

Allelopathy: Current status of research and future of the discipline: A Commentary

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ABSTRACT

The study of allelopathy as a discipline has a long and at times controversial history. Since Hans Molisch coined the term before World War II, allelopathy research has grown from a trickle of papers before 1970 to a burgeoning subdiscipline of chemical ecology represented by hundreds of papers each year. Yet, allelopathy research still suffers from a reputation for papers of poor scientific quality that equate the presence of a phytotoxic phytochemical as proof of an allelochemical function without regard for proving that the compound is bioavailable in soil at sufficient concentrations to affect vegetation either directly or indirectly through effects on soil microbes. Synergism has often been invoked without proof to explain why effects of crude extracts are sometimes greater than even the additive effects of phytotoxins known to be in the extract. Much of this work may be correct, but to be widely accepted more rigorous proof is needed. Much of this literature also makes the assumption that allelochemicals must be highly water soluble, when there are good scientific reasons to hypothesize that the most effective allelochemicals would have very limited water solubility. Very little is known about the mode of action of and mechanisms of resistance to putative allelochemicals. Nevertheless, the quality and quantity of papers on allelopathy has increased steadily over the past several decades and knowledge gaps are being filled at an ever increasing pace. There can be little doubt that allelopathy plays an important role in plant/plant interactions in nature and in agriculture. Translating this growing knowledge to technology to manage weeds in agriculture has been slow. There is only one good case of discovery of an allelochemical (leptospormone) leading to the development of a major class of herbicides (triketones). There are examples of allelopathic cover crops being used for weed management in other crops, as well as other cultural methods to employ allelopathy. However to my knowledge, there are still no cultivars of crops being sold with allelopathic properties as a selling point. Enhancement or impartation of allelopathy in crops through the use of transgenes could eventually be used to produce such a cultivar. Some of the most high profile recent examples of research in our discipline will be discussed. The study of allelopathy appears to have a bright future, especially if we can translate our research into technologies that will reduce our reliance on synthetic herbicides.

Keywords: Allelochemical, allelopathy, benzoxazinones, *m*-tyrosine, momilactone B, sorgoleone

INTRODUCTION

Although people have long been aware of chemical interactions between different plant species (82), Molisch first rigorously outlined the concept and presented us with the term allelopathy (56). Some have tried to expand the definition of allelopathy to include almost all of chemical ecology, but for the purposes of this commentary and review, I define it as chemical interactions between plants via compounds other than primary metabolites, including those involving participation of microbes. This is still an enormous range of interactions. For the most part, the interactions of interest have been those involving chemical interactions that provide an advantage to the producer of the ‘allelochemical’ over the plant affected by that allelochemical.

This brief paper is not intended to be a detailed history of the field of allelopathy, but rather a commentary on the history, current status and potential future of our discipline. As a commentary, my own opinions will perhaps be more apparent than in a normal review or scientific paper.

HISTORY

Even though allelopathic phenomena have been known for many years, it has not been the topic a large number of journal publications until the past twenty years. A search of scientific journals in SciFinder®, using the search term ‘allelopathy and allelochemical’ found a dramatic rate of increase in articles on allelopathy during the past five decades (Fig. 1). This search included only journal articles, but did not include all journals. If *Allelopathy Journal* and book chapters had been included, the magnitude of the increases would obviously have been larger, but the trends would have been the same. Furthermore, many of the early papers on allelopathy had neither the terms allelopathy nor allelochemical in their titles nor abstracts.

