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Review: Allelochemicals as multi-kingdom plant defence compounds: Towards an integrated approach

Running title: Multi-kingdom effects in allelochemicals

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Abstract

The capability of synthetic pesticides to manage weeds, insect pests and pathogens in crops has diminished due to evolved resistance. Sustainable management thus becomes more challenging. Novel solutions are needed and, given the ubiquity of biologically active secondary metabolites in nature, such compounds require further exploration as leads for novel crop protection chemistry. Despite improving understanding of allelochemicals, particularly in terms of their potential for use in weed control, their interactions with multiple biotic kingdoms have to date largely been examined in individual compounds and not as a recurrent phenomenon. Here multi-kingdom effects in allelochemicals are introduced by defining effects on various organisms, before exploring current understanding of the inducibility and possible ecological roles of these compounds with regard to the evolutionary arms race and dose-response relationship. Allelochemicals with functional benefits in multiple aspects of plant defence are described. Gathering these isolated areas of science under the unified umbrella of multi-kingdom allelopathy encourages the development of naturally-derived chemistries conferring defence to multiple discrete biotic stresses simultaneously, maximising benefits in weed, insect and pathogen control, while potentially circumventing resistance.

Keywords: Allelopathy, plant defence, multi-kingdom, secondary metabolites, fitness.

Introduction to the concept of multi-kingdom allelopathy

Allelopathy is defined in a broad sense as a phenomenon encompassing the both positive and negative effects of plants or microbes on other organisms by means of the chemicals, described as allelochemicals, which these species produce.[1] This form of interference is distinct from resource competition, which is regulated by light, water or mineral nutrients.[2] For the purposes of this review, we will consider allelopathy of plant species in a primarily detrimental context, as this provides most promise for crop protection and pest management.

The multi-kingdom effects of some allelopathic plant secondary metabolites have long been acknowledged in definitions and discussions of allelopathy,[3,4] in spite of the original definition solely addressing plant-plant interactions.[5] In the 1980s, multiple examples of compounds exhibiting allelopathy and toxicity to other organisms were defined,[6] and the term ‘allelopathy’ was used in this context by the International Allelopathy Society in the 1990s.[1] Other works have documented multiple ecological roles and applications for specific, individual plant-derived secondary metabolites.[7–10] Works examining multi-kingdom effects in allelopathic compounds nonetheless remain exceptional, with most literature focusing on the identification of inhibitory effects in novel natural compounds rather than their multi-kingdom functions. This affects the scope of their applications for crop protection.

Allelochemicals are plant secondary metabolites, compounds considered non-essential for the direct development of cells, released into the environment *via* root exudation, leaching by precipitation, volatilisation, or decomposition of plant tissues. Around 10,000 secondary metabolites have thus far been characterised from plant root exudates,[11] complicating the isolation and elucidation of putative allelochemicals. There are few consistent terms for allelochemicals which may affect organisms of multiple kingdoms in the existing literature, and those that do exist serve different purposes to satisfy discussion of their individual disciplines. Considering such metabolites for multi-disciplinary applications first requires clear definitions of these compounds.

In this review, the case is made that the existence of allelochemicals as defined above, with multiple ecological functions, necessitates the need for definitions that encompass both generic allelopathic interactions and more specific interactions with plants, animals and microbes. It is hereby suggested that ‘allelopathy’ is used in its wider definition in affecting multiple kingdoms as previously described,[1,3] and the terms ‘phytoallelopathy’, ‘zooallelopathy’ and ‘microbial allelopathy’ are used to describe specific interactions with plants, animals, and microbes, respectively, in support of this. More detailed definitions of these terms as used throughout this review are provided in the text box. Having defined these interactions more clearly, it is now possible to describe the roles they could play in pest management.

Allelopathy: The inhibition or stimulation of the growth or development of an organism through the biological action of secondary metabolites produced by plant species. These chemicals can be described as **allelochemicals** given this bioactivity, and will have effects on competition dynamics, and the stress tolerance of competitors.		
Phytoallelopathy: Allelopathy specifically towards another plant species, mediated by **phytoallelochemicals**.	**Zooallelopathy:** Allelopathy towards an animal species, typically an herbivore and most commonly observed in arthropods. This is mediated by **zooallelochemicals.**	**Microbial allelopathy:** Allelopathy towards a microbial species, such as a bacterium or fungus, mediated by **anti-microbials**, **phytoalexins or phytoanticipins**

Text box 1: Proposed definitions of allelopathy and associated terms regarding potential for multi-kingdom applications.

Driven by the burgeoning issue of herbicide resistance in weeds,[1] there is a growing need to develop more diverse and integrated weed management systems, to which phytoallelochemicals could contribute. As of 2020, herbicide resistance was reported in 262 species, to 167 herbicides, in 70 countries.[12] Parallel to this, there is a growing cohort of insecticide-resistant invertebrate species, with over 600 species resistant to at least one insecticide mode of action in 2020,[13] driving the desire for alternative approaches to their management in the same way. Fungicide resistance is also an issue, occurring in nine modes of action of fungicide by 2015.[14] As a result, the recognition of multi-kingdom allelochemicals which could potentially provide benefits against pesticide-resistant organisms, and the development of control strategies which utilise these allelochemicals should be considered.

