

Molecular regulation of induced terpenoid biosynthesis in conifers*

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Abstract

Conifers have evolved elaborate inducible, terpenoid-based defense mechanisms to deter attacks from bark beetles and other herbivore species. Herbivore damage triggers the production of oleoresin containing monoterpene, sesquiterpene and diterpene components that serve as toxins and physical barriers to herbivore invasion. Induced terpene formation appears to be regulated by specific enzymes of terpene metabolism whose activity increases on herbivore damage. Among the best studied of these are terpene synthases, enzymes which convert acyclic prenyl diphosphates to the parent terpene skeletons. Terpene synthase activity in turn is regulated by the transcription of terpene synthase genes. Induced terpene biosynthesis is also often accompanied by extensive cellular differentiation, including the formation of new resin ducts. The signal transduction cascades that initiate these shifts in conifer metabolism and cell differentiation are poorly understood due to the lack of well-developed model systems and appropriate genetic mutants. However, there are strong indications that octadecanoid pathway metabolites and ethylene have roles in this signaling, as they do in defense signaling in angiosperms. There are still large gaps in our knowledge of the signal transduction networks leading to herbivore-induced terpenoid accumulation in conifers. However, the development of new genomic, proteomic and metabolomic tools, as well as the establishment of convenient *in vitro* systems should facilitate more rapid advances in this field in the near future. The results will have important implications for understanding the evolution of conifer defense mechanisms as well as for the management of commercially important forest tree species, such as spruce, pine, and fir.

Introduction

Terpenes, also known as terpenoids or isoprenoids, are the largest class of natural products known (Connolly and Hill, 1991). Derived from

the five carbon building blocks, isopentenyl diphosphate and dimethylallyl diphosphate, they play essential metabolic roles in all living organisms. The widest structural and functional variety of terpenoids is seen in plants where these compounds have many physiological and ecological roles. Among the physiological roles, terpenoids serve as phytohormones (Swain and Singh, 2005), redox cofactors, photosynthetic pigments (Eckhardt et al., 2004), photo-protectants (Lois et al., 2000), and anchors for membrane associated proteins (Galichet and Grisse, 2003). The ecological functions of

* This contribution celebrates the 60th birthday of Rodney Croteau, who served at various times as a research advisor and mentor for all of the authors of this review. As a result of his creativity, hard work and love of the field, Rod made enormous contributions to our current understanding of the molecular biology and biochemistry of terpene biosynthesis in conifers, and much of the work cited in this article was carried out in his laboratory.

plant terpenoids include acting as attractants for pollinating insects or fruit dispersing animals, anti-herbivore defenses, anti-fungal defenses, agents of plant-plant competition, and as signaling compounds in multitrophic interactions. The many roles of plant terpenoids are reflected in their large chemical diversity which encompasses more than 30,000 unique structures.

Within the plant kingdom, conifers are notable for the production of terpenes in massive quantities and great variety as the major components of oleoresin or pitch. These complex mixtures of mono-, sesqui-, and diterpenes are derived from 10, 15, and 20 carbon prenyl diphosphate precursors (Figure 1), denoted geranyl (C_{10}), farnesyl (C_{15}), and geranylgeranyl diphosphate (C_{20}),

