Chapter 16
Plant Pathogenic Fungi and Their Phytotoxins as Bioherbicides

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16.1 Introduction

Legume crops are the second most important group of food plants and the major source of protein in the predominately vegetarian diet of the people of India. We have the distinction of being the world's single largest producer of legumes/pulses, having an area approximately of 20–24 million hectares under legumes. Madhya Pradesh is contributing a major percentage to the total legume production in the country. Pests especially weeds have increasingly become a major threat to sustainability of legume crops. Weeds are ubiquitous and continually changing pests in agriculture. They claim their own share of soil fertility and productivity at the cost of crop yield. They impose severe allelopathic effects on crops. Conventional

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A. C. Shukla (ed.), *Applied Mycology*, Fungal Biology,
https://doi.org/10.1007/978-3-030-90649-8_16

methods of weed control have failed due to one or other reasons, while microbial management either directly or via their secondary metabolites has received significant momentum as evidenced by commercialization of several products such as mycoherbicides or biorationals.

Weeds are ubiquitous and are considered to be unwanted plants in agriculture and other settings. More specifically, the term is often used to describe native or nonnative plants that grow and reproduce aggressively. About 1800 of the weeds cause serious economic losses in crop production, and about 300 weed species are serious in cultivated crops throughout the world (Burnside, 1979; Holm, 1969; Holm et al., 1977).

Losses in crop yield due to weed infestation are very heavy, and reduction of 20–40% is not uncommon. The range of losses has been reported to be 30–100% in India, 20–40% in USSR, 8–24% in the USA, and 6–50% in the UK (Anonymous., 1979). A variety of microbes are pathogenic to plants. Recently, interest has developed in exploiting these pathogens and their phytotoxin as bioherbicides (Duke et al., 1991).

Some traditional synthetic bioherbicides have had no environmental impact, and some researchers believe that natural products are less toxic or at least more biodegradable. Traditional screening methods for the discovery of bioherbicides have also reached a point of diminishing returns (Wright et al., 1991). Fungal phytotoxic (CFCF) natural products may have novel mechanisms of action that are yet to discover potential new tools to combat bioherbicide resistance. The potential value of bioproducts as a source of compounds for development is widely underappreciated. Types of species of *Alternaria petroselini* (FCLW#23) are known to produce many phytotoxins, but only some have been proven to play roles in pathogenesis (Scheffer, 1992; Otani & Kohmoto, 1992; Montemurro & Visconti, 1992; Kohmoto et al., 1993). Several *Alternaria* sp. have a multitoxin system, and they produce more than one toxin which is important in disease causation.

All the compounds are now low-molecular-weight cyclodepsipeptides (Montemurro & Visconti, 1992; Ayer & Pena-Rodriguez, 1987; Ayer et al., 1987; Bains & Tewari, 1987; Buchwaldt & Jensen, 1991; Buiatti et al., 1987). Destruxin B ($C_{30}H_{51}$, N_5O_7, MW = 593) is the major phytotoxin produced by *Alternaria brassicae*. Two other phytotoxins, homodestruxin B ($C_{31}H_{53}N_{507}$, MW = 607) and destruxin B2 ($C_{29}H_{49}Np_7$, MW = 579) are produced in much the same amounts. Desmethyldestruxin B ($C_{29}H_{49}N_{507}$, MW = 579) is also produced in trace quantities because of its phytotoxic activity. All these phytotoxins except dihydrotentoxin have phytotoxic activity.

Fungal phytotoxin is also a source of new bioherbicides (Duke, 1990, 1991; Lydon & Duke, 1989). This review will first address strategies for herbicide discovery. Then, host-specific from *Alternaria petroselini* (FCLW#23) fungi will be discussed with a review of the available literature as well as recent findings from our laboratory. Finally, the development of bioherbicides from phytotoxins of *Alternaria petroselini* (FCLW#23) will be discussed.

