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Review

Microbially produced phytotoxins and plant disease management

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Pathogenic fungi and bacteria often damage their host (plants) tissues by producing toxic metabolites, which induced various symptoms such as necrosis, chlorosis, wilting, water soaking and eventually the death of plants. These toxic metabolites also known is one of the weapons used by pathogen inducing disease condition in susceptible host plants. Many pathogens are known to produce toxins both *in vitro* and *in vivo* and these toxins have been implicated in the symptom development on the host tissues. Many of these phytotoxic metabolites have also been extracted from diseased plant tissues. Based on the reactions of host crops to the toxic metabolites of respective hosts, methods of rapid screening of germplasm for resistance to plant diseases have been developed. Their application has successfully resulted in resistant lines in some tropical crops like cowpea, cassava, maize, yam, and soybean. Nowadays, these evaluation techniques are becoming an important complement to classical breeding methods. The knowledge of the inactivation of microbial toxins has led to the use of microbial enzymes to inactivate phytotoxins thereby reducing incidence and severity of disease induced by microbial toxins. Considering the increasing awareness of herbicide resistance, and the restriction of the use of chemical pesticides in agriculture against plant pathogens, **novel compounds from microorganisms also provided new environmentally friendly bio-herbicides for the control of parasitic weeds that are normally difficult to control.**

Key words: Microbial toxins, phytotoxins, toxic metabolites, disease managements, pathogenicity , toxigenicity, host specific toxins, non host specific toxins.

INTRODUCTION

Diseases constitute a major set back in crop production world-wide and especially in the tropics. Diseases affect plants right from the planting stage to harvesting and storage of the produce. Sinclair (1982) reported that diseases accounted for world-estimated losses of 7 million tones. Anthracnose diseases of cassava and yam have resulted in an annual loss of 30-70% in both crops (Ikotun and Hahn, 1991; Nwankiti, 1982). The effect of plant diseases is not only on the market value of the produce, but also on the availability of planting materials. Hahn et al. (1989) reported that the most significant effect of cassava anthracnose disease is the reduction of healthy planting materials available to farmers.

Several methods have been employed to manage plant diseases in crops, (Hahn et al., 1988, 1989). But the most effective approach would be the selection and breeding for diseases resistance varieties (Nwankiti et al., 1987). Most of the existing techniques for selecting resistant varieties include evaluation for disease incidence and severity in the field and green houses. However, these screening procedures are very cumbersome, time consuming, labor intensive and require a large amount of land space (Amusa, 1991).

Typical symptoms of most plant diseases revealed the involvement of phytotoxic metabolites, which therefore suggest a role for toxic metabolite secreted by the pathogen in the disease development. Metabolites of many fungi may have adverse or stimulatory effects on plants (Heisey et al., 1985; Rice, 1995) such as suppression of seed germination, malformation, and retardation of seedling growth (Lynch and Clark, 1984). Neergaard (1979) reported that some fungal pathogens often produce phytotoxins that affect seed germination and seedling growth. Betina (1984) reported that some fungi on the surface of seeds often produce mycotoxins that affect food quality. Tey-Rulh et al. (1991) reported

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that the foliar symptoms of Eutypa infected grapevines are not the direct result of the fungus, but rather that of a toxin, eutypine, produced by the fungus and transported through vascular tissue to the affected shoot.

Pathogenic fungi and bacteria often damage their host plants by producing toxins, which cause various symptoms including necrosis, chlorosis, wilting, water soaking and eventually the death of plants (Scheffer, 1983). One criterion of the importance of a toxin in a disease syndrome caused by a pathogen is that toxigenicity is often related to pathogenicity or virulence (Scheffer, 1983).

