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Phytotoxins as potential herbicides

G. Strobel, D. Kenfield, G. Bunkers, F. Sugawara* and J. Clardy +

*Department of Plant Pathology, Montana State University, Bozeman (Montana 59717-0002, USA), * Institute of Physical and Chemical Research, Laboratory of Herbicide and Plant Growth, Riken, Wako-shi Saitama (Japan), and + Department of Chemistry, Baker Laboratory, Cornell University, Ithaca (New York 14853, USA)*

Abstract. Phytotoxins are produced in various culture media by many fungi that are pathogenic to weeds. These phytotoxins belong to a wide array of chemical substances including sesquiterpenoids, sesterterpenoids, diketopiperazines, peptides, spirocyclic lactams, isocoumarins, and polyketides. In most cases, the phytotoxin belongs to a family of related compounds produced by the fungus. These related compounds may or may not be phytotoxins. Phytotoxin production, in some cases, is optimized by the addition of a host extract to the culture medium. Biological activity is usually observed in a range of concentrations from 10^{-3} to 10^{-6} M. The concept of using these molecules, derivatives thereof, or related compounds as herbicides should be explored.
Key words. Phytotoxins; herbicides; weeds; fungi; *Cochliobolus*; *Drechslera*; *Phoma*; *Alternaria*.

Introduction

Plant pathogens, especially fungi and bacteria, are capable of inducing disease symptoms in their respective host(s) by virtue of the phytotoxins that they produce [13, 27]. These compounds vary dramatically in size and also in the chemical class to which they belong, e.g., peptides, terpenoids, macrolides, phenolics, and others. The phytotoxins also vary in host specificity, ranging from showing a host specificity identical to that of the pathogen to having no specificity whatever [13, 27]. Traditionally, most investigators have been concerned with the isolation, characterization and mode of action of phytotoxins from pathogens of crop plants. Sometimes, these phytotoxins have proved useful as tools for screening plants for toxin insensitivity (disease resistance) and as probes of normal physiological plant function.

Virtually all plants – including crop plants, herbs, weed species, ornamentals, tropical species, forest, plants, important land cover forms, and aquatic species – are hosts to a score or more of pathogens. With the exception of crop plants, the disease causing fungi and bacteria of the vast majority of the plants in these groups have not been examined for their ability to produce phytotoxins. Potentially, there is a reservoir of novel biologically important substances awaiting discovery in these organisms.

Over the past eight years we have investigated phytotoxin production in some fungal pathogens which cause disease in important weeds. Our rationale has been that such phytotoxins might prove useful as new probes of plant function and new models for herbicides. Weed pathogens have had millenia to coevolve with their hosts and devise biochemical mechanisms to kill them or influence their gross physiology [7, 11, 12, 27, 28]. Now we need to take advantage of this in order to devise new strategies for weed control. The first step in this process is an examination of the structure and function of phytotoxins from weed pathogens.

Pathogen acquisition and culturing

In some cases, fungal and bacterial pathogens of weeds have found their way into various private and public culture collections and can be obtained from them [28]. Many pathogens of weed species, however, have yet to be

isolated and identified. The standard techniques used in phytopathological research can be used to isolate the causal organism and ultimately show its relationship to the disease.

Once obtained in pure culture, the organism is placed in a defined liquid medium under shaking or stationary conditions. A simple puncture-wound leaf assay is used as a guide to phytotoxin production in the medium, which may take 2–3 weeks[31]. Sometimes, however, a simple aqueous extract made from 1–2 g of the host plant is needed to stimulate the production of phytotoxins by the pathogen in culture. As a general rule it is advisable to keep the addition of various concoctions to the medium at a minimum since these substances only make the phytotoxin purification process more difficult. Phytotoxin isolation proceeds according to the general methods used for the isolation of natural products from any source. One frees the medium of the pathogen, reduces the volume of the medium, and begins solvent partitioning procedures. Flash chromatography, preparative thin layer chromatography, and high pressure liquid chromatography are common techniques used for toxin isolation. These generalized procedures, however, may exclude some proteins that have phytotoxic activity since solvent extraction may inactivate them. Also, very polar or charged compounds may also be excluded since they end up in the rarely-investigated aqueous fractions. Characterization of the phytotoxin is done by a variety of spectroscopic means and an ideal structural determination would include crystallization and characterization by X-ray diffraction in addition to the spectroscopic data.

