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Verticillium-toxins: Their Role in Pathogenesis

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ABSTRACT

Bioassay results confirmed the role of low molecular weight phytotoxin in the pathogenesis of *Verticillium albo-atrum.* The metabolites separated from 21-day-old culture filtrate by adsorption on the resin Amberlite XAD-4, and further chromatographed on Bio-Gel P2 polyacrylamide gel, induced chlorosis and necrosis on the leaflets of tomato and potato cultivars, similar to those caused by the fungus on diseased plants. Leaflets from tolerant cultivars were much less sensitive to the toxin (s) than those from the susceptible ones. In the presence of toxin(s) plant tissues and individual cells showed ion-leakage and cell death to an extent relating to the plants reaction to the fungus. The relative specificity observed during pathogenicity tests between potato and tomato and their related isolates was shown to be related to the action of toxin (s).

Keywords: Pathogenesis, Toxins, Verticillium.

INTRODUCTION

Many different fungi and bacteria are known to produce a wide range of metabolites in culture, which are toxic to plants. These include certain substances with varied biochemical structures, belonging to certain groups such as polypeptides, glycoproteins, aminoacid derivatives, polyketides, terpenoids, sterols and quinones (Kono *et al*., 1981., Stoessl, 1981). Such compounds have been named pathotoxins (phytotoxins) or simply toxins.

The role of a toxin in a plant disease is not always easy to elucidate. A range of criteria is used to evaluate a substance before it is regarded as a factor in pathogenicity or virulence of a pathogen. There is considerable evidence that the pathogenicity of certain fungi such as *Alternaria* and *Helminthosporium* is closely related to the production of host selective toxins by the invading organisms (Arntzen *et al*., 1973). These pathogens produce toxins before penetration. In this case, toxinless mutants are not infectious.

However, it is important that a toxin or its known derivatives be detected in infected plants, induce typical symptoms of the disease itself, and that toxin production across a range of pathogen isolates correlates with their virulence toward the host cultivars. These criteria have been satisfied for very few putative toxins (Yoder, 1980; Daly and Deverall, 1983).

Vascular pathogens, including *Verticillium,* produce toxins which are not needed for infection, but express the symptoms (Daly and Deverall, 1983).The best evidence for a causal role for such a toxin (s) is to reproduce entire syndromes or those which cause particularly distinctive symptoms.

Chlorosis and necrosis on the naturally infected plant are among typical symptoms of toxin effects (Wheeler, 1981).Necrosis of plant tissues is the result of ion-leakage and cell death. However, chlorosis of the leaves, is a common symptom induced by host selective and non-selective toxin as a result of a more complex events. Both are usually

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used in toxins bioassays (Mussel, 1972).

Although *Verticillium* has a wide host range in dicot plants, some sort of relations are observed between isolates and their hosts since symptoms appear faster and more severely than unrelated ones (Bhat and Subbarao, 1999). Specificity is a term used to describe the ability of a host-specific toxin to produce visible and biochemical symptoms typical of the disease only in the related host. In this study, the term is used to show the favorite relationship between the hosts and the isolates of *V. albo- atrum* in the pathogenesis of the fungus. Furthermore, the purpose of the present study was to demonstrate that the toxin(s) elaborated by the isolates of *V. albo-atrum* in a culture, and initially separated by adsorption on the resin Amberlite XAD-4, could produce effects in bioassays which correlate with the virulence of the pathogen on the host plant

MATERIALS AND METHOD

Fungal Strains and Plant Material

Pathogenic strains of *V.albo-atrum* designated as V_2 and V_3 originally isolated from tomato and potato respectively, were obtained from University College of Swansea, Wales, UK. Certified potato tubers-Home Guard (resistant) and Desiree (susceptible)-were obtained commercially. The near isogenic tomato lines, Cragiella, resistant and susceptible, were kindly supplied by Dr. J Mansfield, Wye College, England.

Pathogenicity Tests

Pure cultures of *V.albo-atrum* isolates V_2 and V_3, grown for three weeks on Potato Dextrose Agar (PDA) at 25°C in the dark, were macerated in sterile water to produce a spore/mycelia suspension. The suspensions were adjusted to a concentration of approximately 1×10^6 propagules/ml based on the dilution plate method, and used as inoculums. Potato cultivars were inoculated by dipping a single bud tuber piece in the inoculum suspensions for 10 minutes. Tomato cultivars were inoculated by immersing the roots of four week-old seedlings in the inoculums for 5 minutes. Inoculated potato pieces and tomato seedlings were then transplanted into 35 cm pots containing soil, peat and sand mixture. In addition, 50 mls. of the inoculum suspensions were added to the soil around the roots or tuber pieces at the time of planting. Pots were placed on greenhouse benches at 21± 3ºC, 70% RH, and at 12000 lux. Five potato pieces and tomato seedlings from each cultivar were inoculated with host and non-host isolates. Macerated PDA in SDW was used to control the inoculations. Symptoms were recorded at three day intervals, for nine weeks in potatoes and two weeks in tomatoes.

