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CHAPTER TWO

Allelopathy and the Role of Allelochemicals in Plant Defence

S. Latif[*], G. Chiapusio[§,¶], L.A. Weston[*]
[*]Charles Sturt University, Wagga Wagga, NSW, Australia
[§]Université de Bourgogne Franche-Comté, UMR CNRS 6249, Montbéliard, France
[¶]Savoie Mont Blanc University INRA CARRTEL, Chambéry, France

Contents

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Abstract

Allelopathy is described as the interference to plant growth resulting from chemical interactions among plants and other organisms mediated through release of plant-produced bioactive secondary metabolites referred to as allelochemicals. A number of mechanisms have been studied for the release of allelochemicals from various plant tissues including volatilization or leaching from aerial parts, exudation from roots and decomposition of plant residues in soil. Despite differences in biological activity and mode of action, related compounds commonly share similar biosynthetic pathways while some classes of metabolites can be produced using diverse biosynthetic pathways. Recently considerable research has also been undertaken to critically understand the role of allelochemicals in plant succession and plant invasion in native and nonnative ecosystem. In addition, numerous studies have been performed on the selection and utilization of weed suppressive crops and their residues for weed management in sustainable agriculture systems. A better understanding of allelochemical production with respect to plant defence strategies, both physical and chemical, may also allow us to better protect and manage developing crops, limit the spread of invasive weeds, preserve native plant stands and create strategies for allelochemical development and application as novel pesticides. The use of sensitive analytical techniques associated with performance of metabolomics in concert with other *omics* technologies has led to new advances in the identification of unique allelochemicals, the biosynthetic pathways associated with their production, their complex role(s) in the soil rhizosphere and their production as impacted by a changing climate. Identification of novel plant metabolites, including allelochemicals, may result in a source of biologically based pesticides through the provision of complementary structures for future synthesis and as an aid in the development of new molecular target sites.

1. INTRODUCTION

Plants and associated microflora collectively produce a vast assortment of compounds of diverse chemical nature, the majority of which do not appear to have a role in primary metabolism necessary for growth and development of the plant. These low organic weight compounds are referred to as plant secondary products or secondary metabolites. Certain classes of secondary metabolites called allelochemicals have stimulatory and/or inhibitory effects on the growth, health and behaviour of neighbouring plants (Rice, 1974). This phenomenon is referred to as allelopathy, a term first applied by Molisch (1937), broadly defined by some researchers as direct or indirect biochemical interactions among plants, and potentially also microorganisms, mediated through release of allelochemicals by plants (Weston & Duke, 2003).

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The International Society of Allelopathy (IAS) defined allelopathy as chemical interactions among plants and other organisms, excluding herbivores (Weir, Park, & Vivanco, 2004). Given that most researchers studying allelopathy have focused on interactions among plants mediated by allelochemicals, we have chosen to describe plant–plant interactions in this chapter. However, it is important to note that microbes can both degrade and also activate secondary metabolites, in some cases rendering them more biologically active as plant growth inhibitors (Cipollini, Rigsby, & Barto, 2012). Secondary products exhibiting allelopathic potential are referred to as ‘allelochemicals’ (Whittaker and Feeny, 1971), however, both terms are not interchangeable. Moreover, allelopathic interference due to the action of allelochemicals differs from interference due to competition in which plants compete with neighbouring plants for soil resources including, water, space, light, gases and macro- and micronutrients necessary for growth (Weston & Duke, 2003). However, both competition and allelopathy generally result in growth reduction or interference with plant growth over time.

Allelopathy is not a new phenomenon and has been described for over 2000 years in the literature; in ancient manuscripts, plants were frequently reported to ‘sicken the soil’ and produce toxins that adversely affected the growth of other plants (Weston & Duke, 2003). The Greek botanist Theophrastus (300 BC) first noted that chickpeas exhausted the soil and inhibited weeds. Later, the Roman scholar Pliny described the toxicity of the walnut tree to neighbouring plants (Weir et al., 2004). In 1832, De Candolle performed the first well-described experiment to study toxicity associated with root exudates of allelopathic species (Singh, Batish, & Kohli, 2001). In recent years, following on from these foundational studies, research into the mechanisms associated with allelopathic interactions has increased exponentially. The latest studies on allelopathy have been critical in advancing our understanding of plant ecosystems and their drivers and have focused on the following: the impact of allelochemicals on successful invasion by plant invaders in the nonnative range; the role of allelochemicals in plant succession in dynamic ecosystems; the selection and utilization of weed suppressive crops and their residues for enhanced weed management; and the role of root exudates in driving rhizosphere interactions with plants and/or microbial symbionts.

This chapter aims to present a general overview of allelopathic interactions among plants and plant defence mechanisms, the biochemical classification of allelochemicals, allelochemical mode(s) of action and key allelochemical interactions occurring in the rhizosphere or plant–soil interface.

2. PLANT DEFENCE AND THE ROLE OF ALLELOCHEMICALS

Plants are sessile and therefore cannot easily move away from potential threats or towards beneficial entities. During the course of evolution, plants have developed both physical and chemical mechanisms of defence from pests and pathogens (Bernards, 2010). Traditionally resource competition has been considered as the single most important factor that influences the patterning of plant communities (Niklas & Hammond, 2013). However, recent research has described allelopathy as an important aspect of plant defence that impacts plant community diversity (Fernandez et al., 2013). In this process, plants release a diverse repertoire of low molecular weight secondary metabolites that are considered to interact with the surrounding environment by inhibiting the germination or growth of neighbouring plants (Ben, Jordan, & Osbourn, 2006; Fernandez et al., 2016). The majority of allelochemicals in the plant kingdom are found in vascular plants, but our knowledge of secondary metabolites in ancient terrestrial nonvascular plants such as mosses or liverworts has also increased in recent years. Allelochemicals can, therefore, play an important role in plant succession through their release by pioneer plants (i.e., Bryophytes) which contribute substantially to the accumulation of aboveground biomass, particularly in cold temperate biomes including boreal forests and peatlands (Chiapusio, Jassey, Hussain, & Binet, 2013; Michel, Burritt, & Lee, 2011).

Approximately 100,000 secondary metabolites have been identified to date in plants (Afendi et al., 2012; Croteau, Kutchan, & Lewis, 2000). A smaller number of these are described as bioactive allelochemicals and are generally classified as members of specific chemical families that include phenolics, terpenoids, glycosteroids and alkaloids (Ahuja, Kissen, & Bones, 2012). Plants can allocate large investments in carbon resources to synthesize, regulate and store secondary metabolites including allelochemicals involved in plant defence. However, many plants have evolved cost-effective strategies for production and recycling of these bioactive metabolites; the conversion of one product to another through plant metabolism may result in a completely different biological function. In some cases, a single compound or related family of compounds can have multiple functions from an ecological context. For example, strigolactones are stimulants of germination of parasitic plants while other lactones are potent germination inhibitors. Despite differences in biological activity and mode of action, related compounds commonly share similar biosynthetic pathways while some classes of metabolites including phenolics can be produced using diverse biosynthetic pathways and precursors (Neilson, Goodger, Woodrow, & Møller, 2013).

Plants have further evolved protection from potential predators, including herbivores and pathogens, through specific physical and chemical defence mechanisms. Plants possess specialized morphological structures including trichomes, spines and hairs that provide generalized physical defence. Specialized defence mechanisms include the production and release of bioactive metabolites, some of which are constitutive and found in almost every class of plants while others are synthesized or activated in response to biotic and abiotic stressors (Bartwal, Mall, Lohani, Guru, & Arora, 2013). Precursors which are formed immediately after pathogen attack from preexisting constituents are referred as phytoanticipins or more commonly as phytoalexins (Pedras, Yaya, & Glawischnig, 2011; VanEtten, Mansfield, Bailey, & Farmer, 1994). These low molecular mass metabolites are produced in host plants under diverse stressors and exhibit broad-spectrum inhibitory activity and extensive chemical diversity.