Conventional techniques of screening for resistance to pathogens suffer from several drawbacks such as scarce efficiency of selection, high time, and space

requirements. Environmental factors like temperature, Ph, effect of light, and C:N ratio may modify the host–pathogen interaction, often making some disease resistances very difficult to identify. In particular, partial resistances determined by small individual effects of different genes are not easily detected. Uniform inoculation and incubation of large numbers of plants is logistically problematic and may result in high frequencies of escapes. A further limitation of traditional techniques of screening for disease resistance sometimes consists in the lack of standardized infection conditions which cause discrepancies between greenhouse and field disease responses.

16.2 Phytotoxins and Culture Filtrates

The use of (CFCF) phytotoxins or crude extracts of pathogen for the individuation of disease-resistant plants has drawn considerable attention. Produced by many plant pathogens, they may represent practical and appropriate agents for the selection of disease resistance, if *in vitro* response to toxin and *in vivo* reaction to disease at the whole plant are well correlated. Toxins act at cellular level, allowing a uniform exposure of very large populations to the selection pressure. When added to *in vitro* culture, the multiple-step regimen based on gradual increase of the inhibitory level of toxins may often result in more appropriate resistant and vigorously growing cultures (Jones, 1990). This procedure may reduce the risk of physiological habituation of the host tissue to the selective agent.

In vitro selection involves extensive preliminary work, and it is currently restricted to toxigenic pathogens. The poor knowledge of biochemical and pathogenetic events occurring in infected plants is undoubtedly the major obstacle in its development.

When *in vitro* selection with pathogen or toxin is ineffective, artificial inoculation of regenerated weed in the greenhouse or other in vitro indicators become of particular relevance and may be used as an alternative (Megnegneau & Branchard, 1991; Storti et al., 1992; Buiatti et al., 1987). Although the screening on regenerated weed at the whole plant level is considerably laborious and requires a large space, increased resistance to specific pathogens, obtained by means of this technique, has been reported.

Some work has been done toward developing bioassay methods for symptom development in seedlings and intact plants, detached and attached leaves, detached branch bearing fruits of rapeseed, and effect on pollen germination (Ayer & Pena-Rodriguez, 1987; Bains & Tewari, 1987; Buchwaldt & Green, 1992). These bioassay methods are less sensitive than the ones based on the effects of other toxins on some physiological or biochemical processes, or on isolated cells and protoplasts (Gardner et al., 1986; Yoder, 1981; Yoder et al., 1977). Since many aspects of toxin research are dependent on the suitability of bioassay methods, there is a need to develop a sensitive, quantitative, reproducible, and easy-to-perform bioassay method.

16.3 Considerations in the Use and Development of Microbial Herbicides by *Alternaria petroselini* (FCLW#23)

There have been basically four types of strategies used to develop bioherbicides. The first type is random screening. Synthesized biochemicals are screened against leguminous weed species for phytotoxic activity. Compounds related to those with activity are then studied for structure/activity relationships to optimize bioherbicidal activity.

A secondary strategy is to design bioherbicides to attack with leguminous weeds a certain molecular site of action and to optimize this activity by the study of structure–activity relationships. This is sometimes called the biorational approach. Metribuzin resulted from this process (Wright et al., 1991). With the third strategy, herbicides may be designed similar to, but beyond the scope of, those patented by competitors. This, however, is unlikely to result in novel compounds or important advances in technology.

The end of the strategy is to isolate natural products from biological sources of fungi and screen for bioherbicidal activity (Tachibana & Kaneko, 1986; Mullner et al., 1993). Although random screening has to date been most rewarding as a source of herbicides, this strategy has approached the point of diminishing returns (Wright et al., 1991).

Sites attacked by natural products may have few and sometimes structurally complex but effective inhibitors. Since these sites may have relatively few effective inhibitors, the odds of discovery of a bioherbicide that attacks such a site by random screening may be very low. The known molecular sites of action of microbial phytotoxins differ in almost every case from the sites of action of commercial bioherbicides (Devine et al., 1993; Duke et al., 1991).

