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Phytotoxins as tools in breeding and selection of disease-resistant plants

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Abstract. Conventional plant breeding for resistance to pathogens, although successful, is in many cases still too slow to keep pace with pathogen adaptation, and suffers from the lack of genetic variability in cultivated varieties. Phytotoxins, because of their role in disease development, have been proposed as convenient markers for early screening of resistant genotypes and as selective agents for in vitro selection. The present review summarizes, firstly, the evidence for a genetic correlation between tolerance to toxins and resistance to pathogens, with particular reference to host-selective toxins (HST) and factors affecting early screening. There follows a discussion of results obtained from the use of phytotoxins for in vitro selection of resistant plants. The conclusion is drawn that this practice, while potentially useful in the case of HST, leads to contradictory results when ill-defined toxins or culture filtrates are used. Finally, prospects for future research are adumbrated.
Key words. Resistance of plants to diseases; plant pathogenic fungi; in vitro culture and selection of resistant plants; phytotoxins.

Introduction

Selecting plant genotypes resistant to pathogens has become one of the major tasks of breeders both because of increasing crop losses, and as a result of the general move towards reducing the use of chemicals in agriculture. However, traditional plant breeding for resistance to diseases suffers from several drawbacks, such as the lack of genetic variability, the high costs, the time and space required for screening plant populations under selection,

and a lack of reliable 'out of the field' screening methods. The key to the solution of such problems may be the use of selection methods at the cellular level, screening techniques based on reliable biochemical markers of resistance, and genetic engineering for single genes affecting host-parasite interactions.

The use of purified pathogen toxins or culture filtrates as probes for resistance has been attempted during the last 10–15 years, based on considerable evidence suggesting a correlation between toxin tolerance and resistance to pathogens. Until now, toxins and culture filtrates have been mainly used as tools for early screening of segregating populations within 'classical' breeding programs, or for direct in vitro selection of tolerant cells and subsequent regeneration of putative resistant plants. The aim of the present paper is to summarize the results obtained so far, and to draw some tentative conclusions on the real value of these methods for plant breeding.

The genetic correlation between toxin tolerance and resistance to pathogens and early screening methods

Plant pathogens produce a great variety of phytotoxic compounds. Those involved in disease are classically subdivided into host selective (HST) and non-host-selective toxins.

In the first case only susceptible crop cultivars are damaged, and toxins often play a primary role as determinants of the disease. Susceptibility, therefore, is often the result of the interaction of two genes, one in the pathogen, responsible for toxin production, one in the plant, possibly coding for a receptor.

Targets, if not the receptors themselves, have been identified in some cases. Plasma membranes are affected by most HSTs, with the notable exceptions of AL-toxin and AT-toxin from pathotypes of *Alternaria alternata*, affecting respectively tomato and tobacco[31,40,48].

Other preferential direct or indirect targets may be chloroplasts, as in the case of AM-toxin, whose effect is inhibited by light[41], and ACR-toxin, both from *A. alternata* pathotypes. Well-documented examples of toxic effects on mitochondria are the action of HMT toxin produced by *Cochliobolus heterostrophus*, and of PM toxin produced by *Phyllosticta maydis*[50]. In the case of *C. heterostrophus*, the causal agent of southern leaf blight of maize, race O, which does not produce toxins, is pathogenic but causes only mild symptoms.