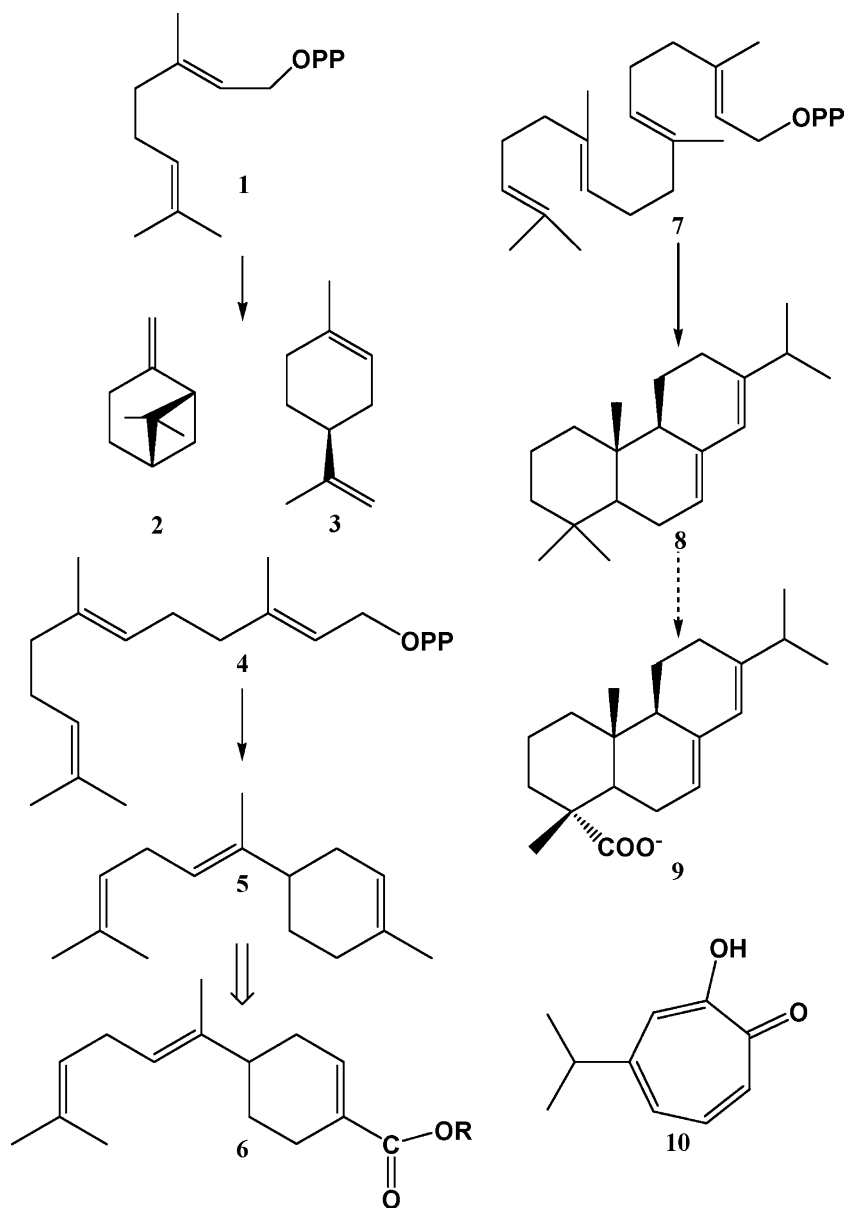


Figure 1. Structures of typical terpene compounds involved in defense. 1, geranyl diphosphate; 2, (-)- β -pinene; 3, (-)-limonene; 4, farnesyl diphosphate (FPP); 5, (*E*)- α -bisabolene; 6, R=H, todomatiuc acid, R=CH₃, juvabione; 7, geranylgeranyl diphosphate (GGPP); 8, abietadiene; 9, abietic acid; 10, β -thujaplicin. OPP denotes the diphosphate moiety.

respectively (Gershenzon and Kreis, 1999). Conifer oleoresin and the roles it plays in plant defense are sometimes considered a major reason for the widespread distribution and evolutionary success of this group. The approximately 600 species in the order Coniferales have colonized some 80% of the available niches on earth, and include some of the longest-lived organisms on the planet (Scagel, 1965). The natural enemies of conifers include insect defoliators (Schopf, 1986), stem-boring weevils (Alfaro et al., 2002), root feeders (Nordlander, 1990), nematodes (Futai and Furano, 1979), and mammalian and avian herbivores (Hohf et al., 1987; Danell et al., 1990). However, the most devastating threats to conifers worldwide are caused by the phloem boring bark beetles and their associated fungal symbionts (Raffa and Berryman, 1983; Seybold et al., 2000). While the majority of the nearly 6000 described phytophagous bark beetles live on dead trees (Franceschi et al., 2005), a small number of aggressive taxa, including species of *Ips*, *Dendroctonus*, and *Scolytus*, attack and colonize living trees. The close interactions between bark beetles and their conifer hosts have been the subject of several recent reviews (Phillips and Croteau, 1999; Trapp and Croteau, 2001; Huber et al., 2004; Raffa et al., 2005; Franceschi et al., 2005; Seybold, elsewhere in this issue).

