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FINAL REPORT

PROJECT NO. I-603-83

Tolerance to Verticillium Wilt of Potato: the Role of a Phytotoxin

A. Nachmias, Y. Burstein, J.R. Davis, J.J. Pavek

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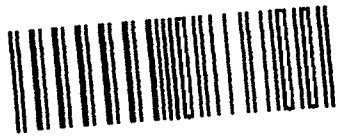

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BARD Research Proposal No. I-603-83

Title of Research Project:

Tolerance to Verticillium wilt of potato: The role of a phytotoxin

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Signature

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ABSTRACT

Verticillium dahliae, the causal agent of fungal wilt disease in many economically important crops in the U.S. and Israel, causes over 50% reduction in marketable yield of potato tubers in arid zones. In Europe and the U.S., breeding for genetically stable Verticillium disease tolerance is imperative because seed is exported to hot climates where V. dahliae thrives. We have defined an "Alpha-type" tolerance as those cultivars showing little or no reduction in yield of marketable tubers, despite colonization by the pathogen and appearance of visual foliar symptoms on the plant, presumably because they are immune to the effects of phytotoxins. Development of new commercial cultivars is not limited by the availability of new genotypes, but rather by the methods and time required for screening the improved varieties for desired commercial characteristics. Phytotoxins have a potential for selection of plants both for resistance in the field and to the toxin in controlled tissue culture conditions. The goal of this research was to improve the efficiency of existing resistance breeding programs by development of rapid laboratory methods for screening of new germplasm in a convenient, controlled environment, far from the breeding center and growing sites. This goal ultimately depends upon how readily a host-selective phytotoxin becomes generally available in a purified form. It is stressed that toxins cannot adequately screen for all types of resistance, but rather only for immunity to the toxin which results in disease tolerance in the field.

Our research program was aimed at investigating the role of a highly purified, chemically characterized pathogen-produced toxin in the pathogenesis of and tolerance to Verticillium wilt, and assessment of the toxin as a potential tool for screening of potato germplasm for "Alpha-type" tolerance to disease. During the course of this research, a low molecular weight phytotoxin of peptidic nature was purified from high molecular weight complexes produced in liquid culture of a potato isolate of V. dahliae, and from potato stems infected by the fungus. The toxin induced symptoms of wilt on potato leaflets obtained from disease susceptible cultivars, but not on leaves from tolerant cultivars, such as cv. Alpha. Thus we denoted this type of cultivar as having "Alpha-type" tolerance, rather than resistance to colonization by the fungus. The toxin purified from culture was very similar to its in vivo counterpart in molecular weight (1000 daltons), purification profile, amino acid composition, biological activity and antigenic cross-reactivity. Visible Verticillium wilt symptoms were observed when either peptide, in amounts as low as 20 ng (2×10^{-6} mol), was injected into detached potato leaves. The differential activity of the peptides on hosts and non-hosts of the fungus, as well as on susceptible and tolerant cultivars, indicated that this V. dahliae toxin shows host specific properties.

Comparison of infection rates of susceptible, tolerant, and resistant potato cultivars, as colony forming units (CFU), demonstrated that the susceptible cv. Nicola was already infected 68 days after planting, and reached a very high level of infection (315 CFU), whereas CFU were detectable in cv. Alpha only three weeks later and showed only minimal infection levels (42 CFU) at the end of the growing season. The resistant cv. Kondor and only 5 CFU very late in the growing season, when natural barriers to penetration become weakened. Severe foliar symptoms of unilateral chlorosis and necrosis were observed on cv. Nicola on day 70, while cv. Alpha, despite being infected at this time, showed

only moderate symptoms. Detection of the toxin in potato stems before extensive colonization occurred, using a a sensitive cytoimmunofluorescence assay (IFA), suggested that the toxin may be translocated in advance of the growing hyphae. IFA and bioassay of stem extracts showed that the amount of toxin increased with time and was present in the tolerant cv. Alpha, as well as in susceptible cultivars.

The data from this study indicated that some symptoms of Verticillium wilt disease are due to toxic effects and not to mechanical plugging of xylem vessels. The toxin may be involved in root stunting because it inhibited in vitro tomato root elongation. Moreover, the toxin reduced the viability of protoplast and caused losses of electrolytes from potato cells in suspension cultures from susceptible, but not from tolerant, cultivars. The exudate obtained from stems of cv. Alpha causes slightly less damage than that obtained from cv. Nicola. The low amount, later appearance and reduced toxicity of the toxin from cv. Alpha suggested that "Alpha-type" tolerance may be due to inhibition of toxin production or its alteration.

Evaluation of potato genotypes for responses to toxin and tolerance to Verticillium wilt in the field showed that in potato breeding programs, the toxin may be an important tool for screening new potato clones for the described "Alpha-type" tolerance. In Israel, tolerant lines which developed marked symptoms in response to infection showed no yield reduction and no response to the toxin, while susceptible lines with a good yield potential suffered a dramatic yield reduction and showed a positive response in the bioassay. The results in Israel indicated that yield reduction parallels sensitivity to the toxin rather than severity of disease symptoms. The highly purified toxin was more reliable than high molecular weight crude forms, because non-specific sensitivity to macromolecules giving false positive bioassay results was not observed. The use of the low molecular weight toxin precluded the necessity of screening the plants for sensitivity.

Results in Idaho confirmed the idea that this V. dahliae toxin is specific for "Alpha-type" tolerance. The cv. Alpha showed immunity to all forms of the toxin. It was concluded that other cultivars showing immunity to the toxin, while being colonized by the fungus, were indeed tolerant rather than susceptible. Resistance based on lack of colonization of the pathogen was not related to toxin sensitivity, therefore the toxin clearly cannot detect this type of resistance. In addition, the results in Idaho indicated that crude forms of the toxin, such as culture filtrates and acetone precipitated material, are not reliable for detection of disease susceptibility, probably due to the presence of materials other than the toxin that cause damage to plants.

D. OBJECTIVES

The objectives of this proposal were to: 1) investigate the role of a highly purified, chemically characterized pathogen-produced toxin in the pathogenesis of Verticillium wilt; 2) assess the toxin as a potential tool for screening of potato germplasm for "Alpha-type" tolerance to disease, in order to select tolerant early varieties with economically important characteristics; 3) investigate in the cv. Alpha the mechanism of immunity to the phytotoxin and its relation to tolerance to V. dahliae infection.

E. REPORT

E.1 INTRODUCTION

Verticillium wilt, caused by Verticillium dahliae Kleb. or Verticillium albo-atrum Reinke & Berth., is a major disease of economic importance in numerous crops, including tomato, potato, cotton, hops, alfalfa, tobacco and eggplant, causing yield losses of up to 50%. Disease symptoms of gradual chlorosis, followed by necrosis of leaf laminar tissue, occur at a relatively long range from the pathogen, suggesting that all or part of the wilt syndrome may be caused by a translocatable phytotoxin. It is now generally accepted that toxins play a major if not exclusive role in tissue necrosis.

In arid zones under irrigation, V. dahliae is the fungus attacking potato, and it may cause over 50% reduction in marketable yield of tubers. In Europe and in several areas of the U.S., breeding for Verticillium tolerance is of paramount importance because seed is exported to hot climates where V. dahliae thrives. V. dahliae elaborates in culture a phytotoxin, which in crude form, was found to induce disease symptoms when injected into many susceptible hosts.
In previous research we partially purified a phytotoxic peptidic fraction from an isolate of the fungus which is pathogenic for potato, and found that it is associated with production of disease symptoms in susceptible hosts. The toxin shows the same host-specificity as the pathogen and is absent from culture fluids of a non-pathogenic mutant strain of the fungus. Cytoimmunofluorescence studies demonstrated that it is present in infected potato tissue and is localized on the walls of the xylem vessels in tubers and stems.

Phytotoxins have a potential for selection of plants both for resistance in the field and to the toxin in controlled tissue culture conditions. Breeding for resistance to Verticillium wilt is a useful method of controlling the disease in most crops because most pesticide programs are very expensive. The bottleneck in the development of new commercial cultivars is not limited by the availability of new genotypes, but rather by the methods and time required for screening the improved varieties for desired commercial characteristics. The root-inoculation screening procedure is laborious, time consuming, restricted to those geographic locations where the disease exists and has slow symptom expression.

In several diseases where toxins play a major role in pathogenicity, disease resistant individuals have been identified by selecting out of a population those which are resistant to the toxin. This approach was used to select for resistance of potato to Alternaria solani and Rhizoctonia solani.