The development of microbial symbiosis is also crucial for the survival and growth of vascular plants. For example, mycorrhizal fungi form symbiotic associations with 90% of vascular plants and are also ubiquitously distributed in a wide range of ecosystems (Finlay, 2008; Read, Leake, & Perez-Moreno, 2004). These organisms often play fundamental roles in belowground processes because they are crucial for the mobilization of nitrogen and phosphorus from the soil to their host plant. These organisms are also sometimes involved in mediation of allelopathic interference as plant-produced allelochemicals, herbicides and other metabolites present in the soil rhizosphere are released into the rhizosphere and later taken up and transported to other plants by way of the endophyte's network of underground hyphal networks, sometimes referred to as the belowground 'superhighway' facilitating interplant transport of allelochemicals (Delaux, Sejalon-Delmas, Becard, & Ane, 2013; Souto, Pellissier, & Chiapusio, 2000).

2.1 Allelochemical Localization and Release Into the Environment

Secondary metabolites including allelochemicals are ubiquitous in nature and can be released over time from all plant tissues including leaves, stem, roots, flowers, seed, rhizomes, pollen, bark and buds (Weston & Duke, 2003). A number of mechanisms including volatilization or leaching from aerial parts, exudation from roots and decomposition of plant residues in

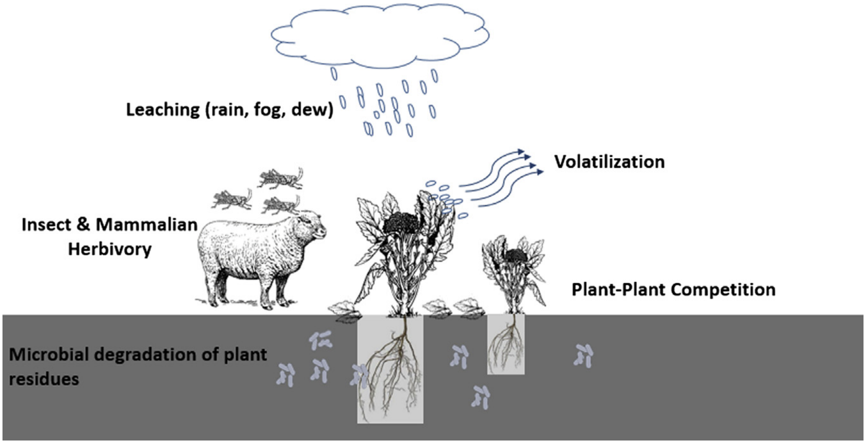


Figure 1 Chemical (leaching, volatilization) and biological (herbivory, microbial degradation, competition) mechanisms of release of allelochemicals from various plant tissues.

soil (Fig. 1) have been well characterized with respect to the release of allelochemicals over time in the environment (Cheng & Cheng, 2015; Putnam & Tang, 1986). Direct volatilization and precipitation, including rain, fog and dew, also play an important role in the solubilisation and release of allelochemicals from foliar parts of the plants, particularly those that possess glandular trichomes containing allelochemicals (Bais, Weir, Perry, Gilroy, & Vivanco, 2006; Inderjit & Duke, 2003). Trichomes are hair-like appendages on the surface of the plant leaf, stem or inflorescence. They provide a physical defence against mammalian herbivores upon ingestion, and potentially also chemical defences due to the unpleasant sensorial experience associated with the allelochemical release. In addition to a physical barrier, glandular trichomes have specialized cells which can synthesize allelochemicals as part of a constitutive chemical defence system (Champagne & Boutry, 2016).

3. CLASSIFICATION OF SECONDARY METABOLITES

Secondary metabolites including allelochemicals can logically be classified according to their carbon skeletal structure and type of functional groups; however, the most useful classification system is based on the biogenetic origin of metabolites (Fig. 2). As presented by Walton and Brown (1999) and Rice (1974) plant metabolites and allelochemicals can be further divided into three major groups: phenolics, terpenoids, and alkaloids.

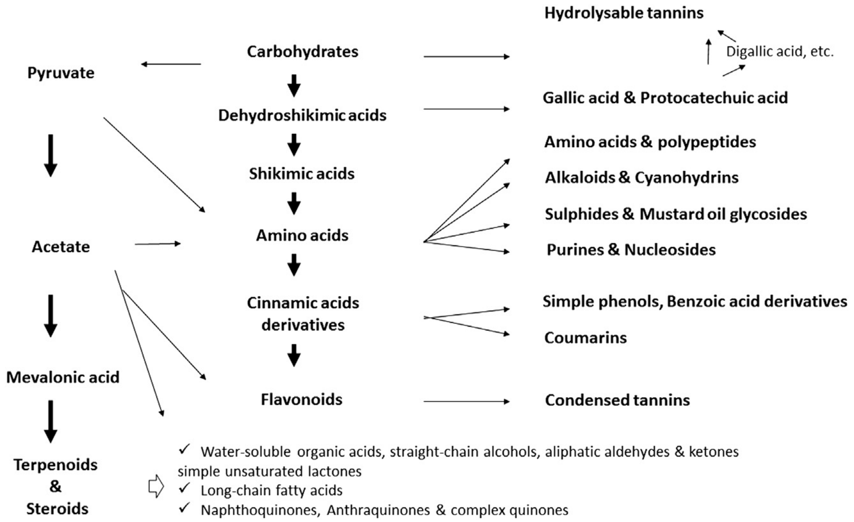
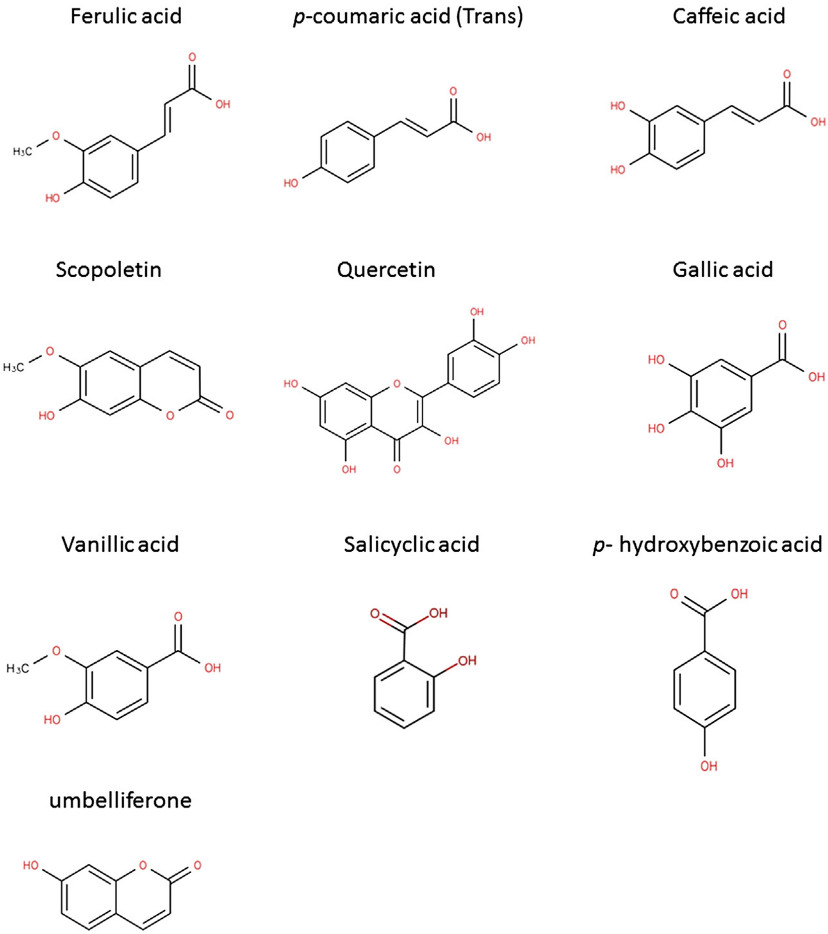


Figure 2 Major families of allelochemicals and their common biosynthetic pathways originating from numerous key precursors (Rice, 1974). Amino acids and polypeptides as well as purines and nucleosides are often categorized as primary metabolites due to their roles in primary biosynthetic pathways of proteins and DNA. However, some of these molecules are also bioactive in their own right and can serve as allelochemicals, thereby impacting higher plant growth.