The turpentine fraction of conifer oleoresin includes the mono- and sesquiterpenes and contains many insect and microbial toxins which often act as repellents or deterrents to discourage insect predation (Raffa and Berryman, 1983). It also serves as a solvent to mobilize the higher molecular weight diterpenoid resin acids (rosin) to injury sites. After the turpentine fraction volatilizes, the resin acids undergo oxidative polymerization entrapping and killing invading insects (Phillips and Croteau, 1999). Terpenoid components of both oleoresin fractions are produced constitutively during normal growth and development in foliage, stems and roots of many species of the pine family (*Pinaceae*). In genera such as *Pinus* (pines), *Picea* (spruce), *Abies* (true firs), and *Pseudotsuga* (Douglas fir) additional oleoresin production occurs after biological challenge in a process known as traumatic resinosis (Figure 2). This strategy of induced oleoresin defense may allow the tree to conserve resources in the absence of insects and pathogens and enhance and modify

its defenses as required upon actual attack. Traumatic resinosis requires coordinated regulation among the participating metabolic pathways needed to produce oleoresin components. These include the cytosolic mevalonate and the plastidic methylerythritol pathways for the production of isopentenyl diphosphate and dimethylallyl diphosphate, the prenyltransferase reactions that construct the appropriate branch point intermediates (Burke et al., 1999; Tholl et al., 2001; Burke and Croteau, 2002a, b; Martin et al., 2002), the terpene synthases that produce the various terpene carbon skeletons (Martin et al., 2004), and downstream modifying enzymes that catalyze principally oxidative transformations including cytochrome P450 diterpene monooxygenases (Funk and Croteau, 1994; Funk et al., 1994; Ro et al., 2005). In addition, up-regulation of terpenoid pathways in the traumatic resin response can be associated with major changes in cell differentiation leading to the *de novo* formation of traumatic resin ducts (Franceschi et al., 2002;



Figure 2. Traumatic resinosis in Norway spruce (*Picea abies*) induced by treatment with methyl jasmonate.

Martin et al., 2002; Hudgins et al., 2004). Induced terpenoid formation can also be associated with emission of terpenoid volatiles from conifers (Martin et al., 2003; Miller et al., 2005).

How conifers perceive biological challenges, distinguish microbial from insect attacks, transduce defensive signals to activate the relevant biosynthetic pathways, regulate flux through each pathway, and control processes of induced cell differentiation and volatile emission is currently unknown. However, a picture is slowly emerging in which specific elicitors from insect or pathogen challenges trigger complex signaling networks which divert resources from growth to defensive metabolism, including the formation of new storage structures. This review will focus on what is known about the molecular regulation of defensive oleoresin biosynthesis in conifers. Most of our knowledge in this area concerns the controls exerted by genes and enzymes of terpenoid biosynthesis. However, it has recently been discovered that signaling networks involving octadecanoids and ethylene activate terpenoid biosynthesis. In addition, there is scattered evidence for some of the early steps in signal transduction that might precede octadecanoids and ethylene.

Induction of genes and enzymes of terpenoid formation

As mentioned above, bark beetles and other insect herbivores are one of the greatest threats to conifer survival. Since insect herbivory causes wounding of conifer tissue, mechanical wounding was used extensively in many of the earliest studies of induced terpenoid defenses in conifers as a convenient treatment to simulate herbivory. However, virtually nothing is currently known about the mechanism of wound perception in conifers and whether there are significant differences in response to herbivory as compared to mechanical wounding. This process probably resembles wound signaling in angiosperms in its general features (Leon et al., 2001). However, because insect feeding and fungal inoculation are tightly linked in conifers, microbial and wounding elicitation are not yet understood as discrete signaling pathways as clearly as in angiosperms. The effect of wounding and insect feeding on induced

terpenoid production has been studied in several conifer species, including Grand fir (*Abies grandis*) (Funk et al., 1994; Steele et al., 1995; Bohlmann et al., 1998a, b), Sitka spruce (*Picea sitchensis*) (Byun McKay et al., 2003; Miller et al., 2005), Douglas fir (*Pseudotsuga menziesii*) (Hudgins et al., 2003), and various pine (*Pinus*) species (Litvak and Monson, 1998; Luchi et al., 2005). Because different conifers rely on constitutive and inducible defenses to different extents, those species with largely inducible systems, namely Grand fir and spruce, have been used more extensively to understand induced resinosis.

