POTENTIAL FOR UTILIZATION OF ALLELOPATHY FOR WEED MANAGEMENT

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Introduction

Theoretically, weeds, crops, rotation crops, companion crops, green manures, and cover crops are capable of producing chemical compounds that can influence the growth and productivity of surrounding plants. This has been recognized for over 175 years. These allelopathic phenomena can affect weed management, either adversely or favorably. Significant potential exists to exploit allelopathy in order to reduce our reliance on synthetic herbicides, especially in reduced or no-tillage agriculture. This review will describe how allelopathy can be identified and utilized as a tool in managing weeds.

Proof of allelopathy

Interference is defined as inhibitory effects of one plant on another, due to direct competition for resources (light, water, nutrients, *etc.*) and/or to allelopathy. Separating these two processes in field experimentation is not possible. So, how does one prove allelopathy? Unfortunately, much of the literature dealing with allelopathy does not rigorously prove that the observed interference between plant species is due to direct or indirect chemical interactions. No one method is totally conclusive, but results of a number of studies, taken together, can allow a reasonable determination of the role of allelopathy in specific plant-plant interactions.

Allelochemicals may reach the target plant by leachates or volatiles from plant foliage or litter, or through root exudation. In production agriculture, leachates and exudates are the only likely sources to be of significance.

One simple method to support a theory of allelopathic interactions is to determine the effects of leachates and exudates from pots containing suspected allelopathic plants and/or their residues on potted target species, when supplied as a soil drench. To more closely approximate the field situation, leachate from the target plants can also be used as a soil drench for the suspected allelopathic species. This method, pioneered by C.S. Tang, eliminates competition for resources, but maintains the most likely method of delivery of the allelochemicals. Objections

to this method are that it might provide greater exposure to allelochemicals than could be expected in the field because of limited root systems in pots and the fact that leached compounds may move out of the root zone in the field. Also, allelochemicals are secondary plant products, and production may be elevated under stress conditions (such as restricted growth in pots). However, this is a quick method to establish that allelopathy may account for some or all of the observed symptoms of interference between two species. Additional experimentation is required to more firmly establish the role of allelopathy.

Identification of the allelochemical is another step in proving allelopathy. This process involves bioassay-directed isolation. That is, chemicals contained in or exuded from different tissues of suspected allelopathic plants are fractionated and tested for phytotoxicity to the target species.

The extraction procedure for potential allelochemicals will profoundly affect the results. For example, grinding fresh tissues in organic solvents and/or heating tissue in these solvents to optimize extraction may result in extraction of high levels of phytotoxins, but this procedure may overestimate the concentrations of extracted chemicals which would be present in the root zone of the target species. Less extreme methods of extraction (such as aqueous extraction over time) should more closely approximate natural loss of the compounds from the allelopathic plant, as it might occur under field conditions.

The proper bioassay is also critical in identification of allelochemicals. One must consider how the target plant is exposed to the putative allelochemical in the field. Generally target plants receive the compound through the roots and/or other plant parts in contact with the soil. If so, a convincing bioassay should be done in the

same soil type in which allelopathy is suspected, to evaluate root uptake. This is often problematic, in that only very small amounts of the allelochemical are generally available. Compounds that are very active in a petri dish, on wet filter paper, often loose much or all of their activity in soil because of binding to organic matter, soil particles, and soil colloids. Several bioassays have been developed to evaluate effect of dissolved allelochemicals on *Lemna* spp. growth over time. Although these assays do not simulate field conditions, they enable the investigator to evaluate effects on plant growth and photosynthesis using low concentrations under controlled conditions. *Lemna* spp. assays are generally quick, inexpensive, and reproducible, factors which are important in selecting a good bioassay system.

After identification of the active (allelopathic) fraction, the active compounds in the fraction are isolated and bioassayed individually. Subsequently, the structures of those that prove to be active are determined. Unfortunately, much of the allelopathy literature involves searching for compounds that the investigator knows how to identify. Often these compounds can be found, but the most active compound(s) may be missed by this method. Modern, more automated isolation and identification methods (*e.g.*, GC-MS, LC-MS, and LC-NMR) allow greater ease in conducting more conclusive searches and identification of allelochemicals.

Perhaps the least desirable method for isolation of allelopathic compounds is extracting the macerated foliar portions of live plants. All plants produce phytotoxins, but evidence suggests that few species are truly allelopathic in plant-plant interactions. Many of these phytotoxic compounds are generally cytotoxic and are probably more functional in combating pathogens, insects, and herbivores than in fighting competing plants. Under normal circumstances, by

the time these compounds reach the soil, their concentration and availability to competing species is often too low to influence interference.

For example, artemisinin, a highly potent sesquiterpenoid with antimicrobial and phytotoxin activity from *Artemisia annua* L. accumulates to a level of 1-2% of the shoot dry weight of some chemotypes of this species. However, allelopathy has not been observed with this species, although it has been recognized in other species of *Artemisia*. As another example, several species of *Hypericum* produce the very potent cytotoxin hypericum. In the presence of both light and molecular oxygen, this photodynamic compound is toxic to all living tissues. However, we are aware of no convincing reports of this compound being an allelochemical in plant-plant interactions. There are many more examples of highly potent phytotoxins apparently being produced for other purposes than plant-plant interactions.