Toxin Production and Isolation

For the production of toxin (s), 5 mm mycelia discs were removed from the margin of an actively growing 3-week culture of the isolates V_2 and V_3 and transferred to 250 ml flasks with modified Czapeck's dox liquid medium, which contained per liter of distilled water: 10 gr glucose, 2 gr $NaNO_3$, 0.5 gr KCl, 0.35 gr K_2SO_4, 0.01 gr $FeSO_4.7H_2O$, 0.5 gr Magnesium glycerophosphate. The pH of the medium was adjusted to 6.7 with KOH before autoclaving. The flasks were incubated at 21 ± 2ºC in the dark for three weeks. The resin Amberlite XAD-4 (BDH Chemicals Ltd, Poole UK) was used for isolation of the toxin (s) from culture filtrate as described previously (Mansoori *et al*.,1995).The residue obtained was dissolved in 2mls distilled water and regarded as concentrated resin- purified toxin (s). The dissolved residue was further purified by filtration through a column (21× 1.6 cm) prepared from a slurry of Biogel-2 (Bio Rad), and equilibrated with a 10 mm phosphate buffer pH 7.2. The column was eluted with distilled water at room temperature at a flow rate of 10 ml/h. Fractions 6-9 (18-27 ml) which showed toxic activity were used

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in bioassays.

Bioassays

Evaluation of the sensitivity of plant cultivars to toxin

1) Detached - leaf bioassay

This method involved cutting the petiole of the leaflets from fully expanded 1st to 4th true leaves of 4 to 8 week old potato or tomato plant cultivars with a razor blade under water and placing them in 2 mls of toxin solutions previously applied to 1/4th pieces of 9cm filter paper disks inside petri dishes (Gilchrist and Grogan, 1976). The toxin solutions were two - fold dilutions made from readjusted volume of concentrated toxin to the original volume of crude culture filtrate. The dishes were incubated at 21 ± 2º C with a 16 hour photoperiod (at 1200 lux) and at 70% relative humidity (RH). Observations were made over a period of seven days to determine the appearance of symptoms. Toxicity scores were 0= no symptoms, 1= chlorosis at the base of the lamina, 2= one side of lamina showing chlorosis, 3= general necrotic spotting and chlorosis of the lamina, 4= marginal necrosis, and spots on the lamina.

2) Cell death

Susceptible and tolerant cell suspensions were generated from potato callus tissue. Callus cultures, in100 ml of the liquid Murashige and Skoog medium (Murashige and Skoog, 1962), were incubated on a rotary shaker at 100 rpm at 25 ± 1ºC in the dark. After five sequential transfers of the cultures, 0.1 ml of the suspension containing approximately 1×10^4 cells were placed at the bottom of a plastic microcentrifuge tube and combined with 0.1 ml of filtered sterile (Sartorius, Minisart NML, 0.22µm) partially purified V_3- toxin obtained from Biogel P-2 column, containing 74.21, 7.42, 3.71 and 0.74 mg/ml of active toxin on a dry weight basis .In control experiments, a sterile inactive preparation of the toxin (refrigeration at 4ºC for one week, Mansoori *et al*., 1995) was used. At 15 minute intervals, the tubes were gently agitated by hand. After four hours incubation at room temperature the cells were allowed to settle and 0.1ml of the medium was replaced with three changes of, 1 ml fresh liquid medium to remove any toxin from the cell suspension. The percentage of cells killed was determined according to the method described by Larkin (1976). Using these methods, the vital dye Fluoresin diacetate (FDA; Sigma) dissolved in acetone (5 mg/ml) was added to the cell suspension to a final concentration of 0.01% (W/V). After five minutes incubation at room temperature, an aliquot of the cell suspension was viewed under a Ziess photomicroscope equipped with IV F1 Epi-Fluorescence condenser, and incidental light from an HB050 mercury-vapor lamp. An excitation filter KP 500 was used with a barrier filter LP 520. Cells that failed to show yellow color luminosity were assumed dead. The percentage of viable cells was determined by direct counting.