Race T, a variant of race O, produces a toxin, HMT-toxin, which selectively affects lines of maize carrying *Tms*, a cytoplasmic gene for male sterility. Plants with normal, N, cytoplasm are not affected by the toxin. Similarly, maternal inheritance of resistance has been shown to occur in the maize/*Phyllosticta maydis* system[42]. More frequent, however, are the cases where single, nuclear genes are responsible for both tolerance to HST and resistance to the pathogens. In the case of the interaction of *Alternaria alternata* f. sp. *lycopersici* and tomato, for example, pathogen resistance and toxin tolerance are due to a single dominant allele at the *asc* locus. Dominance is incomplete in heterozygous plants which are 50 times more sensitive than susceptible homozygotes, but 20 times more sensitive than resistant genotypes[20]. In this case different forms of aspartate-carbamoyl-transferase (ACTase), the putative target for AAL-toxins, may be responsible for resistance and susceptibility[31]. A dominant mutation confers on rice both resistance to brown spot disease and tolerance to crude culture filtrate from *Cochliobolus miyabeanus* race 46[44]. Two genes are responsible for resistance of maize to *C. carbonum* and tolerance to its toxins, susceptibility being correlated with the number of recessive alleles present in the genotype[52,57], while one semidominant allele confers resistance to *Periconia circinata* and tolerance to PC-toxin on *Sorghum bicolor*[63]. In contrast, tolerance of oats to victorin from *C. victoriae* has been found to be recessive, the dominant form of the same gene or a closely linked one being responsible for resistance to another pathogen, *Puccinia coronata*[62].

Only in a very few instances have single genes which produce tolerance to non-HST been shown to confer resistance against pathogens. Tomato isogenic lines with or without the gene *Ph-2* for partial resistance to *Phytophthora infestans* differed in tolerance to *Phytophthora* culture filtrates[60], and *Medicago sativa* and tomato plants regenerated from in vitro cultures were found to carry dominant mutations affecting both resistance to *Fusarium oxysporum* f. sp. *medicaginis* and tolerance to its culture filtrates[37,64].

Tolerance to *Fusarium* toxins was also found to cosegregate with resistance in a progeny from a cross between a resistant and a susceptible carnation cultivar[70]. However, although very little evidence is available suggesting the contemporary control of the two characters by the same gene, a good amount of data has been collected indicating that resistant cultivars from a number of species tend to differ from susceptible ones in the level of toxin tolerance. In table 1 and 2 a series of examples of this behavior are reported for both HST and non-HST toxins, together with the methods used for screening for toxin tolerance.

It should be stressed, however, that results sometimes seem to be contradictory, and there are systems where such a correlation seems to be proven only for some cultivars. For instance, in the interaction of carnation and *F. oxysporum* f. sp. *dianthi*, in two separate series of experiments, explants from resistant and susceptible cultivars were cultured in vitro on media containing a series of culture filtrate concentrations. Out of the five resistant genotypes, only three showed higher tolerance to toxins. The other two were shown to produce high levels of phytoalexin when challenged with fungal elicitor[16,17]. The apparent contradictions may be due to the test used, as one of the latter cultivars was later found to respond in the expected way to an ion leakage test carried out on

Table 1. Some cases where a precise genetic correlation has been found between resistance to a pathogen and HST tolerance.

Pathogen	Plant	Control	Method	Reference
Alternaria alternata	*Solanum tuberosum*	F	a, b	56
A. alternata	*Lycopersicon esculentum*	A	d	20, 40
Cochliobolus victoriae	*Avena sativa*	C	a, b, d	62
C. heterostrophus	*Zea mays*	D	b	26
C. carbonum	*Z. mays*	A	d	26, 52
Bipolaris sacchari	*Saccharum officinarum*	E	a, b, d	41
Periconia circinata	*Sorghum bicolor*	B	a, c	63
C. miyabeanus	*Oryza sativa*	A	b	44
A. brassicae	*Brassica campestris*	F	d	7
A. alternata	*Pyrus communis*	F	b	41
Phyllosticta maydis	*Z. mays*	D	b	42
A. solani	*Solanum tuberosum*	F	a, b	65
Pyrenophora tritici repentis	*Triticum aestivum*	F	d	8

Genetic control: A = dominant; B = semidominant; C = recessive; D = cytoplasmic; F = unknown
Screening method: a = ion leakage; b = growth in culture; c = seedling growth; d = leaf bioassay.

Table 2. Some cases where the resistance to the pathogen has been shown to be correlated with non host-selective toxin tolerance. Screening methods as in table 1.