Eremophilanes

Eremophilanes are bicyclic sesquiterpenoids and over 200 compounds of this group have been characterized in higher plants[22]. Numerous eremophilanes have been found in fungi. These include phomenone (*Phoma exigua*), phaseolinone (*Macrophomina phaseolina*), sporogen AO-1 (*Aspergillus oryzae*), and PR-toxin (*Penicillium roqueforti*)[9, 23, 37, 39]. Phomenone and phaseolinone cause necroses on dicots. Sporogen AO-1 seems to be involved in the physiology of sporulation in *A. oryzae*. PR-toxin is a dangerous mycotoxin produced by the fermentive agent of blue cheese when it is cultured on corn (*Zea mays*) kernels.

Eremophilanes with phytotoxic properties were isolated from *Cochliobolus cynodontis* (anamorph: *Bipolaris cynodontis*), a fungal pathogen of Bermuda-grass (*Cynodon dactylon*)[31]. This pathogen makes two eremophilanes, bipolaroxin (fig. 1) and its reduced analog, dihydrobipolaroxin. Bipolaroxin shows some host selectivity. It causes lesions on Bermuda-grass at 38 μM, whereas a concentration of 0.7 mM is required to produce detectable symptoms on wild oats (*Avena fatua*), sugarcane (*Saccharum officinarum*), and corn. Dihydrobipolaroxin lacks the aldehyde moiety and is totally inactive[7].

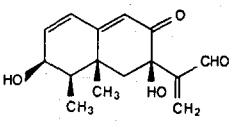


Figure 1. Bipolaroxin.

Drechslera gigantea, a relatively obscure pathogen of Bermuda-grass and quackgrass (*Agropyron repens*), produces grey lesions on its hosts surrounded by dark green borders suggesting that it makes phytoactive compounds (fig. 2)[6]. Organic extracts of culture filtrates contain numerous (at least 13) eremophilanes, many of which have novel structures. The basic structure of eremophilanes from *D. gigantea* includes a rigid bicyclic system with a keto group at C8, unsaturation at C9–C10, a hydroxyl at C3, and *cis*-methyl groups at C4 and C5. Biogenic variability occurs via hydroxylation, epoxidation, and dehydration at C6 through C13. Hydroxylation at C1 occurs in one compound. Organic synthesis of eremophilanes has been accomplished[3, 22].

Our interest in this class of compounds was galvanized by the observation that when dicots were tested for sensitivity, a necrotic reaction developed[17]. When tested on monocots, however, phomenone, petasol, and gigantenone (fig. 3) evoked green islands, localized areas of chlorophyll retention in senescing tissues. Cucumber (*Cucumis sativus*) was a notable exception because this dicot developed green islands (fig. 4). Pumpkin (*Cucumis pepo*), a close relative of cucumber, followed the norm and became necrotic. Further studies revealed that, like

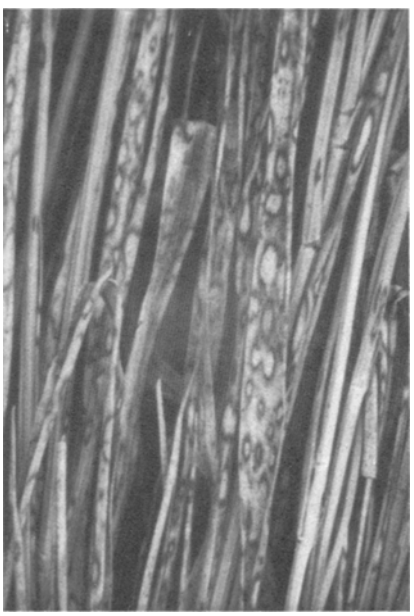


Figure 2. *Drechslera gigantea* infecting Bermuda grass. Note the light colored lesions surrounded by darker borders.

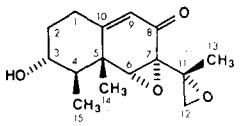


Figure 3. Gigantenone.