Many Verticillium toxin preparations show host specificity. Although apparent host specificity alone is not a sufficient criterion for a role in pathogenesis, Verticillium toxins that have been capable in bioassays of selecting plants that are both immune to the toxin and field-resistant have been used successfully to screen plants for disease resistance. It is important to differentiate between susceptibility to the fungus and susceptibility to disease. There are cultivars which, despite penetration and colonization by the fungus, do not appear to suffer from wilt disease presumably because they are immune to the effects of toxic agents. It has been suggested that this

form of resistance be referred to as "tolerance". It follows that phytotoxins cannot adequately screen for all types of resistance, rather only for tolerance to the toxin. We have found that, although not all resistant potato clones are immune to the toxin in a detached potato leaf bioassay, leaves of the tolerant cultivar, Alpha, do not react to injection of the toxin. We define tolerant cultivars as those which show no reduction in yield of marketable tubers, despite being infected by the pathogen and appearance of visual foliar symptoms on the plant. The results indicate that, in potato breeding programs, the toxin may be an important tool for screening new potato clones for the described "Alpha-type tolerance".

E.2 MATERIALS AND METHODS

E.2.1. Purification of the toxin from culture and infected stems: For one batch, 300 ml culture filtrate was concentrated under vacuum at 50 C and the proteins then precipitated with cold acetone and separated by gel filtration. Peak I was characterized as a protein-lipid-polysaccharide (PLP) complex which contained the majority of the phytotoxic activity. PLP was concentrated to ~1 ml, under vacuum at 50 C, and dialyzed for 48 h against 5% ethanol in distilled water at 4 C. Essentially all of the toxic activity was recovered from the material which passed through the dialysis membrane. The phytotoxic material, denoted peak I out (pIo), was concentrated to dryness by lyophilization. The dried material was reconstituted with 10 ml distilled water, concentrated to 0.5 ml in a Speed Vac Rotary Concentrator (Savant) and subjected to HPLC. The Vd-toxin was purified

at room temperature using a SpectraPhysics SP8750 HPLC system equipped with a Waters Associates Model 441 fixed wave length detector. The column effluents were monitored by uv absorbance at 214 nm and fractions were assayed for biological activity. Analytical HPLC was performed using a SpectraPhysics SP8100 LC and a SP8733 variable wave length detector at 210 nm. Aliquots (10 ul) of active fractions were analysed at high sensitivity (A210 = 0.04) at pH 1.8, using a linear gradient of 0-20% acetonitrile in 10mM sodium perchlorate/0.1% phosphoric acid. The toxin purified from infected stems, as described above, was chromatographed using the same system.

E.2.2. Detached leaf bioassay of toxin activity: Fractions from HPLC were monitored by a detached potato leaf assay. Briefly, the first true leaves of 3-6 week-old potato plants were excised under water and placed in vials containing 20 ml water. Test solutions (100 ul) were injected using a syringe and 22 gauge needle into the intercellular spaces of the leaves. The cuttings were placed under continuous illumination (approx. 7000 lx) for 18-48 h at room temperature, after which the extent of chlorosis and necrosis, beginning at the point of injection, was recorded.

E.2.3. Root culture bioassay: Tomato roots were excised under water and placed into 50 ml flasks containing White's medium and toxin (100 ug-1.0 mg ml-1). The flasks were incubated on a rotary shaker at 2 C in the dark. Root growth was measured after 48 h.

E.2.4. Ion Leakage: Ten leaf disks, 9 mm diameter, were gently washed with double distilled water (DDW). Disks were treated with toxin or control preparations for 2 h on a rotary shaker at 50 rpm, washed again gently with 10 ml DDW and transferred to a 10 ml flask containing DDW, average 2.5 uMho. Electrolyte leakage was measured over a period of 18 h with a conductivity meter. Potassium content of the fluid was measured by a flame photometer.

E.2.5. Molecular weight determination: Molecular weight of the toxins was determined by high performance gel permeation chromatography using an Ultropac TSK-G2000SW Blue column, 7.5 x 300 mm (LKB, Sweden) and by amino acid composition. Molecular weight calibration standards included cytochrome c (12500), insulin (5700), glucagon (3500) and bacitracin (1450).

E.2.6. Amino acid composition analysis: Samples were dried in a Speed Vac Rotary Concentrator (Savant Instruments Farmingdale, NY), hydrolyzed in 6N HCl under vacuum at 110 C for 22 h and analysed with a Biotronik Model LC6001 automatic amino acid analyzer.

E.2.7. Colony forming units (CFU) from plants: One cm of stem, 5 cm above the soil level, was surface sterilized in 1% NaOCl. Three segments were suspended in 5 ml sterile water, homogenized with an ultraturax for 1 min at 4 C; 0.1 ml was plated onto selective media (0.2% sorbose and streptomycin) and incubated at 27 C for 12-14 d in the dark.

E.2.8. Sources of plant and fungal materials: Verticillium-free seed tubers of the potato cv. Blanka, were imported from Holland. Progeny of a cross between potato cvs. Alpha x Maris Piper were kindly supplied by Dr. Brian Costelloe, Loughgall, Ireland. For field experiments, tubers were planted by hand in Verticillium-infested or non-infested soils on 20 February 1982, at the Gilat Experiment Station, Negev, Israel. Verticillium disease symptoms were recorded on 3 June 1982 and the tubers were harvested 12 days later. The yield was calculated as total weight of marketable tubers (tubers >60 g) per plant.

The pathogenic isolate of V. dahliae (G1) and the non-pathogenic mutant (V297), used in an earlier study were used for this investigation. The methods used for culturing the fungus were the same as previously described.

E.3. RESULTS

E.3.1. Purification of the toxin from culture: The highly purified Vd toxin elicited specific symptoms of chlorosis followed by necrosis 48-60 h following injection into leaves from a disease-susceptible cultivar, Nicola. No symptoms were elicited in leaves from the tolerant cultivar, Alpha. The appearance of necrosis was concentration dependent and toxin dilutions which elicited chlorosis did not always cause necrosis. Rapidly appearing necrosis (~24 h), necrotic spots and late-appearing symptoms (after 60 h) were considered to be non-specific. Phytotoxicity of the highly purified fractions was abolished by incubation with protease (Pronase P, Sigma type VI), but not by

treatment with sodium metaperiodiate.

As a first step, pIo was loaded onto the HPLC column and was eluted at a flow rate of 1 ml min-1, using a linear gradient of 0-6% n-propanol (Merck) in 0.1% trifluoracetic acid (TFA). A broad zone of specific phytotoxic activity, showing a prominent peak of absorbtion at 210 nm, eluted at a retention time of ~29-31 min (Fig. 1a). Fractions eliciting non-specific reactions on cv. Nicola and/or positive reactions on cv. Alpha were not investigated further. When 10ul aliquots of VT-1 preparations were subjected to analytical HPLC, as described in Materials & Methods, most batches showed the presence of one major peak, eluting at ~23 1 min (Fig. 2a). The active fraction, denoted VT-1, was separated on the same column, using a gradient of 0-10% acetonitrile in 50mM triethylamine formate, pH 7, and toxic activity eluted at ~13-14 min (Fig. 1b). When the active fraction, denoted VT-2, was desalted on the same column, using a gradient of 0-10% acetonitrile in 0.1% TFA, a double peak, designated VT-3, was resolved (Fig. 3a). The amino acid composition and molecular weight of each half of the peak was identical. The major peak of VT-3, denoted "A", was rechromatographed and purified to homogeneity using a gradient of 2-10% acetonitrile in the same buffer (Fig. 3b). When injected separately under the latter conditions, Peaks "A" and "B" showed identical elution profiles; analytical HPLC showed the presence of a single, sharp peak for each, which eluted at ~23 min (as in Fig. 2). High performance gel permeation chromatography of the two peaks, using a calibrated column of TSK-SW2000, showed that each eluted at the same position as a single, sharp peak, with a molecular size less than that

Figure 1a.

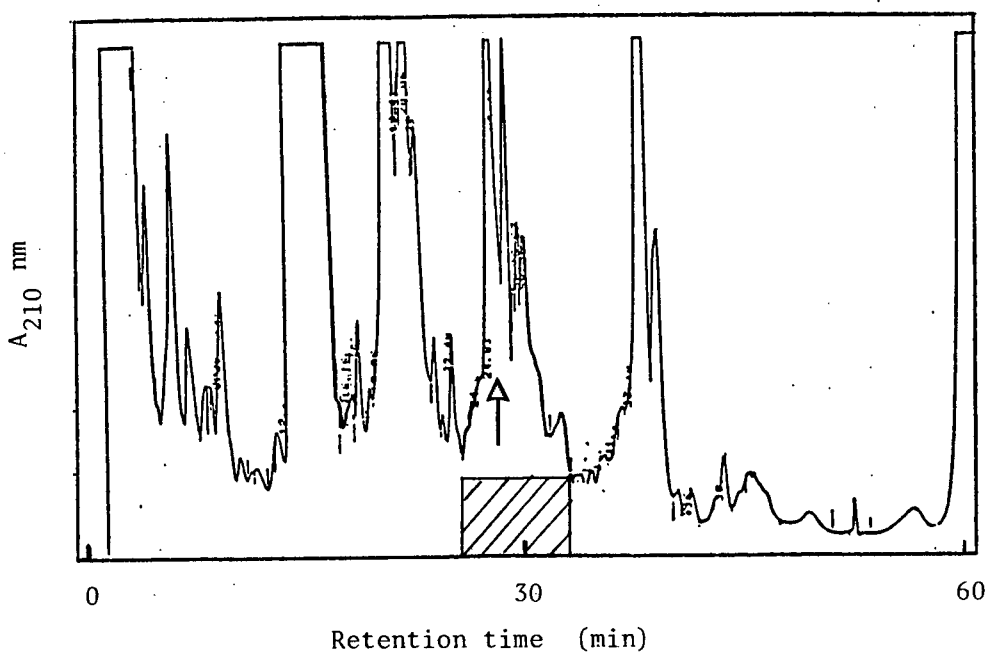


Figure 1b.

