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Biochemical and physiological mechanisms mediated by allelochemicals

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Allelochemistry, the production and release of toxic chemicals produced by one species that affect a receiving susceptible species, has been the subject of diverse degrees of scientific enquiry. Recent advances in plant biology have permitted the revamp of allelochemistry as a biologically and ecologically sound explanation for plant invasion and plant–plant communication in the rhizosphere. Recent progress has been made in understanding the biochemical and molecular changes that are induced by allelochemicals in susceptible plant species, and the complex mechanisms that are used by allelochemical-resistant plants to defend against this toxic insult.

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Current Opinion in Plant Biology 2004, 7:472–479

This review comes from a themed issue on
Biotic interactions
Edited by Maria J Harrison and Ian T Baldwin

Available online 5th June 2004

1369-5266/\$ – see front matter
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DOI 10.1016/j.pbi.2004.05.007

Abbreviations

BOA benzoxazolin-1(3H)-one
DCF dichlorofluorescein
POD peroxidase
PSII photosystem II
Q_A plastoquinone A
Q_B plastoquinone B
ROS reactive oxygen species

Introduction

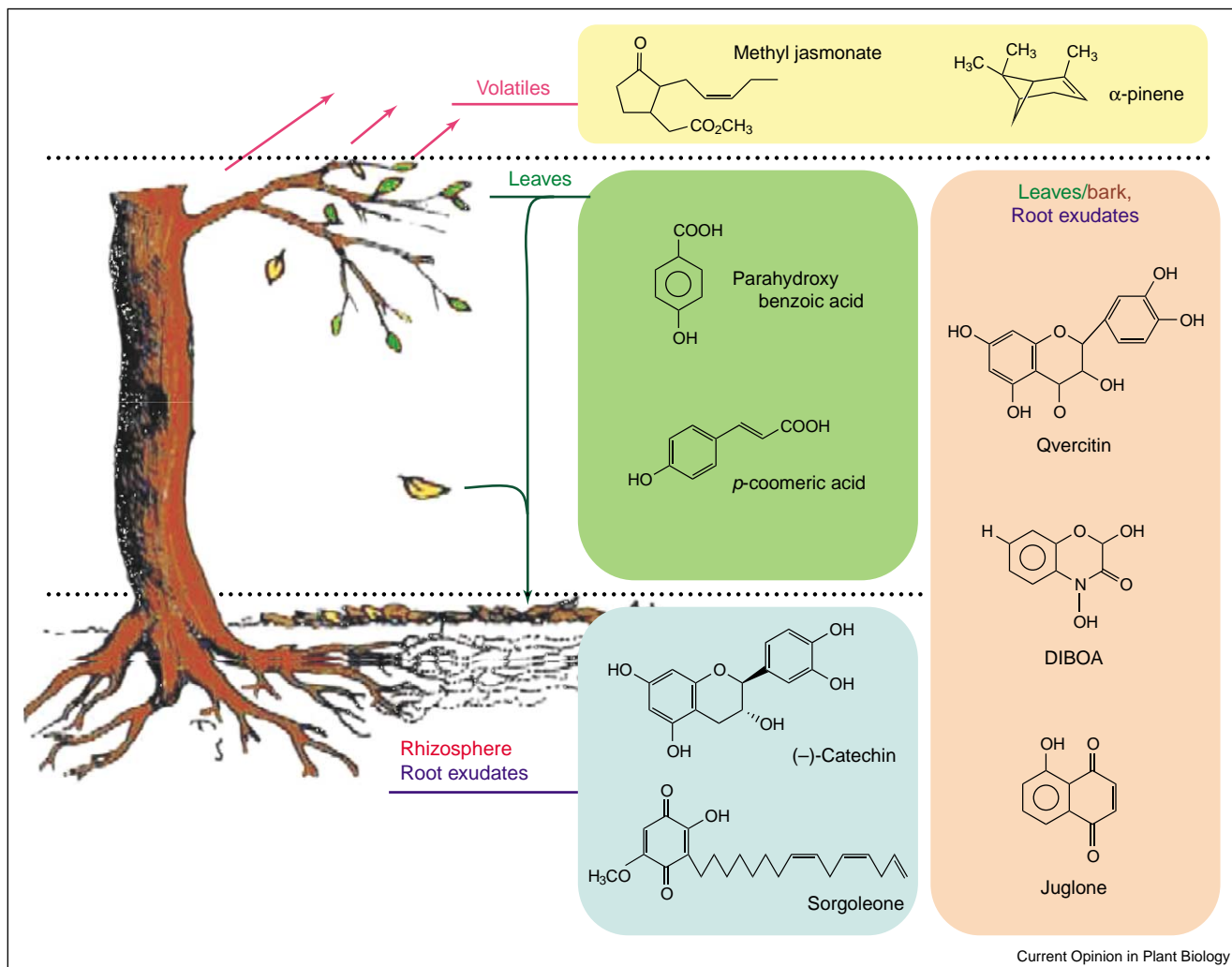
Allelopathy, from the Latin words *allelon* ‘of each other’ and *pathos* ‘to suffer’, refers to the chemical inhibition of one species by another. Although the term allelopathy is most commonly used to describe the chemical interaction between two plants, it has also been used to describe microbe–microbe, plant–microbe and plant–insect or plant–herbivore chemical communication. In plants, allelochemicals can be present in the leaves, bark, roots, root