Quite frankly, allelopathy has not had a good name among many mainstream scientists during the several decades of its scientific study. This has been the case for at least two reasons. First, most of the papers claiming to demonstrate allelopathy or dealing with “allelochemicals” do not prove allelopathy, nor do they prove that the compounds that they call allelochemicals are actually involved in significant plant/plant interactions. Correlative relationships have been interpreted as causal relationships in many cases without adequate proof. The large number of such papers stems partly from the fact that all plants produce secondary compounds that in high enough concentration are phytotoxic, especially in bioassays conducted in the absence of soil. Thus, one can show that any plant produces phytotoxic compounds in bioassays without soil. Crude extracts or preparations of plants are also often phytotoxic in such bioassays. When mixed in soil, plant residues of many plant species inhibit the growth of many other plant species. These effects are a relatively easy phenomenon to demonstrate. But such experiments only suggest, but do not prove allelopathy. Rigorous papers demonstrating allelopathy are rare. But, this is in part due to the extreme difficulty in unequivocally proving allelopathy.

Extrapolation of soil-free experiments to what happens in soil is almost impossible, in that allelochemicals are presumably entering the soil environment continuously from root exudation, leaching from shoots, or release from plant litter. This

process is almost impossible to accurately monitor, especially if the allelochemical(s) is unknown. Furthermore allelochemicals are apportioned between soil moisture and adsorption to soil particles, so that knowing the biologically available amount of an allelochemical is very difficult. Few papers have discussed or dealt with these difficulties.

Second, there have been a few cases in which “high profile” papers on allelopathy have been discounted by later work. For example, the paper by Muller *et al.* (59) claimed vegetation patterning around aromatic shrubs in xeric areas of the western U.S. was due to release of volatile phytotoxic compounds. This conclusion was later disputed by Bartholomew (8), whose experiments explained the sparse vegetation around these plants was due to animal activity. This controversy and others led influential ecologists, such as Harper (38) to argue that allelopathy is seldom a significant component of plant/plant interference, with most of the influence being due to competition for resources.

Much of the early work with allelochemicals did not identify the allelochemicals in crude extracts or tried to correlate allelopathic effects with the amount of ubiquitous (*e.g.* simple phenolic acids) or almost ubiquitous compounds (*e.g.* common flavonoids) in the donor plant instead of using bioassay-directed isolation and identification of the most phytotoxic compound(s). The latter process is not a trivial one. But this procedure can yield very surprising and profound results, such a *m*-tyrosine in fescue (12) and cyanamid in hairy vetch (43). If the compound is a new compound, the discovering laboratory must be equipped with analytical equipment (*e.g.* mass spectroscopy and NMR) required for structural elucidation. Few laboratories attempting to conduct allelopathy research in the past were equipped with the necessary instrumentation to discover novel compounds.

Despite these problems, many good researchers persevered and continued to publish rigorous and thoughtful research on allelopathy. Pioneers of allelopathy during the lag phase of allelopathy research include such pioneers as Rice in the west and Grodzinsky in the east. Occasionally, undisputed papers on allelopathy were published in the most high profile journals (*e.g.*, 65), providing a boost for the image of allelopathy research.

CURRENT STATUS

With this foundation, the discipline of allelopathy has become more organized in recent years. Many journals such as the *Journal of Chemical Ecology* increased publication of papers on allelopathy and in 1994, *Allelopathy Journal*, the first journal exclusively devoted to allelopathy research, began publication. New journals that are highly appropriate for papers on allelopathy have appeared in the last decade: *e.g.* *Plant Signaling and Behavior*, *Journal of Plant Interactions* and *Biological Invasions*.

In 1995, the International Allelopathy Society (IAS) was founded in India and their first congress was held in Cadiz, Spain in 1996, with subsequent congresses held every 3-years in Thunder Bay, Canada (1999), Tsukuba, Japan (2002), Wagga Wagga, Australia (2005) and Saratoga Springs, NY, USA (2008). The 2011 congress will be held in Guangzhou, China. Despite the growth in numbers of published papers, IAS congresses have not grown in size, being attended by 100 to 200 delegates every three years. Nevertheless, the quality of research being reported at these meetings has been high and the participants have been enthusiastic. Regional and national allelopathy congresses and symposia have been held in Europe and Asia over the past two decades, sometimes with

attendance similar to that of IAS congresses. Other scientific organizations, such as the American Chemical Society and the International Association for Ecology have had allelopathy symposia as part of their programmes. Clearly the number of presentations, symposia and meetings based on allelopathy has increased and continues to grow. One can only assume from this activity that funding for allelopathy research is at an unprecedented high. The European Commission (EC) recently funded a large allelopathy project entitled FATEALLCHEM on benzoxazinoids involving researchers from several European countries (33). This project produced a considerable amount of new information on the chemistry and biological activity of this important class of allelochemicals. This was perhaps the largest project in allelopathy's relatively short history.