3) Ion -leakage

The assay was performed according to the method of Scheffer and Livingston (1980). Leaflets from lower fully expanded leaves were removed. Discs (1cm in diameter) were cut out from the leaflets under water with a cork borer, rinsed with distilled water and randomly placed in glass vials. Each vial contained 12-13 leaf discs and 5 mls of toxin solution, or distilled water, and the discs were removed and rinsed several times with a total of 100 ml distilled water over a 10 minute period. After the last rinse, 10 mls of distilled water was added as a leaching solution. The vials were incubated on a shaker and the conductivity was measured after 0.5, 1, 2, 3, 4, 5, 6 and 18 hours of incubation with a PCM 123 electrode (K=1.04) coupled with a Jenway 4010 conductivity meter.

Specificity

1) Detached-leaf bioassay

Potato leaflets are sensitive to a wide range of toxin concentrations. The selective response of susceptible cultivars of potato and

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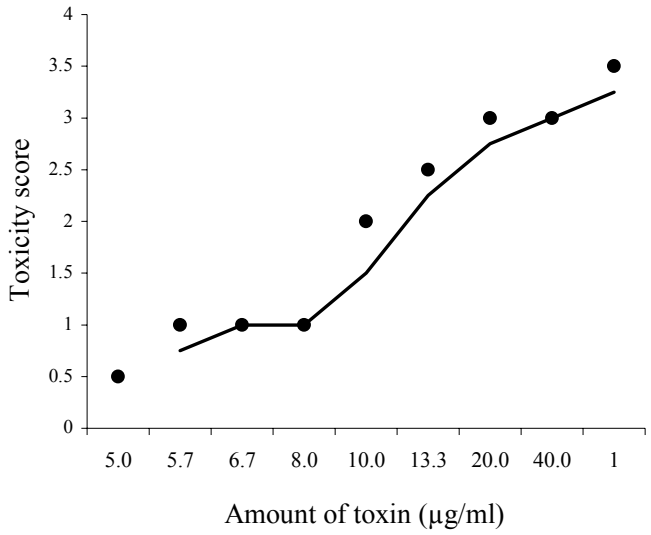

Figure 1. Dose-response relationship between toxicity score and dilution factors. Detached leaflets from susceptible potato cv Desiree were passively infiltrated with toxin dilutions prepared from concentrated (resin) purified V_3-toxin. Symptoms were scored over a time period of 3-7 days. The ED50 corresponds to the toxicity score 2 resulted from in filtration of 11µg/ml of V3-toxin on the basis of the original concentration in crude culture filtrate.

tomato to V_2 and V_3-toxins were evaluated using a standard amount of toxins obtained from the mid-point of the most fitted line of a dose response curve (Yoder, 1981). In a preliminary study, concentrated resin purified toxins isolated from 300 mls of crude culture filtrate were diluted with distilled water to the original volume of the culture filtrate. The Toxicity of a series of dilutions was examined in detached leaflets of susceptible potato cultivar, and scored as described earlier. Symptom scores were used as a qualitative character to draw the most fitted line against dilution factors, according to the method of Roberts and Boyce (1972). The standard toxin dilution, ED50, was derived from the mid-point of the most fitted line. At this point, ED50 corresponded to a dilution of 1:1800th of the original crude culture filtrate, a solution which contained approximately 11µg/ml of concentrated V_3-toxin (Figure 1).

This amount was used to compare the selective response of susceptible potato with tomato cultivars in a detached leaf bioassay. The experiment was repeated twice with different batches of toxins.

A similar experiment was conducted to draw the most fitted line using V_2-toxin and susceptible tomato leaflets. However, leaflets were found not to be dose-responsive at the lower dilutions of toxin employed (1:80-1:4,000th), and the dose-response curve was drawn from the most fitted regression line (r= 0.24) as shown in Figure 2. The mid-point of this line (ED 50) was equal to a dilution of 1:2200th of the original crude culture filtrate and contained approximately 9 µg/ml of concentrated V_2 -toxin. Selectivity of V_2-toxin toward susceptible potato and tomato was assessed in a detached leaf bioassay as described earlier.