exudates, flowers, and fruits. The delivery of allelochemicals into the rhizosphere is often thought to occur through leaching from leaves and other aerial plant parts, through volatile emissions, by root exudation, and by the breakdown of bark and leaf litter (Figure 1).

Around 300 BC, the Greek botanist Theophrastus was possibly the first person to recognize the allelopathic properties of plants when he observed and recorded that chickpea plants exhausted the soil and destroyed weeds. Later, Pliny the Elder, a Roman scholar and naturalist, noted that walnut trees were toxic to other plants, and that both chickpea and barley ruined croplands for maize. Despite these ancient and illustrious beginnings, the study of allelopathy has not enjoyed widespread acceptance in the modern scientific community. One reason for the slow progress in this area is the difficulty of designing field experiments that unquestionably prove that a chemical produced by one plant directly affects a neighboring plant. Another hindrance to the study of allelopathy has been the convincing argument that the toxic compound being transported from one plant to another is not likely to be sufficiently concentrated to have an effect on the recipient plant, and would only be found in much higher concentrations around the producing plant [1]. Even if the chemical were found in sufficient quantities in the rhizospheres of producer and receiver plants, it has been argued that resistance to the toxin would have co-evolved in both plants, negating the effectiveness of the toxin. As a consequence of these obstacles, little is known about the biological mechanisms by which allelochemicals cause phytotoxicity.

The most extensive mechanistic studies have been carried out on sorgoleone, an allelotoxin from *Sorghum bicolor*. Recent insights into the biochemical and molecular effects of some other naturally produced phytotoxins have shed some light in this dark area of rhizosphere interactions [2••]. Another way to elucidate mechanisms of phytotoxicity is to investigate the mode of action of synthetically produced commercial herbicides. Sorgoleone and several other natural allelotoxins most probably share modes of action with synthetic chemicals. In this review, we combine recent biochemical and physiological evidence to explore the mechanisms of phytotoxicity induced in recipient/susceptible plants by plant- and microbe-produced allelochemicals. Furthermore, we outline some possible biochemical mechanisms of detoxification that are present in plants.

Figure 1



Allelochemicals can be found in any part of the plant, including the bark, leaves, roots, or fruits, and can also be secreted as root exudates or released as volatile compounds. This schematic shows examples of some allelochemicals and their mode of release from the plant.