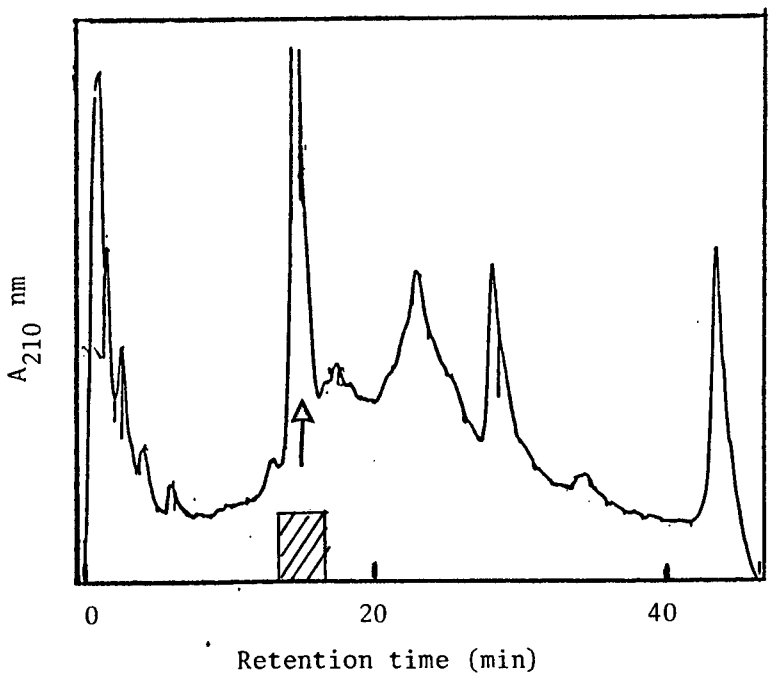


Figure 2a.

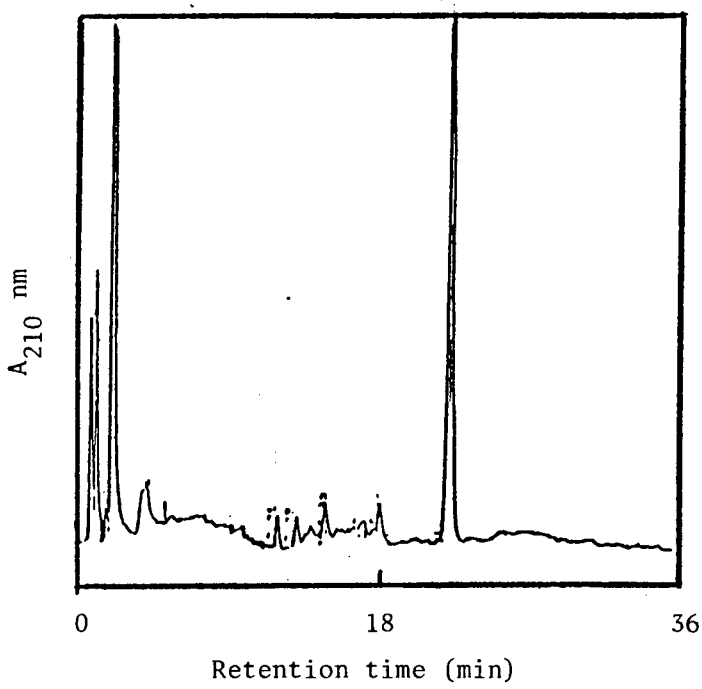


Figure 2b.

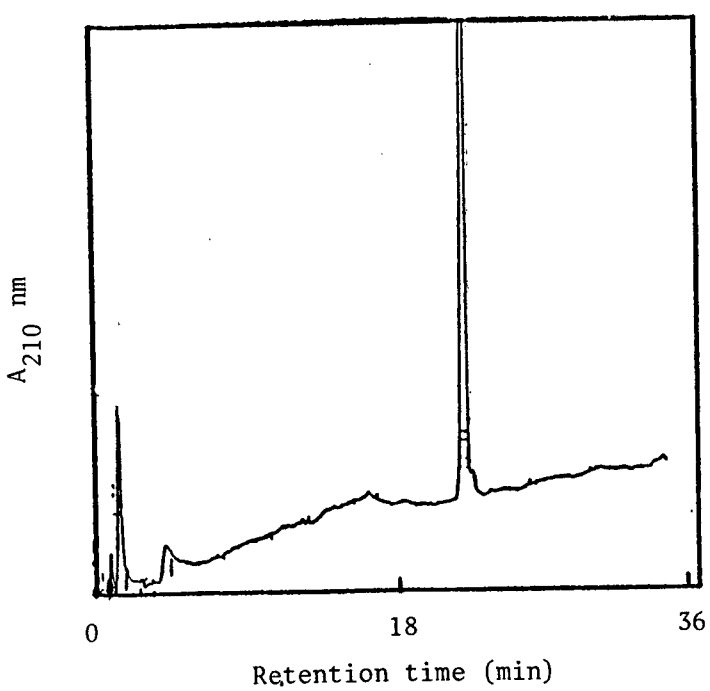
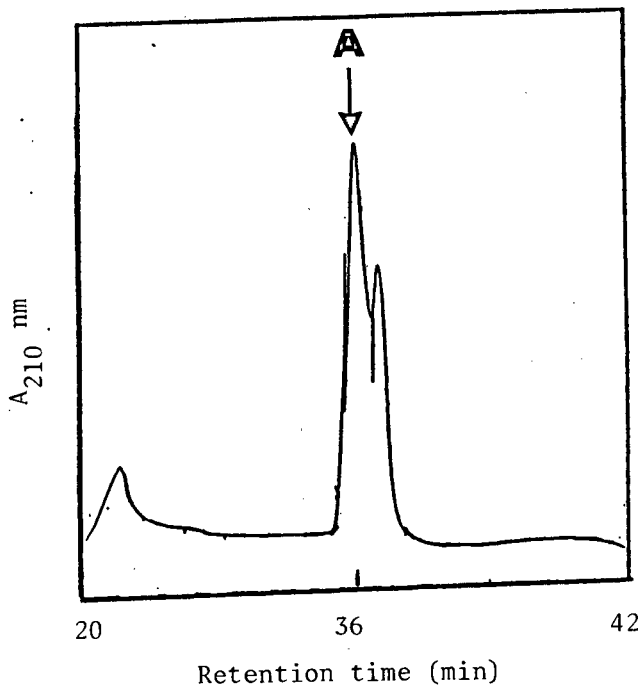


Figure 3a.



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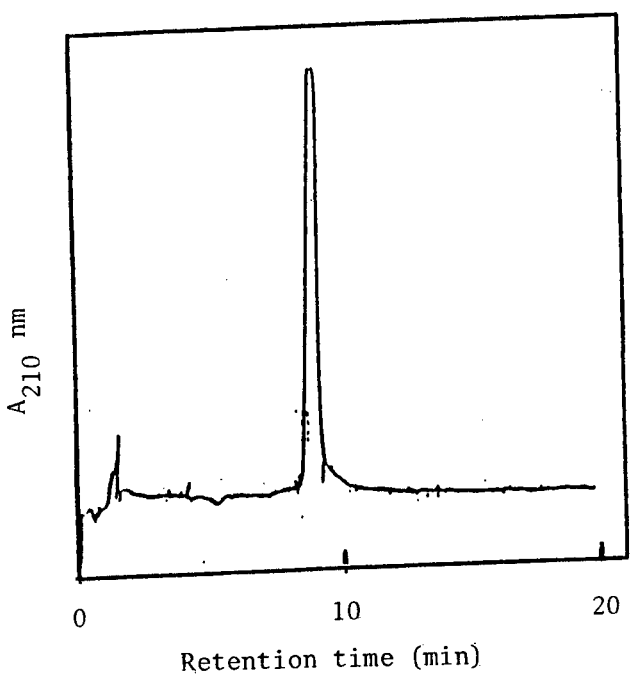


Table 1.

