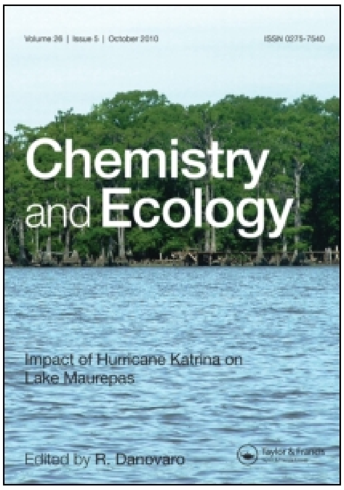

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The Influence of Potassium and Phosphorus in Predisposition of Tomato to Verticillium Wilt

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The effects of varying the concentration of phosphorus and potassium independently in the growing medium of pot-grown tomato plants on their subsequent susceptibility in normal medium to vascular wilt caused by *Verticillium dahliae* Kleb. were studied. The degree of host invasion was measured by estimating the chitin component of diseased tissue. Different levels of potassium had little effect on the degree of invasion of inoculated stems, but this was considerably enhanced in plants raised in the absence of phosphorus. Root inoculated plants were particularly resistant when raised in nutrients totally lacking in potassium, and very susceptible when raised in the absence of phosphorus.

INTRODUCTION

Predisposition has been defined as ‘the tendency of treatments and conditions acting before inoculation or before the introduction of the incitant, to affect susceptibility to biotic and abiotic pathogens’ (Yarwood, 1976). Frequently it is not easy to determine whether the effect of a treatment on disease is through its effect on the pathogen or on the host; if a chemical or physical treatment affects disease through its effect on the host, it is predisposition.

Many experiments have demonstrated that physical or chemical treatments of host plants prior to inoculation can affect subsequent susceptibility to pathogens (Schoeneweiss, 1975). Thus some permanent or semi-permanent change must occur to the hosts physical and/or chemical constitution.

Nutrient stress is probably the most important predisposing factor since it can be practically applied both in the field and under glass. More experiments have been conducted on the effects of mineral nutrients, particularly nitrogen, phosphorus and potassium, on disease susceptibility than on any other potentially predisposing treatments (Yarwood, 1959). Lewis (1953) proposed a balanced hypothesis of parasitism, in which host-parasite relations are governed by a combination of the biochemistry of the host and the nutritional requirements of the parasite. Because nutrients present in the host in metabolic concentrations sometimes cause inhibition of the parasite, a certain nutrient balance may be a necessary prerequisite for infection. Garber (1956) extended this hypothesis and suggested that only a combination of host nutritional environment adequate for the parasite plus an ineffective host inhibitory environment results in the establishment of a pathogenic relationship.

In general it seems that susceptibility is increased in plants treated before inoculation with high nitrogen concentrations, and decreased by high concentrations of potassium, whilst the effects of phosphorus may be varied (Tarr, 1972). However, the increase or decrease in plant susceptibility to the different concentrations of nutrients supplied before inoculation does indeed vary from one plant species to another, and from one plant pathogen to another. This is true of even closely related disease types such as vascular wilts caused by imperfect fungi. Thus, Burge and Isaac (1977) found that *Aster* became more susceptible to *Phialophora* after growth in soil rich in potassium or phosphorus, but low in nitrogen content. Foster and Walker (1947) also noted increased *Fusarium* wilt of tomato plants that had been treated before inoculation with low nitrogen and phosphorus, and high potassium levels as compared with the balanced solution. However, Al-Shukri (1969), who employed the technique used by Foster and Walker, found that a high level of nitrogen or a deficiency in potassium predisposed cotton plants to the development of *Fusarium* wilt.

The severity of diseases such as vascular wilts is often difficult to assess because there are no actual lesions to measure and estimation of disease usually depends on the somewhat subjective assessment of such criteria as reduction in leaf size and number and the degree of yellowing or browning of leaves and/or vascular tissue. Whilst undoubtably suitable for certain types of experiment, these assessment methods are unsatisfactory for determining the degree of actual host invasion by the pathogen. Thus, for this purpose, analysis of the fungal component of the host-parasite complex by chemical means is a valuable technique. Furthermore, it enables measurements of fungal invasion to be made even before the appearance of symptoms.

Chitin, a polymer of N-acetyl-D-glucosamine, has not been detected in higher plants but it is a constituent of the cell walls of most fungi (Tracy, 1955). Thus, although the amount may vary with age, morphology and cultural conditions, glucosamine assay provides a convenient means of measuring the amount of fungus in infected plants. Ride and Drysdale (1972) developed a method originally devised by Bassett (1957), of alkaline hydrolysis of the chitin to chitosan and subsequently to glucosamine, which can be measured colorimetrically with ferric chloride. A similar method was employed by Hepper (1976) in the measurement of vesicular-arbuscular mycorrhizal infection in roots. The conversion of chitin to glucosamine is assumed to remain constant throughout the analyses.

In the present work, chitin analysis is used to assess the effects of different concentrations of potassium and phosphorus as agents of predisposition of tomato (*Lycopersicon esculentum*) to vascular wilt caused by *Verticillium dahliae* Kleb.

MATERIALS AND METHODS

Test plants

Tomato plants used were *Lycopersicon esculentum*, C.V. Moneymaker. Plants were raised from seed in an inert substrate, Vermiculite, so that the nutrient supply to the plants could be regulated from germination. Plants were maintained in a greenhouse at 18±4°C. Illumination was provided by 250 w MBF/U lamps for 16 h per day. Seeds were germinated in a tray of moist Vermiculite, and then transferred at the two leaf stage to individual 7 cm diameter polystyrene pots. Each pot was placed into a 9 cm Petri dish lid which acted as a pot saucer. Water was added to each pot until the saucer was full, thus ensuring saturation of the Vermiculite. The seedlings were then left for a week to become established.