Grand fir was the first species to be developed as a system for studying the regulation of oleoresin biosynthesis, chosen because it had higher fold induced responses than the 10 other conifer species surveyed in a comparative study (Lewinsohn et al., 1991). Time-course analysis of terpenoid accumulation and enzyme activities in mechanically wounded Grand fir stems indicated that monoterpene, sesquiterpene and diterpene resin acid biosynthesis are all induced in response to wounding. All three classes of terpenoid synthases involved in resin formation, mono-, sesqui-, and diterpene synthases, and two of the cytochrome P450 oxygenases involved in abietic acid biosynthesis showed increased activities (Funk et al., 1994; Steele et al., 1995). The monoterpenes are the first to accumulate, consistent with their proposed dual role as insect toxins and as a solvent for the resin acids. The peak of monoterpene production in Grand fir (up to 200 times higher than unwounded controls) occurs about 10 days after wounding (Funk et al., 1994). This is among the slowest of the induced defense responses reported in plants but is nevertheless appropriate for the time scale of bark beetle attack (see below). Western blot analysis with polyclonal antibodies raised against the (-)-pinene synthase of Grand fir suggested that the observed increase in the activity of this terpene synthase results from a corresponding increase in enzyme protein (Gijzen et al., 1992).

Following the isolation of terpene synthase genes from Grand fir (Bohlmann and Croteau, 1999), the time-course of induced oleoresinosis in this species was examined by RNA blot hybridization using a set of 'class-specific' DNA probes designed for monoterpene, sesquiterpene or diterpene synthases (Steele et al., 1998a, b). Gene expression analysis using these three probes

indicated that the monoterpene synthase genes were induced first (transcripts detectable at 4 h after mechanical wounding), while transcription of sesquiterpene and diterpene synthases did not begin until day three. Changes in the terpene synthase activity of this tissue reflected the increase in gene transcript levels, but the increase in enzyme activity lagged behind the rise in mRNA transcripts. Once the peak in transcript levels was reached (day 5–6 through 11–12), enzyme activity continued to rise, suggesting that the terpene synthases are stable *in vivo* and that regulation occurs mainly at the transcriptional level. Although these experiments defined the general features of the induced response and its regulation, individual assays of the various terpenoid synthases revealed that the generic probes had masked some of the details of induction of the individual synthases (Steele et al., 1998a, b). For example, within 24 h of wounding limonene synthase is the principal induced monoterpene synthase, but is replaced by (–)-pinene synthase as the major type by day seven. This differential regulation of two types of monoterpene synthase genes in the induced defense response in Grand fir suggests that the monoterpene products could have different defense functions.

In comparing the trends for different classes of terpenes, the delayed production of diterpene resin acids relative to the monoterpenes (Steele et al., 1998a, b) is appropriate to the time-frame of beetle attack, which usually lasts several days, because a delayed flow of resin could maximize entrapment and killing of beetles involved in boring activity. Alternatively, this response may conceivably be a defense against future bark beetle attacks. If an initial attack is a reliable indicator of further attacks, there may be a selective advantage in a general increase in terpene accumulation.

The delayed induction of sesquiterpene biosynthesis may also have distinct defensive advantages. The initially formed sesquiterpene olefin, (*E*)- α -bisabolene is subsequently converted to todomatuic acid and juvabione (Figure 1), fungicidal metabolites and insect juvenile hormone mimics that could serve as a valuable second line of defense later in the sequence of attack (Bohlmann et al., 1998a, b). It is noteworthy in this context that the induced (*E*)- α -bisabolene synthase produces a single product whereas the constitutive Grand fir sesquiterpene synthases are multi-product enzymes producing in excess of 30

distinct products each (Steele et al., 1998a, b), suggesting that the two enzymes have very different defense roles. Further studies on the mode of action of the various terpenoid oleoresin components on insects and microbes may shed light on why the different constituents appear in the sequence observed.