Multi-kingdom allelochemicals in an evolutionary context

Plant fitness and chemical defence

Plant productivity, and ultimately fitness, is not only impacted by resource competition with other plants but also by herbivory, disease and stresses. Sessile plants cannot flee to avoid hostile organisms, so a key component of plant fitness is the ability to defend themselves by other means. Thus, evolution of generic defence mechanisms that maximise fitness would be of great benefit to plant species when faced with multiple stressor organisms. Indeed, it was posited that secondary metabolites provide general defence against multiple enemy organisms (Figure 1).[6] This assertion is connected to the optimal defence allocation theory, which suggests that allelochemicals are allocated to a greater extent where tissues are of greatest value, albeit encountering trade-offs between growth, fecundity and defence.[15] Allelopathy is thus linked to the ecological roles of these compounds through the vulnerability to different valuable tissues to different antagonistic organisms.

Plant defences are also affected by an evolutionary arms-race, formalised by the ‘Red Queen’ hypothesis (Figure 2).This hypothesis dictates that a species must constantly evolve adaptations to survive and thrive while faced with other species which are evolving similarly, effectively running as fast as it can to maintain its place, in the same manner as its namesake from *Through The Looking Glass*.[16] Natural selection is therefore dynamic, and all species are constantly evolving to counter the defences of competitors, hosts or prey, to such an extent that the fitness of these organisms will decline unless natural selection facilitates the evolution of counter-adaptations. It is thus ubiquitous across biological kingdoms, as it constitutes an element of maximising ecological fitness.

Direct effects of phytoallelochemicals

The prevalence and possible ecological role of phytoallelopathy must first be examined in isolation to provide the basis for the wider phenomenon of multi-kingdom effects. The ecological significance of phytoallelopathy is given weight by the study of invasive plants in natural ecosystems. Some invaders have the capacity to inhibit the development of would-be local competitor plants through their phytoallelopathic interactions which enable them to dominate invaded ecosystems. Examples include *Alliaria petiolata* and *Sonchus oleraceus*.[17,18] In both cases these interactions conform with the ‘novel weapons’ hypothesis (Figure 3); in the case of *A. petiolata* this may be attributable to the action of glucosinolate compounds such as allyl isothiocyanate and benzyl isothiocyanate, while a number of potential allelochemicals have been identified in *S. oleraceus*. The phytoallelopathic potential and resulting disproportionate success of these species exists because resistance or tolerance has not evolved in this invaded ecosystem as would commonly be observed in the invader's native ecosystem.[19] Phytoallelopathy in an agro-ecological context, and the potential applications that this may have for agricultural benefit, have been extensively reviewed.[4,20–22]

Sorghum species, and their phytoallelochemical sorgoleone, constitute an extensively-studied and thoroughly-reviewed example of phytoallelopathy at molecular, physiological, and agroecological scales.[23] The plant is known to have weed-suppressive properties in field,[23] through the exudation of bioactive quantities of sorgoleone from root hairs.[24] Sorgoleone is a potent phytoallelochemical, reducing *Digitalia sanguinalis* shoot growth by 50% at a dose of 10 µM, and reducing *Abutilon theophrasti* and *Echinochloa crus-galli* development by the same degree at 200 µM.[25] Multiple modes of action have been found in this compound, including the inhibition of photosynthetic and mitochondrial electron transport, the photosynthesis-related enzyme *p*-hydroxyphenylpyruvate dioxygenase (HPPD), and root H^+-ATPase activity required for water uptake.[23]

It should be noted that some plant secondary metabolites have indirect effects on dynamics of resource competition. This may occur *via* stimulation of beneficial donor plant-microbe interactions, increasing donor competitive ability, or through phytoallelopathic effects, as reduced growth vigour in target plants culminates in reduced competitive ability. *Carduus nutans* root exudates, for instance, appear to be particularly inhibitory to legume species, starving soil of nitrogen over time and creating conditions to which the plant is comparatively tolerant.[26] These effects may be attributable to the alkatetraene, aplotaxene.[27] It is for this reason that some claim a separation of resource competition from phytoallelopathy to be unrealistic in an ecological context.[26] It has been hypothesised that phytoallelopathy has evolved in reaction to intense resource competition to the detriment of the phytoallelopathic species.[28] Phytoallelopathy and resource competition may thus be components of a complex web of rhizosphere-based interactions involving nutrient availability (governing resource competition), exudation of secondary metabolites (including phytoallelochemicals) and soil microbial communities.[29]

Recognition and induction of allelochemical production

Allelopathic interactions in plants are likely to be influenced by recognition mechanisms, proposed to be mediated by chemical signalling in plant-plant interactions. The fitness benefit of phytoallelochemical exudation is optimised by inducibility,[30] and as such the recognition of other plant species may constitute an important factor in phytoallelopathic behaviour. Such recognition can be influenced by both volatile aboveground and root-secreted belowground stress-related metabolites and proteins which appear to indicate the relatedness of a neighbour. There is growing evidence that allelochemical synthesis or exudation is elevated in response to recognition of neighbouring, competing plant species, a process that has been described as 'allelobiosis'.[31] The presence of root exudates from a number of weeds, specifically *Abutilon theophrasti*, *Aegilops tauschii*, *Amaranthus retroflexus*, and *Digitaria sanguinalis*, all stimulated the accumulation of phytoallelochemicals in wheat.[31] Bioassay of a wider variety of weed species indicated that phytoallelochemical accumulation in wheat varies depending on the identity of the competing species.[32] This indicates that crop-weed recognition is species-specific, mediated by a wide range of diverse, and currently undefined signalling compounds.