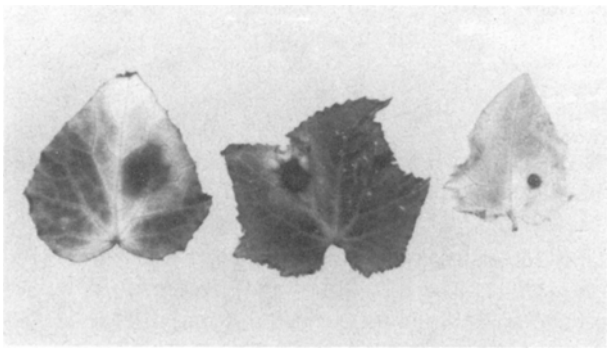


Figure 4. The induction of green island formation on cucumber leaves by the application of gigantenone.

cytokinin-induced green islands, eremophilane-induced green islands retained their photosynthetic ability. Eremophilanes differ from cytokinins, however, by inducing a localized delay of senescence which does not act as a metabolic sink. Furthermore, they are nearly as effective as cycloheximide in inhibiting in vitro and in vivo protein synthesis, making their mode of action dramatically different from that of the cytokinins[4]. In addition, they fail to induce synthesis of chlorophyll in etiolated tissue from both monocots and dicots. Gigantenone and petasol, the two most interesting eremophilanes from *D. gigantea*, stimulate rhizogenesis in cuttings of mung beans (*Vigna radiata*)[4]. In tissue culture, these compounds promote rooting of calli of common sunflower (*Helianthus annuus*). When the compounds were tested on explants of asparagus (*Asparagus officinalis*), a monocot, no effect on rhizogenesis was observed, but shoots were larger and more branched than those of controls.

The mixed phytohormone-like response of plant tissue to these eremophilanes offers another tool for exploring the bioregulation of important physiological processes, ranging from senescence to rhizogenesis. In addition, the differential response of monocots and dicots has implications in agriculture, for selective herbicidal as well as growth-promotive effects. Currently, the fate of these interesting chemicals once they enter the plant is being explored. Preliminary evidence using radiolabeled petasol, also an eremophilane produced by *D. gigantea*, indicates that it is converted to a water-soluble product within 12 h after application to oat leaves[4]. The bioactivity of this conversion product is unknown, but it fails to elicit green islands when extracted from treated leaves and reapplied to fresh tissue. Chemically, it does contain the petasol moiety. Finally, we wish to stress that gigantenone and its chemical relatives join zinniol and polyhydroxamates as the only pathogen-produced compounds known to cause green islands that have been chemically identified[1,24]. Numerous researchers have ascribed the green island effect appearing in some diseased higher plants to purine-based 'cytokinin-like' compounds, but the chemistry supporting this speculation is incomplete[8]. More attention should be addressed to the possible involvement of eremophilanes and their lactone derivatives in the normal physiological functions of plants.

Ophiobolins

A second group of terpenes recognized as phytotoxins are the ophiobolins, a group of sesterterpenoids (fig. 5). Ophiobolin A is the original and most widely studied member of this group, and more than twenty biogenic analogs are now known. Ophiobolins have been implicated in two of the most significant plant disease epidemics of recent times – the Bengal rice famine of 1943 and the southern corn leaf blight epidemic in the USA in 1972[29]. In both diseases, the pathogen was a species of *Drechslera* or *Bipolaris* (with telemorph in the genus *Cochliobolus*) known to produce a contingent of ophiobolins. *B. maydis*, the etiological agent of southern corn leaf blight, has been shown to produce ophiobolin-A, 6-epiophiobolin A, 25-hydroxyophiobolin I, 3-anhy-