All of the terpene synthases mentioned in this section are encoded by genes of the terpene synthase d (TPS-d) gene family, with more than 30 members in conifers (Bohlmann et al., 1998a, b; Martin et al., 2004). The group provides some of the best molecular markers for wound and insect induced defenses in conifers. Functional and phylogenetic analysis of the TPS-d gene family has identified a substantial amount of genomic hard-wiring of terpenoid defenses in long-lived conifer trees accompanied by phenotypic plasticity (Huber et al., 2004; Martin et al., 2004).

Many recent studies on induced terpene formation in conifers have employed methyl jasmonate (MJ), a member of the octadecanoid family of plant growth regulators. The first reports in this series demonstrated that applying MJ, by spraying a solution onto the foliage or painting it onto the surface of the bark, induced terpene accumulation via initiating the formation of new resin ducts and increasing the activity of enzymes of terpene biosynthesis (Franceschi et al., 2002; Martin et al., 2002). An increase in volatile emission of terpenoids was also noted in Norway (Martin et al., 2002, 2003; Fäldt et al., 2003) and Sitka spruce (Byun McKay et al., 2003; Miller et al., 2005). In Sitka spruce, the MJ-induced response was compared to the effects of feeding by the white pine weevil (*Pissodes strobi*), a natural pest of Sitka spruce. MJ treatment was found to induce effects similar but not identical to white pine weevil feeding. Both MJ and weevil feeding caused the accumulation of traumatic resin in stems, but weevil feeding led to more resin (Miller et al., 2005). Additionally, the volatile terpenoid emission patterns were more complex in the case of weevil feeding, consisting not only of (–)-linalool, (*E,E*)- α -farnesene, and (*Z*)- α -bisabolene, also induced by MJ, but other monoterpene olefins more typical of stem resins as well. Transcript analysis of weevil-damaged stems using RNA blots and probes specific for the major terpene synthases of Norway spruce (Martin et al., 2004) showed strong up-regulation

of several monoterpene and diterpene synthases reminiscent of Grand fir. Limonene synthase and (+)-3-carene synthase transcripts were strongly induced within 3–12 h as the major monoterpene synthase types, but were later overtaken by (-)-pinene synthase at 8 days, again implying independent regulation of different monoterpene synthases. Whereas substantial MJ-induced monoterpene synthase transcript accumulation occurred within 3 h of MJ treatment, similar levels of monoterpene synthase transcription were not seen in weevil-damaged trees until 24 h after feeding initiation. This delay may reflect the longer time necessary for the triggering of octadecanoid signaling by insect feeding compared to the direct effect of exogenous MJ application, or could be due to the relatively small wounds inflicted by stem boring weevils and their slow rate of initial feeding.

To study spatial regulation, Miller et al. also analyzed terpene synthase transcript accumulation at sites at some distance from weevil feeding (Miller et al., 2005). Although transcripts were observed to accumulate in inner and outer stem tissue 20 cm away from feeding sites, there was no strong accumulation of resin terpenoids at these positions compared to the feeding sites themselves, implying that mono- and diterpene formation may also be subject to regulation through substrate availability or post-transcriptional regulation of the corresponding synthases. Analysis of needles showed that younger needles were more able to respond to wounding or chemical elicitation than were mature needles; however, the constitutive levels of terpenoids in mature needles were higher (Miller et al., 2005). Diterpene synthase transcript accumulation in needles was not significantly induced by mechanical wounding or weevil feeding, and monoterpene synthase transcript accumulation in needles was only slightly elevated by damage compared to that induced in stem tissue with the exception of (-)-linalool synthase. The induction of (-)-linalool synthase transcripts in needles corresponded well to the induced emission of this monoterpene alcohol by weevil-fed and MJ treated needles of Sitka spruce. The transcript for the sesquiterpene synthase, (*Z*)- α -bisabolene synthase, was also induced in needles after treatment with MJ consistent with the emission of this sesquiterpene following MJ treatment and weevil feeding.