In phytoallelopathic plants, recognition interactions with competitive neighbours may be facilitated by phenotype matching, *i.e.* the ability of a plant to distinguish related individuals compared to those from other populations or species through chemical signatures.[33] In parallel to another biotic kingdom, microbes contain recognition alleles, genes controlling the cues mediating recognition interactions, and therefore interact in a comparable manner in terms of recognition.[33] Recognition interactions in plants, the compounds and systems involved, and how these influence phytoallelopathic mechanisms, are poorly understood, and require further elucidation. Competition stress and other environmental stress factors are also likely to influence allelopathy inducibility,[30] but should be further examined to provide greater understanding.

There is also, conversely, evidence of allelochemical multi-kingdom function in the induction of allelochemical synthesis; some allelochemicals accumulate *in planta* at atypically high levels when under pressure from herbivores, pathogens, or both. For example, tissue disruption or wounding by the aphid *Rhopalosiphum padi* and the northern blight fungus *Setosphaeria turtica* stimulated allelochemical accumulation in maize.[34] Similarly, feeding of *Psylliodes chrysocephala* on oilseed rape promotes the accumulation of multiple glucosinolates.[35] This group of secondary metabolites is recognised for their phytoallelopathic potential.[36] Thus, it is apparent that plants both recognise and react to multiple biotic stresses in a manner comparable to other organisms. Additionally, these inducible allelopathic mechanisms appear to have some consistency between multiple kingdoms of hostile organisms. It is thus logical that the compounds involved in these mechanisms have potential for multi-kingdom effects.

Allelochemical allocation and fitness consequences

The theory of multi-kingdom functionality in allelochemicals is dependent on ecologically rational allocation *in planta*. It is a reasonable extension of the optimal defence allocation theory that the distribution of a compound within a plant may be indicative of its fitness benefits.[15] For example, benzoxazinoids, widely-known as cereal phytoallelochemicals, are found at greater levels in wheat and rye roots than other tissues of these plants.[37] Relative concentrations vary between wheat cultivars, however, and are greatest within a few days of germination, diminishing greatly as the plant develops.[38] Glucosinolates and their isothiocyanate breakdown products, believed to be the primary allelochemicals in brassicaceous species, also accumulate at greater levels in roots.[39] One could thus suggest that root exudate phytoallelopathy or microbial allelopathy to the rhizospheric community are the primary factors driving their selection. This can be disproven, at least in crop species such as wheat, which have undergone selection under unnatural conditions, by variability in phytoallelochemical exudation. Benzoxazinoid exudation was only detectable in 11 of 57 wheat cultivars despite all containing high concentrations within root tissues.[40] It may thus be that allelochemical accumulation in root tissues provides the additional functional benefit of defence against root-feeding herbivores such as the nematode *Pratylenchus neglectus*.[41] Alternatively, the presence of high concentrations of allelochemicals in roots may be indicative of sequestration in root vacuoles, as has been reported with benzoxazinoids.[42] This may prevent *in planta* autotoxic interactions which are harmful to vital plant tissues, rather than providing a direct fitness benefit. The apparent necessity of synthesizing and sequestering these compounds constitutes a fitness cost, which is likely to be overcome by a combination of benefits that confer a net competitive advantage.

Putative allelochemicals can also be found in high concentrations in aboveground tissues. This is particularly common in young tissues, of greater value to the plant due to their active growth, and thus allelochemical accumulation would appear to provide greater functional benefit as a feeding deterrent.[15] This is the case in *Artemisia annua*, where artemisinin accumulates in flowers and buds, and is exuded from glandular trichomes on the surface of leaves and stems.[43] Artemisinin is a potent phytoallelochemical, inhibiting the development of lettuce, as well as the weeds *Amaranthus retroflexus* and *Portulaca oleracea* at a concentration of 33 μM.[44] There is evidence that artemisinin is also zooallelopathic to multiple arthropod species, indicating additive functional benefit to this compound in relief of insect herbivory pressure. The beetle *Epilachna paenulata* and the armyworm *Spodoptera eridania* both suffered significant mortality when fed on pumpkin leaves treated with a dose of 1.5 mg cm^{-2} of artemisinin.[29] One would thus assume zooallelopathy to be the primary fitness benefit conferred by this allocation. Even then, artemisinin may provide phytoallelopathic benefits in nature through leaching from the leaf surface by rainwater. Such an effect would be enabled by its relatively long half-life in soil, around 30 days, ensuring that it would persist sufficiently for uptake by surrounding plant competitors.[43] The influence of persistence of allelochemical persistence on their fitness benefits is further discussed later in this piece.