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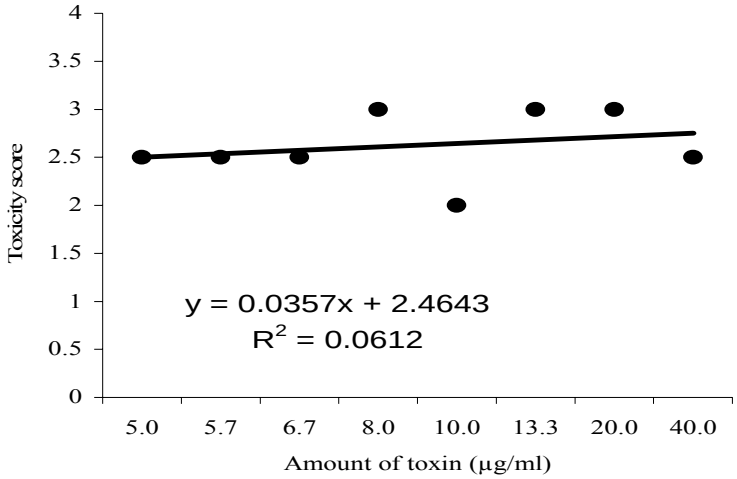

Figure 2. Dose-response relationship between toxicity scores and dilution factors. Detached leaflets from the susceptible tomato cv. Cragiella S were passively infiltrated with toxin solutions prepared from concentrated resin-purified -toxin. Symptoms were scored over a time period of 3 days and the best fitted line drawn from the equation y = 0.0357x + 2.4643 .The ED50 corresponds to the toxicity score 3 and 9 µg/ml of V_2 - toxin on the basis of original concentration in the crude filtrate.

2) Ion-leakage

The extent of the leakage of ions from susceptible tomato leaf discs, treated with either V_2 or V_3-toxin was measured as the conductance of leaching solution. Tomato tissues were more sensitive to toxin than from susceptible potato cultivar and leaching could be measured over a six hour period. In this experiment, 0.69 µg/ml of concentrated V_2 or V_3-toxin were used. This amount of V_2-toxin showed to cause extensive leakage of ions from leaf discs of susceptible tomato cultivar.

RESULTS

Pathogenicity Test

Leaf symptoms developed earlier than other signs of Verticillium wilt, both in susceptible potato and tomato cultivars. In susceptible potato cv. Desiree inoculated with V3 isolate, leaf symptoms appeared 45-48 days after inoculation, as unilateral leaf chlorosis accompanied by the appearance of withered dry patches along the margins of chlorotic leaflets. At the same time necrotic

Table1. Potency and differential toxicity of V_2 and V_3 – toxin on susceptible and tolerant tomato and potato cultivars using detached – leaf bioassay.

Source of toxin	Plants	Dilution end - point[a]		
		Susceptible	Tolerant	Sensitivity(S/T)
V_2	Tomato	1:4000	1:8	500
V_3	Potato	$1:10^{12}$	1:64	$1:10^{10}$

Highest dilutions of concentrated resin purified V_2 and V_3 – toxin inducing symptoms.

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Table 2. The effect of V_3 – toxin on viability of cultured susceptible and tolerant potato cells.

Concentration of toxin (µg/ml)	Viable cells[a] (%)					
	74.21	7.42	3.71	0.74	IN.T. Con.	D.W. Con.
	Potato cultivars					
Desiree (S)	2-10	36-60	70-77	73-90	85-95	88-100
Home Guard (T)	20-35	51-67	80-95	80-95	90-100	85-100

Cell suspension cultures of potato were treated with a preparation of V_3 – toxin obtained from the Biogel p-2. After 4 hours of treatment cells were washed free of toxin, treated with 0.01% Fluorescin diacetate and viewed under fluorescence microscope. The percent of viabel cells was determined on a count of at least 100 cells. The data represent the range of viable cells from two experiments each with two replicates.
S = Susceptible, T= Tolerant, D.W =Distilled water, IN.T.= Inactive toxion

spots were formed on the surface of the younger upper leaves. The onset of leaf symptoms on susceptible potato cultivar inoculated with isolate V3 was one week earlier than with V2. In the tolerant potato cultivar Homequard, mild chlorosis developed on the lower leaves 55 days after inoculation with isolate V3, but no symptoms developed when the tolerant cultivars was inoculated with isolate V2. In the susceptible tomato, cv. Cragiella S, leaf symptoms appeared 15 - 20 days after inoculation of seedlings with isolates V2 and V3 respectively. In tolerant cv. Cragiella R, leaf symptoms developed on the lower leaves of plants inoculated with V2, but not with isolate V3.

Bioassays

Evaluation of sensitivity of plant cultivars to toxin

Detached leaf bioassay: Leaf symptoms including chlorosis and necrosis developed on the susceptible potato leaflets over a period of 3-7 days as passively infiltrated with dilutions made from concentrated resin-purified V2 and V3- toxin (s). In contrast no symptoms appeared on the leaflets which were similarly exposed to either culture media or inactive toxin preparations and distilled water as controls. The rate of development and severity of symptoms in detached leaflets appeared to be a function of relative toxin concentration. Severe symptoms (toxicity scores of 3 and 4 appeared on the leaflets infiltrated with lower dilutions of toxin (s)

Table 3. Conductivity of leaching solutions as change in leakage of ions from susceptible and tolerant tomato and potato cvs. Leaf discs treated with V_2 and V_3 – toxin.