Direct inhibition of PSII components

One of the best-characterized phytotoxic mechanisms induced by allelochemicals is the inhibition of photosynthesis and oxygen evolution through interactions with components of photosystem II (PSII) [3–5]. A major function of many of the components of PSII is to serve as an adaptable, membrane-embedded scaffold. This scaffold organizes the ligands that bind an excitonically linked network of pigments and other cofactors that trap, transfer, and utilize solar energy to drive water-splitting reactions [6]. Sorgoleone, a lipophilic benzoquinone component of the root exudates from *S. bicolor*, is a particularly well-documented inhibitor of PSII [7–9]. In studies using intact chloroplasts and thylakoid membranes, sorgoleone specifically inhibits the chloroplast electron-transport chain, acting in a similar way as triazine herbicides such as atrazine. In fact, sorgoleone acts by competitively inhibit-

ing the atrazine-binding site of PSII [8]. This prevents the reduction of plastoquinone B (Q_B) by disrupting the electron-transfer chain between plastoquinone A (Q_A) and Q_B [7]. Sorgoleone most probably acts as a plastoquinone analog, interfering with plastoquinone binding at the D1 protein of PSII [9]. Several sorgoleone-like compounds, such as 5-ethoxysorgoleone, are minor components of the root exudates of sorghum, have been characterized recently and were found to have similar PSII inhibitory properties [10*]. Sorgoleone also inhibits hydroxyphenylpyruvate dioxygenase (HPPD), which disrupts the biosynthesis of carotenoids, resulting in foliar bleaching [11].

The inhibition of photosynthesis by sorgoleone and sorgoleone-like compounds is one of the best-characterized mechanisms of an allelochemical; however, other phytotoxins have similar activity. Many cyanobacteria produce

allelochemicals that act as algicides by directly inhibiting electron transport by specifically binding to sites in PSII [12]. For example, fischerellin, from *Fischerella muscicola*, probably acts at several PSII sites [13]; and cyanobactrin, from *Scytonema*, is associated with the oxidizing side of the Q_B electron acceptor [14]. The aquatic angiosperm *Myriophyllum spicatum* produces tellimagrandin II, which appears to have a mechanism of PSII inhibition that is slightly different from that of most of the previously described allelochemicals and commercial herbicides. It disrupts PSII electron transport at the non-heme iron that is located between the plastoquinone electron acceptors Q_A and Q_B , facilitating electron transfer between them [15•].

Interruption of dark respiration and ATP synthesis

Many allelopathic chemicals have more dramatic effects on seed germination than on the growth and viability of adult plants. In fact, (–)-catechin from *Centaurea maculosa* inhibits seed germination in plant species whose seedlings are generally tolerant of its phytotoxic effects [16•]. Even the germination of *C. maculosa* seeds decreased in the presence of (–)-catechin, suggesting that *C. maculosa* may use this allelochemical to regulate its own population to avoid competition for limited resources. One suggested mechanism for the inhibition of seed germination is the disruption of ‘dark’ or mitochondrial respiration. Dark respiration is often considered to be a three-phase process, beginning with glycolysis, in which glucose is converted to pyruvate, which is fed into the Krebs cycle to generate CO_2 and NADH. Finally, electron transport and oxidative phosphorylation occur using NADH generated during the Krebs cycle, resulting in the production of large amounts of ATP. During seed germination, there is a rapid increase in glycolytic activity linked to an increased rate of respiration [17]. This glycolytic activity is necessary to mobilize stored carbohydrates to provide the seed with the reducing power, ATP, and carbon products required for the biosynthesis of the roots and aerial parts of the emerging seedling.