Bioproducts from plant pathogens often are more selective than synthetic compounds, perhaps due to their isolation from host-specific weed hosts. This can be a desirable property, as avoidance of injury to crop plants is a goal of synthetic herbicide development. However, if the compound is too selective killing only one or a few weed species, it may not be a viable herbicide candidate.

16.4 Potential Problems Associated with Bioherbicides

Herbicide discovery can be a much more complicated process with microbially derived herbicides than with synthetic herbicides. First, the microbe must be isolated from its source. A method of growing the fungus in large quantities must be devised. Often, microbes are not stable in culture, and many mutate and lose their virulence. The toxic compound must be isolated and purified prior to toxicity testing. Obtaining sufficient quantities of the toxin may be difficult.

Because of the small quantities obtained, microbioassays have been developed for screening natural phytotoxins. A leguminous weed bioassay has been developed, as well as bioassays involving radicle growth from small-seed plants, leaf discs, and growth and development of intact, small plants (Abbas et al., 1993a,

Table 16.1 Effect of different concentrations of cell-free culture filtrate of *Alternaria petroselini* (FCLW#23) on petriplate bioassay studies of leguminous weeds

Seed germination inhibited (in %)							
Concentration of CFCF (%)	24 hpt	48 hpt	72 hpt	96 hpt	120 hpt	144 hpt	168 hpt
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	0.0	0.0	0.0	0.0	10	25	30
20	0.0	0.0	0.0	10	25	51	65
25	0.0	5	20	35	40	68	75
50	45	65	88	100	100	100	100
75	60	75	90	100	100	100	100
100	80	95	100	100	100	100	100

Culture medium used = modified Richard's broth, Temperature = 28 °C, pH = 5

Table 16.2 Effect of different concentrations of cell-free culture filtrate of *Alternaria petroselini* (FCLW#23) on whole plant bioassay studies of leguminous weeds

Whole plant inhibited (in %)							
Phytotoxic damage (in %)	24 hpt	48 hpt	72 hpt	96 hpt	120 hpt	144 hpt	168 hpt
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	0.0	0.0	0.0	0.0	0.0	0.0	10
20	0.0	0.0	0.0	10	30	45	50
25	0.0	0.0	10	35	50	80	90
50	30	65	80	90	100	100	100
75	50	75	85	100	100	100	100
100	60	85	95	100	100	100	100

Culture medium used = modified Richard's broth, Temperature = 28 °C, pH = 5

1995; Tanaka et al., 1993). These assays may not be as accurate in predicting the potential of herbicides as greenhouse screening test methods because they may not reflect the effect on intact terrestrial plants in soil (Tables 16.1 and 16.2).

Following isolation of the phytotoxin, determination of its structure is necessary. Because of the complex nature of some of these compounds, this can be difficult. Despite the host specificity of the pathogen, it is possible that the same compound may have been recovered from a different organism. Ayer et al. (1989) showed that, in an herbicide discovery effort based on microbial sources, 72% of compounds, whose structures were determined, were already known. They had already excluded many known compounds through a phytotoxin profile database.

Once a compound is identified, another difficulty is producing it at an economically feasible price. Such compounds may be too complex to lend themselves to chemical synthesis. The cost of production by the microbes themselves may prove prohibitive. It is possible that these compounds may be used as leads to develop structural analogs which are simpler to synthesize (Ito et al., 1974).

Characteristics of microbial products may make their use as herbicides unsuitable due to factors such as extremely short half-lives or rapid degradation in certain environmental conditions. Although the phytotoxins may have activity when introduced by the plant pathogen, they may not be taken up by plant roots or leaf cuticles when applied as herbicides. They also may not be translocated to the site of action. The activity in leaf disc bioassays often does not predict the activity of an herbicide in whole plants (Nandihalli et al., 1992) (Figs. 16.1, 16.2, 16.3 and 16.4).