Conductance									
	Tomato					potato			
	6 hr		18 hr			6 hr		18 hr	
V_2-toxin µg/ml	S	T	S	T	V_2-toxin µg/ml	S	T	S	T
34.8	31	17	48	28	27	25	17	41	24
0.69	40	17	52	25	.057	23	19	37	25
0.13	33	17	44	26	.010	22	16	35	22
Cont.	20	17	27	25	Cont.	19	16	30	23

Leaf discs (1.5gr) were exposed to V_2 and V_3 – toxin for 2 hours, washed and placed in leaching solution. The conductance (umhos) was measured 6 and 18 hr after treatment. The toxin were active fractions collected from Biogel P-2 column. The values of conductance are the mean of 4 replicates.
S = Susceptible, T= Tolerant, Cont. = Distilled water.

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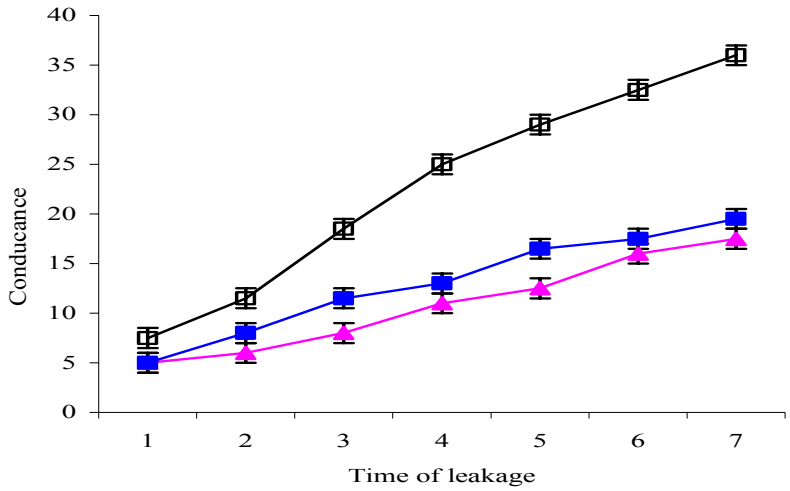

Figure 3. Effect of V_2 and -toxin on loss of electrolyte from susceptible tomato leaf discs. Treatments were distilled water containing 0.69µg/ml V_2 (□) or V_3-toxin (■). The values of conductance are the mean of 4 replicates of separate experiments at indicated time intervals. Control was distilled water (▲); ± standard error.

over a 2–3 day period (Table 1). Concentrated V3-toxin had a dilution-end point of 1:10 for the susceptible compared to 1: 64, for the tolerant potato cultivars. In tomato, V2-toxin (s) had a dilution end–point of 1:4000 for the susceptible and 1:8 for the tolerant cultivars (Table 1).

Cell death

Table 2 indicates that potato cells from susceptible and tolerant cultivars in culture were affected by all the doses (74.21-0.74 µg/ml) of V3-toxin tested. However, a lower percentage of tolerant cells were affected at each dose as compared with susceptible cells. Inactive toxin(s) at7.42 µg/ml was not found to affect cells of either cultivars, as the percentage of viable cells being similar to those treated with distilled water alone.

Ion-leakage method

The results indicate that the potato tissues were only responsive to the highest dose of toxin (27µg/ml) used and, after 18 hours leakage of ions was detected at a level higher than that of the control. Leakage of ions from leaf discs obtained from the tolerant potato cultivar was not evident in any of the treatments. In contrast, susceptible tomato tissues treated with V2-toxin result in the loss of electrolytes in all treatments, which was higher than that of the corresponding controls. Only at the highest dose of V2-toxin (34.8 µg/ml), some leakage of ions from tolerant tissues was measurable after 18 hours leaching periods (Table 3).