Nevertheless, controversy about claims of allelopathy continues to this day. For example, the high profile work on putative allelochemicals of *Centaurea* species (5,6,62,73,76-78,80), has been seriously disputed (13-15,28,29,60,74,75). Two of the papers supporting the identity of allelochemicals from *Centaurea* species have now been retracted (73, 80). In one case, the work could not be repeated (73) and in the other the results were found to have been obtained by questionable means (80). Other papers in this series have been modified by published errata (5,77) or have had later papers published (63,66) that question the results of earlier papers from the same laboratory (5,6,78). This kind of situation is unhealthy for the discipline of allelopathy. Within bounds, the allelopathy community must be critical of its science to have credibility within the wider scientific community.

Nevertheless, the interest in and number of papers in our discipline continues to grow rapidly (Fig. 1). And there are now numerous examples of high profile allelopathy research that appear to be rigorous and significant. Several examples of this work will be discussed. There are many other examples of excellent recent allelopathy work that will not be covered in this limited commentary.

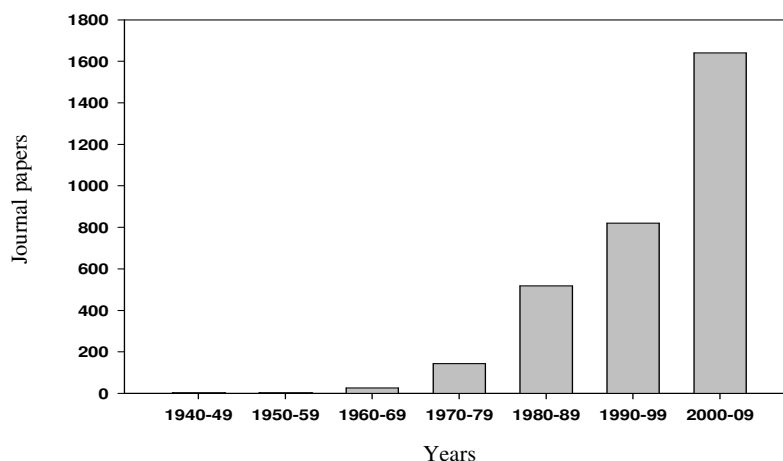


Figure 1. Journal papers accessed on SciFinder® using the search term 'allelopathy or allelochemical' for each decade of the past 70 years.

After describing an apparently highly allelopathic variety of fescue (11), Bertin *et al.* (12) found that the putative allelochemical was *m*-tyrosine, a non-protein amino acid. The compound is exuded into the soil from roots of the fescue plant. Recently, Kaur *et al.* (46) questioned the role of *m*-tyrosine as an allelochemical because of the apparent rapid microbially-mediated loss of the compound in soil, resulting in levels of the compound in soil lower than those causing significant phytotoxicity. However, their paper did not consider the flux of an allelochemical from donor to receiving plant that occurs in a dynamic system. As discussed below, the static concentration of a compound in the soil is less important than the rate of bioaccumulation by the receiving plant from a steady state or nearly steady state concentration that is maintained by constant influx from the donor plant. Clearly, this fescue variety is highly allelopathic and no allelochemical other than *m*-tyrosine has been identified.

