant P450s catalyzing the terminal step in the synthesis of an allelochemical and insect P450s catalyzing the initial step in its degradation. Since features controlling substrate recognition in these "paired" P450s are defined by catalytic site mutagenesis, the biochemical relationships between plant and insect P450s handling similar structures should provide interesting and detailed information on the molecular forces driving insect and plant interactions.

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