Several phytotoxic metabolites have been found associated with bacteria and fungal pathogens, which, causes symptoms similar to those caused by the pathogen. Such toxic metabolites include pinolidoxin from *Ascochyta pinodes* (Antonio et al., 1993), deoxyradicin and maculosin from *Alter-naria helianthi* and *Alter-naria alternata* (Robeson and Strobel, 1986, Stierle et al., 1988). Identified metabolites from other pathogens includes piricularin from *Piricularia oryzea*, victorin from *Cochliobolus vitoriae* (Samddar and Scheffer, 1968), phaseolotoxin from *Pseudomonas syringae* pv *phaseolicola*, toxin from *Periconia circinata,* sacchari-toxin from *Helmithosporium sacchari* (Byther and Steiner, 1971, 1972; Strobel and Hapner, 1978), cercosporin from *Cercospora* spp. (Daub, 1982). Phytotoxic metabolites of most of these pathogens have been reported to play a significant role in pathogenesis (Chandraskhanran and Ramakrishnan, 1973; Walker and Templeton, 1978; Amusa ,1991; Amusa et al., 1993).

Phytotoxic metabolites have been employed in screening crops for disease resistance (Wheeler and Luke, 1955; Hartman et al., 1982; Amusa, et al., 1994, Amusa, 1998, Amusa, 2000). Considering the importance of phytotoxic metabolites in crop protection management's practices, this paper reviews the use of phytotoxic metabolites of pathogens in plant disease management.

MICROBIAL TOXIN PRODUCTION AND CLASSIFICATION

Microbial toxins are metabolites produced by plant pathogens (fungi, bacteria), which play a role in host-pathogen interactions and in disease expression. They are low molecular weight substances produced by some pathogens which are capable of reproducing symptoms similar to that found in natural infections in plants (Bilgram and Dube, 1976) According to Scheffer (1983) phytotoxins are a product of microbial pathogens, which should cause an obvious damage to plant tissue and must be known with some confidence to be involved in disease development. For toxic metabolites of pathogens to be regarded as phytotoxin, when applied at a concentration, which could be reasonably expected in or around the diseased plant, the metabolites should produce in a susceptible host all the symptoms characteristic of the disease. Also the pathogen and the toxic metabolite must exhibit similar host specificity. Furthermore, the pathogen and its toxin must be able to induce similar disease symptoms and finally a single toxin is must be involved.

Toxins differ from enzyme in that they do not attack the structural intergrity of the tissue but they affect the metabolism in a subtle manner (Buddenhanen and Kilman, 1964). Phytotoxins act directly on protoplast of the cell, while other metabolites of pathogens such as high molecular weight polysaccharides secreted by wilt-inducing bacteria which obstruct the flow of fluid in the xylem vessels and may result in death of plant are not toxins.

Several characteristics have been used for the classification of toxins that affect plants. Such features include their chemistry. Based on this, some phytotoxins are regarded as low molecular weight peptides, others have terpenoid structures and still others contain carbohydrates (Amusa, 1991). However, few other structures are known for toxins that play an unquestionable role in plant disease (Scheffer and Briggs, 1981). Another form of classification is based on the producing organism (fungi, bacteria). This is, however, of no predictive value since more than one type of phytotoxins can be produced by one organism. Phytotoxin classification has also been based on biological activities such as enzyme inhibitors, anti-metabolites, membrane-affecting compounds (Scheffer and Briggs, 1981) However, the widely accepted classification is that based on toxic selectivity to plant genotypes (host selective or non-host selective) (Rudolph, 1976; Scheffer, 1976) and on the general role in disease development (Wheeler and Luke, 1963; Scheffer and Pringle, 1967).

Several reports are available on the production of phytotoxic metabolites by species of *Colletotrichum* (Goodman, 1960; Grove et al., 1966; Ballio et al., 1969; Kimura et al., 1973; Gohbara et al., 1978; Goddard et al., 1979). Such toxic metabolites include colletotin from *Colletotrichum fuscum* Laub (Goodman, 1960; Lewis and Goodman, 1962), colletotrichin and colletopyrone from *C. nicotianae* (Gohbara et al., 1976, 1978). Production of phytotoxin by the turmeric (a monocot plant) leaf spot pathogen, *Colletotrichum capsici*, and its involvement in disease induction was reported by Nair and Rama-krishnan (1973). Identified metabolites from other pathogens include piricularin from *Piricularia oryzea*, victorin from *Cochliobolus vitoriae* (Samddar and Scheffer, 1968), phaseolotoxin from *Pseudomonas syringae* pv *phaseolicola*, toxin from *Periconia circinata,* sacchari-toxin from *Helmithosporium sacchari* (Byther and Steiner, 1971; 1972, Strobel and Hapner, 1978) and cercosporin from *Cercospora* spp. (Daub, 1982). *In vitro* production of several fungal phytotoxins by *Mycosphae-*