Amino acid compositon of phytotoxins purified from culture fluids or potato stems infected by Verticillium dahliae

Amino acid	Culture		Infected Stems	
	pmol[a]	Residues[b] (no.)	pmol	Residues (no.)
Aspartic acid	187	2	99	2
Threonine	70	1	70	1
Serine	141	1	87	1
Glutamic acid	186	2	118	2
Proline	ND[c]	ND	ND	ND
Glycine	180	2	104	2
Alanine	144	2	93	2
½Cysteine	ND	ND	ND	ND
Valine	81	1	51	1
Methionine	0	0	0	0
Isoleucine	47	0.6	31	0.5
Leucine	119	1	51	1
Tyrosine	99	1	19	0
Phenylalanine	93	1	36	1
Lysine	30	0	20	0
Histidine	23	0	17	0
Arginine	ND	ND	ND	ND

[a] Picomoles detected.

[b] For estimation of residues, valine arbitrarily chosen as 1.

[c] ND = not determined by this procedure.

of the marker Bacitracin (MW = 1450 daltons).

Dr. Nachmias, in the laboratory of Dr. N. Keen at Riverside, CA, undertook large-scale production of Peak I from Dvir 1 and from various tomato isolates of V. dahliae Race 1 and Race 2, from which we isolated fractions with similar amino acid composition and identical biological activity on detached potato leaves. Amino acid composition of the toxic fractions is shown in Table 1.

A sample of the fraction purified from Dvir I was submitted to microsequence analysis by automated Edman degradation (Gas-phase sequenator, Applied Biosystems, Foster City, CA), which can analyze as little as 20 pmols of sample. The material was not degraded by this method and eluted as one large peak, indicating that the material required further treatment before it can be sequenced. For this purpose, we attempted degrade a sample of the toxin by mild acid hydrolysis. using 6N HCl for 2, 4, 6, 8, 9 and 10 min. at 100 C. HPLC of the hydrolysate revealed that several amino-acid containing fractions were recovered, suggesting that this method might be used to prepare the material for sequencing. Large quantities of crude toxin will be required in order to purify enough material for further chemical characterization. Large-scale production will require special facilities which at present are not available.

Until now, investigations of the wilting phenomenon in Verticillium spp. have been performed using products from in vitro cultures where the pathogen is growing under conditions quite different from those in the vascular system of the host. However, in order to correlate in vitro products with in vivo events, it is important to demonstrate

the presence of the toxin in the vascular tissues of infected plants. A substance produced by the pathogen while growing in the host, which is produced in sufficient quantities to cause production of disease symptoms and is absent from healthy plants has been termed a "vivotoxin". The isolation of a vivotoxin from a diseased plant may be difficult if the toxin is labile or if very low dosages are effective or binding of it occurs to sites of action from which it cannot be liberated without destruction. In order to determine whether the antigenic material that we detected in infected tissue and the toxin produced in culture are the same, we purified phytotoxic peptidic fractions from V. dahliae culture fluids and plants infected by the fungus and compared their physico-chemical properties and biological activities.

E.3.2 Purification of the toxin from V. dahliae infected potato stems: V. dahliae-infected and healthy potato plants were harvested at various times during the growing season. Leaves were removed and discarded and the stems were cleaned from debris then cut into 10 cm lengths. The pieces of stem were then centrifuged at 2500 rpm at 4 C in a Sorvall R3 centrifuge. The fluid which accumulated at the bottom of the bottles (~100 ml/10 Kg stems) was collected, passed through a 0.45u sterilization filter unit (Nalge, Sybron, Rochester, NY) and frozen at -20 C until use. The extract was thawed and concentrated under vacuum at 50 C. Initially, the extract was subjected to agarose gel filtration, as described for culture fluids, omitting the acetone precipitation step. However, in contrast to the elution pattern

observed for the toxin from culture (Fig. 4a), essentially all of the toxic activity was recovered in a fraction which eluted at a position of <10,000 daltons MW (Fig. 4b); therefore, as a first step, the remaining extracts were simply dialyzed for 60 h against water, with three changes of the dialysis fluid. Two liters of the diffusate (exudate outer, designated "EXo") were concentrated to dryness by lyophilization. The dried material was reconstituted with 10 ml distilled water, concentrated to ~2 ml in a Speed Vac Rotary Concentrator (Savant) and 0.5 ml aliquots were subjected to HPLC, using the same steps as described for the toxin from culture.

Fig. 5a shows a representative HPLC profile of a separation of EXo, using a gradient of 0-10% 1-propanol in 0.1% TFA, at a flow rate of 0.5 ml-1. The fraction containing the majority of toxic activity eluted at 63 min and was designated VTX-1. This fraction was rechromatographed on the same column, using a 30 min gradient of 2-30% acetonitrile in triethylamine formate, pH 7, at a flow rate of 1 ml-1. Fig. 5b shows that the toxic activity eluted at ~20 min; when this fraction, VTX-2, was desalted as described above, a double peak was resolved (Fig. 6a), similar to that observed in the toxin from culture (Fig. 3a). Purification to homogeneity (VTX-3) was achieved by rechromatography of peak A, using a gradient of 10-25% acetonitrile in 50mM triethylamine formate, pH 7, at a flow rate of 0.5 ml/min (Fig. 6b). Analytical HPLC of this fraction showed that the retention time was identical to that of the toxin from culture (Fig. 2b) and amino acid compositions of the peptide fractions were very similar (Table 1).

14A

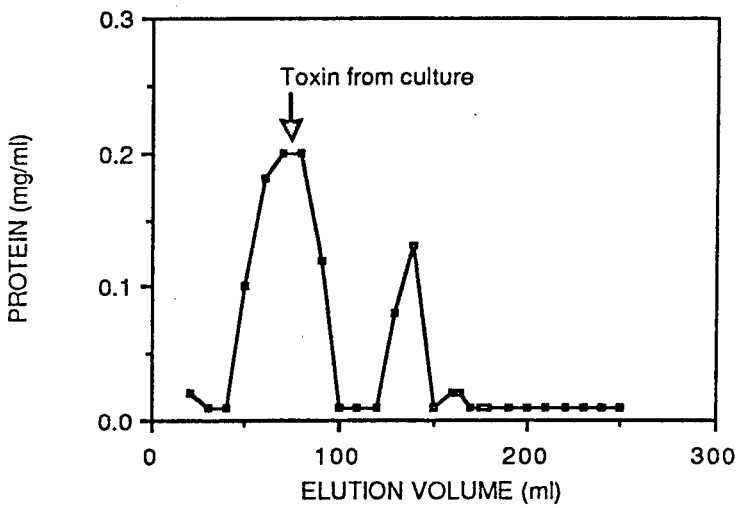


Figure 4a.

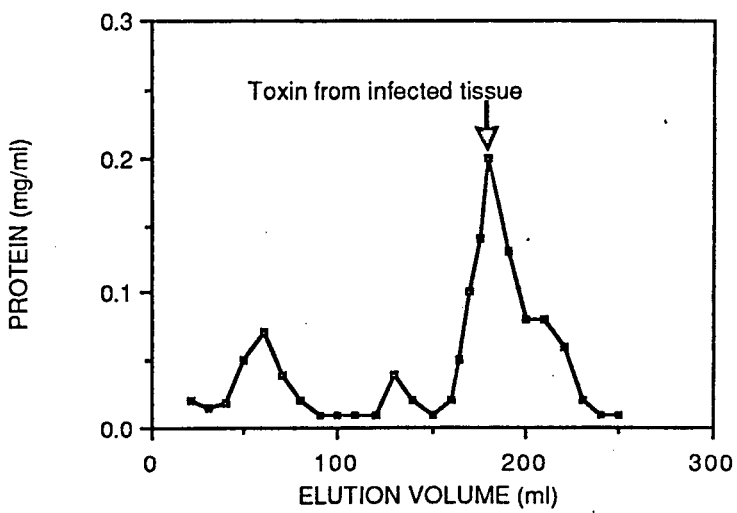


Figure 4b.

Figure 5a.