Seed germination in *Pinus laricio* is inhibited by several phenolic compounds from the soils around *P. laricio* and *Fagus sylvatica* trees. This inhibition has been attributed to a disruption of the activity of metabolic enzymes that are involved in glycolysis and the oxidative pentose phosphate pathway (OPPP), which takes substrates from glycolysis and feeds its products back into glycolysis [18]. However, the exact molecular mechanism of this disruption has not been determined. The glycolytic enzymes aldolase (ALD) and glucosephosphate isomerase (PGI), together with glucose-6-phosphate dehydrogenase (G6PDH), which catalyzes the first step of the OPPP, showed decreased activity in the presence of phenolic compounds found in soil [18]. Several of these phenolic compounds, such as vanillic, *p*-coumeric, *p*-hydroxybenzoic and pro-

tochatechuic acid, tested alone and in combinations, were able to inhibit the enzymatic activity of all or several of the enzymes monitored [18]. This suggests that the decrease in enzymatic activity is a secondary effect of these compounds, which might be caused by general protein damage leading to decreased enzymatic activity.

Some allelopathic compounds are thought to interact with the mitochondrial membrane and to impair mitochondrial respiration directly. The monoterpenes camphor, α -pinene, and limonene all strongly affected the respiratory activity of soybean radicular hypocotyl mitochondria, but apparently have different modes of action [19•]. Camphor stimulated basal respiration but had no effect on oxygen uptake in the presence of ADP, suggesting that it causes mitochondrial uncoupling; whereas limonene only affected oxygen uptake that was dependent on ADP addition, suggesting that it may act on ATP synthase or the adenine nucleotide translocase complexes [19•]. The effect of *a*-pinene may involve more than one mechanism, one of these being interference with the electron flow in the cytochrome pathway [19•,20]. The flavonoid quercetin has also been shown to cause decreased respiration in isolated soybean hypocotyl mitochondria through three potential mechanisms. Quercetin appears to inhibit substrate oxidation, probably by interfering with electron transport directly, to inhibit the uptake of phosphate, and possibly to uncouple oxidative phosphorylation [21]. Unfortunately, it is difficult to measure allelochemical effects on mitochondrial respiration in intact plants because many of these effects are masked by photorespiration that occurs in the chloroplasts. In fact, no correlation between respiratory inhibition in isolated mitochondria and seed germination or root growth was found upon treatment of maize with α -pinene and limonene [22]. Therefore, many of the inhibitory effects of the monoterpenes have been measured only in isolated mitochondria, and their ability to act as allelochemicals on intact seeds and plantlets is probably directly related to their ability to permeate intracellular compartments.

ROS-mediated allelopathic mechanisms

Redox transformations that ultimately result in the formation of reactive oxygen species (ROS) play an important role in interactions between plants and their pathogens, mutualists, and competitors [23]. Controversially, not only have ROS been implicated in signal transduction and in plant defense mechanisms, such as the hypersensitive response, but also ROS accumulate in plant cells in response to compatible pathogen infections and are known to damage cells, often leading to cell death [24]. The toxicity of many quinones and phenols can largely be attributed to the formation of semiquinone radicals that donate electrons to molecular oxygen, forming superoxide anions (O_2^-) [25]. These can undergo a series of further reactions to become the more reactive