rella fijiensis and *Mycosphaerella musicola* has also been reported (Svabov and .Lebed 2005). *Fusarium* species produce a variety of potent phytotoxins such as fumonisins, moniliformin, fusaric acid, 2,5-anhydro-D-glucitol (AhG) and trichothecenes (Abbas et al., 1991; Abbas and Boyette, 1992; Jin et al., 1996; Tanaka et al., 1996)

MICROBIAL TOXIN AND PLANT DISEASE DEVELOPMENT

The ability of a pathogen to infect and invade a compatible host may be facilitated by the production of toxins that induce cell death in the proximity of the invading organism (Baker et al., 1997; Dangl and Jones, 2001). These toxins was also reported to play important roles in inhibiting the physiological processes in cells surrounding the point of infection, enabling the spread of the disease (Feys and Parker, 2000; Staskawicz et al., 2001). Gaumann (1950) has earlier suggested that some pathogens would be unsuccessful if the toxin did not kill the cells in advance of the fungus and permit it to establish itself continually on dead or dying cells and produce more toxins. While Baker et al. (1997) reported that the virulence of an organism is sometimes enhanced by its ability to produce phytotoxins that kill cells in the tissue surrounding the point of infection.

In some plant diseases, especially with yam anthracnose, toxins often produced a more rapid and extensive invasion by the pathogen than would be in the case in the absence of toxins (Amusa, 1991). Amusa et al. (1993) reported the extraction of phytotoxic metabolites from *Colletotrichum gleoesporioides* infected yam leaves. The extracted phytotoxic substance induced necrotic lesion similar to the symptoms induced by the pathogens on healthy yam leaves.

Phytotoxins often act as the initiation factor for successful pathogenesis. Spores of some fungal pathogen have been associated with phytotoxin production, which probably kill cells of susceptible host paving way for the penetration of the germ tube. All known host specific toxins can be detected from the spore germinating fluids of each virulent pathogen but not from those of the avirulent ones (Nishimura and Kohomoto, 1983; Nutsugah et al., 1994; Otani et al., 1998; Quayyum et al., 2003). Thus, specificity found to be characteristically associated with host specific toxin suggests the early participation of toxin at the site of initial contact of inoculated and host surface.

Several phytotoxins are now known, beyond reasonable doubt, to be the determinant factor in pathogenesis and some can even act as reliable surrogates for pathogen that produce them. Amusa (1994) reported that the partially purified metabolites of *Colletotrichum* spp. induced necrotic lesion of varying sizes on leaves and stems of susceptible hosts. While the phytotoxic metabolites of *Colletotrichum graminicola*, *C. truncatum* and *C. lindemutianum* inhibited seed germination in respective host crops (Amusa, 1994).

Results of seedling bioassay revealed that sorghum, millet, maize, cowpea and soybean seedlings treated with 100 µg/ml of the toxic metabolites of the respective pathogens showed symptoms of blight and cessation of growth and the potency of the metabolites also decreased with increased dilution. Secalonic acid A (SAA) isolated from *Pyrenochaeta terrestris* and *Penicillium oxalicum* inhibited the onion seedling elongation by 4, 32, 40, 68, and 94% at concentrations of 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M, respectively (Zeng et al., 2001). Zeng et al. (2001) reported that Secalonic acid F significantly inhibited the seedling growth of radish, rape, cucumber, corn, hairy beggarticks, barnyardgrass and sorghum crops at 0.038 mM, while the roots of rape seedling stopped growing after germination at 0.075 mM of SAF, and the roots of all tested crop seedlings could not grow at 0.3 mM of SAF. The germination rates of rape, radish, and cucumber were inhibited by 54.2, 91.7, and 8.5%, respectively when exposed to Secalonic acid F culture. While the root lengths of rape, radish, and cucumber were 18.6, 27.5, and 25% of the control, respectively. Zeng (2001) reported that when the fungus infects crop seeds and the storage conditions are humid with high temperatures, those crops often fail to germinate or even grow badly because of the involvement of phytotoxic metabolites of the fungus