Specificity

Detached-leaf bioassay

Plates 1and 2 illustrate that 11µg/ml resin-purified V3-toxin induced chlorosis only in the detached leaflets of the susceptible potato cultivar. This amount of toxin (s) failed to induce any symptoms in the leaflets of the susceptible tomato cultivar. A corresponding

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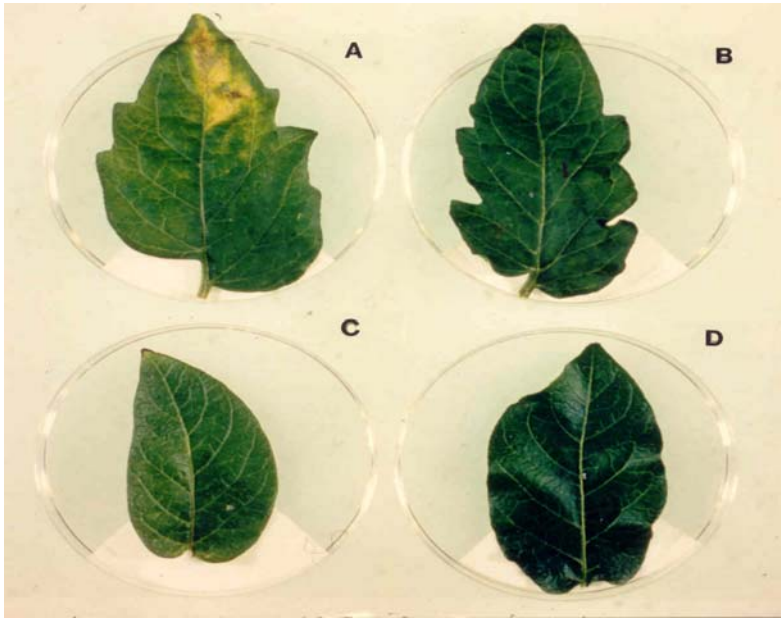

Plate 1. Selective response of susceptible tomato leaflets to V2-toxin. 9µg/ml concentrated resin-purified V2-toxin induced chlorosis in A) susceptible tomato but not C) susceptible potato leaflets. Note this amount of toxin did not induced any symptom in B) tolerant tomato and D)tolerant potato leaflets.

result was obtained with V2-toxin (s) where 9 µg/ml of resin- purified toxin (s) induced symptoms only in the susceptible tomato leaflets. Potato leaflets were unaffected by this amount of toxin (s).

Ion-leakage

Figure 3 indicates that after six hours, significant leakages of ions occurred from tomato tissues treated with 0.69 µg/ml of V2-toxin(s). Leakages of ions were not significant when similar tissues were treated with an equal amount of V3-toxin(s).

DISCUSSION

Pathogenicity tests indicate that the vascular pathogen *V. albo-atrum* causes a severe wilt disease in the susceptible potato and tomato cultivars. It is interesting to note that potato cultivar Desiree, which had been reported tolerant to *Verticillium dahliae* Kleb (Susnoschi *et al*. 1976) was susceptible to *V. albo-atrum.* However the response of the tolerant potato cultivar Home Guard was similar to that described by Fletcher (1972). Tomato cultivars Cragiella S and R responded to *V. albo- atrum* in a manner similar to that described by Tjamos and Smith (1975). These results, indicate that the pathogens exhibit a certain degree of host specificity; in that symptoms appeared ealier ,and the diseases was more severe in susceptible cultivars inoculated with their related isolates i.e. V2- tomato, V3- potato, than with unrelated ones.

The results reported here confirm the earlier observations of Green (1954), that *V.albo-atrum* produces an extracellular metabolite in liquid culture which induces foliar symptoms on detached leaflets, indistinguishable from those observed on parts of the leaf lamina which do not harbour the fungus. The biological activities of the toxins were determined in a number of ways, depending on the apparent effect it induces in susceptible plants. Mussel (1972) selected

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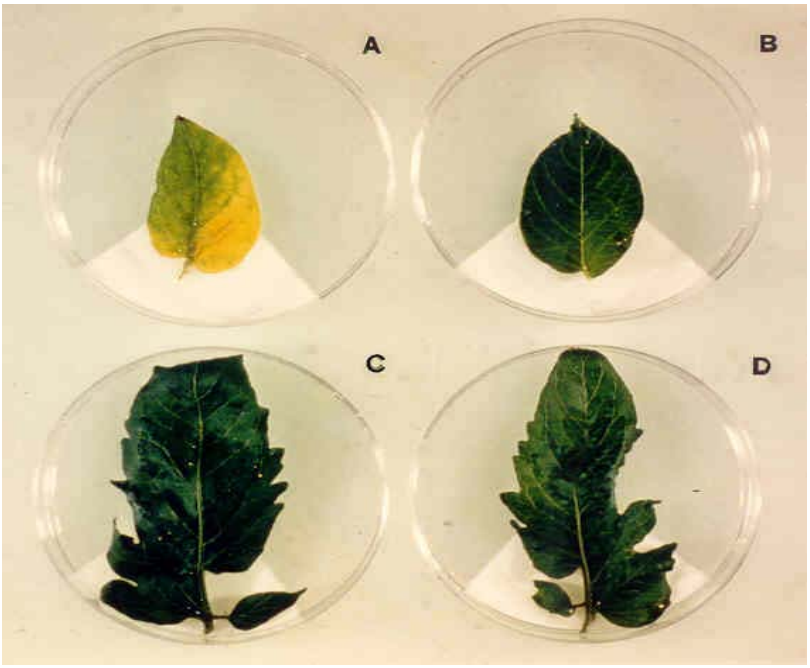