In summary, the major benefit of allelochemical synthesis is likely to be defence against multiple hostile organisms, as would be suggested from the phenomenon of multi-kingdom functionality. The resources required to produce such compounds and their tendency towards autotoxicity are major costs. Both appear to be minimised by the inducibility of synthesis in response to stress, and their tissue localisation. The development of tolerance by a plant to the allelochemicals exuded into the environment is another potential adaptation to minimise fitness costs, as will be discussed at the end of this section.

Autotoxicity as a fitness cost

A further element in the discussion of multi-kingdom allelochemicals is the existence and potential ecological role of autotoxicity, which disproves the specificity of these compounds to putative antagonistic species. Indeed, it should not be taken for granted that phytoallelopathic species are tolerant or resistant to their allelochemicals, and must thus still overcome autotoxicity in these compounds.

Some of these compounds appear to have a degree of specificity in terms of their phytoallelopathy, but others do not, so their producers reduce associated fitness costs through inducibility, localisation and tolerance. Multiple plant species still exhibit a degree of autotoxicity, including wheat,[45] and *Sonchus olearaceus*.[17] These species produce root exudates with both phytoallelopathic and autotoxic potential. Few studies have successfully elucidated autotoxic compounds, but where they have, interspecific phytoallelochemicals are among such compounds; In alfalfa, for instance, the compounds of greatest effect were coumarins, *trans*-cinnamic acid and *o*-coumaric acid.[8,46] This would suggest that some phytoallelochemicals may also act as autotoxins, although their effects are likely to have evolved to confer some fitness benefit to their target. Artemisinin also represents an autotoxic phytoallelochemical, a dose of 33 µM significantly reducing *Artemisia annua* germination and seedling development.[44] In this case, autotoxicity is avoided by localisation, protecting the producing cell's cytoplasm through restricting the compound to the subcuticular space of the glandular trichomes while *in planta*.[43]

The reasons for the evolution of autotoxicity are not clear, although explanations have been posited which rationalise the phenomenon in spite of the existence of the aforementioned adaptations which would seemingly prevent it. A commonly-suggested hypothesis is that of biochemical recognition, which postulates that intraspecific inhibition of germination provides selective advantages for population fitness in the avoidance of intense intraspecific competition, favouring later germination and establishment when conditions are more suitable.[47] This can be compared to phytoalexin-regulated hypersensitive cell death to contain pathogenic infection, one example being in response to resveratrol in pathogen-infected grape plants.[48] Another hypothesis concerning the existence of autotoxicity in an ecological setting is more simplistic; it is possible that there is an unavoidable fitness cost associated with the production and maintenance of more effective defences against other, more pressing stresses. The compounds involved must be conferring considerable fitness benefits in this case, which may be explained by their multi-kingdom potential.

Hormesis and the dose question

A possible alternative explanation for the existence of autotoxicity is that it is an undesired fitness cost relating to the promotion of hormesis, *i.e.* the stimulation of growth at low concentrations by compounds that are known or suspected to be detrimental at higher concentrations. Hormesis specifically occurs at around one-tenth of an effective inhibitory dose.[49] Several reasons for hormesis of autotoxins have been discussed, including the theory that exudation of these compounds is intended to stimulate, rather than inhibit, further growth of the species.[50] In the case of hormesis, inhibitory effects would occur due to unnaturally high plant density, such as in a planted monoculture field. Alternatively, exudation may be over-stimulated to the detriment of the producing species by other stress factors, including the presence of competitors, underpinned by the recognition interactions described earlier . The occurrence of autotoxicity would therefore be a consequence of the dose-dependency of phytoallelochemicals. Hormesis was reported in some wheat lines,[45] as well as in a number of cases where pure phytoallelochemicals were applied to target species.[49]

Hormesis is additionally known to occur in synthetic herbicides such as glyphosate and bromoxynil.[49] It also appears to occur in inhibition of arthropods by zooallelochemicals, as has been observed in *Azadirachta indica*-derived azadirachtin applied to the bean weevil, *Zabrotes subfasciatus*.[51] The phenomenon manifests itself as a trade-off in this case, however, with the effect of increasing fecundity but reducing longevity in an apparent case of r-selection.[51]

Hormesis and autotoxicity exemplify two extreme outcomes in the governance of the ‘Paracelsus axiom’ over allelochemical interactions. This is the theory that toxicity is only ever determined by dose, and by extension, all compounds can exhibit stimulatory and inhibitory interactions towards an organism at the correct dose.[49] In the case of hormesis, allelopathic behaviour is not likely to be detrimental; indeed it would be of ecological and evolutionary benefit for a plant to evolve the synthesis of a compound stimulatory to growth of kin and inhibitory to competitors at low concentrations, allowing their benefit from plentiful resources in their environs while inhibiting competitors, but which became autotoxic at higher concentrations where seed germination is inhibited at times of intense intra-specific competition.