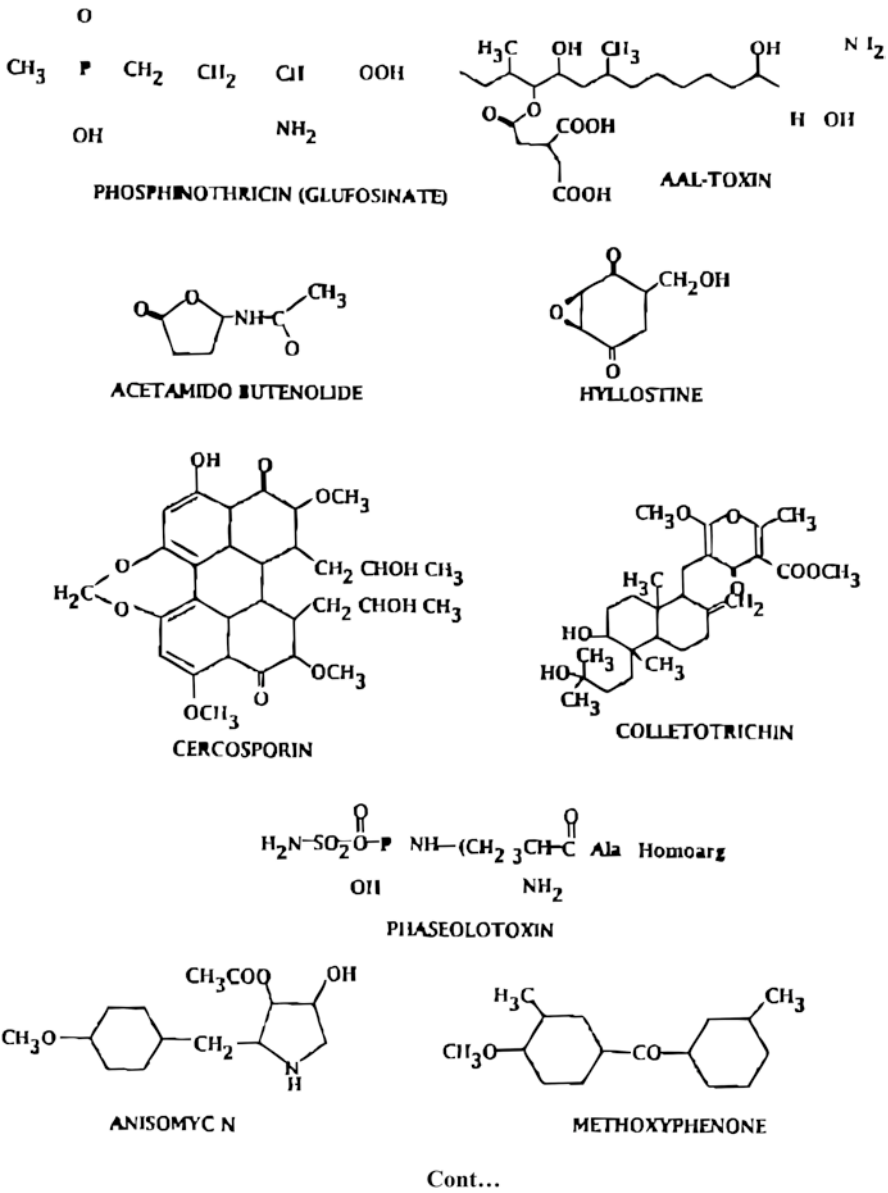
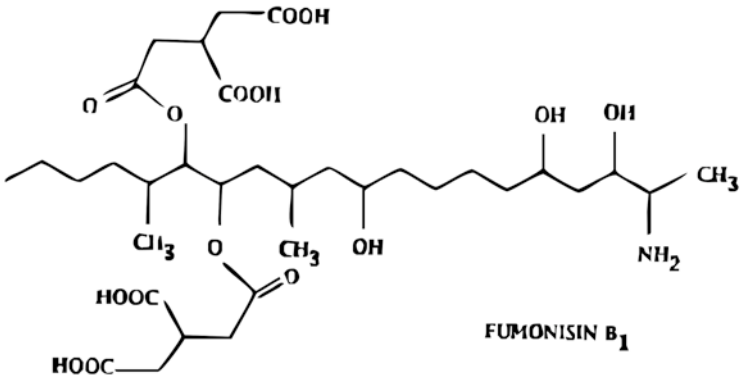
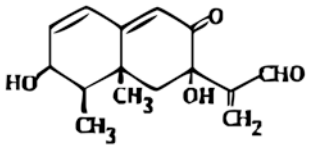