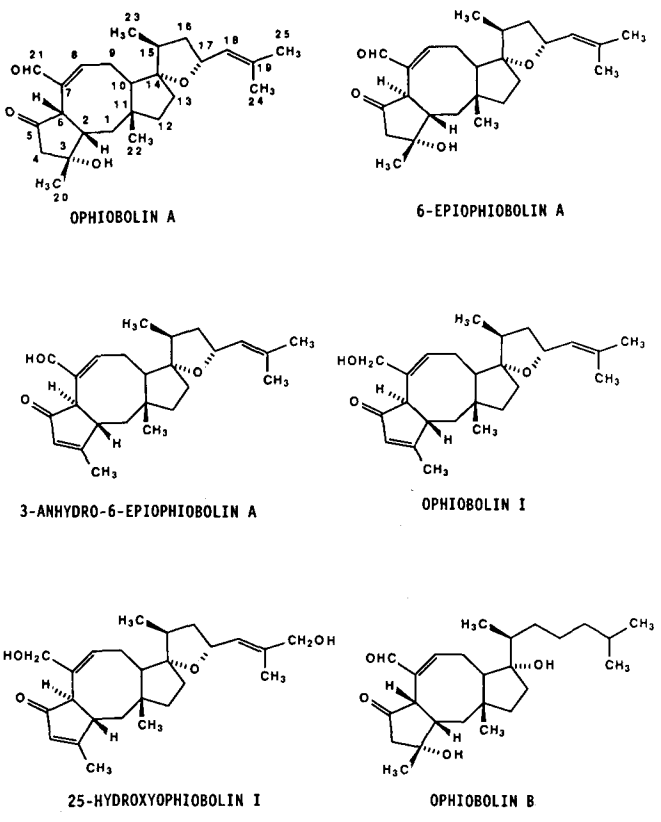


Figure 5. Some ophiobolins produced by various species of *Cochliobolus*.

dro-6-epiophiobolin A, ophiobolin I, and the previously known ophiobolin C [34].

6-Epiophiobolin-A, which differs from ophiobolin A only in the orientation of the proton on C6, can selectively inhibit CO_2 fixation in corn bearing Tms (Texas male sterile) cytoplasm at concentrations three orders of magnitude below that required for the same effect in corn bearing normal cytoplasm [34]. Mitochondria from corn bearing Tms cytoplasm are also selectively inhibited by 6-epiophiobolin-A [5]. Ophiobolin A is not discriminating in its toxicity towards these two germplasms of corn. Thus, in corn, 6-epiophiobolin-A is cultivar selective, showing that the most subtle of chemical changes can dramatically alter the bioactivity of a molecule.

This notion of host-specificity has to be tempered, however, because of the following example. *D. heveae* is a pathogen of the rubber tree (*Hevea brasiliensis*), a relative of such noxious weedy euphorbs as leafy spurge (*Euphorbia esula*) and wild poinsettia (*Euphorbia heterophylla*). In an attempt to find euphorb-specific toxins, we analyzed metabolites of this fungus and found that it, too, produces a number of ophiobolins, including ophiobolin-A and 6-epiophiobolin-A. Although 6-epiophiobolin-A was the most toxic, all of the ophiobolins examined were phytotoxic to a variety of grasses and dicots [29]. *Drechslera sorghicola*, a pathogen of Johnson grass, produces all of the ophiobolins found in *C. heterostrophus* except ophiobolin-C and 6-epianhydro-ophiobolin-A [34]. *C. miyabeanus* (anamorph: *B. oryzae*), causative agent of brown spot on rice, also produces the ophiobolins found in *C. heterostrophus*, and, in addition, several novel ophiobolins including ophiobolin J which showed modest host selectivity on rice varieties [32]. The genetic compatibilities of all three species of *Cochliobolus* should make these fungi amenable to mating experiments and subsequent assessment of ophiobolin production correlated with pathogenicity.

Curvulins

Curvulin is a cyclic polyketide produced by numerous fungi. Its chemistry had been worked out in the late 1960s, but nothing was known of its biological activity. Curvulin was isolated from *D. indica*, a pathogen of common purslane (*Portulaca oleraceae*) and spiny amaranth (*Amaranthus spinosus*), and the structure confirmed by X-ray crystallography (fig. 6) [16]. A related compound, *O*-methylcurvulinic acid, was also obtained [16]. At nanomolar concentrations, curvulin was somewhat selective towards purslane and spiny amaranth. As the concentration increased, curvulin became more broadly toxic. *O*-methylcurvulinic acid, the methylated free acid of curvulin, was generally toxic although a few plants were insensitive. A dozen analogs of curvulin are known from various fungi offering excellent prospects for assessing structure-activity relationships of these ketides. Also, curvulin is readily synthesized, making it amenable to structure/activity manipulations.