3.1 Phenolic Compounds and Their Derivatives

Plant phenolics (Fig. 3) represent an extremely diverse group of organic compounds which share a common structure; they consist of an aromatic ring possessing at least one hydroxyl group and possibly other substituents (Stalikas, 2007). They are generally categorized as phenolic acids, flavonoids, stilbenes, coumarins, lignins and tannins (Cheynier, Comte, Davies, Lattanzio, & Martens, 2013; Dinelli et al., 2009). The term phenolic is descriptive as phenolics are secondary metabolites derived from the phenylpropanoid-acetate biosynthetic pathway (Croteau et al., 2000; Haig, 2008, pp. 63–104; Quideau, Deffieux, Douat-Casassus, & Pouysegu, 2011). Approximately 8000 naturally occurring secondary metabolites belonging to this group have been identified so far. They perform a broad array of structural and physiochemical roles *in planta* (Croteau et al., 2000). Phenolics are the most commonly reported metabolites known to play a role in defence mechanisms in higher plants; with variable toxicity, they target cellular functions at multiple sites (Haig, 2008, pp. 63–104). They also represent 40% of the structural matrix of plants in form of lignins



Ferulic acid

p-coumaric acid (Trans)

Caffeic acid

Scopoletin

Quercetin

Gallic acid

Vanillic acid

Salicyclic acid

p- hydroxybenzoic acid

umbelliferone

Figure 3 Some examples of diverse plant phenolics with known allelopathic properties.

and tannins, and through these molecules which degrade over time in the soil rhizosphere, maintain their abundance through the important ecological cycle of synthesis, transformation, release and decomposition (Dalton, 1999, pp. 57–74; Hattenschwiler & Vitousek, 2000).

The mechanism of allelopathy associated with phenolic compounds has been studied extensively and reports indicate that phenolics interfere with several key plant enzymes and physiological processes. For example, cinnamic and benzoic acids (Fig. 3) interfere with hormone activity, membrane permeability, photosynthesis, respiration and synthesis of organic compounds. However, there has been no evidence to date for phenolics impacting cell division or gene translation (Einhellig, 2004).

Perennial legumes including alfalfa (*Medicago sativa* L.) and clovers (*Trifolium* spp.) are known for their allelopathic properties following pasture establishment (Weston & Mathesius, 2013). Some species including alfalfa and clover can also prove autotoxic once they are established. These perennial plant stands tend to inhibit the growth of plants of the same species over time. Carlsen, Pedersen, Spliid, and Fomsgaard (2012) studied the phenomenon of phytotoxicity following clover establishment and reported that release of several secondary metabolites, including flavonoids and their glycosides, causes weed suppression and negative plant–plant interactions associated with replanting following legume establishment.

3.2 Terpenoids

Terpenoids are specific allelochemicals that are biosynthetically derived from the mevalonic acid and isopentenyl pyrophosphate pathways (Haig, 2008, pp. 63–104). The term 'terpene' or the preferred term 'terpenoid' comes from the German word 'terpentin' (terpentine) due to the fact that first reported terpenoid was isolated from turpentine (Herz, 1963). Naturally occurring, approximately 24,000 terpenoids are comprised of five carbon isoprene subunits (Fig. 4) linked together through the common head-to-head or less common head-to-tail linkages but some are also characterized by head-to-middle ring closure (Croteau et al., 2000). Based on the relative number of isoprene subunits possessed, they are classified into hemiterpenes (single five-carbon isoprene unit) monoterpenes (two isoprene units), sesquiterpenes (three isoprene units), diterpenes (four isoprene units), triterpenes (six isoprene units), tetraterpenes (eight isoprene units) and polyterpenes (with more than eight isoprene subunits). Terpenoids are typically volatile compounds having multiple biological activities in plants as signalling molecules, photoprotective agents, reproductive hormones but also allelochemicals (Croteau et al. 2000).

Monoterpenes, the main constituents of plant essential oils, are widely known for their strong inhibitory effects on plant growth and seedling germination (Haig, 2008, pp. 63–104). For example, the monoterpenes 1,4-cineole and 1,8-cineole (Fig. 5) are well studied as growth inhibitors

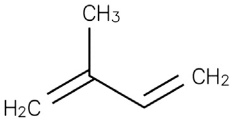


Figure 4 The structure of an isoprene unit, the backbone of terpenoids.

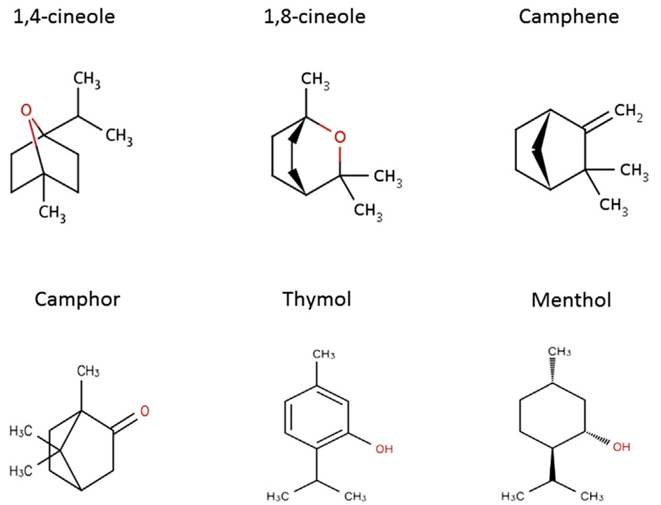


Figure 5 Some examples of plant monoterpenes with known allelopathic properties.

and potential candidates for herbicides (Duke et al., 2004). Despite structural similarities, these monoterpenes have very different modes of action where 1, 8-cineole affects all stages of mitosis and 1, 4-cineole causes growth abnormalities in shoots.

Sesquiterpenes and their associated metabolites along with the monoterpenes in essential oils have been well characterized with respect to their phytotoxic effects on plants. The sesquiterpene *β*-caryophyllene (Fig. 6) is

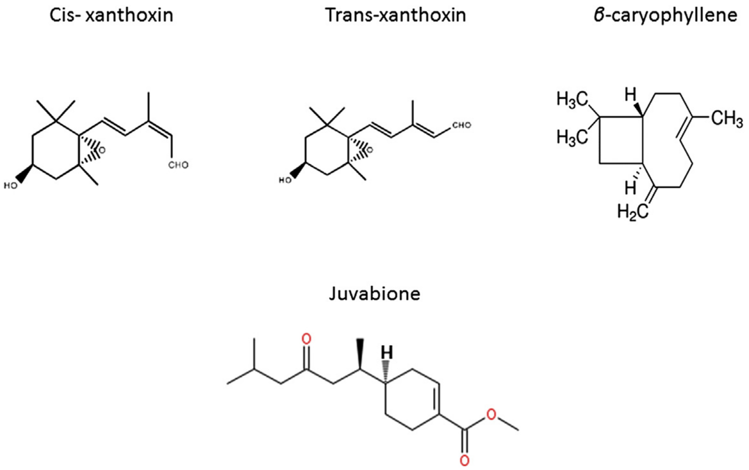


Figure 6 Some examples of plant sesquiterpenes with known allelopathic properties.

present in numerous plant volatiles and inhibits germination and seedling growth of *Brassica napus* L. and *Raphanus sativus* L. at very low concentrations (Wang, Peng, Zeng, Ding, & Xu, 2009). Geometric isomers of xanthoxin (Fig. 6) present in leaves of *Pueraria thunbergiana* L. (Sieb. and Zucc.) Benth., also known as the invasive kudzu vine, inhibited seedling root growth of numerous small-seeded annual broadleafs and grasses at very low concentrations (Kato-Noguchi, 2003; Rashid, Asaeda, & Uddin, 2010).

3.3 Alkaloids

Alkaloids (Fig. 7) are heterocyclic nitrogen-containing basic compounds of plant origin and are named accordingly, due to their alkaline chemical nature. Plant alkaloids predominate in four families of plants including the Asteraceae, Apocynaceae, Boraginaceae and Fabaceae (Haig, 2008, pp. 63−104).They have been important since antiquity due to their pharmacological properties and are among the largest group of secondary metabolites with approximately 20,000 compounds identified to date representing great structural biosynthetic diversity (Yang & Stöckigt, 2010). Based on their biosynthetic origin, alkaloids are classified into different classes; e.g., indole alkaloids are derived from tryptophan, pyrrolizidine alkaloids are derived from ornithine or arginine, and quinolizidine alkaloids are derived from lysine (Seigler, 1998, pp. 546−567).

