

Natural products as herbicides

Stephen O. Duke

*United States Department of Agriculture,
Agricultural Research Service, Southern Weed
Science Laboratory, Stoneville, MS 38776, U.S.A.*

Summary

Plants and microbes contain a largely unexploited storehouse of phytotoxins that might be used directly or as structural indicators for new synthetic herbicides. Several factors have increased the interest of the herbicide industry in this source of natural products as herbicides. Natural compounds have made a large impact in the area of insecticides, whereas relatively minor successes have been obtained with these compounds as herbicides. The number of options that must be considered in the discovery and development of a natural product as a herbicide is larger than for a synthetic herbicide. Furthermore, the molecular complexity, limited environmental stability, and low herbicidal activity of many phytotoxic natural products are discouraging. However, advances in chemistry and biotechnology are increasing the speed and ease with which we can discover and develop natural products as herbicides.

Need and rationale

The need for new herbicides will continue into the foreseeable future. There is a need for more environmentally and toxicologically safe herbicides that are equally, or more, efficacious and selective than currently available herbicides. Increased emphasis on reduced-tillage and no-tillage agriculture will make adequate control of weeds more dependent on herbicides. The accelerating incidence of herbicide resistance is creating a need for new herbicides with novel mechanisms of action. New herbicides will be required to solve the dilemma of the need for increased herbicide use while older herbicides are removed from the market for toxicological and environmental reasons.

The number of synthetic compounds screened to discover a commercially useful herbicide has increased dramatically due to diminishing returns with this method and more stringent requirements for new herbicides. Therefore, new discovery strategies, such as using natural products as leads, must be considered. Natural product-based herbicides may have shorter environmental half-lives than synthetic herbicides that are predominantly halogenated hydrocarbons with relatively long environmental half-lives. Furthermore, synthetic halogenated hydrocarbons generally have more suspect toxicological properties than most natural compounds. Thus, natural compounds have increasingly become the focus of those interested in discovery of herbicides (1-3,5,6,8-11).

New sites of herbicide action are of great interest to the herbicide industry. There are less than 15 known molecular sites of herbicide action (4). There is little overlap between these sites with the known molecular sites of action of microbial phytotoxins (3). Thus, natural products offer compounds with activity at new sites of action, and information on the feasibility of targeting particular sites of action in herbicide discovery efforts.

There is little interest within the herbicide industry in utilizing crude extracts of organisms as herbicides, even though there are many reports of crude plant extracts with herbicidal activity (7,12). The toxicological, quality control, economic, and patent obstacles to development of crude extracts as herbicides are severe. Therefore, this short review will mention only individual natural compounds that have been utilized or considered for weed control.

Plant-derived compounds with herbicidal potential

There are many reports of the production of phytotoxic symptoms by specific secondary compounds (allelochemicals) isolated from plants. A role of the compound has been

clearly established in plant-plant interactions in relatively few cases. Although most of the simple, ubiquitous phenolic acids and flavonoids reported to be allelochemicals are weakly phytotoxic, they are ineffective phytotoxins in soil and have little selectivity. Several of these phenolic compounds are effective against weeds only at high rates and are relatively non-selective (13). However, synthetic modification of these compounds can increase their efficacy and selectivity. For example, the halogenated benzoic acid herbicides (e.g., dicamba, chloramben, and picloram), are analogues of benzoate, a phenolic plant product. Some phenolic compounds, such as the coumarins, are much more phytotoxic than others. Synthetic derivatives of coumarins have been reported to be good herbicides in patent applications. Other aromatic compounds, such as 2,4-dihydroxy-1,4-benzoxazin-3-one, are as active in reducing plant growth as many herbicides.

Some of the most convincing reports of allelochemical involvement in plant competition involve terpenoids. For example, the monoterpene 1,8-cineole is strongly phytotoxic. A 1,8-cineole derivative, cinmethylin, has been seriously considered for development. A mixture of about 200 different compounds produced by polychlorinating camphene was used both as an insecticide and a herbicide. Many plant-produced terpenoids are very phytotoxic (e.g., artemisinin; see ref. 5), although they are not being developed as herbicides.

Plants produce many photodynamic compounds, such as hypericin. These compounds are strongly phytotoxic, but they are unlikely to be developed as herbicides because, in the presence of light, they are toxic to all organisms. Plants can be caused to generate phytotoxic levels of photodynamic porphyrins by treatment with both 5-aminolevulinic acid, a natural porphyrin precursor, and 2,2'-dipyridyl, a synthetic compound (10).

Certain alkaloid and terpenoid plant products that stop growth of plants by interference with mitosis (15) have modes of action identical to certain synthetic herbicides. Little attention has been given to their potential as herbicides, due, in part, to difficulty in synthesizing these complex molecules and the low yield from the producing plants. Simpler

alkaloids such as caffeine can, in some cases, act as selective herbicides at high application rates.

Unaltered, plant-produced phytotoxins are generally less active than commercial herbicides. Most known allelochemicals would have to be applied at rates of more than 10 kg ha⁻¹ to achieve significant weed control, whereas many recently introduced herbicides would achieve the same level of control at levels three orders of magnitude smaller. That plants do not produce large numbers of highly phytotoxic compounds is not unexpected, because production of such compounds would lead to strong autotoxicity unless the producing plant developed metabolic or physical mechanisms to cope with its own phytotoxins. Some of the more potent allelochemicals are toxic to the producing species and this autotoxicity has been implicated in vegetation shifts during ecosystem succession. Microbial conversion of relatively non-phytotoxic plant-derived compounds in the soil to highly phytotoxic derivatives has also been documented.