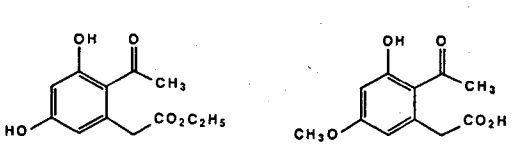


Figure 6. Curvulin (left) and *O*-methyl curvulinic acid (right).

De-O-methyldiaporthin

D. siccans is a pathogen of perennial ryegrass (*Lolium perenne*) and oats (*Avena sativa*); the telemorph is *Pyrenophora lolii* [19]. Toxic extracts from this fungus contained de-*O*-methyldiaporthin (fig. 7), a novel isocoumarin [14]. The toxin is selective and effective when applied in nanomolar amounts to abaxial surfaces of leaves. De-*O*-methyldiaporthin is notable because relatively few coumarins have been characterized in fungi. Indeed, much of the literature on coumarins stresses their role as phytoalexins, as anti-fungal compounds from higher plants [20, 38] and as allelopathic compounds [12].

Resorcyclides

Resorcyclides were first discovered in an unidentified species of *Penicillium* [21]. Two isomers, *cis* and *trans*, were identified, which differed in the stereochemistry of the alpha, beta unsaturation adjacent to the ketone group. The *cis* isomer is relatively inactive; however, *trans*-resorcylide (fig. 8) is cytotoxic and antimicrobial, and inhibits growth of roots of rice seedlings at concentrations approaching 1 ppm. These two isomers and the saturated analog have been identified recently in extracts of *D. phlei* [15]. The *trans* isomer caused necrosis on corn and crabgrass (*Digitaria sanguinalis*) at 0.06 µg per leaf. At 2 µg/leaf, timothy (*Phleum pratense*), wild poinsettia, and sunflower (*Helianthus annuus*) were also very sensitive. The *cis*-isomer was inactive at 1 µg per leaf. The saturated resorcylide fell midway between the two and retained activity at 0.5 µg per leaf. Again, modest chemical alterations affect the bioactivity of these compounds and

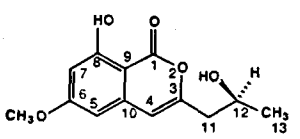


Figure 7. De-*O*-methyldiaporthin.

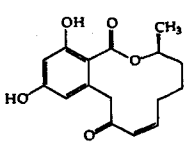
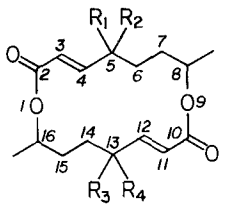


Figure 8. *Trans*-resorcylide.



1: R_1 = H, R_2 = OH, R_3,R_4 = 0 [(−)dihydropyrenophorin];
2: R_1 = H, R_2 = OH, R_3 = H, R_4 = OH [pyrenophol].

Figure 9. Pyrenophorins of *P. avenae*.

demonstrate the potential for developing important herbicides from these toxins.

Dihydropyrenophorin

(−)Dihydropyrenophorin (fig. 9) is a novel macrolide phytotoxin produced by *Pyrenophora avenae* (anamorph: *Drechslera avenae*) causal agent of leaf blotch of oats[30]. Pyrenophol (fig. 9), totally inactive in leaf assay tests, is also produced by this fungus. While none of the plants tested responded to (−)dihydropyrenophorin at concentrations much below 10^{-4} M, when applied to Johnson grass (*Sorghum halepense*), reddish flecks were produced at the point of application at 6.5×10^{-8} M[30]. These observations stress the importance of screening phytotoxins isolated from crop pathogens, such as *D. avenae*, on a wide range of plants, including common weed species. It may be the case that although the pathogen does not attack a given weed species, the plant may be sensitive to its toxin.