In some instances, very weak phytotoxins are claimed to be significant allelochemicals. For example, common cinnamic- and benzoic-derived acids have frequently been claimed to be responsible for field effects that appear to be due to allelopathy. This is possible if compounds accumulate in large quantities and especially if microbes convert these relatively benign compounds to more potent phytotoxins. An example of this conversion of the hydroxamic acid, BOA, by actinomycetes to a much more toxic azoperoxide, AZOB.

Some have argued that several very weak phytotoxins can act synergistically to produce an allelopathic effect. However, when proper dose-response analyses are done, compounds claimed to be synergistic have been found to be antagonistic.

Following identification of the suspected allelochemical, the compound can be tested in the field to see if its effects are similar to those of the putative allelochemical effect caused by the suspected allelopathic species. If the results are not similar, this does not rule out the involvement of the compound in allelopathy. Effects of the allelochemical may be modified by environmental changes, interaction with other compounds, or by microbial transformation.

Finally, proof of direct involvement of an allelochemical in plant-plant interactions requires that it be detected in the soil at levels that are phytotoxic. This is complicated by the fact that soil-bound compounds might be slowly made available to a target plant, but very difficult to extract from the soil using current technology. Further research to develop appropriate field assays and soil extraction techniques for natural products are needed.

Correlation of selectivity of the suspected allelochemical with selectivity of interference by the producing species can provide strong support for allelopathic interference.

Genetic evidence can also be used to suggest allelopathy. Correlation of varying levels of suspected allelochemicals in different chemotypes or varieties of a species with their level of interference sometimes provides support for allelopathic interference. However, different chemotypes are generally different ecotypes that may vary in other characteristics in addition of allelochemical production. Mutants that vary dramatically in the amount of the suspected allelochemical, but are isogenic in other traits, would be ideal for determination of the role of the chemical in plant-plant interactions. However, even with such biological material, loss of the allelochemical could produce pleiotrophic effects which would affect competitive ability of the phenotype.

Indirect effects of allelochemicals on competing plants could further confuse the question of whether a species is allelopathic. For example, microbial conversion of plant-produced "pro-allelochemicals" to effective phytotoxins will be dependent on the

presence and abundance of the proper microbes. Compounds that are mildly phytotoxic may have a more important function in protecting the plant from soil-borne pathogens and nematodes, making the producing plant more competitive toward other species.

Clearly, proof of allelopathic plant-plant interactions is generally not trivial and has rarely been demonstrated conclusively. Nevertheless, the phenomenon does exist and can be harnessed as an aid in the management of weeds.

Allelopathy in the field

Adverse effects. Many of the allelopathic effects found in the field adversely influence crop production. Some of the earliest observed effects were the inhibitory effects of certain crops on succeeding crops. For example, rye (*Secale cereale*) residues have been implicated in suppression of both crops and weeds during the next growing season. Small seeded vegetable crops are often stunted by residue of grass crops or covercrops. Lower than expected yields in continuous monocultures of some crops (*e.g.*, rice, coffee, maize, and sugarcane) have been attributed, in part, to autotoxicity of allelochemicals. Differences in weed populations following different crops have been attributed in part to varying allelopathic effects of different crop residues.

Other adverse effects are those of allelopathic weeds on crops. Many weed species commonly found in agroecosystems are known or suspected to be allelopathic. For example, yellow nutsedge (*Cyperus esculentus*) is alleged to release allelochemicals detrimental of maize. Residues of such species in no-tillage farming have the potential to reduce yield. Nodulation and nitrogen fixation of legumes seems to be particularly sensitive to allelopathic inhibition. This was demonstrated with effects of quackgrass (*Agropyron repens*) on nodulation of snap beans (*Phaseolus vulgaris*).

Beneficial effects. Crops have been screened for allelopathic traits, although this approach has not resulted in revolutionary approaches or new cultivars for weed management. Superior weed suppressive cultivars of sorghum, oat, sunflower, soybean, and rice have been selected. Over five hundred cucumber varieties were screened for allelopathic potential. Inconsistent results were obtained in fine-textured soils or after heavy rainfall.

Little has been done to genetically transfer the allelopathic traits mentioned above to high-yielding varieties. Currently, there are no published reports of allelopathic traits being incorporated into commercial cultivars. Clearly, if crops that were both highly productive and strongly allelopathic could be produced by conventional breeding, such traits would have been co-incorporated into crops long ago.

Although little data exists on the productivity of allelopathic crops, one might expect that any level of autotoxicity by the allelochemicals could reduce yield. In general, the more phytotoxic the allelochemical, the more probable autotoxicity might be. Little work has been conducted to date to study the mechanisms of self-protection which could be utilized by various plants producing copious quantities of allelochemicals. However, in general, the efficacy of allelochemicals compared to that of synthetic herbicides, is generally quite poor. Even if an allelochemical is very potent when applied as a herbicide, it may not be delivered naturally by the producing plant at the right time and place in sufficient quantities for an allelopathic effect.