Allelochemical persistence in the environment

The environmental fate of allelochemicals in soil is also a noteworthy factor in their evolution and activity towards multiple kingdoms. A degree of persistence is necessary for a compound to induce phytoallelopathy or microbial allelopathy in nature, albeit not to the degree that resistance would evolve. Many phytoallelochemicals are degraded by microbial action, such as simple phenolic acids, benzoxazinoids, juglone, quercetin, rutin, and *m*-tyrosine,[52] some of which exhibit multi-kingdom effects, which will be reviewed in the next section. The effect of degradation on phytoallelopathic bioactivity can be profound. For example, of nine weed species reported in one study to have phytoallelopathic root exudates, only one, *Ageratum conyzoides*, maintained its bioactivity in unsterilised soil.[53] For this reason, many bioassays investigating the potency of phytoallelochemicals in artificial conditions such as sterile soil could overestimate their effects.[52,53] Difficulty in proving in-field phytoallelopathy gives credence to the perspective that studies in these artificial conditions are ecologically irrelevant.[54] Rather, the ideal study of a putative allelopathic species or compound should begin with a simplified lab model which is necessary to elucidate its effects and modes of action. This should be followed with assays in more ecologically relevant conditions, culminating in in-field bioassays to ensure their applicability.

The benzoxazinoid allelochemicals DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) and DIBOA (2,4-dihydroxy-1,4-benzoxazin-3-one) degrade quickly in unsterilised soil, with half-lives of around 24 hours[45] and 43 hours[55], respectively. Rapid degradation of allelochemicals can sometimes result in more persistent compounds with greater bioactivity, and therefore may be an ecologically rational strategy in this case. For example, phytotoxic APO (2-amino-phenoxazin-3-one), a degradation product of DIBOA, persists for up to 90 days in biologically active soil, part of the reason for its acknowledgment by some as an important component in cereal phytoallelopathy.[55]

Resistance and tolerance to allelochemicals

There is propensity for resistance to allelochemical compounds to evolve, in much the same manner as resistance to synthetic pesticides. It is for this reason that multi-kingdom effects are not universal at uniform concentrations. Evolution of resistance occurs as a natural ebb and flow of the evolutionary arms race in a natural ecosystem, but by extension, evidence which will be discussed in this section suggests that such developments could facilitate the use of allelochemicals as naturally-inspired crop protection compounds. Indeed, evolution of tolerance or resistance by the intended target species in its natural setting may be the primary reason for limitations in the universality of such compounds.

Multiple fungal wheat pathogens, including several *Fusarium* species,[37] and several plant species have evolved the ability to detoxify benzoxazinoids, for instance.[42] Similarly, the presence of low concentrations of glucosinolate compounds from *Alliaria petiolata*, as a result of partial degradation by the native rhizosphere community, is linked to eventual resistance of these microbes to these compounds.[56] Insect herbivores can similarly evolve tolerance to secondary plant metabolites, circumventing zooallelopathic defences through counter-resistance evolved in the manner suggested by the Red Queen hypothesis. This is particularly apparent where host resistance is only encoded by one gene, with selectively bred lettuce resistant to the aphid *Pemphigus bursarius* for just 10 years before the aphid evolved counter-resistance.[57] Similar dynamics are apparent in various lepidoptera that evolved mechanisms to glycosylate DIMBOA back to its non-toxic storage form.[58] The DIBOA degradation product BOA (benzoxazin-2-one) can furthermore be detoxified by glutathione transferase (GST) and cytochrome P450 monooxygenase (CYP-P450) activity in *Arabidopsis*.[59] Thus, from an ecological perspective, the benefit of novel weapons will ultimately be overcome by counter-selection. The vulnerability of native ecosystems to the allelochemicals of an invading plant species will be overcome by the evolving resistance of native species in time, but this first requires the invader to become dominant and disrupt the ecosystem, thereby creating an intense selection pressure.

There also appears to be further association in the form of cross-resistance, as insect pests of allelopathic herbaceous species have a greater likelihood of evolving resistance to synthetic pesticides. A recent example of this can be found in the cotton bollworm (*Helicoverpa armigera*), which exhibited reduced larval sensitivity to the synthetic insecticide methomyl when fed with a number of allelochemicals including coumarin and DIMBOA. This metabolic cross-resistance was correlated with elevated activity of both GSTs and CYP-P450s, which often confer resistance.[60] This is connected with the theory of pre-adaptation, that the mechanisms to detoxify zooallelochemicals of insect pests may incidentally provide a degree of pre-adaptation to synthetic insecticides.[61] In a parallel to synthetic herbicides, control of resistant insect species is becoming more difficult due to an overreliance on these insecticides. The dynamics of pre-adaptation need to be further explored in order to facilitate more effective application of allelochemical-derived biocides.