One of the first physiologically detectable event induced by phytotoxin is an increased loss of electrolytes from susceptible leaves (Kohomoto et al., 1987) Nishimira and Kohomoto (1983) reported that *Alternaria kikuchiana* toxin released during spore germination on leaves caused an almost instantaneous increase in electrolyte loss from susceptible but not resistant tissue. Different phytotoxin are known to have different modes of action. Kimura et al. (1973) reported that a phytotoxin produced by *Colletotrichum lagenarium* was found to function as an anti-auxin. Sensitivity of sugarcane clones relating to electrolyte leakage caused by *Helminthosporium sacchari* toxin has been reported (Scheffer and Livingston, 1980).

Most of the phytotoxic metabolites acts by modifying the metabolism of the host plants, while some are toxic to the plant tissues once accumulated and poison the plant tissues. A phytotoxin secreted by *Psuedomonas syringae* pv. *Tabaci*, the pathogen inducing wild fire disease of tobacco, drastically modifies the amino acid metabolism of the plant with the eventual accumulation of ammonia in tobacco leaves, which causes extensive blighting. Interestingly, the pathogens that synthesize the phytotoxin remain unaffected by the toxin (Balasubramanian, 2003).

MICROBIAL TOXIN AND PLANT DISEASE MANAGEMENT

Several researches on phytotoxin and their role in pathogenesis have lead to a remarkable scientific break through in some areas such as disease and weed cont-

Table 1. Response of 21 cassava clones to natural infection by *Colletotrichum gloeosporoides* f sp *manihotis* and to the toxic metabolites of the pathogen *in vitro* .

Cassava clone	Field screening				*In vitro* screening			
	Disease score		Canker number		Lesion sizes on leaves (mm)		Lesion sizes on stem cuttings (mm)	
95/0084	3.60a	S	20.00	S	20.00	S	18.17a	S
95/0076	3.40ab	S	18.94ab	S	19.11ab	S	16.33b	S
95/0087	3.40ab	S	18.67ab	S	16.00cd	S	15.00c	S
95/0045	3.40ab	S	16.67c	S	16.00cd	S	15.33bc	S
95/0018	3.20ab	S	16.50bc	S	16.00cd	S	16.33b	S
95/0078	3.20ab	S	15.67cd	S	17.33bc	S	16.83ab	S
Isu nikan kiyan	3.00ab	S	17.00bc	S	15.67cd	S	15.33bc	S
95/0079	3.00ab	S	16.93c	S	18.00b	S	15.33cd	S
95/0088	3.00ab	S	12.00d	MS	15.67cd	S	15.00c	S
95/0065	2.80b	MS	17.03bc	S	14.67d	MS	12.50d	MS
95/0069	2.80b	MS	16.27c	S	15.50cd	S	15.33bc	S
95/0073	2.40bc	MS	7.00e	MS	10.50e	MS	8.33e	MS
95/0010	2.40b	MS	6.47e	MS	10.67e	MS	11.17d	R
95/0025	1.60cd	R	4.00f	R	7.50f	R	7.67e	R
95/0027	1.60cd	R	2.00g	R	7.00f	R	7.00e	R
TMS30211	1.60cd	R	2.00g	R	8.00f	R	7.00e	R
95/0014	1.40d	R	2.00g	R	7.00f	R	7.00e	R
95/0030	1.40d	R	2.00g	R	3.00d	R	4.00e	R
95/0022	1.20d	R	0.99g	R	8.00f	R	8.00e	R
95/0056	1.00d	R	0.00h	R	6.67f	R	5.88f	R
95/0057	1.00d	R	0.00h	R	7.00f	R	5.19g	R