Plate 2. Selective response of susceptible potato leaflets to V3-toxin. 11µg/ml concentrated resin-purified V3-toxin induced chlorosis in A) susceptible potato but not in C) susceptible tomato leaflets. Note this amount of toxin did not induce any symptom in B) tolerant potato and D) tolerant tomato leaflets.

necrosis and chlorosis as a more valid signs of toxin activities than wilting in Verticillium infected plants. The technique of passive infiltration of detached leaflets with toxin solution under fluorescent light, a 16 hour photoperiod and 70% RH was both convenient and reproducible. It was thus used for routine determination of toxin activity (Mansoori, *et al*., 1995). Uninoculated autoclaved liquid media, distilled water and inactive toxin preparations had no effect on the leaflets, indicating components of the culture filtrate or other metabolites of the fungus are not the active factor, and confirming that the symptoms were selectively induced by genuine toxin (s). However, since impure toxin preparations were used in the bioassays the most proper control would be an inactivated preparation of the toxin. To give the most reliable results (Scheffer, 1983). Similar treatment was followed in the present study.

Although the detached-leaf bioassay was useful because of its reproducibility, simplicity and sensitivity, other types of bioassays, such as cell death and ion-leakage, are also used to show the effect of toxin at cellular level. These methods are rapid ones in which toxins lesions are measured directly.

Differential responses of the cultivars in the bioassays indicate the role of V2 and V3-toxins in pathogenesis of the *V. albo-atrum*. There was a positive correlation between the susceptibility of plants and sensitivity to the toxin (s). Dilution end point experiments indicated that detached leaflets from susceptible potato and tomato cultivars responded to a much lower amount of toxin(s) than tolerant ones. Similar effects were observed in other bioassays, cell death and ion-leakage. Indeed, some degree of specificity that was observed between susceptible potato and tomato cultivars and their related isolates also occurred in the

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bioassays. In the detached- leaf bioassay, 9µg/ml V2 and 11µgml V3-toxins (= ED 50) induced symptoms only in the leaflets of related plant species. Similarly, in tomato, leaf discs were only responsive to 0.69 µg/ml V2, but not to an equal amount of V3-toxin (s).

Notable included the following results, (1) Detection of nanograms of toxin(s) by the detached leaflets, and (2) the lack of response in several bioassays to an inactive toxin preparation. (3) The differential response of susceptible and tolerant tissues and cells to and (4) the selective response of susceptible potato and tomato leaflets to the related toxin(s) in the ED 50 region (s). (5) The higher amount of leakage of ions from tomato leaf discs in response to a specific amount the related V2-toxin (s) rather than the unrelated V3- toxin (s). All these indicate the role the metabolite (s) initially separated from crude culture filtrate by adsorption on the resin Amberlite XAD-4, in pathogenesis of *V. albo-atrum.*