Alkaloids are widely distributed across the plant kingdom and frequently reported to play key defensive roles in plant interactions against herbivores,

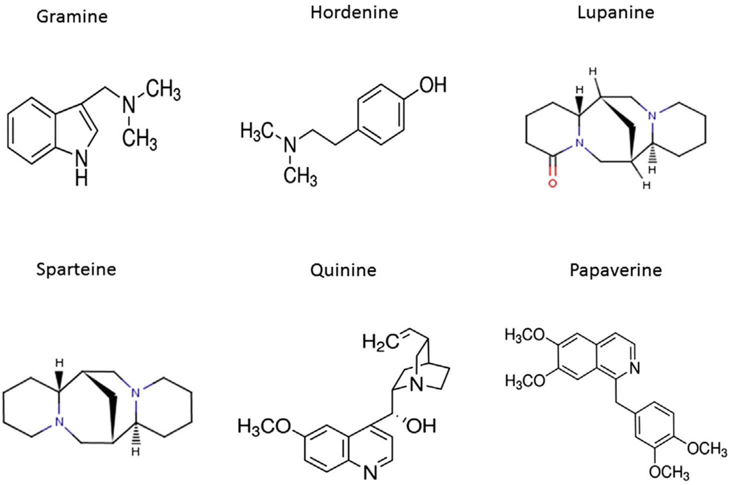

Gramine
Hordenine
Lupanine
Sparteine
Quinine
Papaverine

Figure 7 Some examples of plant alkaloids with known allelopathic properties.

microorganisms, fungi, and in some cases, neighbouring plants. Several naturally occurring pyrrolizidine alkaloids have been evaluated for hepatotoxicity to humans and grazing livestock (Quinn, Kessell, & Weston, 2014) and also deter herbivory as part of the plant's aboveground defence system. However, other alkaloids have in some cases shown activity as inhibitors of plant growth. Nicotine production in wild tobacco is upregulated after grazing or damage by herbivores; interestingly nicotine also possesses allelopathic activity against common annual weeds. Alkaloids are thought to inhibit plant growth by several mechanisms including interference with DNA, enzyme activity, protein biosynthesis and membrane integrity in developing plants (Wink, 2004). Lovett and Hoult (1995) studied defence mechanisms in barley (*Hordeum spp.)* through the release of the plant-produced alkaloids gramine and hordenine (Fig. 7) from living roots; these were shown to have allelopathic effects in seedling bioassays. The quinolizidine alkaloids studied by Wink (2004), including lupanine and sparteine, are produced by legumes and are thought to cause potent inhibition through interference with membrane permeability and protein synthesis.

3.4 Hydroxamic Acids of Benzoxazinoids: An Agronomic Case Study

Hydroxamic acids are phenol derivatives, known as cyclic 2-hydroxy-2H-1,4-benzoxazin-3-(4H)-ones, which are a basic skeleton of a broader family of bioactive plant secondary metabolites referred to as benzoxazinoids (Fig. 8). They have been well characterized by numerous scientists and have antimicrobial, antifungal, antifeedant and phytotoxic activities (Chiapusio et al., 2005, pp. 139–155).

Hydroxamic acids of benzoxazinoids present in cultivated cereals of the Graminaceae including maize, rye and wheat have been widely studied for their allelopathic potential and their abundance has been highly correlated with resistance to insects and other pathogens (Elek et al., 2013; Niemeyer, 2009). 2,4-Dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-one (DIMBOA)

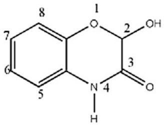


Figure 8 The structure of 2-hydroxy-2H-1, 4-benzoxazin-3-(4H)-ones, also known as hydroxamic acids of the benzoxazinoid family of allelochemicals.

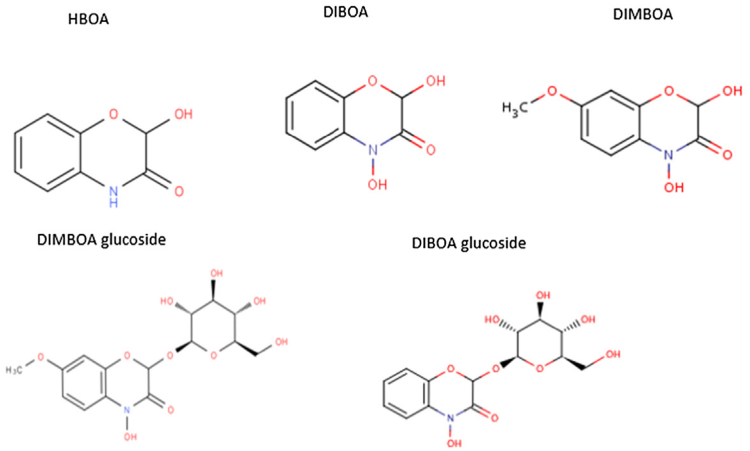


Figure 9 Common examples of benzoxazinoids with known phytotoxic properties.

and 2,4-dihydroxy-2H-1,4-benzoxazin-3-one (DIBOA) and their associated microbial conversion products (Fig. 9) are prominent hydroxamic acids with potent allelopathic effects on numerous broadleaf weeds and crops lettuce (*Lactuca sativa* L.) and wheat (*Triticum aestivum* L.) (Macías et al., 2003, pp. 239–252). They are typically released into soil from injured plant tissues or by degradation of crop residues after harvest. The glucosides of DIBOA and DIMBOA, together with their respective aglycones and degradation products released from rye (*Secale cereal* L.) root residues, are mainly responsible for allelopathic properties on developing broadleaf crops and weeds and several annual grasses (Schulz, Marocco, Tabaglio, Macias, & Molinillo, 2013).

4. ALLELOCHEMICAL MODE OF ACTION

One of the current challenges in allelopathy is to determine the specific mode(s) of action of allelochemicals in association with their diverse chemical nature and multiple target sites in higher plants. Various bioassays have been designed to carefully study the direct effects of allelochemicals on higher plant growth as well as evaluate their underlying mechanism of action. This can be a particularly difficult task, as bioassays for specific enzymes that may be potential target sites of allelochemicals are often challenging to devise and require prior knowledge of the structure of the catalytic or target site. In some cases, the actual target site of allelochemical action is not evident by visual observation of affected plant growth and/or morphology. In addition, the relevance of some in vitro bioassays with respect to the provision of conclusive evidence related to allelochemical mode of action has come under scrutiny, and the relationship of in vitro effects to those seen in a natural setting has also been questioned (Inderjit & Weston, 2000).

It has also been suggested that the relative concentration of allelochemicals typically released into the environment is often quite low and transient; therefore, the concentration of an allelochemical or complex mixtures of allelochemicals encountered in the soil or in the environment is often difficult to estimate or measure (Weir et al., 2004). Therefore, it is particularly important to use appropriate bioassays at relevant concentrations to assess the potential inhibitory effects of allelochemicals on higher plant growth. In many cases, it may be critical to use more than one bioassay to assess impacts on plant growth. For example, assays utilizing local soils, plant species of importance in natural systems and those providing uniform, repeatable results are critical for the development of useful data sets. Other investigators, including those assessing mode of action of synthetic and natural products such as allelochemicals in the agrochemical industry have developed robust models to assess plant growth using very sensitive assays. One can typically use several assay conditions to generate data including the use of an aqueous plant known as pondweed or duckweed (*Lemna spp.*) that is rapidly growing and uniformly reproduces additional plantlets over a one week period. The duckweed assay allows the testing of very low concentrations of allelochemicals as well as mixtures of metabolites, provided the compounds can be solubilized in an aqueous solution. In addition, various small pot assays using local soils and/or agar have been adopted to assess impacts both above- and belowground on shoots and roots, respectively (Blum, 2014, pp. 77—129). These small pot or container assays typically require a longer period to generate plant growth for assessment. Finally, a bioassay to assess direct impacts of decomposing plant residues has also been utilized to assess phytotoxicity associated with plant residues following a crop or a weed infestation using recovered plant tissue (Weston, Harmon, & Mueller, 1989). By use of appropriate assays for screening, a number of different mechanisms of action have been postulated and later confirmed using very specific assessment at the site of action; these include membrane permeability, water and nutrient uptake, respiration, photosynthesis, protein and nucleic acid synthesis, and growth regulation in susceptible plants (Einhellig, 1995).