Pathogen	Host	Method	Reference
Phoma tracheiphila	*Citrus* spp.	b	51
Phytophthora infestans	*Lycopersicon esculentum*	a, b	60
Pyrenophora avenae	*Avena sativa*	c	43
Fusicoccum amygdali	*Prunus* spp.	a, b	73
Fusarium oxysporum f. sp. *dianthi*	*Dianthus caryophyllus*	a, b	17, 70
Fusarium oxysporum f. sp. *lycopersici*	*Lycopersicon esculentum*	a	pers. comm.
Fusarium oxysporum f. sp. *medicaginis*	*Medicago sativa*	a, b	5
Ophiostoma ulmi	*Ulmus carpinifolia*	a	49
Leptosphaeria maculans	*Brassica napus*	b, d	67
Xanthomonas campestris pv. *pruni*	*Prunus persica*	b	35
Phialophora gregata	*Glycine max*	b	77
Phytophthora parasitica	*Nicotiana tabacum*	b	34

the leaves, and this result was confirmed in segregating progenies from crosses involving the same genotype [70]. Comparable results were obtained in the case of the interaction of *F. oxysporum* f. sp. *lycopersici* and tomato, where differential toxin tolerance was observed using a callus growth system [61], while ion leakage from leaf disks was later shown to be an efficient test (Storti et al., unpublished data). Similarly, Witsenboer et al. [78] tested the effect of AAL toxins produced by *A. alternata* f. sp. *lycopersici* on leaves, leaf disks, roots, calli, suspension cells, minicalli and protoplasts of resistant and susceptible tomato genotypes. In this case, differential toxin effects could be observed on leaf necrosis and root growth, but not on shoot induction from leaf disks or on growth of callus, in cells suspension, or protoplasts. These results suggest that differential sensitivity to toxins may depend upon the degree of differentiation and the physiological state of plant cells used in the test. This hypothesis is supported by growing evidence of a clear influence of phytohormone levels on toxin tolerance.

Recent experiments carried out in our laboratories suggest that ion leakage induced by culture filtrates of *A. alternata* may be altered in the potato through the integration in the plant genotype of *Agrobacterium tumefaciens* genes coding for isopenteniltransferase, an enzyme involved in cytokinin synthesis [18]. Reports are also available on the modification of toxin effects through changes in auxin/cytokinin equilibria induced by exogenous treatments [33, 34].

Similar phenomena may also explain some other contradictory situations, although lack of toxin tolerance in some resistant cultivars cannot be excluded. For instance, a negative correlation between toxin tolerance and resistance of *Citrus* spp. to *Phytophthora citrophthora* was found by Vardi et al. [74] using a callus growth test, whereas Boudart [12] and Pedras et al. [55] failed to show the correlation observed by Sjödin [67] in the *Leptosphaeria maculans*/*Brassica* system. It is probable that some other negative results have been obtained but have not necessarily been reported in the literature. Notwithstanding these few failures it can be concluded that toxin sensitivity tests may indeed be of real value to the breeder, as suggested by Yoder [80] and shown for the first time in mass selection experiments by Wheeler and Luke in 1955 [76], provided that the right test is used under the best differentiating conditions.

Several tests are available, as described in the review by Yoder [80] already quoted. The ones most widely utilized, and most amenable to fast and cheap use, are root growth, leaf necrosis and chlorosis, protoplast and cell survival [66], cell aggregate growth, and ion leakage from leaves, cotyledons and calli. In a classical breeding exper-

iment these tests should be tried first on parental cultivars and species, adapted, and then used under standard conditions on the progenies of crosses, products of asymmetric protoplast fusion experiments, and segregating pollen grains.

Phytotoxins as tools for the in vitro selection of novel resistant genotypes

As stressed in the introduction to this review, one of the main drawbacks in selecting plants for resistance is the lack of genetic variability for this character in cultivated crops. Plant cell and tissue cultures are known to be highly variable and to show a somewhat different mutation spectrum from that observed in vivo [15, 29] and therefore have been suggested as a potentially useful source of mutants resistant to pathogens. Selection, in plant species where a reliable regeneration technique has been developed, may be carried out at the level of the whole plant, using all the known screening techniques including those mentioned above. Encouraging results have been obtained in this way in the series of host-parasite interactions shown in table 3.