Source: Amusa (1998).
Each value is a mean of 5 replicates (% measurement/plant), each is a transformation from $\log_e$ value used for the analysis. Each value within the same column is a mean of three replicates (30-plants/clone). Mean value followed by the same letters are not significant (P=0.05) by Duncan's multiple range test. S = Susceptible, MS = Moderately susceptible, R = Resistance, HR = Highly resistance. TDa = Tropical *Dioscorea alata*; TDr = Tropical *Dioscorea rotundata*; Tde = Tropical *Dioscorea esculenta*; TDc = Tropical *Dioscorea cayenesis.*

rol. In cases where toxins act as the sole determinants of the disease in plant, knowledge of such phytotoxins may be used to control such diseases. The discoveries of the role of phytotoxins in plant disease development are for practical and conceptual reasons. The practical significance of the pathologically important toxins is that it can act as reliable surrogate for pathogen that produces them (Yoder, 1980). This greatly simplifies biochemical analysis by permitting elimination of the pathogen from the system. It also enhances screening for resistance among population of cells (Gengebach et al., 1977). Toxins can be used for screening of resistance, selection for resistance in tissue culture for which resistant plants may be regenerated and genetically engineered plants to destroy the toxic compound e.g. genotypes of oats that were resistant to the victorin toxin produced by *Helminthosporium victoria* were also resistant to fungus (Wheeler and Luke, 1955) Partially purified toxins of *H. oryza*e, the pathogen of brown spot of rice, have been used to select resistant calli. With the use of the toxic metabolites of *H. oryzae*, it has been possible to regenerate resistant plants that were heritable and stable (Vidhyasekaran et al., 1986). Also, with the use of these metabolites, rapid screening of a large population of the crops within a short period of time (24 h) is possible (Hell and Weber, 1986).

Amusa (1996a) evaluated 21 cassava clones for resistance to cassava anthracnose disease using both natural infection and phytotoxic metabolites of *Colletotrichum gloeosprioides* f sp *manihorts* in the laboratory. Results indicated the 8 cassava clones were resistant while the other 13 clones were susceptible at varying degrees (Table 1). Results from in vitro screening using phytotoxic metabolites compared favorably with

Table 2. Response of 24 yam clones to in vitro inoculation with phytotoxic metabolites of *Colletotrichum gloeosporioides*.

Yam clone	*In vitro* screening Necrotic lesion size on leaves (mm)		Field screening Disease score	
TDa 87/01116	22.618a	S	3.61a	S
TDc 289	22.492a	S	3.61a	S
TDa 86/001115	22.492a	S	3.60a	S
TDa 86/00258	22.450a	S	3.57a	S
TDa 85/01093	22.112b	S	3.43a	S
TDa85/00272	20.112b	S	3.32ab	S
TDa85/00601	20.069b	S	3.19abc	S
TDr85/00272	19.906b	MR	2.54cd	MR
TDr87/00203	18.871c	MR	2.51cd	MR
TDa291	18.810	MR	2.39de	MR
TDr 86/00057	18.563c	MR	2.47de	MR
TDa87/00293	18.437c	MR	2.16de	MR
TDa297	17.228d	MR	2.26de	MR
TDa86/00057	17.011d	MR	2.08ef	MR
TDr87/00340	16.790d	MR	2.12e	MR
TDr 293	15.770e	MR	2.10e	MR
TDa294	15.347e	MR	2.01ef	MR
TDe	15.085e	MR	2.00ef	MR
TDa 87/01117	12.585f	MR	1.60fg	R
TDr87/00211	12.585f	MR	1.58fg	R
TDa5	12.355f	MR	1.58fg	R
TDc750	9.858gh	R	1.54g	R
TDr179	8.755h	R	1.41g	R
TDr89/01750	8.587h	R	1.30g	R

Source: Amusa (2001).