4.1 Membrane Permeability

Many secondary metabolites including allelochemicals alter cell permeability and membrane function with exposure at adequate concentrations.

Exposure to such metabolites may result in leakage of cellular contents and consequently cell death through apoptosis and necrosis (Li, Wang, Ruan, Pan, & Jiang, 2010). This process eventually leads to tissue death and loss of specific function. For example, several phenolic compounds are reported to exhibit allelopathic effects by altering plasma membrane permeability. The compounds are easily able to cross cellular membranes either by diffusion or assisted transport mechanisms. Once membranes have experienced altered permeability, potassium channels are impacted and decreased permeability to chloride ions is often encountered. Yoon et al. (2000) and Singh, Batish, Kaur, Arora, and Kohli (2006) reported that the monoterpenoid, α-pinene, caused oxidative stress to the plasma membrane and disruption of membrane structural integrity which led to cell death. Chai, Ooh, Ooi, Chue, and Wong (2013) also found that concentrations of phenolic compounds greater than 1 mM and mimosine (a nonprotein amino acid) in *Leucaena leucocephala* (Lam.) leachates caused increased membrane permeability in leaf tissue of hyacinth (*Hyacinthus orientalis* L.). Membrane injury is typically assessed in vitro by measuring relative electrolyte leakage in treated plant culture bioassays in contrast to an untreated control.

4.2 Water and Nutrient Uptake

To further investigate the potential mode of action of allelochemicals released into the environment, it is important to focus upon the relative health and function of the plant's root system. In the living roots, allelochemicals can frequently affect the activity of Na^+/K^+ pumps involved in the absorption of ions across the plasma membrane. For example, Abenavoli, Lupini, Oliva, and Sorgonà (2010) found that nitrate uptake and membrane H^+-ATPase activity in *Zea mays* L. (corn) roots were inhibited by cinnamic acid, ferulic acid and *p*-coumaric acid. Franche, Lindström, and Elmerich (2009) also reported that the phenolic compounds cinnamic and ferulic acid reduced the nutrient uptake of P and Fe and thereby inhibited the growth of target plants. Phytotoxins produced by *Beta vulgaris* subspp. *vulgaris* (sugar beet) make zinc more available for zinc-sensitive crops such as beans (*Phaseolis vulgaris* L.) and corn when planted following sugar beet (Boawn, 1965).

4.3 Respiration

Allelochemicals released from donor plants can also seriously affect the growth of plants by impacting the process of respiration including electron transfer in the mitochondria, oxygen uptake, CO_2 generation and oxidative phosphorylation for ATP generation (Cheng & Cheng, 2015). For example, Abrahim, Takahashi, Kelmer-Bracht, and Ishii-Iwamoto (2003) studied the monoterpenes camphor, α-pinene and limonene and determined that each influences respiratory activity in hypocotyl mitochondria of various donor plants through different mechanisms i.e., mitochondrial uncoupling, electron flow in the cytochrome pathways and oxygen uptake, respectively. Czarnota, Paul, Dayan, Chandrashekhar, and Weston (2001) found that the inhibitor sorgoleone, exuded by living sorghum roots in copious quantities, is both an inhibitor of electron transport in photosystem II at the D1 binding protein and also inhibits mitochondrial electron transport by uncoupling electron flow in all plant species evaluated, including weeds and crops. Allelochemicals typically exhibit a stronger effect on seedling growth and germination in contrast to the growth of mature plants, and it has been suggested that one of the underlying mechanisms of germination and seedling growth inhibition is through frequent disruption of mitochondrial ability to carry out respiration (Weir et al., 2004).

4.4 Photosynthesis

Allelochemicals can significantly influence the process of photosynthesis in several ways, including disruption of electron flow in PS I and II, impacting the synthesis of photosynthetic pigments or stimulation of the decomposition of photosynthetic pigments. Consequently, decreased concentrations of photosynthetic pigments in plant shoots can lead to reduced synthesis of ATP, primarily in photosystem II (Cheng & Cheng, 2015; Fengzhi, Kai, Fengming, & Xuedong, 2004; Weir et al., 2004). For example, sorgoleone reacts at the D1 binding protein site in photosystem II and has shown strong competitive interference with electron transport (Einhellig, 1995; Gonzalez, Kazimir, Nimbal, Weston, & Cheniae, 1997). Weeds (*Amaranthus retroflexus* L.) that were resistant to PS II herbicide inhibitors by mutational changes at the D1 binding site were also resistant to the impact of sorgoleone at this site, providing further evidence for activity at the D1 binding protein in PS II (Nimbal, Yerkes, Weston, & Weller, 1996). Poonpaiboonpipat et al. (2013) observed that essential oil from *Cymbopogon citratus* (DC.) Stapf. (lemongrass) significantly decreased the concentration of chlorophyll a and b in the seedling barnyard grass, thereby reducing photosynthesis and subsequent plant growth. These findings suggest that essential oils have a direct impact on chlorophyll biosynthesis. Studies performed with the allelochemical citral, a volatile monoterpene found in many plants, has shown that citral is effective not only on inhibition of seedling metabolism through respiration and reduced photosynthesis but also on adult plants by inhibiting growth and altering the plant oxidative status (Graña, Sotelo, Díaz-Tielas, Reigosa, & Sánchez-Moreiras, 2013). This suggests that mode of action of certain allelochemicals is age and tissue dependent and also dependent on the site of uptake in the plant.

4.5 Protein and Nucleic Acid Synthesis and Growth Regulation

Allelochemicals can impact the growth of neighbouring plants by targeting key regulatory mechanisms affecting plant growth. These mechanisms include intercalation of DNA, inhibition of DNA polymerase I and inhibition of protein biosynthesis and have been associated with reduction in plant growth due to allelochemical exposure (Wink & Latz-Bruning, 1995). Phenolic allelochemicals can also interfere with the formation of nucleic acids, critical in cellular metabolism and gene expression. Li et al. (2010) determined that the phenolic compounds ferulic and cinnamic acid adversely affected total protein synthesis. Allelochemicals from donor plants have also been reported to regulate the expression of other defence genes in the target plant. For example, synthesis of enzymes which are involved in the biosynthesis of phenolic compounds in rice are upregulated in the presence of barnyard grass (*Echinochloa crus-galli* L.), a common weed in cultivated rice (He et al., 2012). In the case of seedling rice, the growth of rice with the weed competitor barnyardgrass results in an increase in production of the allelochemical momilolactone in rice (Kato-Noguchi, 2011). This suggests that stress caused by plant competition or interference due to allelopathy alters a target plant's ability to produce defence compounds and response may occur in a matter of hours to days as measured by enhanced allelochemical production. Similar phenolics have also been shown to oxidize indole-3-acetic acid, a hormone required for cell elongation in higher plants (Yang, Ye, Liao, & Yin, 2005).

5. LOCALIZATION AND TRANSPORT OF ALLELOCHEMICALS IN DONOR PLANTS

Plants have specialized tissues for synthesis and release of secondary metabolites in the environment including stomata and glandular trichomes in plant shoots, and root hairs, border cells, epidermis and periderm in roots. The root hair is a single cell extension of the root epidermis and plays an important role in complex interactions at root—soil interface (Weston, Ryan, & Watt, 2012). Root hairs have the ability to exude both low and high molecular weight organic molecules including ions, amino acids, growth regulators and allelochemicals (Table 1). Some compounds are synthesized within the root hair and directly exuded into the environment, such as sorgoleone (Weston et al., 2012) and shikonins which are directly exuded by *Echium plantagineum* L. seedlings (Zhu et al., 2016). Recently, the localization of red-coloured bioactive naphthoquinones (also known as shikonins) from the outer periderm of living roots of the highly invasive

Table 1 Diverse organic metabolites known to be released in root exudates of living plants (Bertin, Yang, & Weston, 2003)