Many grass species (*e.g.* wheat, rye and maize) and few non-Poaceae species produce a variety of benzoxazinoids known to be herbicidal, fungicidal, antimicrobial and insecticidal. Numerous papers on their properties and biological activities, including their possible roles in allelopathy are available. As mentioned earlier, the papers resulting from the recent large EC grant to study these compounds has added considerably to our knowledge of these compounds. The roles of these compounds in allelopathy, including synthesis and behavior in soil is perhaps better understood than any other group of allelochemicals.

Benzoxazinone glucosides are exuded from roots of the producing species into soil where the sugars are hydrolyzed, releasing the generally more active benzoxazolinone aglycones (54). Some of these (*e.g.* BOA and DIBOA) have soil activity at high application rates (7) and there is a high correlation between root exudation of DIBOA and DIMBOA and allelopathic activity of different wheat varieties (9,40). An excellent summary of the source, soil conversions and half-lives and phytotoxicity of most benzoxazinoid compounds was published by Belz (10). Perhaps the most important recent finding is the discovery that APO, a degradation product of BOA, is more phytotoxic than the parent compound and has a relatively long soil half-life, an indication that it may account for most of the phytotoxicity of benzoxazinoids in the field (51,52).

Sánchez-Moreiras *et al.* (71) reviewed what was known as the mode of action of BOA up until about 2003, but its mode of action and that of its analogues are still unknown. BOA can inhibit mitochondrial function by interfering with both electron transport and mitochondrial ATPase activity (59). A correlation between inhibition of plasma membrane H⁺-ATPase activity and inhibition of growth by BOA and DIBOA (34), suggests that their effects on nutrients uptake and electrolyte leakage could be caused by this primary effect (68). DIMBOA stimulates its own degradation by peroxidase, whereas DIBOA and MBOA did not have such an effect (70). We agree with the conclusions of Macías *et al.* (54) that there have been no definitive mode of action studies with this group of compounds, nor has there been evidence that the different phytotoxic members of this chemical family have a common mode of action. Baerson *et al.* (2) examined whole genome transcriptome changes induced by sub-lethal concentrations of BOA on *Arabidopsis thaliana*. The transcriptional effects were too complex to provide a clear clue to the mode of action. However, the up-regulation of genes encoding detoxification enzymes was profound. Target plants can detoxify benzoxazinoids by both glucosylation

and hydroxylation (72,81). Enzymatic products predicted by the transcriptome responses in *A. thaliana* were identified (2). Different detoxification rates might account for differential sensitivity between species.

The biochemical pathway and genes for the enzymes involved in the synthesis of DIMBOA have been characterized (35,36,42). Although, there are no publications yet on manipulation of these genes to probe the role of these compounds as allelochemicals, a patent exists that covers manipulation of the genes to make crops more pest resistant (17).

The apparent allelopathic property of sorghum (*Sorghum bicolor*) was observed for many years by its negative effect on the growth of other crops grown in rotation with sorghum (*e.g.* 1) without a clear determination of what the primary allelochemical(s) is. Sorgoleone was first discovered as a cue for parasitic weed germination in sorghum (16). Sorghum root hairs exude substantial amounts of sorgoleone and sorgoleone appeared to be a primary source of the allelopathic properties of sorghums (*e.g.* 58). The fate of sorgoleone in soil has been characterized, with a half-life of more than 77 days in some soils (37), longer than for many soil-applied synthetic herbicides. This finding further supports the view that sorgoleone is the primary allelochemical of sorghum. Sorgoleone inhibits growth of many weeds (*e.g.* 1,31,58) and acts as a strong inhibitor of PSII in isolated chloroplasts (69) mitochondrial functions in isolated mitochondria (67), and *p*-hydroxyphenylpyruvate dioxygenase (55) *in vivo*. It also interferes with root H⁺-ATPase and water uptake (39). Although the primary mechanism of action of sorgoleone in allelopathy is still unclear, having multiple potential modes of action is desirable from the standpoint of slowing evolution of resistance in target species if resistance is due to target site insensitivity. Recent work by Dayan *et al.* (24) in experiments designed to test the *in planta* mode of action of sorgoleone demonstrated that it has no effect on the photosynthesis of older plants, but inhibits photosynthesis in newly germinated seedlings. Sorgoleone was not translocated acropetally in older plants, but can apparently be absorbed through the hypocotyl and cotyledonary tissues. Therefore, the mode of action of sorgoleone may be the result of inhibition of photosynthesis in young seedlings in concert with inhibition of its other molecular target sites in older plants.