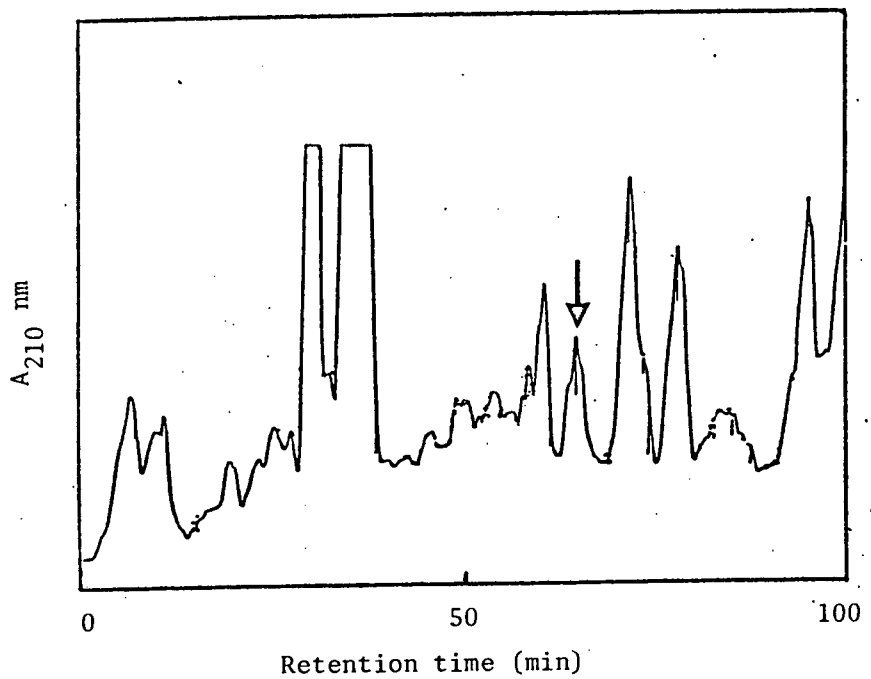
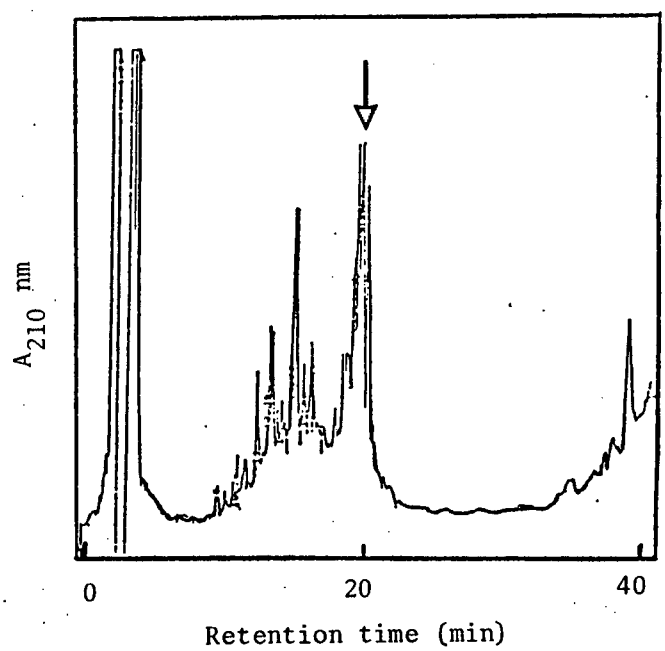


Figure 5b.



14c

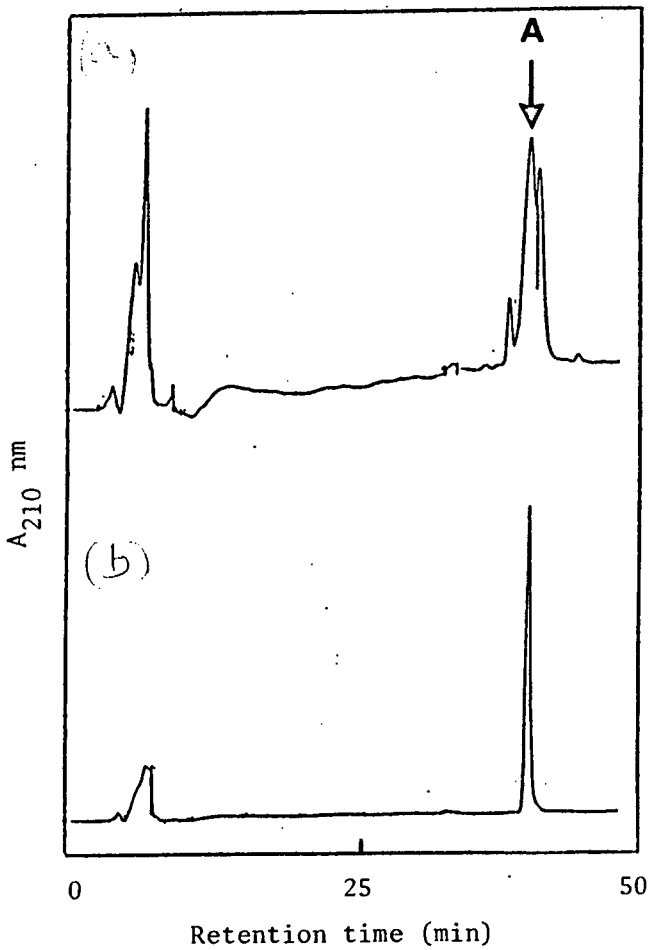


Figure 6.

E.3.3 Host-specificity of the toxins from culture and infected plants. The highly purified fractions VT-3 and VTX-3, at a concentration of 200 ng ml-1 were toxic for leaves from disease-susceptible potato varieties but not for those from tolerant plants (Table 2). Differences between reactions on susceptible and tolerant cultivars were assessed using a toxin concentration at which a known field tolerant cultivar, Alpha, did not show sensitivity.

E.3.4 Uptake of the purified toxins into detached potato leaves: When detached potato leaves were placed in microwells containing 100ul (~1ug ml-1, assessed by amino acid composition) of toxin partially purified from culture (VT-1), symptoms of wilt were induced that were similar to those observed on Verticillium-infected leaves. Infected leaves first exhibit unilateral interveinal chlorosis and later, marginal necrosis, in that order. Excised leaves taking up solutions of either toxin showed specific symptoms of unilateral interveinal chlorosis between 48-60 hours, usually followed by marginal necrosis.

E.3.5 Investigations on the mode of action of the low molecular weight fraction (pIo) of the vd toxin.

E.3.5.1 Effect on potato cell suspensions of toxins from culture and different cultivars: Cell suspensions of potato, cv. Russet Burbank (supplied by Dr. Pavek) were established by placing sterile leaves and stems into liquid MS medium containing 2,4D, 5 mg/L. Undifferentiated callus material that developed was transferred 3 times on solid medium followed by suspension in liquid medium on a rotary shaker. Cells

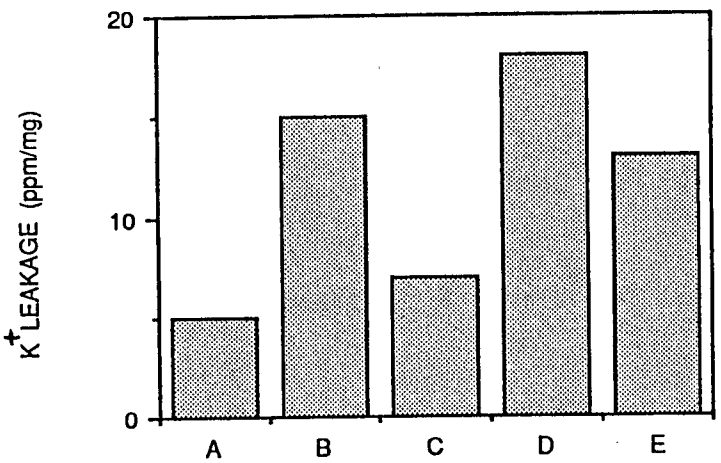


Figure 7a.

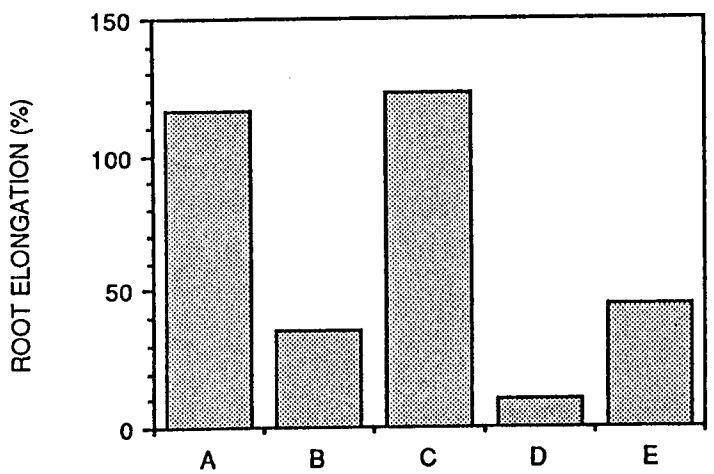


Figure 7b.