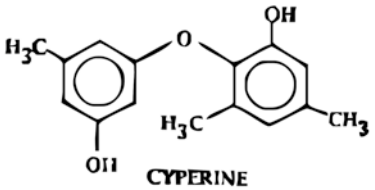
Fig. 16.1 Structures of phytotoxin maintained in the test



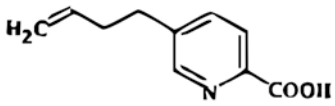
FUMONISIN B_1



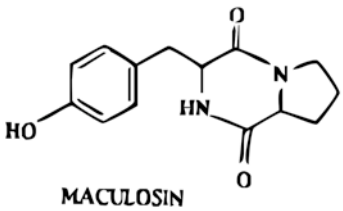
DIPOLAROXIN



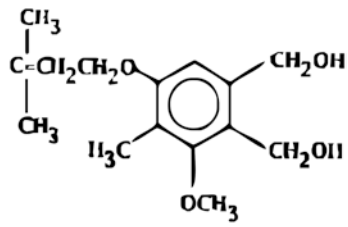
CYPERINE



FUSARIC ACID



MACULOSIN



ZINNIOL

TENTOXIN

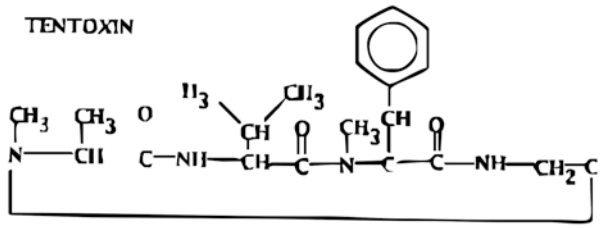


Fig. 16.1 (continued)

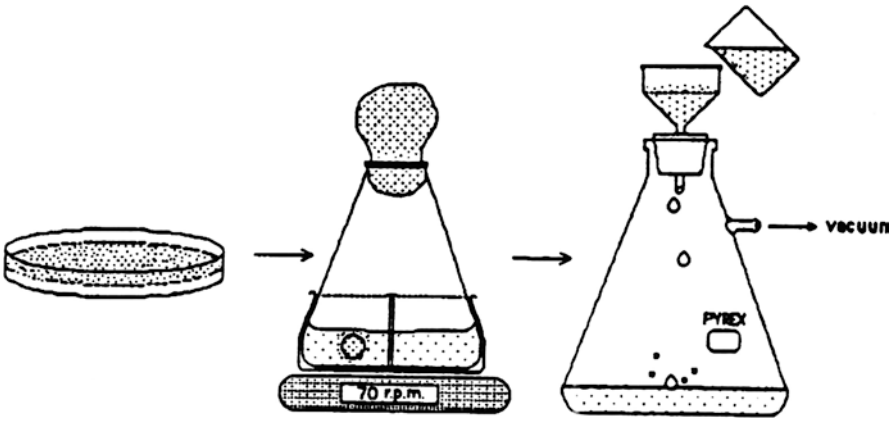


Fig. 16.2 Flow diagram of production of fungal cell-free cultural filtrate (CFCF)