Microbial compounds with herbicidal potential

Microbial compounds have been more successfully exploited either as herbicides (e.g., 1,3,8) or leads for new herbicides than plant-derived compounds. Several strategies may be employed in utilizing microbial products as new herbicides. Either non-pathogenic microbes such as soil microflora (e.g., many of the actinomycetes) or plant pathogens can be utilized as sources of phytotoxins. Non-pathogenic microbes have been the source of natural compounds most commonly utilized by industry in pesticide discovery. They are relatively easily cultured compared to pathogens, and they produce a multitude of bioactive products. A major problem with these organisms is eliminating known phytotoxins such as cycloheximide or gabaculine early in the screening process.

Nevertheless, the only known commercial successes of herbicides originating from microbial products have been compounds derived from non-pathogens. Two of these compounds, bialophos and glufosinate, are related by the fact that bialophos must be metabolically degraded to glufosinate by the target plant in order to be active. Bialophos,

glufosinate, and related microbial products such as phosalacine, are all potent inhibitors of glutamine synthetase. This metabolic site is not targeted by any herbicide developed by other strategies (4).

A large proportion of the metabolites of microorganisms patented as herbicides are from non-pathogens. Most of these patents are of Japanese origin. The majority of the phytotoxins of microbial origin for which no mechanism of action is known, are from non-pathogenic microbes. Examples of these compounds are toyocamycin and several close analogues, the herbimycins, and the herbicidins. These and many others are discussed in more extensive publications (e.g., 3,8).

Plant pathogens often produce several phytotoxins as virulence factors. Thus, the probability of a plant pathogen producing a phytotoxin is good. However, plant pathogens are generally more difficult to culture and to induce to produce toxins in culture than non-pathogens. Some of the compounds are too specific in their selectivity to have any commercial appeal as a herbicide. For these reasons, the herbicide industry has committed relatively few resources to plant pathogens as sources of herbicides. Most of the activity in this area has been the testing of compounds discovered by those outside the pesticide industry.

Although phytotoxins from pathogens that infect crops are likely to be toxic to some weed species, in most cases, there is no published record of the effect of already discovered phytotoxins on a range of weed species. Little effort has been expended in studying pathogens that infect weeds or the phytotoxins that weed pathogens might produce. All known host-specific phytotoxins, except one, are produced by pathogens that infect crop species. These compounds are often highly complex in structure and too selective for herbicidal use. An exception is maculosin, a relatively simple cyclic diketopiperazine analogue of cyclic L-tyrosine-L-proline, which is a host-specific phytotoxin from *Alternaria alternata* that appears to affect only spotted knapweed (*Centaurea maculosa*) (14).

Non-host specific toxins are of considerably more interest. An example of such a phytotoxin is tentoxin, a cyclic tetrapeptide

produced by several *Alternaria* species. It causes severe chlorosis in many of the problem weed species associated with soybeans and maize, without affecting either crop (6). Furthermore, it has two unique mechanisms of action. The synthesis of tentoxin and its production by fermentation are currently too expensive for commercialization. Extensive structure-activity research has not produced a simpler, less expensive compound with similar activity. However, efforts have been made to understand the genetics of tentoxin biosynthesis so that the cost of its production by fermentation technology can be reduced. Fumonisin B₁, a non-host specific toxin of *Fusarium moniliforme*, causes rapid loss of plasmalemma and tonoplast integrity, resulting in cellular leakage and eventual cell death to a wide range of weed species.

Factors influencing discovery and development

Isolation and identification of the compound are required steps in the discovery of natural compounds for use as herbicides. Improving methods continue to reduce the cost and time associated with these steps.

Many natural products are structurally too complex to be synthesized economically for agricultural use. For example, these compounds commonly have several chiral centers. Two strategies are available to overcome this problem. Structure-activity studies might result in discovery of a simpler molecule with a commercially viable ratio of synthesis cost to herbicidal activity. An example of a synthesized herbicide that was developed from a more complex microbial product is methoxyphenone, which was derived from anisomycin, a *Streptomyces* metabolite. Alternatively, the molecule might be biosynthetically produced by fermentation (e.g., bialophos).

Even if a natural product could be synthesized economically, an analogue rather than the native compound might be more desirable to market. If the phytotoxic nature of the natural phytotoxin or a related natural compound has been reported previously, the patent may be less defensible than that of the synthetic analogue, especially if there is no mention of its source.

Two factors are peculiar to microbial products. Culture methods (e.g., liquid or solid

culture) and culture conditions, both nutritive and environmental, can have a profound influence on production of secondary products by microbes. Thus, two laboratories may screen the same microorganism and obtain entirely different results. In addition to new compounds and chemical classes, microbial phytotoxins are sources of new sites of action. Considering the relatively few potential sites of action of synthetic herbicides, this aspect of microbial toxins may become more important. There is little overlap between the known sites of action of microbial phytotoxins and commercial herbicides (2,3). Some screening programs for microbial compounds with herbicidal action have been mechanism-of-action based.

Good microbioassays are often required in screening natural products for herbicidal activity because of extremely limited amounts of available test compound. These types of assays are not as standardized as those used for more traditional herbicide screens. Examples of such bioassays are *Lemna* sp. grown in microtitre plates, germination and root growth of small-seeded species, and coleoptile growth assays (1,2).

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