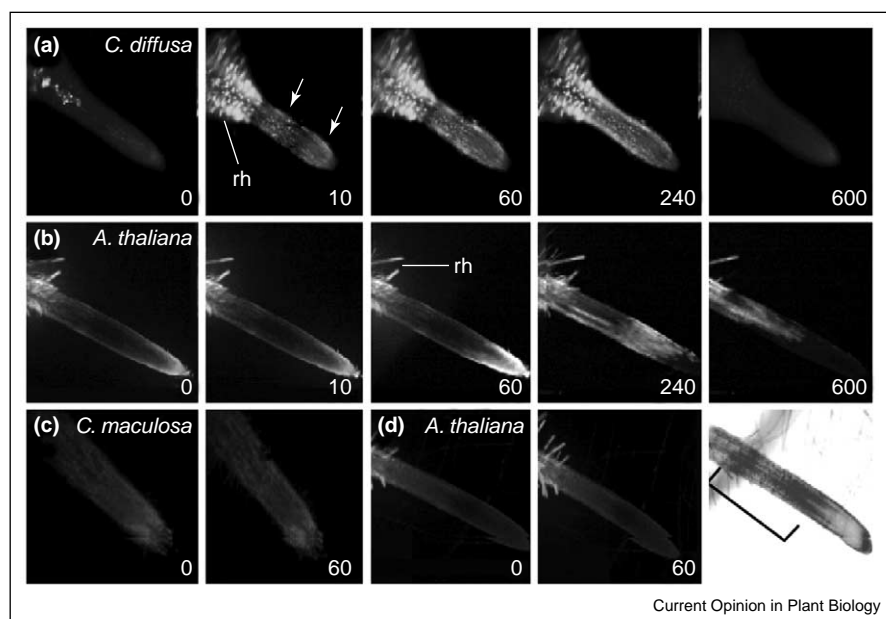
hydroxyl (OH^\bullet) or hydroperoxyl (HO_2^\bullet) radicals [26]. Subsequently, these radicals can affect membrane permeability, cause damage to DNA and proteins, and generate lipid peroxide signaling molecules. Some allelochemicals rapidly depolarize the cell membrane, increasing membrane permeability, inducing lipid peroxidation, and causing a generalized cellular disruption that ultimately leads to cell death [27–29,30^{*}]. Conversely, H_2O_2 is thought to be directly toxic to microbes, to contribute to the structural reinforcement of plant cell walls, and to coordinate the activation of defense genes and phytoalexin production [31–33].

Allelopathic cucumber root exudates/extracts and phenolic acids have an autotoxic effect on the roots of cucumbers [30^{*}]. Exposure to these allelopathic agents reduced stomatal conductance, leaf transpiration and net photosynthesis, and significantly increased root peroxidase (POD) and superoxide dismutase (SOD) activities. SOD activity increased gradually with exposure to increasing concentrations of root extracts/exudates from cucumber, suggesting that the presence of superoxide anions also increased in treated roots as the concentrations of root extracts/exudates increased. SOD converts superoxide anions into hydrogen peroxide (H_2O_2), which can then be converted into harmless H_2O molecules in a reaction catalyzed by catalase or POD. Conversely, it has also been reported that some allelochemicals reduce SOD and POD activity. The phytotoxic allelochemical seca-

lonic acid F, from the fungus *Aspergillus japonicus*, significantly reduced SOD and POD activity in several plants [29]. Likewise, aqueous extracts from rice blocked SOD and catalase activity in *Echinochloa crus-galli* (barnyardgrass) [28]. These results suggest that, in some cases, an allelochemical may be directly involved in the production of ROS whereas the increase in oxidizing enzymes is a secondary response to the increase in free radicals. In other cases, the allelochemical might directly inhibit oxidizing enzymes in some way, leaving the plant vulnerable to oxidative damage.

A transient oxidative burst in plant cells in response to elicitation by pathogens has been reported many times, but a correlation between allelopathic chemicals and a transient increase in ROS has been elucidated only recently [34^{**}]. Using the ROS-sensitive fluorescent dye dichlorofluorescein (DCF), ROS generation was visualized in roots of *Arabidopsis thaliana* that were in direct contact with (–)-catechin, an allelochemical from the invasive weed *C. maculosa* [35^{**},36^{**}]. Within 10 s of contact with (–)-catechin, the roots of *A. thaliana* generated a wave of ROS that moved backwards from the root meristematic region, into the central elongation zone, and finally into the mature region of the root (Figure 2). Subsequent experiments showed that the addition of ascorbic acid along with (–)-catechin blocked the ROS response, supporting the hypothesis that increased activity of antioxidants and antioxidant