Class of Compounds	**Metabolites**	**Functions**
Carbohydrates	Arabinose, glucose, raffinose, rhamnose, ribose, fructose, galactose, maltose, sucrose and xylose	Provide favourable environment for the growth of microorganisms
Amino acids and amides	All 20 proteinogenic amino acids, aminobutyric acid, homoserine, cystathionine, mugineic acid	Inhibit nematodes and root growth of different plant species
Aliphatic acids	Formic, acetic, butyric, isocitric, oxalic, fumaric, propionic, maleic, citric, tartaric, oxaloacetic, pyruvic, oxaloglutaric, glycolic, shikimic, acetonic, valeric, gluconic	Plant growth regulation and inhibition
Aromatic acids	*p*-hydroxybenzoic, caffeic, *p*-coumaric, ferulic, gallic, gentisic, protocatechuic, salicylic, sinapic, syringic	Stimulation depending on concentration
Miscellaneous phenolics	Flavanol, flavones, flavanones, anthocyanins, isoflavonoids	Plant growth inhibition or stimulation depending upon concentration
Fatty acids	Linoleic, linolenic, oleic, palmitic, stearic	Plant growth regulation
Sterols	Campestrol, cholesterol, sitosterol, stigmasterol	Plant growth regulation
Enzymes and miscellaneous		Unknown

weed *E. plantagineum* L. has also been studied; this plant accumulates these antimicrobial and phytotoxic naphthoquinones in the periderm of the primary taproot over time and also releases these molecules from seedling root hairs as red-coloured droplets. These localization studies were facilitated through the use of confocal microspectrofluorometry (Zhu et al., 2016).

In the shoot, allelochemicals can be directly exuded by glandular trichomes on the leaf surface, while in other cases they are translocated to the trichomes from other parts of the plant (Eom, Senesac, Tsontakis-Bradley, & Weston, 2005). In the case of catmint, *Nepeta x faassenii*, 21 volatile constituents were noted in the glandular exudates of the plant and proved to potently inhibit seed germination and seedling plant growth, when assays were conducted with foliage suspended in the proximity of germinating seedlings but not in contact with seedlings. This suggested that volatilization is important for dispersal and inhibition of plant growth processes. Three components, 2-(2-ethoxyethoxy)-ethanol, alloaromadendrene and chi-cadinene, were not only detected in both the volatile mixture and the methanolic extract, but also in an aqueous foliar extract that exhibited potential allelopathic activity on curly cress (*Lepidium sativum* L.) growth. Eom, Yang, and Weston (2006) studied the allelopathic potential of perennial groundcovers and found that foliar volatile compounds from *N. x faassenii* (catmint) significantly decreased the growth of *L. sativum* (curly cress) and also common roadside weeds under field conditions. Lipophilic or volatile compounds such as those produced by catmint can directly diffuse through the lipid bilayer while more polar compounds require specialized membrane-bound transport proteins to assist the transport process (Weston et al., 2012).

Once synthesized and accumulated in plant tissues, most allelochemicals are excreted from the plant to prevent autotoxicity or stored in vacuoles and small membranous vesicles or storage organs, such as the periderm, waxes and glands on the leaf surface or in extracellular spaces or compartments in plant tissues. They can also be transported to other plant tissues using diffusion or more likely specific transport proteins which are embedded in the plasma membrane and actively assist in the transport of a particular class of compounds, including allelochemicals (Fig. 10) (Weston et al., 2012).

5.1 Root Exudation of Allelochemicals

Root exudation is a common mechanism of release of allelochemicals from living plant roots, specifically through root hairs, which are individual cells

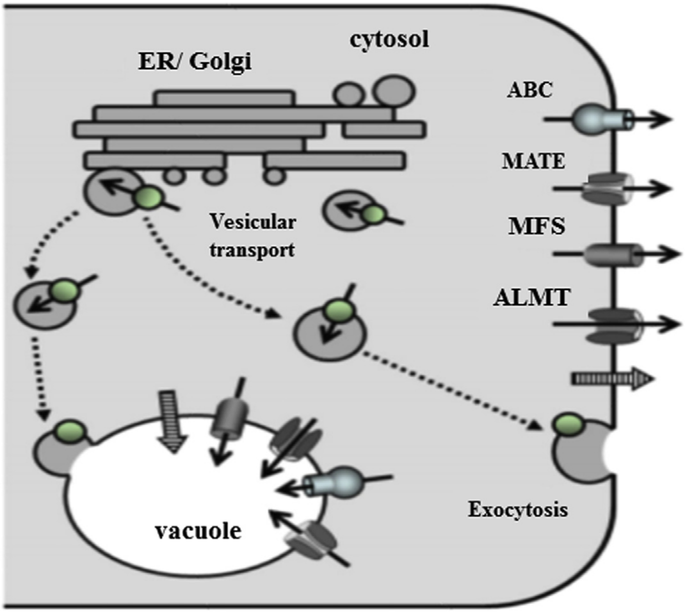


Figure 10 Transport of organic compounds in root cells through membrane-bound proteins including ATP binding cassette family (ABC), multidrug and toxic compound extrusion family (MATE), the major facilitator superfamily (MFS) and the aluminium-activated malate transport family (ALMT) (Weston et al., 2012).

associated with synthesis and excretion of bioactive metabolites, or by the actively growing tips of primary and secondary roots (Table 1) (Weston, Weston, & Hildebrand, 2013; Zhu et al., 2016). The soil-root interface or rhizosphere is considered as the site of greatest activity in the soil matrix as a myriad of organic compounds in varying concentrations are released from living roots, decomposing plant material and by associated microorganisms. The rhizosphere is an extremely complex environment to chemically survey due to actively changing the concentration of secondary metabolites and relative difficulty in extraction from soil matrix. Most recently, the development of silicone microprobe tubing and solid phase root zone extraction techniques have provided an opportunity to precisely profile nonpolar to moderately polar allelochemicals released from living plant roots in the soil (Zhu et al., 2016). In this case, a silicon tube is placed in the soil around the root surface and less polar molecules adhere to the silicone exterior of the tube and can be selectively removed by later solvent extraction (Weidenhamer, Boes, & Wilcox, 2009).

The composition of root exudates is complex and generally consists of carbon-containing compounds including polysaccharides (for example, arabinose, glucose, fructose, maltose), amino acids (for example, arginine asparagine, cysteine, glutamine), organic acids (for example, acetic, ascorbic, benzoic, ferulic acids), phenolic compounds, alkaloids, tannins, terpenoids, flavonoids, growth regulators, vitamins and nutrients and to a lesser extent, noncarbon compounds including ions, water and electrolytes (Table 1) (Baetz & Martinoia, 2014; Bertin et al., 2003). Once released, allelochemicals undergo physical, chemical and biological changes in the soil. Therefore, the biological activity of an allelochemical might be altered before reaching the potential target due to microbial degradation, oxidation or immobilization by irreversible binding to soil particles (Cheng, 1995).

Neumann and Römheld (2007) and Weston et al. (2013) described various mechanisms of root exudation including diffusion, vesicle transport and ion channels. A brief description of these mechanisms is given below.

5.2 Diffusion

Low molecular weight organic compounds such as sugars, amino acids, carboxylic acids and phenolics are released passively through a gradient of concentration between the cytoplasm of living root cells and the surrounding soil. This particular type of excretion depends on the physiological state of the root cells and the polarity of the organic compounds. The hydrophobic nature of plasma bilayer generally facilitates the transport of lipophilic compounds by diffusion.

5.3 Vesicle Transport

Transport of high molecular weight organic compounds (e.g., mucilage polysaccharides) is mediated through the formation of Golgi vesicles from secretory cells of the root cap. These cells are subsequently degenerated or sloughed off from the surface of the roots (Bertin et al., 2003). Enzymes such as acid phosphatase and peroxidase, synthesized at the level of membrane-bound polysomes, enter the lumen of endoplasmic reticulum using vectorial segregation (Neumann & Römheld, 2007). This exocytosis of vesicles depends on intracellular and extracellular calcium level.

5.4 Ion Channels

Exudation of some compounds (e.g., citrate, malate, oxalate) at higher concentrations, particularly under stressful conditions, cannot take place though simple diffusion in certain plant species (Neumann & Römheld, 2007). The use of patch clamp approaches and ion channel antagonist studies indicated the presence of ion channels on the plasma membrane of epidermal root cells involved in the release of malate and citrate in the *Triticum spp* (wheat) and corn (Weston et al., 2013).