However, a more efficient approach would be to screen for novel mutants in vitro, before plant regeneration. This might allow a scale of selection that would be difficult to achieve in other ways, resulting in a significant saving of space, time and money.

It has been estimated that a potato breeder, working with seedlings, can screen only 100,000 individuals for resistance each year, whereas $20 \cdot 10^6$ protoplasts may be screened in vitro in a single experiment [46]. There are thus very strong arguments for the development of efficient in vitro screening procedures.

Phytotoxins have been suggested, on the basis of the correlations already discussed, as possible selective factors [25]. This approach was first tested by Carlson in 1973 using haploid cell lines of *Nicotiana tabacum* [19]. Single cells and protoplasts treated with EMS (ethylmethanesulfonate) were cultured in the presence of a putative analogue of tabtoxin, methyl-sulfoxamine (MSO). Three out of many calli showing a transient tolerance to MSO gave rise to plants resistant to the pathogen. This result was later questioned, since the supposed relationship between tabtoxin and MSO is far from being proven.

Table 3. Some cases of heritable (up to second generation) genotypes obtained through screening genetic variability in regenerated plants and their progenies without preselection on toxin-containing media.

Plant	Pathogen	Reference
Apium graveolens	*Cercospora apii* *Septoria apiicola* *Pseudomonas cichorii* *Fusarium oxysporum* f. sp. *apii*	79
Stylosanthes spp.	*Colletothricum gleosporiaides*	32
Solanum tuberosum	*Alternaria solani* *Sclerospora sacchari*	65
Lycopersicon esculentum	*Verticillium albo-atrum*	29

Table 4. Some examples of heritable resistant genotypes (second generation included) obtained after in vitro selection on toxins or culture filtrates.

Plant	Pathogen	Reference
Brassica napus	*Leptosphaeria maculans*	59
Oryza sativa	*Cochliobolus miyabeanus*	44
Avena sativa	*C. victoriae*	58
Prunus persica	*Xanthomonas campestris* pv. *pruni*	35
Lycopersicon esculentum	*Alternaria solani*	65
Medicago sativa	*Fusarium oxysporum* f. sp. *medicaginis*	5
Nicotiana tabacum	*Alternaria alternata*	28
Lycopersicon esculentum	*Fusarium oxysporum* f. sp. *lycopersici*	64
Solanum tuberosum	*Phytophthora infestants*	10
Zea mays	*Cochliobolus heterostrophus*	30
Oryza sativa	*Xanthomonas campestris* pv. *oryzae*	72

A large number of experiments have been carried out since this early work, and in several cases positive results have been claimed (table 4). These results, however, need to be analyzed more thoroughly, as in several cases the reports did not include a comparison between frequencies of resistant genotypes obtained with and without selection. In other words, the real efficiency of the method in comparison with direct selection in the field has to be further evaluated. We shall attempt here to draw some tentative conclusions on the subject, on the basis of some of the best documented case-histories from our laboratories and from the literature.

Experiments with host-selective toxins

Gengenbach et al. [30] and Brettell et al. [14] used selection of cell cultures to select maize cell variants tolerant to HMT-toxin. Such variants were obtained from susceptible *Tms* corn lines and shown to maintain a high level of tolerance to HMT-toxin even after several months of culture on toxin-free medium. Furthermore, the tolerance expressed by cell cultures was correlated with tolerance of isolated mitochondria of HMT-toxin. Most significantly, plants regenerated from tolerant Tms cultures were fully resistant. The character was transmitted to the progeny through the female parent and appeared to be located on the mitochondrial genome [14, 24, 30]. Rines and Luke [58] regenerated oat plants on media containing victorin, the toxin of *Cochliobolus victoriae*, from calluses of the genotype VB/vb, susceptible to the pathogen. Nine out of the eleven regenerated plants were shown to be resistant to the pathogen and the resistance was found to be heritable. Moreover, resistant plants were, as expected, susceptible to *Puccinia coronata*. None of the 30 plants regenerated from control cultures (not exposed to victorin) were found to be resistant.