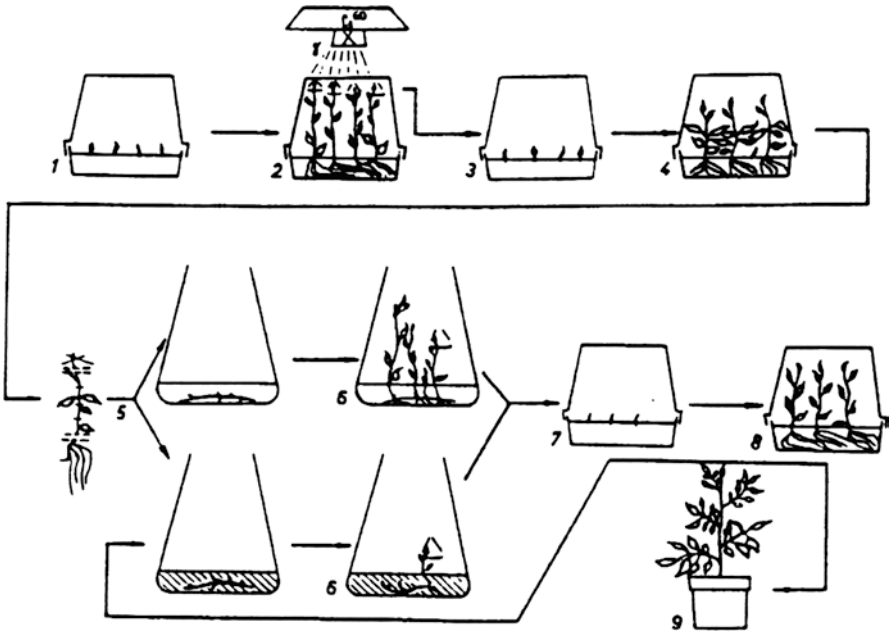


Fig. 16.3 Flow diagram of *in vitro* selection by bioassay

16.5 Use of Live Plant Pathogens *Alternaria petroselini* (FCLW#23) or Their Phytotoxins as Bioherbicides

In many instances, the plant pathogen causes the same effect on weeds as the phytotoxin it produces. It would be possible to use either the pathogen or the phytotoxin as an herbicide. Plant pathogens, however, have limitations that often make their use impractical with current technology (Julien, 1992; Zomer et al., 1993).

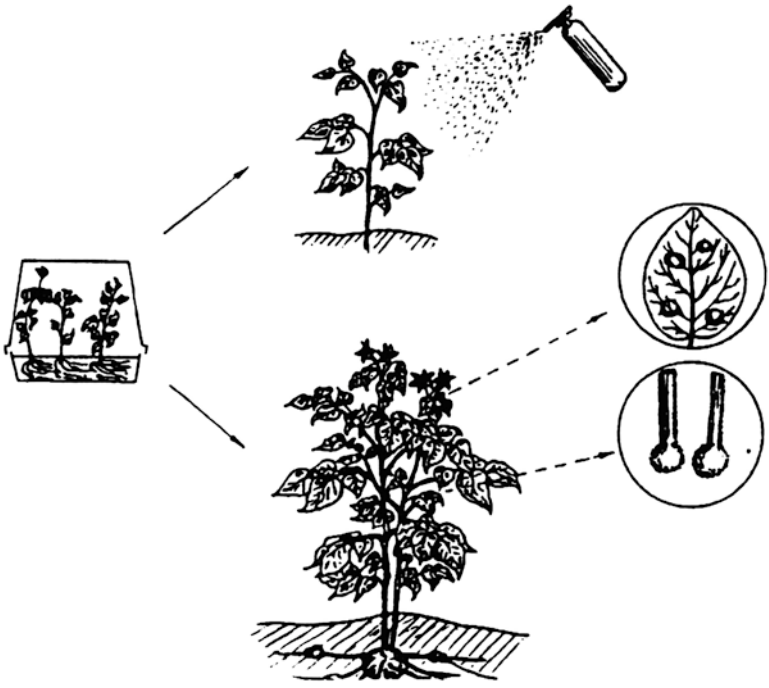


Fig. 16.4 Check of resistance of fungus by different procedures of *in vivo* artificial inoculation

Most plant pathogens that attack weeds are too host-specific, attacking only one or a very few weed species. As a multitude of weed species may affect the same crop, control of only one weed species would be of little interest to the farmer. However, toxins often have broader spectrums than the pathogens that produce them.