Mature root hairs of sorghum exude droplets containing a high fraction of sorgoleone and analogues with similar activity (19,20,23,83), along with a similar amount of a lipophilic resorcinol (24). Labeling studies showed that biosynthesis of sorgoleone involves the convergence of the fatty acid and polyketide pathways (21,32). Every step of the pathway has been confirmed in isolated root hair preparations (23). The genes for this pathway have been identified and the substrate specificity of the enzymes that they encode have been verified for the O-methyltransferase and the desaturases (3,4,18,61). Manipulation of gene expression with RNAi and other methods is now in progress.

Synthesis of phytoalexins is induced by the presence of the target pathogen. This saves the plant from the metabolic cost of synthesis of compound(s) and in some cases, may prevent autotoxic effects. A requirement for such a response to a pathogen is some mechanism for sensing the pathogen's presence. This is generally through detection of the presence of a unique chemical signature of the pathogen. If allelochemicals play an important role in giving a plant species an advantage over other plant species, this same strategy would seem to be advantageous. At least two studies have recently found evidence to support the view that chemical detection of a competing plant species can induce the production of an allelochemical. Dayan *et al.* (22) found that the production

of sorgoleone is for the most part constitutive and not affected by abiotic stresses, however, a crude extract of velvetleaf (*Abutilon theophrasti*) did increase the amount of sorgoleone produced by *Sorghum* spp. Similarly, Kong *et al.* (48) found that rice growing in the presence of *Echinochloa crus-galli* exuded significantly more phytotoxins from its roots than when growing alone. The question that arises is whether the increased allelochemical production is due to stress induced by the competing plant or phytotoxins from that plant or due to specific chemical elicitors from the competing plant.

These are only a few of the many examples of recent research that has made great progress in understanding allelopathy.

THE FUTURE

Clearly, allelopathy is an important phenomenon in nature and agriculture. Understanding the mechanisms of allelopathy in the many situations in which it occurs is essential in understanding plant/plant interactions. We have only discovered a small fraction of the information, but clearly our knowledge is growing exponentially (Fig.1). After understanding the information that is acquired, utilizing it to manage invasive plants in natural ecosystems and to manage weeds in agriculture will be another challenge.

Considering how little research has been done in allelopathy by laboratories with the capability of bioassay-directed isolation and structural elucidation of phytotoxins from plants, we have probably found only small fraction of the compounds actually involved in allelopathy, especially in non-crop plant species. The growing number of laboratories with such resources that have taken an interest in allelopathy promises to fill this knowledge gap at a faster rate than in the past. Perhaps finding these compounds will be less problematic than determining their true roles in chemical ecology. However, molecular biology methods provide powerful tools in such studies (30).

Another straightforward endeavor is determination of the genetics and biosynthetic pathways of allelochemicals. Modern methods in molecular genetics, molecular biology and biochemistry have made this type of research more rapid and more direct than in the past, and the facilities and training for this type of research are becoming more common. This type of research will be useful in harnessing the potential utility of allelopathy in agriculture through transgenically imparting or enhancing allelopathy in crops (26). In some cases (*e.g.* sorghum), it might be advantageous to eliminate allelochemical production to avoid replant problems. A recent paper that uses such knockout methods to determine the potential role of the plant hormone ethylene in plant/plant interactions is that of Inderjit *et al.* (41). Similar studies with compounds suspected of being allelochemicals should provide profound insights into the true ecological role of the compounds.