Early events in signal transduction leading to terpenoid biosynthesis

While the early steps of defense signaling are largely unexplored in conifers, it is likely that the induction of terpenoid defenses begins with the perception of either mechanical wounding or a microbial pathogen and then proceeds via a tightly regulated signal transduction network to the expression of the relevant genes involved in defensive metabolism. Separate signaling pathways for insect wounding and microbial infection have not been demonstrated in gymnosperms. Since pathogen infection in conifers usually occurs as a result of insect attack, as in the case of bark beetles, separating the herbivory and pathogen-induced defense responses is difficult. However, work performed on angiosperms infected by pathogens provides clues as to what may be expected. Based on studies performed on soybean (Fliegmann et al., 2004), cereal grains (Okada et al., 2002), Arabidopsis (Bauer et al., 2001), tomato (Meindl et al., 2000), and tobacco (Meindl et al., 2000), a general model of defense response to pathogens in plants has emerged in which plasma membrane bound receptor proteins recognize a pathogen-specific polypeptide or oligosaccharide and then activate effectors, such as ion channels, GTP binding proteins (G proteins), or protein kinases (Figure 3). The next steps of signal transduction may include cytosolic Ca²⁺ spiking, plasma membrane depolarization, acidification of the cytosol, NADPH oxidase activation and subsequent production of reactive oxygen species, production of jasmonates and ethylene, expression of defensive genes and secondary metabolite accumulation (Zhao et al., 2005). Studies on monoterpene-producing cell cultures of a conifer, Mexican cypress (*Cupressus lusitanica*), have supported the role of calcium influx and G protein receptors in the activation of this signaling cascade (Zhao and Sakai, 2003). The production of the potent anti-microbial monoterpene β -thujaplicin (Figure 1) in cell cultures was enhanced by treatment with calcium ionophores, suggesting an essential role for this ion in elicitation and signaling. The production of β -thujaplicin was inhibited by suramin, a G protein inhibitor, consistent with G proteins playing a role in gymnosperm signaling as they do in angiosperms (Zhao and Sakai, 2003). The involvement of cAMP as a second messenger

in this signaling cascade has likewise been demonstrated in *C. lusitanica* cultures (Zhao et al., 2004a, b). cAMP and several analogs were shown to elevate β -thujaplicin production in *C. lusitanica* cultures, as did cholera toxin and forskolin, potent activators of adenylyl cyclase, the enzyme which synthesizes cAMP. Clearly, further studies are warranted to determine the extent of the similarities between gymnosperm and angiosperm signaling pathways, whether separate pathogen and mechanical wounding signaling pathways are operational in conifers, and whether similar signals can activate distinct secondary metabolic pathways in these two major groups of plants.

The role of jasmonates in the induction of terpene biosynthesis

The induction of terpene resin biosynthesis by MJ treatment (Franceschi et al., 2002; Martin et al., 2002; Hudgins et al., 2003, 2004; Huber et al., 2005) argues for an essential role of endogenous jasmonates in conifer defense signaling networks and activation of genes of secondary metabolism. In fact, the properties of MJ have been exploited to produce cDNA libraries from *Taxus cuspidata* (Jennewein et al., 2004) and *Pseudotsuga menziesii* (Huber et al., 2005) enriched in defense transcripts for the isolation of genes involved in induced

terpenoid metabolism. Jasmonates are cyclopentanone ring bearing, lipophilic plant hormones derived from the octadecanoid pathway that are often involved in plant responses to biotic stresses (Creelman and Mullet, 1997), especially wounding (Creelman et al., 1992). Good evidence for the involvement of jasmonates in conifer defense signaling also comes from the fact that octadecanoid pathway inhibitors, such as ibuprofen, diethylthiocarbamate and proadifed (Zhao et al., 2004a, b), block induction of monoterpene production by yeast elicitor in *C. lusitanica* cultures. Although the exact chemical identity of the octadecanoid signal *in vivo* has not been thoroughly established in conifers, *cis*-jasmonic acid (considered more active than the *trans*-isomer) was detected in fungal cell wall-elicited *Taxus* cultures (Mueller et al., 1993). Further evidence for the involvement of octadecanoid signaling in conifers comes from the sequencing of more than 180,000 ESTs from white spruce (*Picea glauca*), Sitka spruce (*P. sitchensis*), and interior spruce (*P. glauca* \times *engelmannii*) cDNA libraries which showed sequences representing all enzymes of the octadecanoid pathway (Ralph and Bohlmann, unpublished results). In related work, these sequences were induced by mechanical wounding and weevil feeding in Sitka spruce as determined by RNA gel blot experiments (Miller et al., 2005). For these reasons, the octadecanoid pathway can be assumed to be operational in defense signaling