Triticones

Triticones are novel toxins containing a rare spirocyclic gamma-lactam moiety[34] (fig. 10). These compounds have been found in Pyrenophora tritici-repentis which causes tan spot on wheat (*Triticum aestivum*) and in *Curvularia clavata*, a pathogen of turfgrasses. Other related species of fungi, causing diseases of monocotyledenous weeds, are also likely to be found as producers of the triticones. Currently, 8 triticones are known. Only triticones A and B, which have an exocyclic double bond adjacent to a ketone, are toxic. Triticone A causes necroses on numerous plants, kills protoplasts of wheat, and inhibits esterase activity and CO_2 fixation in wheat[18]. Weed species which are sensitive include common lambs-quarters (*Chenopodium album*), redroot pigweed (*Amaranthus retroflexus*), leafy spurge, and dandelion (*Taraxacum officinale*). When tested on isolated chloroplasts of wheat, oats, or spinach, triticone A inhibits photosynthetic electron transport (PS-ETS)[18]. However, with purified ferredoxin-oxidoreductase, the terminal enzyme in PS-ETS, it is stimulatory. Another seeming paradox is that triticone A quenches protease activity in the fungus that produces it. Neither beta-glucosidase nor esterase activity in this fungus are affected. In the test tube, triticone A reacts stoichiometrically with cysteine in less than 1 min. This affinity for sulfhydryl groups is thought to be responsible for some of its biological activity – a premise reinforced by the ability of triticone A to inhibit ficin, a known sulfhydryl protease. Thus, when assaying tissue or organelles, triticone A appears to be non-selectively toxic. At the molecular level, however, it is selective and has great potential as an analytical tool for studying active sites on enzymes and site-specific sensitivity in multi-component systems such as PS-ETS.

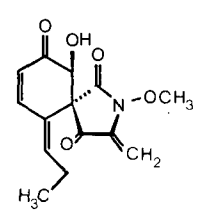


Figure 10. Triticone A.

Amino acid derivatives

Tryptophol

Tryptophol (fig. 11) is a major metabolite in culture filtrates of *D. nodulosum*, a pathogen of goose grass (*Eleusine indica*)[36]. At a concentration of 0.6 mM, tryptophol is selectively toxic to young leaves of goose grass. As the concentration is increased to 6 mM, selectivity is lost and tryptophol becomes toxic to many grasses and dicots.

Diketopiperazines

Exserohilum holmi is a pathogen on crowsfoot grass (*Dactyloctenium aegyptium*), an annual weedy grass of the Old World tropics. This fungus makes two novel diketopiperazines. A p-bromobenzoate derivative was made of one, exserohilone, which was crystallized, and its structure determined by X-ray crystallography[33] (fig. 12).

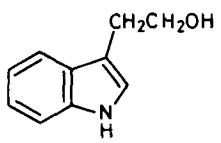


Figure 11. Tryptophol.

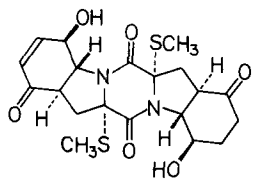


Figure 12. Exserohilone.

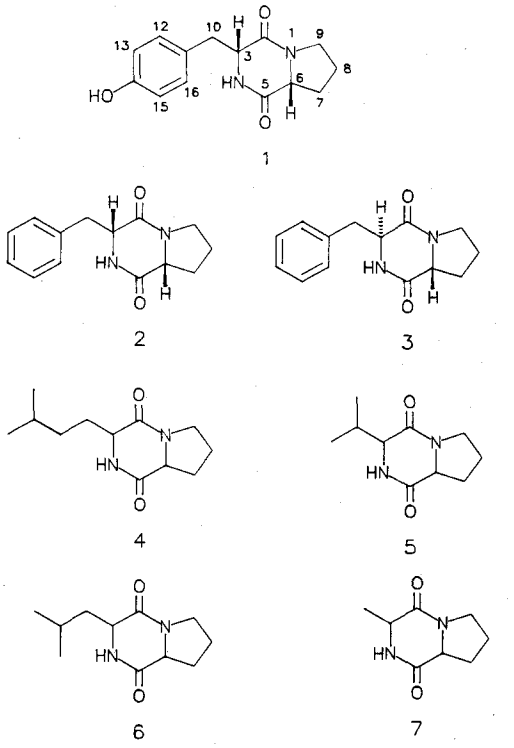


Figure 13. Maculosin (**1**) and related diketopiperazines produced by *Alternaria alternata*.