Examples of allelochemical multi-kingdom functionality

An integrated approach which takes account of the multi-kingdom behaviour of allelochemicals could optimise benefit in terms of crop yield. It is important to consider individual compounds within this multi-kingdom framework. To this end, the examples of benzoxazinoids, *meta*-tyrosine and juglone, are presented as multi-kingdom allelochemicals that give credence to this recurring concept. Such examples are not exhaustive, and also include momilactones in rice, which are both phytoallelochemicals[62] and phytoalexins[63], and parthenin from *Parthenium hysterophorus*, which is both phyto-[64] and zooallelopathic[65]. Table 1 summarises the multi-kingdom effects presented in this section.

Table 1: Summary of multi-kingdom effects in allelochemicals discussed in this section.

Allelochemical	Plant producer	Phytoallelopathy	Zooallelopathy	Microbial allelopathy
Benzoxazinoids	Various[66]	*Sinapis alba, Lolium rigidum, Avena fatua*[66,67]	*Ostrinia nubilalis, Diuraphis noxia, Meloidogyne incognita*[68–70]	Various[71,72]
***Meta*-tyrosine**	*Festuca rubra*	*Digitaria sanguinalis*, *Trifolium repens*, *Taraxacum officinale*[73]	*Coptotermes formosanus*[74]	*Bacillus* spp.[75]
Juglone	*Juglans nigra*[9]	Various[76]	*Callosamia promethea*[77]	Various[78]

Benzoxazinoids

Benzoxazinoids are a family of cyclic hydroxamic acids synthesised by a range of plant species, and long studied for their biological activity. Benzoxazinoids are widespread in nature, occurring in Acanthaceae, Ranunculaceae, Scrophulariaceae and Poaceae,[66] including wheat, rye, barley and maize.[37] The two benzoxazinoids most commonly attributed to conferring wheat allelopathy are DIMBOA and DIBOA, and their breakdown products.[79] DIBOA was discovered in 1959, and DIMBOA in 1962, although their phytoallelopathic potential was not discerned until the 1990s.[79]

These compounds degrade to MBOA (6-Methoxy-2-benzoxazolinone) and BOA respectively, which then degrade further into AMPO (2-amino-7-methoxy-phenoxazin-3-one) and APO respectively, as has been previously reviewed[80].

All of these compounds have been tested on multiple target species and considered as putative phytoallelochemicals.[67,81,82] Elevated benzoxazinoid exudation by multiple cereal species correlates with the suppression of *Sinapis alba* development, indicative of phytoallelopathy.[66] A 500 µM dose of DIMBOA is sufficient to inhibit root length in *Avena fatua* by around 70% and *Lolium rigidum* by around 55%, compared to controls.[67] DIMBOA isolated from wheat root exudates reduced dry weight of *Alopecurus aequalis* by around 20%.[83] Since a similar biomass reduction (21%) in test plant species was caused by crude wheat root exudates,[84] DIMBOA would appear to be the primary phytoallelochemical exuded by the species.[83]

DIBOA is similarly phytoallelopathic to DIMBOA, also inhibiting *Lolium rigidum* at a dose of 500 µM and *Avena fatua* at 100 µM.[67] When DIBOA was applied axenically to oat and broad bean plants, H^+ATPase activity in roots was reduced.[85] This is likely to be related to the electrophilicity of DIBOA, its attraction to electrons and electron-dense molecules.[7] Therefore, benzoxazinoids appear to limit supply of adenosine triphosphate (ATP) by inhibiting electron transport, hindering the mechanisms by which cells release energy.

Conversely, this is only one of many suggested modes of action posited for benzoxazinoid allelochemicals. Treatment with these compounds has led to a number of effects, including reduced activity of other enzymes such as papain, *α*-chymotrypsin, and GSTs.[86] The mode of action has not been conclusively identified for DIMBOA or DIBOA, or their respective degradation products,[87] and has only been elucidated in APO and AMPO.[88] These compounds bind to and inhibit the action of highly-conserved histone deacetylase (HDAC) enzymes, which are necessary for amino acid transcription and therefore cell development.[88] Such effects occur at concentrations as low as 3.25 µM, sufficient for physiological relevance.[87] This explains the notable allelopathic potency of APO in particular, being a much more potent phytoallelochemical than DIMBOA or DIBOA.[81]

Some benzoxazinoids confer zooallelopathy against invertebrate herbivores, known long before their phytoallelopathic potential was discovered. DIMBOA is inhibitory to larval development in the European corn borer *Ostrinia nubialis*, translating to a 25% mortality rate at a concentration of around 1.5 mM kg^{-1} in no-choice diet assays.[68] Similarly, DIBOA in wild barley species negatively impacted development of the aphid *Diuraphis noxia*,[69] and when exuded from rye, also inhibited egg development of the nematode *Meloidogyne incognita.*[70] This suggests that both DIMBOA and DIBOA are broadly toxic to invertebrate species. This assertion seems reasonable given that higher benzoxazinoid content in wheat leaves correlated with enhanced resistance to various aphid species at naturally relevant concentrations, around 3 mM kg^{-1} fresh weight.[89]

Benzoxazinoids additionally have well-documented anti-microbial potential. BOA, the primary degradation product of DIBOA, was first discovered as an anti-fungal agent against pathogenic *Fusarium* species.[71] Moreover, multiple bacteria and yeasts are sensitive to DIMBOA, DIBOA and BOA at concentrations typically below 3 mM,[72] suggesting that this family of compounds have applications as broad-spectrum antimicrobials. As benzoxazinoids have been suggested to inhibit ATP synthesis, central to all life excepting viruses, it is logical that they would be toxic to multiple taxa of plants, animals and microbes.