(5x103.) in 200ul were transferred to wells of Microtiter plates, sterile toxin solution was added, and after incubation for 4-6h at room temperature, sodium fluorescein was added. After 30 min at room temperature, the medium was removed, the preparations were rinsed with fresh medium and cells were examined under a UV microscope; cells which failed to fluoresce were assumed to be non-viable (Fig. 1). Table 4 shows that at concentrations of 10 ug ml-1, the in vitro- and in vivo-produced toxins reduced equally well the viability of Russet Burbank cells, but the exudate from cv. Alpha caused slightly less damage than that obtained from cv. Nicola.

E.3.5.2. Ion leakage of toxin treated tissue: Leakage of electrolytes from leaf disks is one parameter used for measuring cell membrane damage. Electrolyte leakage from leaf disks of cv. Blanka treated with toxin preparations from culture or infected tissue was greater than that from untreated leaf disks or those treated with exudate from healthy tissue (Fig. 7a). Increased ion leakage could be detected in disks as early as 4 h following initiation of treatment. Ion leakage from treated leaf tissue increased with time and increasing concentration of toxin.

E.3.5.3. Effect of the toxins on elongation of tomato roots: Fig. 7b shows that toxin from culture (pIo) inhibited the elongation of cultured tomato roots obtained from a susceptible variety (ve), Hosen-Eilon, but not those obtained from the resistant (Ve) cv. VF-134. The exudates from stems of V. dahliae-infected cv. Nicola inhibited to

same extent. The minimum amount of toxin required to cause inhibition was 100 ug ml-1 protein, while maximal inhibitory activity was achieved using about 10-fold higher concentrations.

E.3.5.4. Time-course study of appearance of wilt symptoms, V. dahliae colony forming units (CFU) and serological evidence of toxin in different infected cultivars: We compared the infection rate of susceptible, tolerant and resistant potato cultivars as colony forming units (CFU)/cm stem, 5 cm above soil level. The results are shown in Table 3. The susceptible cultivar, Nicola, was already infected at 68 days after planting, whereas CFU were detectable in the tolerant cv. Alpha only three weeks later. At the end of the growing season, 96 days after planting, cv. Nicola reached a very high level of infection (315 CFU), while cv. Alpha showed only minimal infection levels (42 CFU). Cv. Alpha did not reach a high infection level and cv. Kondor had only 5 CFU very late in the growing season, when natural barriers to penetration had become weakened. Table 4 shows that on cv. Nicola, severe symptoms of unilateral chlorosis and necrosis were observed on day 70, while cv. Alpha, despite being infected at this time, showed only moderate symptoms. An indirect immunofluorescence assay (IFA) using antiserum to pIo, performed on pieces of infected potato stems, demonstrated that the low molecular weight toxin was detectable in at least one bundle when the infection level was still extremely low (2 CFU) and before the toxin could be extracted in amounts sufficient to elicit specific symptoms in the bioassay. Both IFA of stems and bioassay of stem extracts showed that the amount of Vd toxin increased

Table 3.

Time-course study of appearance of wilt symptoms, Verticillium dahliae colony forming units (CFU) and serological evidence of toxin in infected cultivars

		TOXIN			FUNGUS	
Cultivar (day)[d]	Symptoms[a]	Bioasssay[b] (titer)	Serology[c] Double Diffusion	IF *in situ*	Isolation Saboraud	CFU
Nicola (susceptible)						
35	0	Negative	Negative	Negative	Negative	0
50	0	Negative	Negative	Negative	Negative	0
55	0	Negative	Negative	+	Negative	2
65	1.0	1:2	Negative	+	Negative	5±1
75	2.5	1:8	Positive	++	Positive	129±4
85	3.0	1:64	Positive	+++	Positive	315±14
90	4.0	1:1024	Positive	+++	Positive	270±11
Alpha (tolerant)						
35	0	Negative	Negative	Negative	Negative	0
50	0	Negative	Negative	Negative	Negative	0
55	0	Negative	Negative	Negative	Negative	0
65	0	Negative	Negative	+	Negative	0
75	0	1:2	Negative	+	Negative	6±1
85	1	1:2	Positive	+	Positive	42±5
95	1.5	1:8	Positive	++	Positive	12±10
100	2.0	1:64	Positive	+++	Positive	34±9

[a] Disease rating: 0 = no symptoms; 4 = plant dead.

[b] The bioassay was performed by injecting 100μl extract into detached leaves from a susceptible cv. Blanka. Results expressed as highest dilution causing chlorosis and necrosis recorded 24-48 h following injection.

[c] Serology was performed using antiserum to pIo (see text).

[d] Day following planting

with time and was present in tolerant as well as in susceptible cultivars. Since the water content of different stems was not constant, it was not possible to calculate toxin yield; nevertheless the amount of toxin in an equivalent amount of stems appeared to be slightly lower in cv. Alpha than that detected in the susceptible cv. Nicola and appeared 10 days later.

E.3.5. Evaluation of potato genotypes for response to toxin and tolerance to Verticillium wilt:

E.3.5.1. In Israel:

The high molecular weight PLP and the low molecular weight fractions derived from it, pIo and Fr9, were phytotoxic to leaves of potato and other host plants but not to leaves of non-host plants (Table G1). Wheat, a non-host plant, showed non-specific sensitivity to PLP, but not to pIo or Fr9. Table G2 shows the reactions of leaves of a representative sample of progeny of lines from a cross between the tow potato cvs. Alpha and Maris Piper. The reactions of the lines to PLP and pIo were identical, except for lines 20, 30, 122, 135 and 205 which gave a positive reaction to the PLP fraction from the mutant strain V297 thus indicating non-specific sensitivity to the high molecular weight PLP fraction. Because of this non-specific reaction the five lines were excluded from the examination of relationships between disease reaction and reaction to toxin. Line 259 is typical of a resistant line, showing very few visible symptoms to infection or to toxic fractions PLP or pIo and almost no reduction in yield. Line 252 is typical of a tolerant line, which, despite the development of marked

Table G1. Specificity of Verticillium dahliae toxic fractions obtained from the different stages of purification

		Bioassay symptoms[b]			
	Disease index[a]	Control	PLP	pIo	Fr9
Host plants					
Potato					
cv. Blanka	+ +	-	+	+	+
cv. Alpha	±	-	-	-	-
Tomato					
cv. Hosen Eilon	+ +	-	+	+	+
cv. VF-134-1	±	-	-	-	-
Peanut	+ +	-	+	+	NT
Melon	±	-	+	NT	NT
Watermelon	+ + +	-	+	+	+
Eggplant	+ + +	-	+	+	+
Pepper	+ +	-	+	+	NT
Chinese cabbage	+ +	-	+	NT	NT
Olive	+ +	-	+	+	+
Avocado	+ +	+	+	NT	NT
Almond	+ +	-	+ +	+	NT
Non-host plants					
Wheat	0	+	+	-	-
Corn	0	-	-	-	NT
Pea	0	-	-	NT	NT
Bean	0	-	-	NT	NT
Citrus					
Lemon	0	-	-	-	-
Sour orange	0	-	-	-	-
Volka mariana	0	-	-	-	-

a Disease index: ± chlorosis; + + + - plant dead.

b The bioassay was performed by injecting 200 ul of test solution containing 0.1 mg of PLP, 0.4 mg of pIo or 0.6 ug Fr9. Symptom production: +, chlorosis and necrosis recorded 18-36 h following injection; -, no reaction. NT = not tested. Control = V297 PLP.

c Tolerant variety (infected by V. dahliae but no reduction in yield).

Table G2. Reactions of some progeny from a cross between cvs Alpha x Maris Piper to infection by Verticillium dahliae and to toxic fractions obtained from culture filtrates

Progeny	Marketable yield of tubers (kg/plant) Control	Infected	Disease[a] rating	Reaction to toxin fractions, in the bioassay[b] Control	PLP	pIo	Relation of toxin reaction to yield
4	2.35	1.00	1.5	-	-	-	N
13	1.70	1.70	1.5	-	-	-	P
18	1.90	1.30	2.0	-	+	+	P
20	0.18	0.10	1.5	+	+	NT	O
25	2.10	1.80	1.0	-	-	-	P
28	1.50	1.00	1.5	-	-	-	N
30	0.40	0.18	1.5	+	+	NT	O
36	1.15	1.15	2.0	-	-	NT	P
86	2.00	1.50	1.5	-	-	-	P
114	0.90	1.15	1.5	-	-	NT	P
122	1.00	1.00	2.0	-	-	-	P
129	1.45	0.35	3.0	-	+ +	+	P
133	1.43	1.20	1.5	+	+	NT	O
135	1.25	1.00	1.5	+	+	NT	O
140	2.40	1.00	1.5	-	+	NT	P
141	1.72	1.40	2.5	-	+ +	+	N
165	2.15	1.60	2.0	-	+	NT	P
205	1.10	1.10	2.0	+	+	-	O
211	1.28	1.00	1.5	-	-	-	P
214	1.17	0.90	2.5	-	+	+	N
220	1.30	1.05	2.5	-	+	+	N
221	1.25	1.00	2.5	-	+	+	N
222	1.70	0.80	2.5	-	+	+	P
225	1.40	0.90	1.5	-	+ +	+	P
230	1.30	0.70	1.5	-	+	+	P
231	2.10	1.05	1.5	-	+	+	P
246	1.10	0.50	3.0	-	+ +	+	P
247	2.00	1.35	2.0	-	+	+	P
249	1.30	1.00	1.0	-	-	-	P
251	1.80	0.95	3.0	-	+ +	+	P
252	2.60	2.60	2.5	-	-	-	P
259	2.10	2.00	1.0	-	-	-	P

[a] Disease rating: 0 = no symptoms; 4 = plant dead.