6. FACTORS INFLUENCING THE RELEASE OF ALLELOCHEMICALS FROM THE PLANT

The nature and concentration of allelochemicals released by the plant into the environment, including the rhizosphere, is dependent on the plant itself and several other biotic and abiotic factors as shown in Fig. 11.

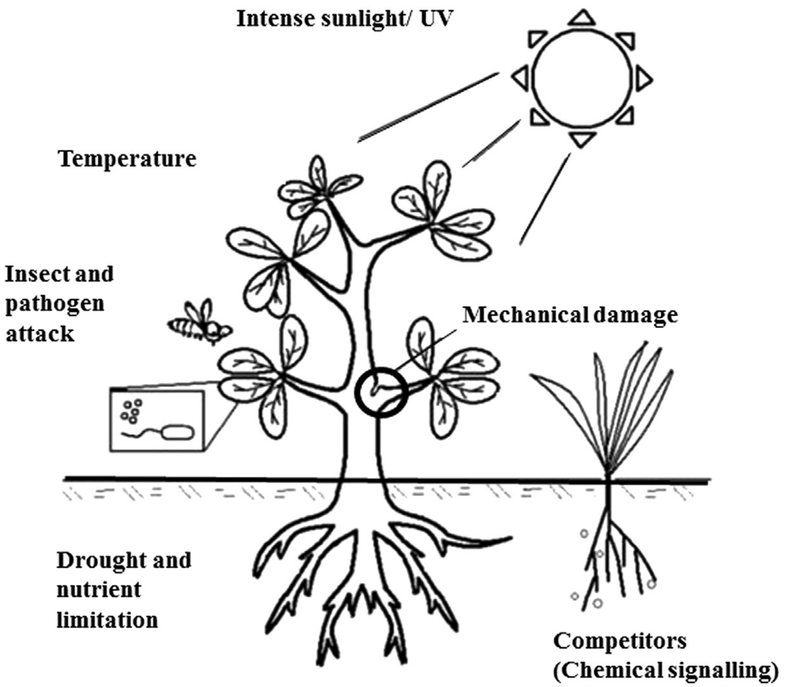


Figure 11 The release of allelochemicals in the environment under the influence of various factors (Albuquerque et al., 2011).

Plant factors include plant species and cultivar, plant age and the type of tissue under consideration for allelochemical production. Environmental factors regulating allelochemical production and release include pathogen infestation or physical injury or abiotic factors including drought, temperature, soil characteristics, rainfall, nutrient deficiency, irradiation, competitors and exposure to ultraviolet radiation (Brimecombe, Leij, & Lynch, 2001; Cseke & Kaufman, 1999; Einhellig, 1996; Iannucci, Fragasso, Platani, & Papa, 2013; Mahmood et al., 2013). A changing climate may also influence allelochemical production and this has been an area of particular interest in recent years (Jassey et al., 2013; Putten et al., 2013; Weston et al., 2013).

Taxonomically related species do not always release similar allelochemicals or concentrations of metabolites into the environment (Imatomi, Novaes, & Gualtieri, 2013). The release and dispersal of allelochemicals into the external environment are influenced by the chemical nature of allelochemicals including their respective molecular weight, polarity and concentration in the plant. Allelochemicals are most often released in mixtures in conjunction with other closely related metabolites. It is important to note that these metabolites can serve more than one biological role in the plant, and one compound can also defend its host from several unrelated competitors or predators (Macías, Molinillo, Varela, & Galindo, 2007). For example, phenolic compounds are known to stimulate and/or inhibit fungi in association with higher plants; certain fungi utilize phenyloxidase activities to metabolize and thus mitigate the toxicity of phenolic compounds produced by higher plants (Jassey, Chiapusio, Gilbert, Buttler, Toussaint, & Binet, 2011; Sinsabaugh, 2010).

The selection and use of bioassays to investigate the allelopathic potential of related metabolites must be carefully planned in any experimentation performed. Allelopathic activity may be seriously impacted by the mixture of metabolites present in complex mixtures, and therefore activity might be associated with complex molecular interactions including synergy, antagonism and enhanced effects in the presence of other metabolites (Albuquerque et al., 2011; Einhellig, 1996; Neilson et al., 2013). To estimate the bioactivity of allelochemicals, a dose/response study is essential to establish their potential effects on the environment. When studies utilize a dose significantly higher than the concentration(s) naturally present in the soil, the results are generally difficult to interpret from an allelopathic perspective. Moreover, the mode of action of allelochemicals can differ when applied at a dose well beyond that encountered in nature (Fujii & Hiradate, 2007).

7. ROLE(S) OF ALLELOCHEMICALS IN THE RHIZOSPHERE, IN NEIGHBOURING PLANTS AND OTHER ORGANISMS

The rhizosphere is a narrow zone of soil around living plant roots that is also inhabited by diverse groups of microorganisms; here plant roots compete with root systems of neighbouring plants and with other soilborne organisms, including bacteria and fungi (Weston et al., 2012). The rhizosphere itself is not easily defined by size but rather by its physical and biochemical properties, with a decreasing chemical gradient away from living plant roots. Newman, Fitter, Atkinson, Read, and Usher (1985) estimated that the roots of a typical higher plant can release 10–40% of photosynthetically fixed carbon in the form of organic and inorganic compounds into the rhizosphere. These root-produced products are collectively called rhizodeposits and include exudate, mucilage, sloughed off border cells and root cap cells (McNear Jr, 2013). As presented in Table 1, the majority of the root exudate consists of diverse low molecular weight organic compounds (Baetz & Martinoia, 2014).

Allelopathic interference in the rhizosphere is well documented under controlled conditions. However, allelopathy remains difficult to separate from competition among plants of different species for growth limiting nutrient resources under field conditions (Weston & Duke, 2003). However, recent advances have increased our knowledge about physiochemical properties of roots. Belowground interactions between roots and microbes are continuously occurring in the rhizosphere and are often difficult to study due to their presence in a complex soil matrix (Cipollini et al., 2012; Inderjit, Weston & Duke, 2005; McCully, 1999). There has been increasing evidence which suggests that root exudates provide a system of simultaneous communication with neighbouring plants, as well as with symbiotic and pathogenic organisms through biological and physical interactions (Fig. 11) (Bais, Park, Weir, Callaway, & Vivanco, 2004). Root exudates have been shown to regulate symbiotic relationships with microorganisms in the rhizosphere, protect against herbivory, alter the chemical and physiochemical properties of the soil and affect the growth of other plants in the rhizosphere (Mathesius & Watt, 2010; Nardi et al., 2000). Recently, secondary metabolites produced by plants have been shown to inhibit microbially mediated denitrification in the rhizosphere of invasive plants, thereby impacting growth and available N for the invasive community (Bardon et al., 2014). Effective symbioses between plants and microorganisms are generally facilitated by electrostatic and chemotactic responses created by the root and its exudates (Van West et al., 2002; Zheng & Sinclair, 1996).

Root exudates can also be associated with stimulation of plant growth, through the attraction of useful microbial communities to the plant rhizosphere having subsequent positive effects on plant growth or by direct effects of the plant-produced metabolites (Bais et al., 2006). The impact of allelochemicals in root exudates on neighbouring plant growth is dependent on the concentration of allelochemicals in the exudate, their relative rate of release, the vegetative stage of the target plant, as well as on the biotic and abiotic environmental conditions (Gniazdowska & Bogatek, 2005; Hill, Ngouajio, & Nair, 2006; Putnam, 1988). It is important to consider that allelochemicals can only mediate negative interference if they are released in sufficient quantities and persist long enough in the environment to remain active. Weidenhamer (1996) has demonstrated in controlled conditions that growth stage, biomass and density of target plants are important factors that alter the availability of allelochemicals released into the rhizosphere and should be considered when performing studies with allelochemicals. A greater density of receiver plants will thus require higher concentrations of allelochemicals provided by the donor to generate similar results in contrast to a reduced density. Therefore, interactions that are documented in one location may not be easily replicated in another natural setting due to varying conditions, including plant densities, encountered.