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REFERENCES

1. Arntzen, C.J., Koeppe, D.E., Miller, R.J. and Peverly, J.H. 1973. The Effect of Pathotoxin of *Helminthosporium maydis* (race T) on Energy-linked Process of Corn Seedlings. *Physiol. Plant Path.* **3**: 79-89
2. Bhat, R.G., and Subbarao.1999. Host Range Specificity in *Verticillium dahliae. Phytopathology*, **89**: 1218-1225.
3. Daly, J. M., and Deverall B. J. 1983. *Toxins and Plant Pathogenesis*. Academic Press, New York. pp. 181.
4. Fletcher, P. 1972. Early-Dying *Verticillium* sp. Disease of Potato. *Ph. D. Thesis,* University of Wales. UK.
5. Gilchrist, D.G. and Grogan, R.G. 1976. Production and Nature of a Host –specific Toxin from *Alternaria alternata f.sp. lycopersici. Phytopathology.*, **66**. 165-171.
6. Kono, Y., Knoche, H. W. and Daly, J.M. 1981. Structure of Host–specific toxin. In: "*Toxins in Plant Diseases".* (Ed): Durbin, R. D. Academic Press. pp. 221-257.
7. Larkin, P. J. 1976. Purification and Viability Determination of Plant Protoplasts. *Planta*., **28**: 213-216.
8. Mansoori, B., Milton, M. and Smith, C. J. 1995. Isolation and Partial Purification of a Phytotoxin Related to pathogenic *Verticillium* species. *J. Phytopathology.,* **143**: 33-36.
9. Murashige T., and Skoog, F. 1962. A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Cultures. *Physiol. Plant.,* **15**: 473-497.
10. Mussel, H.W. 1972. Toxic Proteins Secreted by Cotton Isolates of *Verticillium albo-atrum*. In:*" Phytotoxins in Plant Diseases"*. Wood R. K. S., Ballio A. and Graniti, A. PP. 443-445.
11. Robert, M. C. and Boyce, B.C. 1972. Principles of Biological Assay. In: *Methods in Microbiology".* (Eds): Norris J.R. and Ribbons D.W. Academic Press. pp. 153-190.
12. Scheffer, R. P. 1983. Toxins as Chemical Determinants of Plant Disease. In: *"Toxins and Plant Pathogenesis".* (Eds): Daly, J. M., and Deverall B. J. Academic Press. pp. 1-34.
13. Scheffer, R. P. and Livingston, R. S. 1980. Sensetivity of Sugarcane Clones to Toxin from *Helminthosporium sacchari* as Determined by Electrolyte Leakage. *Phytopathology.,* **70**: 400-404.
14. Stoessl, A. 1981. Structure and Biogenetic Relations: Fungal Nonhost-specific.In *"Toxins in Plant Diseases"*(Ed) Durbin. R. D. Academic Press. pp.109-219.
15. Susnoschi, M., Krikun, J., and Zuta, Z.1975. Variety Trial of Potatoes Resistant to Verticillium Wilt. *American Potato Journal*., **52**: 227-231.
16. Tjamos, E. C, and Smith, I. M. 1975. The Expression of Resistance to *Verticillium albo atrum* in Monogenically Rresistant Tomato Varieties. *Physiol. Plant Pathol.,* **4**: 215-225.
17. Wheeler, H. 1981.Role in Pathogenesis.In: "*Toxins in Plant Disease".* (Ed): Durbin R.D. Academic Press. pp. 477-494.
18. Yoder, O. C. 1980. Toxins in Pathogenesis. *Annu. Rev. Phytopathol.,* **18**: 103-129.
19. Yoder, O. C. 1981. Assay. In:" *Toxins in Plant Disease."* (Ed): Durbin R. D. Academic Press. pp.45-78.

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نقش گیازهر بیماریزایی قارچ ورتیسیلیوم

ب. منصوری و سی. جی. اسمیت

چکیده

نتایج بدست آمده از اعمال چند روش زیست آزمون (Bioassays) نقش گیازهرهایی را با وزن مولکولی کم در بیماریزایی قارچ Verticillium albo-atrum به اثبات می رساند. موادی که با استفاده از نوعی رزین (Amberlite XAD-4) از عصاره 21 روزه کشت مایع قارچ بدست آمد و پس از عبور از نوعی ستون ژلاتینی (Biogel P-2) به خلوص نسبی رسید، قادر بود علائم زردی و نکروز را در برگچه‌های جدا شده ارقام حساس سیب‌زمینی و گوجه‌فرنگی مشابه علائم طبیعی برروی گیاه آلوده در آزمون برگ جداشده (Detached-leaf bioassay) ایجاد نماید. برگچه‌های ارقام متحمل حساسیت بسیار کمتری نسبت به ارقام حساس نشان دادند. در آزمون تراوش یونی (Ion leakage bioassay) میزان یون تراوش شده از بافت ارقام حساس بسیار بیشتر از متحمل بود. در آزمون مرگ سلولی (Cell death bioassay) درصد کمتری از سلولهای پینه (Callus) تشکیل شده از سیب زمینی رقم متیمل تحت تأثیر توکسین قرار گرفتند. در آزما یشات بیماریزائی بروز علائم در ارقام حساس نسبت به سویه‌های جدا شده از خود زودتر اتفاق افتادخ و شدیدتر بود. این رابطه (Specificity) در زیست آزمون ها نیز مشاهده شد، چنانچه مقدار معینی از گیازهر جدا شده از هر یک از سویه ها قادر بود تنها در برگچه‌های ارقام حساس میزبان خود عارضه زردی را ایجاد نمایند. این پدیده،در آزمون تراوش یونی نیز مشاهده شد.

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