[b] The bioassay was performed by injecting 200 ul of test solution containing 0.1 mg of PLP, 0.04 mg of pIo or 0.6 ug Fr9. Symptom production: +, chlorosis and necrosis recorded 18-36 h following injection; -, no reaction. NT = not tested. Control = V297 PLP.

[c] P = correlated, N = not correlated, O = not included.

symptoms in response to infection, showed no yield reduction and no response to the toxin, while line 129 is a good example of a line with a good yield potential but which is very susceptible to Verticillium wilt in the field and suffers a dramatic yield reduction in consequence. Out of 32 lines, 21 showed toxin reactions which paralleled yield (either a positive reaction and reduced yield or a negative reaction with no yield reduction), six did not show such a relationship and five were not included.

E.3.5.2. In Idaho:

A. Evaluation of additional potato genotypes (mainly commercial cultivars) for resistance to Verticillium wilt, degree of stem colonization by V. dahliae and response to crude toxin preparation:
Continuing the investigation of the response of the potato genotypes to the crude toxin preparation as related to the genotypes' level of resistance to V. dahliae, 24 potato cultivars and advanced selections were evaluated during 1987.

The resistance of the genotypes to V. dahliae was evaluated in a naturally infested soil (with a history of Verticillium wilt incidence) in a randomized block design with four replications. Each plot consisted of a row of 35 plants at 10" spacing. Slopes of severe wilt (75% of stem with wilt symptoms) incidence (%) were calculated from the data on 85, 113, 117 and 126 days after planting (x = days after planting; y = mean severe wilt incidence). The degree of colonization of V. dahliae in the stem apices (7.6 cm) was verified by the method of Davis et al. (1983).

The sensitivity of each of these genotypes to the crude toxin preparation from strain V-5RI was verified following the method of Nachmias et al. (1982).

The results presented in Table I1 show:

1. Among the genotypes evaluated, Superior, Russet Norkota, NDA8694-3 and Norland were the most susceptible and the selection A66107-51 was among the most resistant to Verticillium wilt.

2. The degree of colonization of apical stem tissue by V. dahliae was highly correlated ($P = 0.001$) with the slope of severe wilt incidence ($r = 0.86$) and percent incidence of severe wilt at 117 days after planting ($r = 0.84$).

3. That genotypic factors other than resistance to Verticillium wilt were also involved in determining yield.

4. Sensitivity to V. dahliae toxin did not have any consistent relation to the wilt reaction of the genotype. All the four genotypes most susceptible to Verticillium wilt (Superior, Russet Norkota, NDA 8694-3, Norland) were not sensitive to the toxin as were two (TC 582-1, BR7093-24) of the most resistant genotypes. On the other hand, some of the most wilt resistant genotypes (ex. A7805-8, A7816-14, A66107-51) were sensitive to the toxin.

B. Verticillium wilt resistance and response to toxin of selected wilt relatives of potato

Five non-cultivated tuber-bearing species of Solanum (Corsini et al., 1988) related to cultivated potato and one of S. tuberosum, all of them raised as seedlings from true seed, were evaluated in the greenhouse for resistance to Verticillium wilt, in a randomized block

with five replications. Wilt severity was evaluated periodically using a 0-7 scale. Leaves from additional plants of each of these species, along with check genotypes (Russet-Burbank, Alpha, NDA8694-3 and A66107-51), were tested for sensitivity to the V. dahliae toxin.

The results presented in Table I2 show that:

1. S. chacoense, S. sparsipilum and S. tarijense are the most resistant of the six species tested, while S. tuberosum, and S. demissum were the most susceptible.

2. There was no relation between Verticillium wilt resistance and response to toxin of these species.

Table I1. Response of potato genotypes of Verticillium dahliae and its toxin. Aberdeen, 1987

Genotype	Slope x 10 of Severe Wilt 1/	Severe Wilt Incidence (%) 117 days After Planting	Log cfu V. dahliae/g apical Stem Tissue 2/	Yield (t/ha)	Sensitivity to Toxin
Superior	26.9 A 3/	90.0 A 3/	4.01 A 3/4/	15.2 I 3/	-
Russet Norkota	26.7 A	86.5 A	4.00 A	29.9 EF	-
NDA8694-3	26.6 A	87.6 A	3.87 A	26.7 FG	-
Norland	25.9 A	75.6 B	4.07 A	32.1 E	-
Centennial Russet	23.2 B	60.1 C	3.89 A	22.2 H	-
Norchip	22.2 B	55.1 C	4.12 A	29.3 EF	-
Red LaSoda	22.1 B	57.3 C	3.54 ABCDE	36.2 BCD	+
Buntje	19.9 C	56.9 C	3.65 ABC	36.7 BCD	-
Shepody	19.9 C	46.2 D	3.81 AB	37.9 B	-
Lemhi Russet	17.8 D	43.0 D	3.80 AB	37.5 BC	-
Russet Burbank	17.3 D	45.0 D	2.81 F	33.2 CDE	+
Atlantic	14.4 E	34.5 E	3.60 ABCD	24.6 GH	-
Monona	13.1 EF	20.4 GF	2.57 FG	31.6 E	-
Norking	12.5 F	24.8 FG	2.17 G	36.1 BCD	-
White Rose	12.4 F	29.7 EF	3.19 BCDEF	49.7 A	-
Nooksack	11.0 G	29.4 EF	2.97 DEF	29.4 EF	-
Sangre	6.5 H	12.6 I	2.05 G	39.9 B	+
NDA1725-1	6.2 H	19.7 GH	2.89 EF	31.7 E	-
Red Pontiac	6.1 H	17.4 HI	3.07 CDEF	39.9 B	NT
A66107-51	1.7 I	0.0 J	0.00 I	46.1 A	+
BR7093-24	1.0 I	2.4 J	1.98 G	36.6 BCD	-
A7816-14	0.8 I	2.4 J	2.07 G	39.3 B	+
TC582-1	0.6 I	0.0 J	1.18 H	32.9 DE	-
A7805-8	0.6 I	0.0 J	0.73 H	36.9 BCD	+

1/ Slope calculated from arc sine % stems dead or nearly dead (75% with wilt symptoms) at 85, 113, 117, and 126 days after planting (x: days after planting; y: mean percent stems with severe wilt).
2/ Stems collected 31 Aug - 2 Sept by replicate
3/ Different letters denote differences to 0.05 P level
4/ Log cfu were highly correlated (0.001 P level) with both slope and wilt incidence - r-values = 0.86 & 0.84, respectively.

Table I2. Response of some non-cultivated tuber bearing species of Solanum (related to potato) to Verticillium wilt and V. dahliae toxin

Genotype	Reaction to V. dahliae*	Sensitivity to Toxin
S. berthaultii (PI265858)	2.3	+
S. chacoense (PI473402)	0.9	-
S. demissum (PI186561)	4.9	-
S. sparsipilum (PI311000)	1.0	-
S. tarijense (PI473228)	1.3	-
S. tuberosum (PI234585)	4.1	-
Russet Burbank	NT**	+
Alpha	NT	-
NDA8694-3	NT	-
A66107-51	NT	+

* Mean of 5 reps on a 0 (no disease) to 7 (severe symptoms) scale, 57 days after inoculation

** NT = Not tested

Legends to Figures

Fig. 1. HPLC profiles of purification steps 1 and 2 of the phytotoxin from V. dahliae culture fluids. Reverse-phase HPLC of pIo (see text) on a Nucleosil-5μ column (4 x 200mm); the sample was injected via a 500 μl sample injection loop and eluted using a linear gradient of 0-6% n-propanol in 0.1% trifluoroacetic acid, at a flow rate of 1 ml min^{-1}. (a) Arrow indicates position of active fraction (VT-1). (b) Rechromatography of VT-1 of (a) on the same column, using a gradient of 0-10% acetonitrile in 50mM triethylamine formate, pH 7. Arrow indicates position of active fraction (VT-2).

/// , Fractions with phytotoxic activity.