Less convincing are the data reported by Ling et al. [44], who recovered two rice plants resistant to *C. miyabeanus* out of 34 regenerated on media containing toxin, but also obtained one resistant regenerant out of 64 under control culture conditions.

Clearly much more research is required before the full potential of screening plants for tolerance to purified

host-selective phytotoxins can be ascertained. However, two important points require emphasis. First, the number of plant pathogens known to produce an HST with a significant role in disease is relatively small[39,41], although new examples are constantly being discovered. Secondly, very careful studies of the inheritance of putative novel tolerance to phytotoxins and resistance to disease must be carried out, as a resistant phenotype may also be due to epigenetic unstable variation. Notwithstanding these caveats, it may be cautiously concluded that purified HST may have a clearly-defined role as in vitro selection agents, although proof is still required for each host-parasite interaction.

Experiments with non-HST, ill-defined toxins, or culture filtrates

A selection strategy similar to that in which purified HST are used is one in which an unpurified or partially purified culture filtrate, or a toxic compound from it, is employed as the screening agent. This strategy was first described by Behnke[10]. Culture filtrates of *Phytophthora infestans*, a pathogen not known to produce a true phytotoxin, were used. Calluses surviving on media containing culture filtrate concentrations known to give a 90% kill rate were transferred five or more times to media supplemented with the same culture filtrate concentration. Plants were regenerated and tested for resistance to the pathogen. Although in Behnke's experiments regenerants were apparently more resistant to *P. infestans* than unselected control plants, subsequent field tests provided inconclusive results and suggested quantitative inheritance of the trait[75]. An explanation of these contradictory results may be that selection may have been for tolerance to minor toxic components of the culture filtrate. Tolerance to such factors might not confer complete resistance to disease but could influence in the development of symptoms in a positive way, and thereby the development of the disease itself, particularly in favorable environmental and physiological conditions.

A better assessment of the potentialities of such a technique for breeding may, however, come from experiments particularly designed to test and compare resistant genotype frequencies in populations derived from regenerants grown in the presence or absence of toxic media. Studies were carried out with this aim in our laboratories using the systems *Leptosphaeria maculans* (anamorph: *Phoma lingam*) oilseed rape and *Alternaria brassicicola* oilseed rape, and are briefly summarized here. *L. maculans*, an important pathogen of oilseed rape, produces two phytotoxins, sirodesmin PL and deacetylsirodesmin PL[11,13,27], whose action is non-specific and whose role in pathogenesis has still to be properly defined[1,11,36]. These toxins may inhibit the active defence mechanisms of the plant and therefore toxin-tolerant variants may show an increased resistance to the disease. Preliminary experiments showed that variant lines carrying sirodesmin PL tolerance could indeed be selected on toxic media from secondary embryogenic cultures of oilseed rape[38,45]. Sirodesmin PL was used in these and later experiments because Férézou et al.[27] found it to be the major toxin of *L. maculans*, and because it was readily available in purified form. However, as Sacristan[59] had obtained encouraging results (although not fully confirmed through careful heritability tests) using crude culture filtrates, experiments using such filtrates were also carried out[54].

In the experiments in which pure sirodesmin PL was used as a selection agent, secondary embryoids derived from spontaneously resistant oilseed rape cultivars were little or no more capable of growth in vitro in the presence of the toxin than embryoids from susceptible genotypes. This finding was confirmed by tests on germinating seeds[53] and is consistent with the suggestion by Boudart[11] that sirodesmin PL may not have a central role in disease. It should be noted however that Sjödin and Glimelius[67] obtained opposite results in tests with resistant and susceptible *Brassica* spp. (which did not include oilseed rape) for tissue and cell aggregate tolerance of the same phytotoxin. The results with sirodesmin PL also contrast with those obtained in experiments where *L. maculans* culture filtrates were used. A clear correlation was found between the ability of seeds of cultivars of oilseed rape to grow in the presence of culture filtrates from a highly pathogenic isolate of the pathogen and the known resistance of those cultivars to *L. maculans*[2-4]. Furthermore, Delwiche[22] also found a correlation between the toxicity of culture filtrates to germinating seeds of oilseed rape and the degree of pathogenicity of different isolates of *L. maculans* from which the culture filtrates were obtained. These results support the idea that factors contained in culture filtrates, although not necessarily sirodesmin PL, play a role in overcoming resistance to the pathogen and are therefore codeterminants of pathogenicity. Thus, toxin-tolerant plants may be more able to resist infection.