Each value is a mean of 5 replicates (% measurement/plant), each is a transformation from $\log_e$ value used for the analysis. Each value within the same column is a mean of three replicates (30-plants/clone). Mean value followed by the same letters are not significant (P=0.05) by Duncan's multiple range test. S = Susceptible, MS = Moderately susceptible, R = Resistance, HR = Highly resistance. TDa = Tropical *Dioscorea alata*; TDr = Tropical *Dioscorea rotundata*; Tde = Tropical *Dioscorea esculenta*; TDc = Tropical *Dioscorea cayenesis*.

field screening based on natural infection (Table 2). Out of the seven maize germplasm screened for maize anthracnose using toxic metabolites of *C. graminicola,* five were found susceptible, one of the cultivars was moderately susceptible and the other resistant. The result of the *in vitro* screening corresponds to the result obtained from the screen house and field experimentation using *C. graminicola* (Amusa, 1996b).

Fourteen soybean cultivars were also evaluated for reaction to soybean anthracnose diseases using toxic metabolites from *C. truncatum*. Two of the cultivars PI-17144 and TGM236 were considered very resistant to the toxic metabolites of the pathogen, while the remaining 12 cultivars were susceptible (Amusa et al., 1994). The soybean cultivars PI-17144 and TGM236 have been reported to be resistant to anthracnose (IITA, 1987). While the reactions of the other cultivars to the toxic metabolites of *C. truncatum* corresponded to the green house and field screening experiments (IITA, 1987).

As early as 1955, the use of phytotoxic metabolite of *Helminthosporum victoriae* in screening 100 bushels (approximately 45 million seeds) for resistance to *H. victoriae* infection was reported (Wheeler and Luke, 1955). This was accomplished by drenching 12-bushel batches of germinated seeds in *H. victoriae* toxin-containing solutions. Incubation was done for two days and all germinated seedlings were planted. There were about 50 survivors/bushel. A total of 933 seedlings survived such screening and were found to be resistant to the disease. Schertz and Tai (1969) also used *Periconia circinata* toxin to identify homozygous susceptible and heterozygous intermediate plants in progenies of sorghum crosses.

Phytotoxic metabolites do not normally affect the producing pathogen and the genes conferring insensitivi-

ty to the pathogen have also been cloned into susceptible plants, which now conferred resistance to the diseases caused by the pathogens. *Psuedomonas syringae* pv. *tabaci* induced a serious disease called wild fire on tobacco. This pathogen secretes a phytotoxin which drastically modifies the amino acid metabolism of the plant with the eventual accumulation of ammonia in tobacco leaves, leading to extensive blighting. However, the pathogen that synthesizes the phytotoxin remains unaffected by the toxin. This formed the basis for a search of the candidate gene from the pathogen itself. Toxin resistance genes can be isolated from the pathogen itself, as well as from other microbes. A toxin-inactivating gene, which was named '*ttr*', was successfully isolated from the pathogen and the same was cloned into tobacco cultivars, which showed excellent wildfire resistance (Balasubramanian, 2003).

The potential of using phytotoxins of plant pathogens as agents for selection for disease resistance or tolerance in tissue culture systems and problems involved in such attempts have been highlited by Daub (1986). In sugarcane, toxin produced by the eye spot pathogen *(Helminthosporium sacchari)* has been used to develop genotypes resistant to the disease (Steiner and Byther, 1971). Although there are several reports available on the use of pathogen toxins in tissue culture to evolve disease resistant genotypes of host plants, a few reports are available on similar attempts with species of *Colletotrichum*. Narain and Das (1970) have used the phytotoxin produced by *Colletotrichum capsici* to study disease resistance in *Chillies* in tissue culture. Similarly, in coffee studies have been carried out on the generation somaclones resistant to the anthracnose pathogen, *Colletotrichum kahawae*, in tissue culture using the pathogen toxin (Nyange et al., 1995). Responses of embryogenic mango cultures to a partially purified phytotoxin produced by *Colletotrichum gloeosporioides* have been reported to be useful criteria in the selection of disease resistant genotypes (Jaisankar et al., 1999).