Fig. 2. Analytical HPLC profiles of peptides purified from culture (a) and infected plant (b). Aliquots (10 μl) of active fractions were analysed at high sensitivity (A_{210} = 0.04) using a linear gradient of 0-20% acetonitrile in 10 mM sodium perchlorate/0.1% phosphoric acid, at pH 1.8.

Fig. 3. Final purification of toxin from V. dahliae culture fluids. VT-2 was desalted on the same column, using a 45 minute gradient of 0-10% acetonitrile in 0.1% TFA; a double peak was resolved (a). Arrow denotes position of active fraction (VT-3). The major peak, "A", was purified to homogeneity using a 24 minute gradient of 2-10% acetonitrile in the same buffer (b).

Fig. 4. Comparison of agarose gel filtration profiles of V. dahliae-produced phytotoxins from (a) culture fluid and (b) infected potato stems. Acetone precipitate of V. dahliae culture filtrate or extract from V. dahliae-infected potato stems were loaded onto a column (3 x 70 cm). The column was eluted with distilled water at room temperature at a flow rate of 7 ml h^{-1}. Arrow denotes position of active fraction.

Fig. 5. HPLC profiles of purification steps 1 and 2 of the phytotoxin from V. dahliae-infected potato stems. Extracts were concentrated and loaded onto a Nucleosil-5μ column (4 x 200mm) and eluted using a linear gradient of 0-10% n-propanol in 0.1% trifluoroacetic acid, at a flow rate of 0.5 ml min^{-1}. (a) Arrow indicates position of active fraction (VTX-1). (b) Rechromatography of VTX-1 of (a) on the same column, using a gradient of 0-10% acetonitrile in 50mM triethylamine formate, at pH 7. Arrow indicates position of active fraction (VTX-2). /// , Fractions with phytotoxic activity.

Fig. 6. Purification of toxin from V. dahliae infected potato stems. VTX-2 was desalted on a Nucleosil-5μ column (4 x 200mm), using a gradient of 0-10% acetonitrile in 0.1% trifluoroacetic acid; a double peak was resolved. (a) Arrow denotes position of active fraction (VTX-3). The major peak, "A", was purified to homogeneity by rechromatography under the same conditions (b).

Fig. 7. (a) Effect of V. dahliae phytotoxin on permeability of susceptible potato leaf disks as measured by K^+ leakage. Ten leaf disks, 9 mm diameter, were treated with toxin or control preparations for 2 h on a rotary shaker at 50 rpm, washed again gently with 10 ml DDW and transferred to a 10 ml flask containing DDW, average 2.5 μMho. K^+ leakage was measured over a period of 18 h with a flame photometer. Disk treatments were control (A), toxin from culture (B), extract from healthy plant stems (C), extract from infected plant stems (D), EXo from infected plant (E). (b) Effect of V. dahliae phytotoxin on root elongation of tomato (cv. Hosen Eilon). Toxin preparation was added to root culture of tomato in White's medium. Root elongation was measured after 48 h incubation. Treatments were as described above (7a).

F. DESCRIPTION AND RESULTS OF COOPERATION

1) The Gilat Experiment Station planted 0.4 hectares of cvs. Nicola and Alpha in &svd.-infested plots for an attempt to isolate the toxin from infected potato tissue. Exudates were prepared from healthy and infected stem and leaf tissue.

2) The Weizmann Institute group purified small amounts of a toxic peptide from infected potato tissue exudate which was sent to the Gilat Experiment Station for biological characterization. Dialysates of infected tissue exudates were prepared and sent to Gilat for mode of action studies.

3) The group in Idaho compared toxin prepared from &svd isolates obtained from Israel to those prepared in Idaho.

4) Prof. J. Davis and Dr. D. Corsini visited Israel for exchange of information, inspection of field screening trials and discussions of the best ways to accomplish the goals of the project.

G. EVALUATION OF THE RESEARCH

We have purified to homogeneity V. dahliae phytotoxins produced in culture fluids and in infected potato tissue. The toxin purified from culture was very similar to its in vivo counterpart in molecular weight, purification profile, amino acid composition, biological activity and antigenicity. The highly purified toxins contained no detectable contaminants because very sensitive HPLC analysis using three different solvent systems revealed a single, sharp peak; amino acid composition of the two peptide fractions was similar and revealed almost integer numbers; and a single peak was resolved by HPLC.

When detached leaves of disease-susceptible plants were allowed to take up the toxin, specific unilateral symptoms of Verticillium wilt appeared within 48 hours. The specific activity could not be calculated because the bioassays for toxin activity could at most be considered as semiquantitative. Visible symptoms were observed when either toxin was injected into detached leaves at amounts as low as 20 ng (2 x 10-6 mol), an amount greater than that reported for certain host-specific toxins. Nevertheless, the differential activity of these fractions on hosts and non-hosts of the fungus, as well as on susceptible and tolerant cultivars, demonstrated that this Vd toxin indeed shows host specific properties.

Although the molecular size of the toxin is quite small, it is antigenic and is easily detectable in infected plants. Immunofluorescence was more sensitive than bioassay of extracts for early detection of the toxin in infected tissue; the results suggested that the toxin may be translocated in advance of the growing hyphae because

it was easily detectable in segments where CFU were sparse.

Potato cultivars that show "Alpha-type" tolerance to the disease did not show sensitivity in the bioassay to the toxin purified from cv. Nicola. Material which was antigenically cross-reactive with the toxin and toxic for detached leaves of cv. Nicola was present in stems of cv. Alpha. Although this toxic substance has not yet been isolated or characterized, the results suggest that one of the functions of the gene(s) for the "Alpha-type" of disease resistance is to confer tolerance to a phytotoxin. It is possible that the toxin may be altered or its production inhibited in cv. Alpha because the bioassay titer was lower than that of the extract from Nicola and a strong positive immunofluorescence reaction was observed in cv. Alpha only after the cv. Nicola was already dead.

The observation that toxin treatment caused losses of electrolytes from susceptible but not from resistant potato cells, supports the hypothesis that symptoms of Verticillium wilt are due to toxic rather than vessel plugging effects of the Vd toxin.

The role of pathogen-produced phytotoxins in production of foliar symptoms has been well established, but little is known about its effect on roots. In potato, histological studies have shown that root infection by V. dahliae results in stunting of root growth, and experiments using a split-root culture system demonstrated that it is a systemic response to infection which occurs independently of root colonization by the fungus. The data presented here suggest that the toxin may be involved in root stunting because treatment with it inhibited tomato root elongation in vitro. In a previous study, we

found that the PLP inhibited the elongation of roots from susceptible host plants, ve tomato and eggplant, and augmented elongation of those obtained from resistant Ve tomato, but had little effect on those from a non-host plant, onion. Since stunting cannot be detected by detached leaf bioassays, the root inhibition assay could be an additional tool for resistance screening studies using the Vd toxin as probe.

The fact that peptide fraction purified from culture was not degraded in an automatic gas-phase sequencer suggests that it may be cyclic, blocked, or bound to a non-peptidic moiety. Analysis of the HPLC profiles of the two highly purified toxins supports the latter hypothesis because the absorption at 214 nm was greater than could be accounted for by the amino acid content of the fractions. Moreover, mild acid hydrolysis revealed the presence of a large peak which did not contain amino acids. It is not a sugar because it was not hydrolyzed. In an attempt to ascertain more information regarding this moiety, we prepared a large amount of highly purified toxin from culture and submitted it to fast atom bombardment mass spectroscopy. The results were inconclusive because not enough of the peptide was present for significant detection. As stated above, large quantities of crude toxin will be required in order to purify enough material for further chemical characterization, which will require special facilities for mass production.

H. PUBLICATIONS ON THE RESEARCH

Buchner, V., Burstein, Y. and Nachmias, A. (1988) Comparison of Verticillium dahliae-produced phytotoxic peptides purified from culture fluids and infected potato stems. Physiological and Molecular Plant Pathology (in press).

Mohan, S.K., Davis, J.R., Corsini, D.L., Sorenson, L.H. and Pavek, J.J. 1987. Comparison of methods for evaluation of potato genotypes for resistance to Verticillium wilt. Phytopathology 77: 1702.

Nachmias, A., Buchner, V. and Burstein, Y. 1985. Biological and immunochemical characterization of a low molecular weight phytotoxin isolated from a protein-lipopolysaccharide complex produced by a potato isolate of Verticillium dahliae Kleb. Physiological Plant Pathology 26: 43-55.

Nachmias, A. and Krikun, J. 1985. Verticillium wilt of potato in Israel. American Potato Journal 62: 201-205.

Nachmias, A., Buchner, V., Tsror, L., Burstein, Y. and Keen, N. (in press). Differential phytotoxicity of peptides from culture fluids of Verticillium dahliae Races 1 and 2 and their relationship to pathogenicity of the fungus on tomato. Phytopathology.