In our experiments it was initially possible to select secondary embryoids showing either resistance to sirodesmin PL or tolerance to the toxic effects of a culture filtrate of *L. maculans*. In both cases the percentage of embryoids showing resistance increased with each successive selection cycle on toxic culture medium, but resistance proved to be completely unstable in culture after the removal of selection pressure[54]. One possible explanation of the transient nature of the toxin-tolerant phenotype is that tolerance in culture and in regenerated plants observed by others[59] may have been due to semipermanent epigenetic changes in gene expression rather than to mutations or stable epigenetic variation[15]. Despite this observation, it was necessary to determine the resistance to *L. maculans* of plants regenerated from secondary embryogenic cultures submitted to either sirodesmin PL or to culture filtrate and to compare it with that of plants from control, unselected cultures, in order to ascertain whether resistance to the pathogen,

rather than tolerance to its toxin, had arisen during culture independently of the selection process. Plants from selected and unselected cultures were found not to differ in their resistance to *L. maculans* and in some cases, as in previous experiments[45], appeared to be more susceptible to the pathogen – especially when selected – than plants grown from seed. There was no evidence for the presence of novel resistance to *L. maculans* in any of the regenerant material examined. It should be stressed, however, that our experiments do not offer conclusive evidence concerning the question whether a plant with genetically stable tolerance to sirodesmin PL or culture filtrate would be resistant to *L. maculans*. Moreover, it remains to be explained why, using the system described, stable resistance either to toxic factors or to the pathogen was not detected. Possible explanations of this finding could be that the toxin concentrations used were too low, the wrong culture conditions were used, or somatic genetic variation was reduced.

However, it is important to note that Ingram et al.[38] and MacDonald and Ingram (unpublished data) have observed a relatively high rate of stable somatic variation for characters other than resistance, including abnormal morphology in secondary embryogenic cultures of oilseed rape. As already noted, Sacristan[59] was able to generate stable resistance to *L. maculans* in the same system, although chemical mutagenesis may have been responsible for this variation.

Also MacDonald and Ingram[46] observed apparently stable variation again in oilseed rape embryogenic cultures for tolerance to the toxic factors of *A. brassicicola*, while experiments by Loh[45] and Newsholme et al.[54] suggested the possibility of obtaining variation for increased susceptibility to *L. maculans*.

A final explanation for the finding may be that the culture medium on which secondary embryogenic cultures are maintained and regenerated contains factors which act as elicitors of phytoalexins or other compounds involved in resistance[23] and that such substances, many of which are known to be phytotoxic[6], act as negative selection agents, tending to eliminate from cultures cells that are resistant to the pathogen.