The structure of dihydroexserohilone, also produced by *E. holmi*, was determined by spectroscopic analyses[33]. Both compounds showed non-selective toxicity towards several plant species at 10^{-4} and 10^{-5} M. These phytotoxins produce necrotic lesions surrounded by reddish brown borders.

Maculosin is a fascinating cyclic dipeptide belonging to the chemical class of diketopiperazines all containing L-proline (**1** in fig. 13)[25]. A number of these compounds were discovered in an isolate of *Alternaria alternata* cultured from diseased spotted knapweed (*Centaurea maculosa*)[25]. Numerous, non-toxic, diketopiperazines were identified, but one, cyclo(L-Pro-L-Tyr) (maculosin) caused necrotic lesions on spotted knapweed at 10 µM. Even at 1 mM, maculosin-1 did not affect 19 other grasses and dicots that were tested or other *Centaurea species*. This makes maculosin the first phytotoxin produced by a pathogen of weeds that shows such a high degree of host specificity – a pleasant surprise, because the genetic diversity of weeds has been considered to preclude the occurrence of host-specificity[10]. Maculosin has been synthesized from t-BOC-L-proline and the methyl ester of L-tyrosine. The synthetic product retains the host-specificity of the natural compound[25].

Of the diketopiperazines isolated from *A. alternata* and tested it is apparent that certain functional groups are necessary for activity (fig. 13). Maculosin, the most active substance, possesses a phenolic moiety, not uncommon in phytotoxins (**1** in fig. 13). The minor activity of cyclo(L-Pro-L-Phe) (**2** in fig. 13), compared to the inactivity of its diastereomer, suggests the importance of structure conformation in bioactivity. Studies on diketopiperazines containing proline clearly showed that L, L compounds assume an extended conformation that would render both the aromatic moiety and its hydroxyl group accessible for interaction with a biological site[25]. The other diketopiperazines (**3**–**7** in fig. 13) were biologically inactive.

In biological studies on maculosin, knapweed plants were grown from seed collected from a variety of geographic locations in order to ascertain the limits of activity of maculosin[25]. Some diversity was observed in the response of the plants, depending on the locale from which the knapweed was obtained. Knapweed reproduces, in typical composite fashion, by wind-borne seeds carried from the parent plant. Seeds collected from plants within a 20-foot radius of the original diseased specimen were those most affected by maculosin[25]. Indeed, plants grown from seeds of this plant actually exhibited necrotic lesions at 10^{-6} M in leaf bioassay tests. Seeds of knapweed were also collected from various locations in Gallatin County and the Bitterroot Valley, Montana. One plant, from a source 100 miles from the original diseased plant, was actually somewhat resistant to maculosin and necrosis was only induced at 10^{-3} M. These results were not surprising, since a wide genetic diversity probably exists between and among populations of this plant, and such diversity could be expected to be expressed in the potential of the plant to be reactive to maculosin[25].

Understanding the mode of action of a host-specific toxin might provide insight into the development of plant pathogen-host plant dynamics. Also, the nature, location, and function of the toxin receptor-site might be discovered. This could eventually lead to a molecular genetics approach to weed control. A critical step in doing such studies is to have available isotopically-labeled toxin possessing a high specific radioactivity. ^{14}C-Maculosin meeting these requirements was prepared using radiolabeled tryosine and proline as precursors in the synthesis scheme[25]. After the application of ^{14}C-maculosin to an unwounded leaf surface there was little or no movement of radioactivity from the inoculation site in 3–4 days. Even in a previously wounded leaf, there was only slight movement of labeled material from the point of application. However, radiolabel was taken up by knapweed roots suspended in a solution of ^{14}C-maculosin. These initial studies provided useful leads for the practical application of maculosin as a knapweed control agent: derivatization of the compound may be necessary for entry and distribution of maculosin in the plant.