The knowledge of the fact that microbial toxins like any other toxins can be inactivated or detoxified has been exploited in plant disease (involving toxic metabolites) management based on the fact that reduction in the toxicity of metabolite produced by plant pathogens will confer resistance or tolerance on the host plant. Microorganisms form an exotic source of enzymes, which are capable of inactivating synthetic chemicals that are potentially phytotoxic (Malathi et al., 2002). The detoxification of pathogen toxin combined with biocontrol efficacy has been well established with *Pantoea dispers*a, which offered an excellent biocontrol against sugarcane leaf scald disease caused by *Xanthomonas alblinean*s (Viswanathan et al., 2001). In this host-pathogen interaction, the antagonistic bacterium detoxified albicidin toxin produced by the pathogen. Several bacterial strains capable of degrading oxalic acid associated with pathogenesis were able to protect *Arabi-dopsis thaliana* from infection caused by *Sclerotinia sclerotiorum* .(Dickman et al., 1993) The red-rot pathogen *Colletotrichum falcatum* Went is known to produce a phytotoxic metabolite identified as an anthroquinone compound, which is host-specific and produces part of the disease symptoms. Recently, strains of *Pseudomonas* spp. and *Trichoderma harzianum* effective in detoxifying the anthroquinone of the pathogen have been identified (Malathi et al., 2002).

MICROBIAL TOXIN AND WEED MANAGEMENT

Weed, beside its role in competing with crops for nutrients and sunlight rays, also harbor pathogenic pathogens thereby serving as reservoirs of pathogens. Hence weed management is important as far as agricultural business is required to continue and the need for use of new herbicides is inevitable. Currently, about two-third, by volume, of the chemicals used in agricultural production are herbicides (Duke and Lydon, 1993). The present emphasis on reduced- or no-tillage agriculture will depend heavily on herbicides for weed control. On the other hand, the increasing incidence of herbicide resistance is creating a demand for new herbicides with unexploited mechanism of action. The potential for undesirable environmental contamination from herbicides is relatively high, and there is a need for environmentally safe herbicides that are equally or more effective and selective than currently available synthetic herbicides. Thus, the need for new herbicides becomes obvious to solve the dilemma of the continued demand for herbicides while older herbicides are being removed from the production fields for environmental or toxicological purposes

Considering the increasing awareness of herbicide resistance, and the restriction of the use of chemical pesticides in agriculture against plant pathogens, novel compounds from microorganisms may provide new chemistries for weeds that may otherwise be difficult to control, e.g. parasitic weeds. Boyetchko (1999) submitted that microbially-derived compounds may be pursued either as templates for new synthetic chemical herbicides or as pathogens applied directly to the target weed. The use of microbially-derived compounds in biological control of weeds may represent a promising alternative to the use of chemicals.

Amusa and Ikotun (1995) reported that the toxic metabolites of *C. gramnincola* and *C. gloeosporioides* induced necrotic lesion on 14 different weeds species found associated with yam plantation in the humid forest of western Nigeria. Stems of broad leave weeds sprayed with the toxic metabolites became flaccid, wilted and blighted within 24 h of treatment. Shoots of monocotyledonous weeds which are *Cypenus esculentus, Eleusine indica, Penisetum polystachion, Paspalum orbiculare, Andropogon tectarum* and *Androp-*

Table 3. Effect of phytotoxic metabolites of two *Colletotrichum* species on some commonly encountered weeds in cassava, cowpea and yam plantation in south western Nigeria.