Figure 2



(–)-Catechin ($100 \mu\text{g ml}^{-1}$) elicits intracellular ROS generation in (a) *C. diffusa* and (b) *A. thaliana*, as visualized by DCF fluorescence. The generation of ROS originates at the root tip and progresses back to the elongation zone (arrows). Numbers represent seconds after treatment. (c) *C. maculosa* roots showed low stable concentrations of ROS throughout the (–)-catechin treatment. (d) Simultaneous treatment with ascorbic acid (50 mM) resulted in the failure of (–)-catechin to induce ROS production in *A. thaliana* (scale bar, 200 μm), and (e) kept roots viable even 600 seconds after catechin treatment. This figure is a modified version of Figure 3 from [34^{**}]. Rh, root hair.

enzymes is probably a secondary effect of many allelochemicals. It seems that the receiving plant increases the activities of these enzymes in an attempt to counteract the harmful effects of ROS generated either by the various oxidative states of allelochemicals themselves or by a plant signaling cascade that is induced by the allelochemical.

Alternative allelopathic mechanisms

The phytotoxicity of some allelochemicals is attributed to their ability to disrupt normal metabolic processes in the plant. Disruption of amino acid metabolism is an important mechanism for commercial herbicides such as glyphosate and sulfonylurea derivatives, and may be an important mode of action for some allelochemicals. For example, the phytoinhibitory property of L-canavanine, an L-arginine analog found in *Canavalia ensiformis*, is probably due to its ability to block L-arginine metabolism, leading to a deficiency in compounds that are derived from L-arginine [37]. In insects, the incorporation of L-canavanine rather than L-arginine into proteins leads to altered conformation and disrupted function [38]. Although the disruption of amino-acid metabolism by allelochemicals has not yet been shown in plants, it is certainly another potential mechanism for allelochemicals that act as amino acid analogs.

Regulation of the concentrations of hormones, such as auxins and gibberellins, is also important for normal plant cell growth and morphogenesis. Some flavonoid aglycones act to inhibit polar auxin transport, leading to a disturbance in normal auxin levels and resulting in the induction of lateral roots and the suppression of ageotropic growth [39]. Naturally occurring benzoic acids may be involved in auxin catabolism. At low concentrations, the naturally occurring benzoic-acid derivative 3,4-dihydroxybenzoic acid (3,4-DHB) mimics auxin by stimulating callus formation, whereas higher concentrations of this molecule inhibited root growth in tobacco [40]. Because of the ability of auxin to induce ethylene synthesis in root tissues, auxin concentrations of 10^6 M and above are considered to inhibit root growth [41]. If 3,4-DHB acts as an auxin analog, it may also be able to stimulate ethylene synthesis, and this could explain why higher concentrations of this molecule inhibited tobacco growth. In fact, this may explain the concentration-dependent effect of allelochemicals that mimic or influence the synthesis of auxins. A few phenolic compounds have also been reported to have auxin-protecting activity, which leads to the accumulation of auxins. These allelochemicals act by inhibiting the peroxidase- and oxidase-catalyzed oxidation of auxin [42,43].

Indirect allelopathic mechanisms of allelopathic compounds

It is difficult to discern whether some of the specific physiological effects that have been reported are the

primary or secondary consequences of exposure to an allelochemical. We do know that these effects are generally due to direct physical interactions between susceptible plant cells and a phytotoxin, which can generally be tested in a laboratory setting. Once the interaction between plants and allelochemicals is placed in a more dynamic ecological situation, there are several factors that interact to influence the phytotoxicity of an allelochemical. In some cases, allelochemicals may have an effect on plants that is independent of direct contact with them. For example, glucose and phenylalanine are not typically considered to be allelopathic. However, these compounds increase the allelopathic ability of *p*-coumaric acid to inhibit the growth of morning glory (*Ipomea violacea*) by reducing the microbial utilization and sorption onto soil particles of *p*-coumaric acid [44,45].