Transgenic methods have been used to confer crop resistance to both insects and

pathogens by insertion of genes that encode proteins that can either directly or indirectly fight these pests. Herbicide-resistant crops have also been generated by transgenic methods. However, this technology has not been used to produce crops that produce their own herbicides. Potential problems with such an approach are those of yield reductions due to resources spent in producing allelochemicals and autotoxicity of the allelochemicals. However, genes to confer resistance to the allelochemicals could also be inserted into the crop.

For example, genes that protect crops from glufosinate (phosphinothricin when produced by bacteria) have already been inserted into all major crops and many minor crops. The bacterial genes for production of bialaphos, the tripeptide precursor of phosphinothricin are known. Expressed in the roots of glufosinate-resistant crops, these genes could possibly provide the crop with its own bio-herbicide, provided it is leached or exuded from the roots in sufficient quantity. Root-specific expression would be desirable, since presence of the herbicide or pro-herbicide in the harvested portion of the crop might pose health problems. Genes for resistance to other natural phytotoxins are known.

Cover crops offer an alternative approach for the delivery of specific allelochemicals for weed control. These cover crops can accumulate naturally one or more allelochemicals in the rhizosphere. Following desiccation, by completion of the plant's life cycle or through herbicide treatment, the crop of interest is planted through the cover crop residues. Allelochemicals which had accumulated in the soil, or were released by degrading cover crop residues, can act to suppress weed emergence and/or growth. Numerous studies have demonstrated that grain sorghum residues reduce weed populations in fields the year after grain sorghum is grown. Also, herbicide-desiccated residues of fall-planted rye, wheat, and barley in the spring reduce weed densities by 80 to 90%, compared to areas lacking residues.

The level of weed suppression by cover crops depends on many variables. In cooler versus warmer temperate areas, cover crop residues provide longer and more effective weed suppression. This may reflect a longer half-life of the allelochemicals under cooler conditions. The level of rainfall can also impact distribution of allelochemicals, with greater rainfall moving allelochemicals below the zone of optimum weed germination. Sensitivity of weeds to allelochemicals varies, but generally, smaller seeded weeds are more sensitive than larger seeded weeds, and monocotyledonous weeds are more tolerant than dicotyledonous weeds. Conservative estimates for weed suppression using cover crops varies from 21 to 60 days following desiccation.

In addition to allelopathic suppression, cover crops benefit weed management and crop production in other ways. Residues of cover crops act as a physical barrier for light reaching the soil surface, which may minimize germination of weeds requiring light for germination. Residues also minimize soil erosion, conserve soil moisture, reduce fluctuations in soil temperature, and build soil organic matter, all beneficial to developing crops.

Aside from the use of cover crop residues for weed suppression in succeeding crops, pasture grasses which can interfere with weed emergence and growth have been selected with some success. Some of the competitive ability of these species and varieties within a species has been attributed to allelopathy.

The advent of precision agriculture and integrated weed management provides opportunity for the better employment of allelopathy in agriculture. A better understanding of weed-crop interactions, including allelochemical interactions, incorporated into computer-driven decision

aides might allow farmers to exploit allelopathy in an integrated weed management strategy.

In addition to providing direct benefit for weed management, allelopathy also has the potential for use in weed management by providing leads for new herbicides. For example, the herbicide cinmethylin, a commercial herbicide sold in Europe, was apparently derived from one of the first allelochemicals identified, 1,8-cineole. This monoterpenoid essential oil component was found to be involved in vegetation patterning around *Salvia* spp. in arid environments. Structural modification of 1,8-cineole led to the production of a herbicide with more desirable physical and biological properties than the natural compound, including enhanced persistence and selectivity.

Sorgoleone, a quinone exuded from roots of several *Sorghum* species, is as potent as an inhibitor of photosystem II as most commercial herbicides that attack the same molecular site. Interestingly, this molecule appears to fit in the binding pocket of the Qb binding site in a manner very similar to that of other PSII inhibitors such as metribuzin when examined using 3D modeling. The electrostatic charge distribution of the molecule is apparently very similar to that of other PSII inhibitors, allowing tight binding in the Qb pocket.

A greater understanding of the mechanism of action of allelochemicals may provide new information for the biorational design of herbicides. For example, the investigation of microbial phytotoxins has resulted in the identification of numerous unexploited molecular sites for herbicides. Much less is known of the mechanism of action of allelochemicals involved in plant-plant interactions than of herbicides. However, the pesticide industry has an interest in this topic as a strategy for discovery of new molecular target sites for herbicides.

Conclusions and the future

Allelopathy has not yet been of major consequence as a weed management tool for production agriculture. However, technological advancements may change this. Integration of allelopathic crops, cover crops, and companion crops into integrated weed management systems has the potential to reduce use of synthetic herbicides. Molecular genetics has the potential to produce highly allelopathic crops with no autotoxicity problems. Finally, allelochemicals are a virtually unexploited source of novel herbicides and a tool to discover new herbicide molecular sites.