Some successful attempts have been made to discover the site or sites in the plant with which maculosin interacts. The binding of ^{14}C-maculosin was noted in the cytosolic (soluble) fraction of a spotted knapweed leaf extract, and little if any in the insoluble fraction. Binding activity was reduced or destroyed by treatment of the soluble fraction

with heat or proteases, suggesting that the toxin receptor is a protein. Purification of the receptor has been pursued successfully using size-exclusion column chromatography combined with affinity chromatography. The affinity column support consists of epoxy activated Sepharose 6B to which synthetic maculosin has been attached. Further work should reveal the role of the receptor in the process of toxin-induced symptom production (Sang Ho Park and G. Strobel, unpublished observations).

Discussion

Results of studies on pathogens of weed species have shown them to be rich in novel structures and intriguing bioactivity. Even previously known chemicals such as curvulin have a tremendous impact on the biology of the interacting organisms. The future of this field is exciting, especially in associations where one eukaryote – a fungus – produces toxins which regulate or kill another eukaryote – a higher plant. The chemistry of the attack must be finely tuned or the pathogen may become autotoxic. These are examples of pathogenic associations which offer tremendous potential to the searcher for natural products for use as selective herbicides.

Numerous inferences can be drawn from the results in this report. First, it appears that toxins are generally found as groups of related analogs, which usually encompass a range of biological activities. Sometimes, these 'phytotoxins' may be biosynthetic intermediates which play some role in microbial physiology. Their activity as phytotoxins could have arisen by a chance which proves to be fortunate for the natural-products researcher, or they could have been selected because of some environmental advantage they offer the producing organism. In systematic analysis of extracts of pathogens, especially those involved with dramatic or unusual symptoms, will usually yield a number of interesting, potentially useful compounds. A good example is the work cited herein on *A. alternata* from knapweed (*Centaurea* spp.)[25]. Isolates of *A. alternata* usually produce an abundance of known phytotoxins such as tenuazonic acid and perylene quinones[26]. Because a systematic search was made for all necrogenic activity present in the culture fluid of this fungus, a host-specific toxin was discovered. In contrast to the better known, more complex toxins produced by this fungus, maculosin can be synthesized readily, adding a commercially favorable dimension to its potential utility. The same is true of curvulin and its analogs.

A second general conclusion is that, even when studying toxins from pathogens of crops, substances which are candidates for use as herbicides can be discovered. The cultivar-specific 6-epiophiobolin-A has a broad range of activity when one looks outside the microcosm of the *C. heterostrophus* corn interaction. This observation can be tied to a general strategy for development of crops resistant to specific herbicides, such as the current attempts to make important crops resistant to glyphosate (*N*-phosphonomethyl-glycine). Unlike resistance to the synthetic herbicides, resistance to naturally-occurring toxins is already present in many germplasms. This resistance may be exploited by traditional breeding approaches or bioengineering (wide hybridization or transposon mutagenesis), thus circumventing the problems inherent in effecting expression of prokaryotic genes in higher plants.

A further extension of this strategy is suggested by our observations that toxin production by pathogens is often regulated by chemicals produced in the host. Biosynthesis of toxins usually declines the longer a pathogen is cultured on artificial media. Addition of tissue or extracts from the host causes the pathogen to revert to a highly toxicogenic state. In a cropping system such as corn, where Johnson grass is a problem, incorporation of resistance to ophiobolins in corn could serve two purposes: 1) to maintain high yields in the presence of a dangerous pathogen; and, 2) to allow the crop to serve as an inoculum source for toxicogenic pathogens which would help control the weed. The genetic compatibility of *D. sorghicola* and *C. heterostrophus* should allow the development of cross-infective pathogens which produce these potentially useful chemicals, thus avoiding problems of synthesizing such organically complex phytotoxins. Admittedly, these ideas are speculative, but the strategies suggested could well be feasible, given the technical expertise available today. The major point is that they represent a rational biological approach to agricultural problems based on fundamental knowledge of the pertinent molecular biology. Such approaches can be both profitable and environmentally sound.

In conclusion, recent studies have uncovered both novel and previously known phytotoxins that should elicit widespread interest as potential herbicides. Curvulin and Maculosin can be readily synthesized, and offer possibilities for direct application both as non-selective and as extremely selective herbicides. More complex organic compounds like the eremophilanes, resorcylides, triticones, and ophiobolins are useful both as tools for understanding biochemical and physiological processes in plants and as selective, biologically rational agrochemicals. Many other microbes that attack weeds are candidates for study.

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