The examples offered here form a strong case for phytoallelochemicals having applications in other areas of plant defence, and strongly indicates that benzoxazinoids offer leads for potential development of pesticides with multiple applications. This is further corroborated by the considerable research into the various functions of these compounds, as well as the relationship that chemical structure has on these functions, which has already been reviewed in great detail elsewhere.[7,90]

***Meta*-tyrosine**

Grasses such as *Festuca rubra* exude *meta*-tyrosine, the active compound inhibiting root growth in bioassays of crude root exudates from the species. *Meta*-tyrosine inhibited a number of species including weeds such as *Digitaria sanguinalis*, *Trifolium repens*, and *Taraxacum officinale*.[73] The compound also inhibited *Arabidopsis* root length of by 50% at a concentration of 25 µM, a potent phytoallelopathic effect.[73] *Arabidopsis* root tip browning was observed in the phytoallelopathic activity of *m*-tyrosine, indicative of cell necrosis.[91] Leaf necrosis has also been reported in *m*-tyrosine treated *Arabidopsis* at a concentration of 40 µM.[92] Non-protein amino acids are thought to have phytotoxic properties through their substitution of protein amino acids during translation, modifying protein folding as a result.[73] This mode of action has recently been verified for *m*-tyrosine, which is specifically misincorporated in place of phenylalanine.[92]

Despite its apparent specificity to plant proteins in terms of their mode of action,[73] there is evidence of allelopathy towards other organisms by *m*-tyrosine*.* A higher concentration than those required to confer phytoallelopathy (50 mM) results in antifeedant and toxic effects on the termite *Coptotermes formosanus*.[74] The development and sporulation of multiple *Bacillus* bacterial species was inhibited by 500 µM of *m*-tyrosine.[75]

It is likely that *m*-tyrosine is capable of providing multi-kingdom toxicity. This is in spite of an apparent specificity to plant proteins which would explain evidence that zooallelopathy may be an unrealistic expectation at natural concentrations. It may be thus that the observed wider allelopathic effects could potentially be conferred by other, yet undiscovered mechanisms.

Juglone

The phytoallelopathy of juglone, a naphthoquinone produced by walnut trees, particularly *Juglans nigra*, was discovered in the late 1800s.[9] The inhibitory effects of juglone on other plant species have been widely explored and documented.[9] For example, assay of the effects of juglone on sixteen herbaceous and woody plant species both on blotter paper and in soil, found dry weight of five species to be significantly inhibited by a concentration of 10 µM, while a further ten species were affected at a concentration of 100 µM.[76] Dry weight of *Lemna minor* was also significantly reduced at a 10 µM dose of juglone, with a reduction in net photosynthetic activity seemingly related to mitochondrial disruption.[93] While a number of modes of action have been theorised and none confirmed for juglone, inhibition of corn and soybean development at similar concentrations were associated with mitochondrial inhibition in root cells through the reduction of H^+ATPase activity, and the disruption of plasma membrane function.[94] It is therefore apparent that juglone is phytoallelopathic to a wide range of plant species, as past reviews have discussed.[95]

The growth rate of the promethea silkmoth (*Callosamia promethea*) was reduced 3.6-fold when fed on leaves treated with 0.05% juglone (w/w), similar to the concentration in black walnut leaves.[77] It would therefore seem apparent that the compound has additional zooallelopathic potential. Juglone also exhibits a degree of microbial allelopathy to a wide range of plant pathogens, which were significantly inhibited at a concentration of 75 µM.[78] Fungal species in particular seemed highly sensitive to the compound, to the extent that effects of juglone are comparable to those of some commercial anti-fungal agents.[78] It would therefore appear that juglone exhibits a degree of multi-kingdom functionality, the full range of which is apparent from extensive review of its biological effects.[9]

What does multi-kingdom functionality mean for crop protection?

Potential applications of multi-kingdom allelochemicals

As the examples provided throughout this review indicate, a number of crop species are involved in multi-kingdom allelopathic interactions. Bringing such multi-kingdom effects to application for the benefit of agroecosystems first requires consideration of factors influencing in-field crop allelopathy, and broader ecological impacts, both of which have been reviewed by a number of important works.[4,22,96] Ancestor varieties of domesticated crops often appear to be more potent producers of allelochemicals, so there is interest in assessing and re-introducing this material into breeding programmes to augment their natural defences.[97] Few such breeding programmes have been explored, even solely for weed suppression. The prime example in this case is rice, where weed suppression related to competitive and phytoallelopathic potential have been widely characterised.[98]

Such multi-kingdom allelochemicals would also provide leads for the development of future pesticides. They are often multi-target site inhibitors,[99] and may thus provide defence against multiple biotic threats as a result. Prioritising the development of such multi-site inhibitors has recently been advocated given the greater difficulty of evolving resistance against multiple targets.[100] It is hereby suggested by extension that multi-kingdom functionality may be an added, ecologically rational benefit, and provides a broader-ranging basis for pesticide development and deployment in crop protection. From a practical perspective, developing naturally-inspired biocides protective against multiple biotic pressures is economically and agronomically rational.