After release from plant tissue, other processes including leaching, oxidation, biodegradation and uptake by neighbouring plants can influence interference of allelochemicals upon target plants (Inderjit, 2001). Root exudates produced under the influence of chemical and biological elicitors may also act as antimicrobial agents against pathogenic bacteria in the rhizosphere (Zhu et al., 2016). Plant roots typically defend themselves against bacteria and fungi through the action of detachable border cells; these cells located at the tips of actively growing roots produce a vast assortment of bioactive secondary metabolites including allelochemicals. As an example, a newly germinating plant uses products generated by border cells to aid in plant defence, particularly during this sensitive period before the root system is well established in a field setting. Root border cells also produce extracellular DNA and associated proteins which can act as a trap for soilborne pathogens which are later negatively impacted by associated allelochemical production (Hawes et al., 2016). Oats produce avenicin, an important allelochemical and metabolite with potent activity against plant pathogenic organisms. This interaction has been well characterized by the Osbourn laboratory which has shown that triterpenes produced by oats play critical roles in defence against soil microbes and plant pathogens as well as abiotic stressors (Owatworakit et al., 2013). Interestingly, oats have diverged chemically from other cereal grasses which do not produce this family of compounds.

7.1 Tolerance to Allelochemicals

Some plant species are intrinsically insensitive to specific phytotoxins or have evolved mechanisms of defence from allelochemicals produced by the plant itself or from other species (Shitan, 2016). Plants producing allelochemicals as well as those absorbing them can resist toxicity by actively sequestering toxins in membrane-bound structures, vacuoles or vesicles, secreting the compounds immediately after absorption, or altering their chemical structure by primary and secondary metabolism (Bais et al., 2006). For example, *Polygonella myriophylla* (Small) Horton releases hydroquinone and benzoquinone allelochemicals in the form of arbutin, a glycoside of hydroquinone, which prevents autotoxicity (Weidenhamer & Romeo, 2004). Likewise, corn (*Z. mays* L.) produces inactive *N*−glycosylated forms of DIMBOA, DIBOA and BOA (Fig. 9), characteristic benzoxazolinones produced by several *Poaceae* spp (Schulz et al., 2016). Over millions of years, evolution has endowed selected plant species with the capacity to tolerate or metabolize commonly encountered phytotoxins. However, those species which do not encounter phytotoxins frequently are predicted to be less likely to develop resistance (Bais et al., 2006). A recent hypothesis suggests that greater success of invasive weeds is associated with their allelopathic potential to negatively affect native species by the production of unique allelochemicals or 'novel weapons' which can successfully interfere with the growth of native plant communities that are not well adapted to the presence of these bioactive metabolites (Callaway & Aschehoug, 2000).

8. METABOLIC PROFILING OF ALLELOCHEMICALS IN COMPLEX PLANT OR SOIL EXTRACTS OR MIXTURES

The biochemical interactions occurring in the rhizosphere are the least well characterized in all of the biotic zones studied in terrestrial ecology. Despite these challenges, new technological advancements in metabolite detection and identification are proving useful when studying complex systems and interactions, and solving ecological questions about regulation of allelochemicals and their roles in ecosystem function. Metabolic profiling is proving to be an important tool when studying these complex plant interactions and is utilized to study the plant metabolome (the total collection of primary and secondary products produced by a plant). By performing an extraction of plant tissue and evaluating the metabolome, one can assess the functional state of a biological system at a particular point in time (Roessner & Bacic, 2009).

Weston, Skoneczny, Weston, and Weidenhamer (2015) utilized a type of metabolomics referred to as metabolic profiling that is a set of analytical procedures designed to study targeted compounds of interest, including specific allelochemicals, in a biological system in response to a particular treatment. Metabolic profiling can provide a strong insight on the biochemical status of an organism so one might further understand complex interactions following the release of allelochemicals in the rhizosphere, or in some cases their intermediate metabolites (Putnam, 1988; Rice, 1974). It can also be used in conjunction with other *omics* technologies (proteomics and transcriptomics) to fully construct the biosynthetic pathways of targeted compounds of interest. Metabolic profiling of specific allelochemicals may also be employed to study possible mechanisms associated with invasion of weeds. Skoneczny et al. (2015) described significant upregulation of pyrrolizidine alkaloids and their N-oxides in the highly invasive species, *E. plantagineum* L., in contrast to the less successful invader, *Echium vulgare* L., in both field and laboratory settings, through the use of liquid chromatography coupled to mass spectrometry (LC-MS) for evaluation of a series of related pyrrolizidine alkaloids, including the toxins echiumine, leptanthine and echimidine.

Metabolomics is a high throughput approach which produces results in a relatively short period of time when analyzing large sample sets (Scognamiglio, D'Abrosca, Esposito, & Fiorentino, 2015). Progress in this field has been associated with the development of a diverse range of analytical platforms including gas and liquid column chromatography coupled with high-resolution mass spectrometry for targeted and nontargeted metabolic profiling of secondary metabolites and allelochemicals (Kim, Choi, & Verpoorte, 2010; Weston et al., 2015). More recently, the development of triple quadrupole MS or sensitive ion trap MS has facilitated the precise and accurate profiling for putative annotation of thousands of bioactive compounds present in very low quantity in complex matrices (Weston et al., 2015). A thorough description of how allelochemicals may be profiled by using metabolomics approaches is discussed in the review prepared by Weston et al., (2015). Most recently, metabolic profiling has also been applied to studies of the plant rhizosphere. One study was performed to evaluate the release of diverse flavonoids from clover (*Trifolium spp*.) in the soil rhizosphere when clovers have been utilized as cover crops, pointing to the role of the allelochemical kaempferol which persisted in the soil rhizosphere and was likely to be associated with consequent allelopathy in perennial stands of white clover (Weston & Mathesius, 2013). Additional studies to evaluate the role of a complex group of over 15 bioactive naphthoquinones, the shikonins, in plant defence and allelopathy in the rhizosphere, have also been facilitated by the use of sensitive metabolic profiling performed by UPLC QToF-MS (Zhu et al., 2016). Recovery of some of the more persistent allelochemicals including acetyl shikonin and shikonin in ppm levels in infested soils was also successful using LC/MS technologies. The increasing use of metabolomics to study metabolite dynamics and biochemical pathways in plants will no doubt result in an improved understanding of the presence and the role of allelochemicals in the soil environment.

After analysis by metabolomics, processing of substantial amounts of mass spectral data and subsequent characterization of metabolites are attempted (Smith et al., 2005). Although it is never possible to identify all plant metabolites in a complex mixture, and many remain currently unidentified or are undescribed. Such challenges are dealt with using efficient throughput computational and analysis tools which often involve data conversion, features detection, normalization and recently developed quality control of data processing (Sugimoto, Kawakami, Robert, Soga, & Tomita, 2012). The molecular features of the compounds present are normalized for baseline variation and aligned with available structural libraries. The METLIN library is one of the largest databases used for identification of natural products (Weston et al., 2015). Greater flexibility across its catalogues and multipurpose search options make it very convenient and widely used database (Smith et al., 2005).

9. CONCLUSIONS

Allelopathy, the study of complex plant–plant and plant–microbial interactions, is a very active field of research in the plant sciences. Recently, the number of researchers and research papers in the field has increased exponentially. Enhanced collaboration among soil scientists, chemists, ecologists, geneticists and molecular biologists will undoubtedly lead to new insights as to the factors regulating the production of allelochemicals in higher plants and how these metabolites impact invasion ecology, plant competition, plant interference, vegetation dynamics and crop production. The use of new analytical techniques associated with metabolomics in concert with other *omics* technologies has led to new advances in the identification of unique allelochemicals, the biosynthetic pathways associated with their production, their complex role(s) in the soil rhizosphere and their production as impacted by a changing climate. A better understanding of allelochemical production with respect to plant defence strategy may also allow us to better protect and manage developing crops, limit the spread of invasive weeds, preserve native plant stands, and create strategies for allelochemical development and application as novel pesticides.

Currently, increasing public awareness regarding safety and environmental issues associated with the use of herbicides, as well as profitable markets for organic commodities has resulted in greater emphasis on the development of natural product-based pesticide discovery programs (Duke et al., 2002; Weston & Duke, 2003). Identification of novel plant metabolites, including allelochemicals, may result in a source for future development of biologically based pesticides (Chiapusio et al., 2005, pp. 139–155), through the provision of complementary structures for synthetic compounds (Ghisalberti, 2007) and as an aid in the development of new molecular target sites (Duke et al., 2002).

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