Weeds	Colletotrichum gloeosporioides	Colletotrichum graminicola
Sida acuta	+++	+++
Tridax procumben	+++	++
Synedrella nodiflora	++	+++
Spigelia anthelmia	+++	+++
Chromoleana odorata	++++	++++
Phyllanthus amarus	++++	++++
Cyperus esculentus	+++	+++
Eleusine indica	++++	++++
Pennistum polystachion	++++	++++
Paspalum orbisculare	++++	++++
Andropogon tectorum	+++	++++
Andropogon gayanus	++++	++++
Euphorbia heterophylla	+++	++++
Boerhevia difusa	++	++

Source: Amusa and Ikotun (1995).
++: necrotic spots on the leaves
+++: Leaf blight
++++: Severe leave blight

ogon gayanus wilted with their leaves drooping downward 12 h after treatment which later became blighted 24 h after treatment (Table 3). The above also agreed with the report of Wheeler and Templeton (1978) for toxic metabolites of *Colletotrichum gloeospoioroides* f sp *aeschynomenes* on *Aeschynomene virginia*.

Recently, three new phytotoxins, ascaulitoxin, aglycone and trans-4-aminoproline, having promising herbicidal activity have been isolated and characterized from the culture filtrate of *Ascochyta caulina*, a potential mycoherbicide for *Chenopodium album* biocontrol (Mikhail et al., 2000). A phytotoxin isolated from the black leaf blight fungus (*Alternaria alternata*), identified as maculosin, was found to be the active ingredient of the pathogen inducing spots in knapweed. Maculosin appears to be highly toxic only to spotted knapweed and is being researched for potential field efficacy. Studies by Charudattan and Rao (1982) had shown that two phytotoxic compounds (bostrycin and deoxy-bostrycin) produced by *Alternaria eichhorniae* are phytotoxic to water hyacinth and could be used for the control of the weed. Toxins produced by *Fusarium* spp. have also been reported to be phytotoxic to several plants (Duke, 1986), and their bioherbicidal effects have been tested against various weeds and crops (Hoagland, 1990; Abbas and Boyette, 1992; Savard et al., 1997). Further investigations showed that fumonisin B_1, which is produced by *F. nygamai* isolated from *S. hermonthica*, has a herbicidal effect on *Striga* spp., especially when applied as post-emergence (Kroschel and Elzein, 2003). Fortunately, fusaric acid, 10-11-dehydrofusaric acid and their methyl esters, which do not present health risks, were also produced by *F. oxysporum* isolated from *Striga* and *Orobanche* (Savard et al., 1997; Thomas, 1998; Amalfitano et al., 2002) making these isolates very interesting candidates for the biological control of parasitic weeds. The toxic effect of these metabolites on *Striga* seeds was previously reported by Zonno et al. (1996).

The future development of biocontrol of weeds requires an increase in our understanding of the potentials of the controlling agents such as phytotoxins and their chemistry and interaction with host tissues. While the future of biocontrol appears greater in currently underdeveloped countries, social pressures to reduce use of synthetic chemicals because of their unintended side effects will increase pressure to seek alternative approaches to chemicals in developed countries.

CONCLUSION

Disease conditions in plant usually result from the interaction between the host plant, the pathogen and the environment, also known as the disease triangle. These factors often limit plant disease development as well as the use of microbes directly in biological control of plant diseases. Factors such as low inoculum levels, weakly virulent of biocontrol agents, poor spore dispersal mechanisms, unfavorable moisture and/or temperature conditions, susceptibility of the host and widely dispersed host populations often limit disease control by biological

agents. The phytotoxic metabolite approach is an attempt to bypass many of these restraints on disease management using biocontrol agents, since same quantity of the metabolites will be applied to the host tissue directly. The application will be applied at most susceptible stages of plant growth. Mass production of microbial toxins via fermentation or otherwise will probably be much easier than spore production. The commercial world is now interested in extracting phytotoxins from microorganisms to use as herbicides, rather than using living organisms with their inherent problems of sensitivity to the environment. Bialophos is in fact, an example of this approach. It is a metabolite of the soil microbe *Streptomyces viridochromogenes* and is produced by fermentation. It is marketed as Herbiace in Japan (Auld and Mcrae, 1997)

As a consequence, the development of an effective phytotoxin for use in plant disease control will require a comprehensive understanding of the pathogen(s) involved including its virulence and the biology of the target host plant/ weed(s).

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