Plants that display allelopathic potential against other organisms induce net changes in the ecosystem that may have long-term impacts on plant communities [46]. Secondary metabolites that are deposited into the soil through exudation or from decaying plant matter provide a rich source of carbon that is utilized by soil microbial populations. The composition of these metabolites influences the microbial composition of the rhizosphere, potentially affecting the plants with which they interact. The addition of a 1% solution of saponins from *Gypsophila paniculata* was sufficient to change the population dynamics of the rhizosphere of clover (*Trifolium subterraneum*) [47]. After treatment with saponins, the two dominant bacteria in this rhizosphere, *Chryseomonas* spp. and *Acinetobacter* spp., were significantly decreased, while *Aquaspirillum* spp. (the dominant bacteria in *Gypsophila paniculata* rhizospheres) became the major genus. No effect on the biomass of *T. subterraneum* was noted in this study but the amount and species composition of rhizosphere bacteria is important for the plant's ability to obtain nitrogen or nutrients from the soil. In fact, invasive *C. maculosa* in North America cultivates soil biota that positively effect its growth whereas, in its native habitat (Eurasia), the soil biota surrounding *C. maculosa* negatively impacts this species [48].

Mechanisms of resistance to allelochemicals

Some plants have found ways to reduce the effects of allelochemicals produced by neighboring plants. Detoxification mechanisms that are used by plants include the conjugation, sequestration or secretion of carbohydrates, and the oxidation of the phytotoxic compounds [49]. Plants that are equipped to metabolize the benzoxazinone degradation product, benzoxazolin-1(3*H*)-one (BOA), detoxify this allelopathic compound through *N*-glucosylation, through the addition of a pentose sugar moiety, or by hydroxylation followed by glucosylation [50–53]. These activities result in several structurally

similar but less toxic products, such as BOA-6-*O*-glucoside. Detoxification products are then released into the environment, where they are presumably metabolized by soil microorganisms, in root exudates [52]. Another detoxification mechanism used by plants is the glutathione *S*-transferase-catalyzed glutathionation of toxins and their subsequent transport out of the cytoplasm. In *Arabidopsis thaliana*, a Mg²⁺-ATPase transporter is responsible for the removal of glutathione *S*-conjugates from the cytosol [54]. The sequence of the gene that encodes this transporter suggests that it is an ATP-binding cassette transporter that is proficient in the transport of glutathione *S*-conjugates of xenobiotics and endogenous substances.

In some cases, rhizosphere soil and microorganisms may be credited with decreasing the phytotoxicity of allelopathic compounds, particularly phenolic acids. Soil microorganisms rapidly mineralize phenolic compounds because these compounds have greater energy/weight than simple sugars [55]. Soil that was rich in individual phenolic acids was able to induce colonization by bacteria that utilize phenolic acid, and these bacteria reduced the inhibition of seedling growth normally attributed to phenolic acids [56]. Finally, phenolic acids react with soil via sorption and oxidation, decreasing their phytotoxicity [57–59].

Conclusions and future directions

In this review, we have presented evidence indicating that certain allelochemicals, such as (–)-catechin, are able to induce stress responses in specific parts of the root, such as the root tip [34**]. Furthermore, this specific plant response to the allelochemical triggers a genetically tractable cell-death cascade in susceptible plants. This observation indicates that the chemical conformation of certain allelochemicals is very favorable for the manipulation of genetic cell-death cascades that are conserved among plants. It argues that allelochemicals may not necessarily be inherently toxic compounds but rather compounds that induce toxic responses. It is also very apparent that plant responses that are induced by microbes are related to phytotoxic responses induced by allelochemicals. It is possible that avirulence/virulence factors that are produced by microbes may have receptors that are similar to those that perceive allelochemicals; thus, molecular and genetic tools that are available for the study of plant–microbe interactions could be used to study the plant–plant interactions that are mediated by allelochemicals.

Acknowledgements

This work was supported by grants from the Colorado State University Agricultural Experiment Station (JMV), the Environmental Protection Agency (JMV), the National Science Foundation (grant no. NSF-IBN 0335203, JMV), USDA-WRIPM (grant no. 2003-05060, JMV), and USDA-NRI (grant no. 2003-02433, JMV). JMV is a NSF Early Career Development Faculty Fellow (grant no. MCB 0093014).

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