There are, by comparison, multiple examples of insecticides developed from zooallelochemicals.[101] Examples include pyrethroids developed from the pyrethrins found in *Chrysanthemum* species, and insecticides derived from *Azadirachta indica*, which have been extensively reviewed.[102] Even then, this is an underdeveloped tool in crop protection. More pertinently to this review, there are no records of allelochemicals which have inspired the development of multi-kingdom pesticides, in spite of the examples of multi-kingdom functionality posited throughout.

Barriers to development of natural product-based pesticides

There are a number of contributory reasons for the underdevelopment of natural product-based pesticides, particularly herbicides. A major caveat of harnessing phytoallelochemicals is their potential for nontarget effects. *Poecilus cupreus* larvae and *Folsomia candida* springtails are beneficial soil organisms detrimentally affected by these compounds.[103] APO is also inhibitory to the growth and development of the water flea *Daphnia magna*, used as an indicator of aquatic pollution.[104] It is of course a necessity to fully determine the full environmental impact of a new crop protection compound, which is not excused by the perceived environmentally benign nature of allelochemicals or allelochemical-inspired formulations. High concentrations of allelochemicals may be required to elicit the desired inhibitory effects, moreover, as a result of some degree of tolerance. This issue can be minimised by the identification of a maximum relevant dose, be it in terms of how much can be synthesised while remaining economically viable, or in terms of the concentrations of these compounds occurring in the allelopathic plant. The correct dose is further necessitated by hormesis, as there becomes a concern that the incorrect dose could stimulate, rather than inhibit, the growth of a detrimental species.

The development of a breeding programme for phytoallelopathic potential is dependent on a huge amount of knowledge.[4] The germplasm of a given species must be explored widely for phytotoxic potential, and this must be proven consistently on multiple relevant target species.[98] Myriad (in some cases poorly-understood) factors which can influence allelochemical synthesis and exudation, including the recognition interactions described earlier in this piece , as well as the influences of pest insects, pathogens, and environmental factors; all of these must be understood for a breeding programme to succeed and provide agronomic benefit.[90] Dynamics of allelochemical degradation in field soil must be characterised to ensure that there is no detriment to succeeding crops, but also that said compound persists sufficiently to have biological effects,[101] which means that the active allelochemicals must therefore be identified.[4,90] Crops produced by a breeding programme need to maintain comparable yield to those currently commercialised, which must be extensively examined prior to release.[98] There is therefore a large amount of interdisciplinary work attached to the development of a viable agronomic outcome, and this is increased significantly when multi-kingdom effects are desired. It is for this reason that crop protection products based on allelopathy are rare, but not impossible to produce.

Perspective

Given the number of existing examples of apparent phytoallelochemicals with anti-microbial or zooallelopathic properties, it is apparent that these compounds exhibit a degree of multi-kingdom functionality. This must be a result of these defences co-evolving to confer an overall net fitness benefit in natural habitats, likely to constitute tolerance to herbivores, plant competitors, and soil microbes.

Therefore, it is acknowledged that phytoallelochemicals are a sub-class of multi-kingdom inhibitors, and all of these compounds are allelochemicals. It is unlikely that biosynthesis and release of currently-recognised allelochemicals has evolved entirely due to the functional benefit of phytoallelopathy, given the distribution of a number of these compounds aboveground *in planta* and the dynamics associated with such allocation.

From a practical perspective, this means that allelochemical compounds, delivered as weed management tools either through enhanced production and delivery *in planta via* crop breeding or genetic engineering, or through the production of pesticide formulations using these chemicals as leads, may in fact have application in plant defence to multiple biotic stresses. Testing would be required, however, given that resistance, tolerance, or other factors may exist detrimental to the multi-kingdom functionality of some allelochemicals. It remains highly likely that there exist other examples of previously-researched phytoallelochemicals which have currently not been examined for multi-kingdom effects, but which exhibit them.

Conversely, the area of phytoallelochemical discovery is currently hindered by its reliance on the demonstration of phytoallelopathy, a notoriously difficult phenomenon to demonstrate in isolation; it is hereby argued that it would benefit from greater consideration of compounds with proven allelopathic effects on herbivorous pests or microbial pathogens. The hope is that the identification and development of such multi-kingdom inhibition, naturally-derived pesticides would delay the evolution of further resistance to existing synthetic chemistries while also providing effective new tools for weed, arthropod, and pathogen management.

The future outlined here would be realised by the testing of potent allelochemicals with little documented evidence of multi-kingdom functionality for this effect in problematic target species. The adoption of such a multidisciplinary outlook in informing the discovery of potential crop protection compounds has the potential to reduce the considerable time and economic cost required to bring new natural product formulations to market[105] by reducing the likelihood of producing and testing ineffective compounds, thereby benefitting both consumers and industry.

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