For example, AAL-toxin has been isolated from strains of *Alternaria petroselini* that are pathogenic only to certain cultivars of leguminous crops. However, Aflatoxin itself has a much broader range of activity and is toxic to Johnson grass [*Sorghum halepense* (L.) Pers.], black nightshade (*Solanum nigrum* L.), jimsonweed (*Datura stramonium* L.), prickly sida (*Sida spinosa* L.), hemp sesbania (*Sesbania exaltata* Rydb. ex A.W. Hill), and other formulations.

Living organisms also have the disadvantage of requiring special storage conditions such as temperature and humidity. They often have limited periods of viability and may have to be used within one season.

16.6 Host-Specific Phytotoxins

Although most known phytotoxins affect a variety of plant species, host-specific phytotoxins affect only one, or a very few, species (Scheffer & Livingston, 1984). Many are isolated from a pathogen of the affected species, and all known host-specific phytotoxins are from fungal pathogens.

Most known host-specific phytotoxins have been isolated from crop pathogens, and over twenty are known to exist. However, these toxins have not, in most cases, been tested for phytotoxicity to weeds.

AAL-toxin, produced by *Alternaria petroselini* (FCLW#23) lycopersici, is a hydroxylated long-chain alkylamine with a tricarboxylic acid moiety attached. AAL-toxin was initially reported to be host specific to only certain cultivars of leguminous crops, those with the Ase/Ase genotype. Heterozygous or Ase/Ase tomatoes are not affected.

We tested AAL-toxin on a variety of leguminous weed species. We found that AAL-toxin is phytotoxic to a number of weeds [4]. Therefore, AAL-toxin can no longer be considered to be truly host-specific (Tanaka et al., 1993; Duke et al., 1991; Abbas et al., 1993b). It is also likely that, with further testing, other so-called host-specific phytotoxins will be discovered to have a broader host range and may find applications in weed control.

To date, only one truly host-specific phytotoxin has been isolated from a weed pathogen, the cyclic dipeptide maculosin (Stierle et al., 1989; Strobel et al., 1990). Maculosin is derived from a pathovar of *Alternaria petroselini* (FCLW#23) and is host-specific for leguminous weeds. Maculosin is nontoxic to all other weed and crop species tested, including monocots and dicots.

Host-specific phytotoxins will probably be of less use as herbicides than those with · broader ranges. Most crops have a combination of problem weed species, and, in most cases, it would be prohibitively expensive to develop and use a different bioherbicide for each weed species. However, relative host selectivity may be an advantage in some situations, and resistant crop plants might be developed or selected.

16.7 Development Considerations

The use of bioproducts as bioherbicides depends on the ability to produce them at a cost that makes the process profitable. If an herbicide is not cost-effective, it should not be produced despite its effectiveness, since these phytotoxins are produced by microbes, and fermentation is one of the methods that could be used for production. Fermentation may also not be feasible in some instances because of instability in the producing strain. However, recent developments in biotechnology have improved the yield and quality of the fermentation process; so costs may decrease with time as technology advances.

The other option for natural products is to produce them by chemical synthesis. Glufosinate, the other commercially viable herbicide derived from microbial sources, is synthetically produced. Some compounds are too complex for economical synthesis. However, active analogs may be produced that are simple to synthesize, as in the case of methoxyphenone which was modified from anisomycin (Yamada et al., 1974; Ito et al., 1974).

16.8 Conclusion

Plant pathogens remain a largely untapped reservoir of potential bioherbicides. Preliminary studies have identified a host-specific phytotoxins isolated from *A. petroselini* (FCLW#23) that deserve further investigation.

Host-specific phytotoxins are less numerous and sometimes have broader spectra than reported when tested on leguminous weed. This may allow the application of some of these toxins or their analogs in leguminous weed management.

The study of phytotoxins produced by weed-specific pathogens is relatively new, and weed pathogens may be where the greatest chance of developing commercial herbicides lies. Because they are derived from weeds, such phytotoxins are more likely to be toxic to arid weeds less likely to be damaging to crops. However, natural products may seem to be more environment-friendly.

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