The conclusions that may be drawn from the study are that, firstly, heritable somatic variation may be insufficient to make secondary embryogenic cultures a system of value for in vitro selection of novel resistant genotypes; secondly, that the extent and nature of such variation may be conditioned by the parental material and culture and treatment conditions[15]; finally the encouraging results obtained by Sacristan[59] may not have been due to the presence of sirodesmin PL in the medium but to some other compound or synergistic mixture of components. This does not rule out, however, a possible use of tolerance to sirodesmin PL and/or toxic filtrates as markers for resistance in crosses or segregating progenies once it has been proved that it is associated with resistance in one of the parental genotypes. This seems to be the case in the recent work by Sjödin and Glimelius[68], who used sirodesmin PL as a selective agent with asymmetric fusion products between *Brassica juncea*, *B. nigra*, *B. carinata*, all naturally resistant and insensitive to the toxin, and *B. napus*. In these experiments, 19 out of 24 asymmetric and toxin-selected hybrids between *B. napus* and *B. juncea* were found to be resistant to the pathogen while all unselected asymmetric hybrids were susceptible. In the system *B. napus*/*A. brassicicola*[46], as in experiments with toxic factors of *L. maculans*, it was possible to select secondary embryogenic lines of oilseed rape carrying novel tolerance to crude phytotoxins of the pathogen. An important difference, however, was that this new tolerance was apparently completely stable over many generations of subculture. Nevertheless, plants regenerated from such cultures were no more resistant to disease than were plants derived from cultures that had not been subjected to the selection process. Indeed, as in the case of *L. maculans*, plants regenerated from both selected and unselected cultures were more susceptible than plants grown from seed. Apart from these experiments, data reported in the literature on selection on media containing ill-defined toxins or culture filtrates are very contradictory. Arcioni et al.[5] reported obtaining alfalfa plants resistant to *Fusarium oxysporum* f. sp. *medicaginis* only from calli selected on fungal culture filtrates, but not from unselected material. Hammerschlag[35] obtained a substantial increase in peach variants resistant to *Xanthomonas campestris* pv. *pruni* with a similar selection method. On the other hand, Shahin and Spivey[64] recovered seven tomato plants resistant to *F. oxysporum* f. sp. *lycopersici* out of 73 from protoplasts cultures selected on fusaric acid, but obtained only a slightly worse result (6/126) on control regenerated material. In both cases resistance to the pathogen was later shown to be due to a single, dominant gene. All these data taken together do not allow any conclusive verdict on the real potential of in vitro selection with non-HST or culture filtrates, but suggest that success with this technique, if any, is dependent on the system and the technique utilized.

Conclusions and future prospects

There is no doubt that toxins, particularly HST, may play an important role in pathogenesis. In a few cases targets of toxin action have been identified and their modification correlated with resistance; conversely, a direct relationship has been established between virulence and toxin production. However, the data reported in this review seem to suggest that toxin tolerance is not necessarily sufficient by itself for resistance to pathogens. Resistance is probably due in most cases to an interaction between passive and active defense mechanisms. This is suggested by experiments in our laboratories aiming at the dissection, through the isolation of mutants, of the interaction in vitro between tomato cells and *F. oxysporum* f. sp. *lycopersici*[70, 71] (unpublished data). It has been shown

that incompatible reactions are determined by a hierarchy of factors in which recognition, and probably membrane and cell wall structure and composition, play a dominant role. It may be suggested therefore that toxins, particularly non-HST, act primarily by inhibiting active defence processes[41]. Therefore, though toxin tolerance is generally a good marker for resistance in existing cultivated varieties, where it may have been coselected with genes controlling active defence, it may not be sufficient, in the genotypes where the active defence 'machinery' is inadequate, to prevent pathogen penetration and proliferation. This hypothesis may help in understanding the contradictory results sometimes obtained even in different experiments carried out on the same host-parasite system, and the fact that a genetic correlation between toxin tolerance and resistance in existing germplasm seems to be much more frequent than success in selecting genotypes resistant to pathogens on toxin-containing media.

A critical experiment to be done would therefore be to select at the same time for pathogen recognition[18, 71] and toxin tolerance from the same, susceptible genotype and compare the behavior in vivo of plants obtained in this way with others alternatively selected for the two characters. As things stand at present, toxins and culture filtrates seem to offer the possibility of speeding up plant breeding through fast and cheap screening procedures, and they may be useful for selecting in vitro novel mutant genotypes in the case of HST. However, they may only be of help in a few, specific systems, as direct selective agents when non-HST are involved in host-parasite interaction. Finally, an avenue that has so far been little explored is the isolation and integration into susceptible genotypes of genes conferring toxin tolerance as a tool for increasing resistance to pathogens. The lack of work in this field probably stems from insufficient information about putative specific protein targets of toxic action, and about detoxifying enzymes. However, the work in progress reported here and in other contributions to this multi-author review concerning the isolation of possible toxin receptors, and a better understanding of the modes of action of toxic compounds may open new, interesting ways of changing plant genotypes through the use of recombinant DNA techniques.

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Phytotoxins as potential herbicides

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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