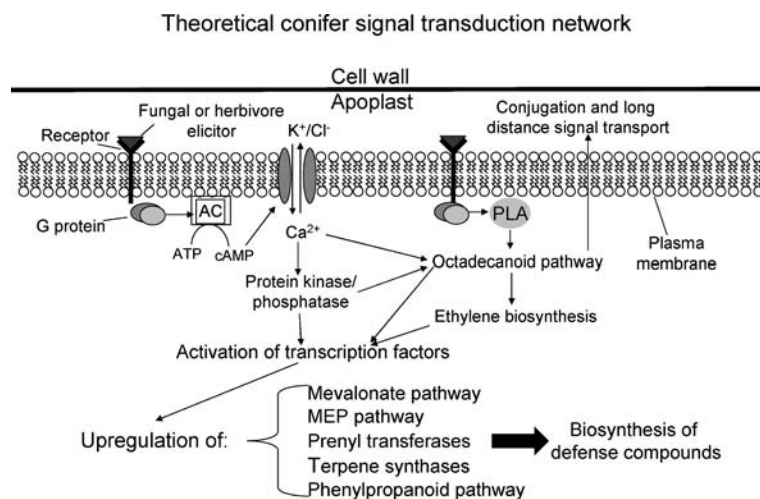


Figure 3. Outline of a possible signaling network in the formation of induced conifer defenses based on evidence from angiosperm systems and reports cited in this chapter. Abbreviations: AC, adenylyl cyclase; PLA, phospholipase A.

in gymnosperms, although further studies are needed to determine if there are any major differences from the way this pathway operates in angiosperms.

Methyl jasmonate treatment also induces anatomical changes in conifers by reprogramming the cambial zone to form traumatic ducts (TDs) instead of xylem cells (Martin et al., 2002; Hudgins et al., 2003, 2004; Hudgins and Franceschi, 2004). TDs are specialized anatomical structures for the accumulation of resin terpenes, which are formed in species of spruce and other conifers. Like other resin ducts, TDs are lined with epithelial cells thought to be the site of terpene biosynthesis. MJ treatment induces the formation of TDs in the xylem of Norway and Sitka spruce in a fashion similar to that caused by insect attack, fungal elicitation, and mechanical wounding (Martin et al., 2002). These morphological changes are accompanied by an increase in monoterpene and diterpene synthase activity peaking at about 10 and 15 days, respectively, after MJ treatment. GGPP synthase activity also increased markedly and simultaneously with diterpene synthase activity (Martin et al., 2002).

Chemical analysis showed significant increases in all classes of resin terpenoids following MJ treatment in bark and wood of Sitka and Norway spruce, both in saplings and mature trees (Martin et al., 2004; Miller et al., 2005; Zeneli et al., 2006, in press). While no major compositional changes were observed in the wood, MJ treatment induced an increase in terpene emission from needles with a shift towards oxygenated monoterpenes, such as linalool, and sesquiterpenes such as (*E*)- β -farnesene (Martin et al., 2003). Changes were also observed for terpene synthase transcript accumulation in MJ treated Norway spruce and in Sitka spruce (Fäldt et al., 2003), supporting the view that regulation of the *TPS* genes largely occurs at the transcriptional level. MJ appears to have a similar effect on terpene biosynthesis in cell cultures since treatment of *Taxus* cultures with MJ was shown to increase the accumulation of taxoids, natural products derived from the diterpene taxadiene with well-known anti-tumor properties (Yukimune et al., 1996; Ketchum et al., 1999; Croteau, elsewhere in this issue). In *C. lusitanica* cultures, MJ increased the production of β -thujaplicin 2- to 3-fold in 48 h (Zhao et al., 2004a, b).