In nature, soil plays an important role in allelopathy. Many of the compounds claimed to be allelochemicals have little or no biological activity on plants in soil, due to their instability, rapid degradation by microbes, or other interactions with soil. Many highly water soluble compounds rapidly leach out from the root zone of potential target species. Nevertheless, some allelochemicals are clearly active in soil. Soil considerations are under a great deal more scrutiny than in the past. Recent work by Tharayil *et al.* (74) has shown that, in combination, one compound can make the bioavailability and half-life

of others greater in soil, because of co-competitive sorption and preferential degradation, increasing the persistence of allelochemical mixtures in a soil matrix. This type of synergism had not been looked for before. This highly important finding complicates the study of allelopathy, but provides evidence that may redeem some of the compounds that had been excluded as allelochemicals by some because of their rapid loss from soil when applied singly.

Other processes that deserve further study are the dynamics of allelochemicals in soil. For example, Dayan *et al.* (24), found that as the allelochemical sorgoleone was removed from root hairs of the producing plant, the root hairs responded by making more. So, plants that exude or secrete allelochemicals from roots may maintain a nearly constant level of the allelochemical in the rhizosphere as the compound is lost by leaching, degradation, or uptake by target species. The mechanics of proving this in a field situation could be daunting. However, Weidenhamer's laboratory is developing methods (50) that could be used to monitor fluxes of allelochemicals in soil, making this type of study possible.

Another area of needed research is that of microbial involvement in allelopathy. We know that soil microbes can both transform less phytotoxic compounds to more phytotoxic one as with the case of conversion of BOA to APO (52) and/or more often degrade them to less phytotoxic metabolites. Do soil microbes adapt to more efficiently degrade allelochemicals to which they have long exposure, as is sometimes the case with herbicides (*e.g.* 79)? Do allelochemicals change the soil microflora populations and composition? This area is fertile ground for those with training in soil microbiology. Much more needs to be done.

Some of the new information that will be generated on allelopathy in the future has the potential for use in understanding and controlling invasive plant species. If allelochemicals are involved in the success of an introduced species in a new environment, there are new technologies that could be used to target this aspect of their success. RNAi directed specifically toward a gene that encodes an enzyme unique to the biochemical pathway for the synthesis of the allelochemical would remove this advantage of the invasive species.

Much of the renewed interest in allelopathy mentioned above may be due to the desire to reduce synthetic chemical input into agriculture. There are efforts to generate more allelopathic cultivars of crops like rice and sorghum (26,27) by manipulation of genes involved in synthesis of allelochemicals. The recent discovery of the more potent allelochemicals in rice (44,47) should help in this effort. But, compared to efforts to use transgenes for herbicide resistance in weed management (25), the work on genetically altering allelopathy of crops to enhance weed management is miniscule. Peters and Xu (64) have recently patented genes in the momilactone B pathway that could be manipulated to alter rice allelopathy. Kato-Noguchi (45) recently reported that chemical-induced stress can induce higher synthesis of momilactone B in rice, but whether this phenomenon could be used for weed management has not been determined.

The use of allelochemicals as compounds to apply as natural herbicides is often touted as the reason for discovery of allelochemicals, but there is very little realistic research to bring this idea to fruition. One major group of synthetic herbicides, the triketones, was derived from leptospermone, a natural triketone that may be involved in allelopathy of plants that produce it and related compounds (49). Others have made

considerable effort to alter benzoxinones to produce commercial herbicides (*e.g.* 53). There are other examples of this approach, but compared to the interest in microbially produced natural products, the interest in phytotoxins from plants is low. There is still good reason to pursue this strategy, especially with respect to new allelochemicals that are discovered.

In summary, despite the tremendous growth in allelopathy research in recent years, we have much more to discover, understand, and apply to solve problems. The coming decades should provide many exciting discoveries and significant examples of the utilization of allelopathy to solve weed management problems.

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