In addition to the induction of traumatic resin terpenoids and terpenoid emission, MJ treatment may also cause other effects on conifer defense metabolism. Global gene expression profiles of spruce as determined with cDNA microarrays showed the significant up-regulation of several enzymes of phenolic metabolism, protease inhibitors, (Ralph and Bohlmann, unpublished), and members of a large family of dirigent proteins in Sitka spruce (Ralph et al., 2006). While triggering defensive responses, there is evidence that MJ down-regulates primary metabolism based on reduced expression of photosynthetic genes as seen in microarrays (Ralph and Bohlmann, unpublished results), reduction in Rubisco levels (Tholl and Gershenzon, unpublished results) and a decline in seedling growth rates (Zeneli and Gershenzon, unpublished results). Taken together these findings support an important role for octadecanoids in reshaping conifer metabolism in response to biological stresses.

The role of ethylene in defense response signaling and its interaction with methyl jasmonate

Ethylene is another plant hormone which functions in defense signaling and whose production is stimulated by wounding, herbivore, and pathogen attack (Wang et al., 2002). Ethylene treatment by itself induces the formation of TDs in *Pseudotsuga menziesii* and *Sequoiadendron giganteum* (Hudgins and Franceschi, 2004) and stimulates the accumulation of β -thujaplicin in *C. lusitanica* cultures (Zhao et al., 2004a, b) at low levels. Both of these studies have indicated that ethylene production is activated by jasmonate treatment, but conversely that ethylene treatment does not induce jasmonate biosynthesis. However, this view may oversimplify the interactions between these two hormones. Zhao et al. (2004a, b) showed that while ethylene treatment does not stimulate jasmonate biosynthesis in *C. lusitanica* cultures, a block in the octadecanoid pathway completely abolishes ethylene-induced β -thujaplicin accumulation (Zhao et al., 2004a, b). Curiously, higher concentrations (>300 ppm) of ethylene can even inhibit β -thujaplicin accumulation. Further studies are necessary to determine the role of jasmonates and ethylene in the signal transduction networks controlling resin terpene biosynthesis in conifers.

Conclusions

Recent research has begun to shed light on some of the regulatory mechanisms by which terpenoid oleoresin formation is induced following herbivore attack. After herbivore damage is recognized by a signal cascade possibly involving receptor-linked G proteins, calcium fluxes and cAMP, the octadecanoid pathway is activated. Octadecanoid metabolites and ethylene then mediate a signaling transduction network resulting in the activation of genes encoding enzymes of terpenoid metabolism (Figure 3) and leading to the differentiation of new resin ducts. In broad outline, this regulatory scheme is similar to that involved in defense signaling in angiosperms, although it remains to be seen whether it will also be similar in details. Given the large structure and long lifetimes of many conifers and their evolutionary persistence, their defense networks may have unique signaling features not found in angiosperms, especially in the herbaceous, annual angiosperms that are the subjects of most current studies.

Future work on molecular regulation of conifer defense systems should benefit from a suite of new tools under development in our laboratories. A cell suspension culture system for Norway spruce has recently been established at the Max Planck Institute for Chemical Ecology which shows induced terpene responses, allowing defense signaling to be investigated with a large, uniform population of cells. The development of genetic transformation protocols for Norway spruce will circumvent some of the difficulties inherent in working with slow growing species which lack appropriate genetic mutants. The Genome Canada and Genome British Columbia funded Treenomix and Conifer Forest Health projects (<http://www.treenomix.com>) at the University of British Columbia have recently developed a suite of metabolite profiling, proteomics, microarray, and marker genomics tools for *Picea* species which facilitate a systems approach to conifer defense (Rungis et al., 2004; Lippert et al., 2005; Ralph et al., 2006, in press; Ralph and Bohlmann, unpublished results). The combination of all of these tools should help identify all of the major components involved in regulation of induced terpene defenses in conifers and help test their roles in response to insects, pathogens and other enemies.

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