The pathogen

An isolate of *Verticillium dahliae* from tomato was grown on Czapek-Dox's agar in 9 cm Petri dishes at 23°C in darkness. For inoculation a twenty-two day old colony (8 cm diameter) was homogenized in 50 ml of sterile distilled water and subsequently made up to 150 ml. This gave a concentrated suspension of propagules composed of conidia, hyphal fragments and microsclerotia.

Nutrient solutions

The basic nutrient solution used was that of Hoagland (1938) with the potassium and phosphorus concentrations adjusted to give six different media: 0, 2× and 4× potassium, and 0, 2× and 4× phosphorus. All solutions were sterilized by autoclaving immediately before use.

Predisposition period and inoculation

The tomato plants were grown in the six different nutrient solutions for a period of 23 days in groups of 14 plants per treatment. After this period, the plants in each group of 14 were subdivided into two groups of six and one group of two. Both subgroups of six plants were inoculated with the propagule suspension; one by root inoculation and one by stem inoculation. The four remaining plants were reserved to examine the effects of predisposition on xylem vessel size.

Root inoculation. Twenty ml of the propagule suspension was poured evenly over the surface of the Vermiculite in each pot. This method gave no disturbance or damage to the roots which was regarded as an important consideration in that the different treatments may variously influence the subsequent susceptibility to penetration of the undamaged roots.

Stem inoculation One of the problems associated with stem inoculation is ensuring that each plant receives an equal amount of inoculum. The usual methods of inserting the inoculum into stem incisions, or syringe injection of spores seldom satisfy this requirement: in the former method, standardization of the depth and surface area of the incision and quantity of applied inoculum is difficult to achieve; in the latter method leakage of a varying proportion of the inoculum occurs.

A novel method of inoculation was therefore employed. Fifteen cm of cotton thread was attached to a small sewing needle and both were sterilized by autoclaving inside a glass Petri dish. The needle and thread were then immersed in the propagule suspension for 15 seconds. Excess inoculum was removed by drawing the (doubled) thread across the edge of the Petri dish. The needle was then immediately pushed through the diameter of the stem 1 cm below the cotyledonary nodes, and the thread drawn slowly through. This was repeated so that the needle passed twice through the stem at precisely the same point. Some of the inoculum was inevitably squeezed from the cotton on to the stem surface. This was considered to be the same in each inoculation and the excess was removed with absorbent cotton wool.

After inoculation, the plants were supplied with complete Hoagland's solution for two weeks, after which they were harvested, examined for signs of disease and subject to chitin analysis.

Effects of predisposition on the host

Some of the effects of preinoculation treatments which may conceivably affect disease development were assessed immediately prior to inoculation. Fresh weight was measured as a general indication of plant vigour; mean root length and the number of laterals were obtained as possible variables which may affect the number of potential sites for penetration, and xylem vessel diameter mas measured as a factor which may influence the rate of fungal invasion through the shoot. The xylem vessels measured were those seen in hand cut transverse sections taken from mid-way between the cotyledonary and first leaf nodes and mounted in water.

Examination of symptoms

The two week period proved insufficient time for the appearance of any external symptoms of disease. However, examination of the vascular system of each stem inoculated plant indicated that this had become discoloured and measurements were made of the length of discoloured xylem after splitting each stem longitudinally. No discolouration was apparent in root inoculated plants.

Chitin analysis

The whole of each root-inoculated plant was analysed, whereas only the stems of the stem-inoculated plants were analysed as it was thought that if adequate penetration of the roots of the former plants had occurred, then subsequent stem invasion would have resulted. In the stem-inoculated plants it was considered unlikely that the fungus would have invaded the root system since this is against the direction of flow of substances within the xylem vessels. The dried tissue of each plant was ground to a fine powder in a mortar and pestle and samples of known weight, between twenty and fifty mg retained for analysis. The procedure outlined by Ride and Drysdale (1972) was then followed for each sample. Varying concentrations of glucosamine were also subjected to the final part of the analytical procedure to obtain a graph of optical density v. glucosamine concentration. This gave a straight line relationship which enabled correlation between the absorbance reading obtained in the chitin analysis procedure with the amount of glucosamine present in the samples tested. Since the glucosamine content per unit dry weight of tomato plant sample is a direct measure of chitin content, it is possible to indicate the relative proportions of fungal material in the samples.

RESULTS

Effects of predisposition treatments on the host prior to inoculation

The effects of treatments on host fresh weight, root length, number of lateral roots and xylem width are summarized in Table I.

The classic symptoms of phosphorus and potassium deficiency were visible in the plants raised in the absence of these nutrients. The leaves of phosphorus deficient plants lacked lustre and had a bluish tint. Leaf margins tended to be necrotic and rolled under. Potassium deficiency also resulted in a bluish discolouration of the leaves, mostly between the veins,

TABLE I

Effects of predisposition on aspects of growth and morphology of the host prior to inoculation

	Fresh wt. (g)						Root lenght (mm)					
	P			K			P			K		
	0	2	4	0	2	4	0	2	4	0	2	4
	3.79	8.23	8.9	6.95	5.75	5.18	118.2	115.0	104.1	97.4	87.7	95.7
F		44.53			4.72			1.81				
P		0.005			0.025			0.25			NS	
LSD		1.125			1.33			0.411				

	No. of lateral roots						Xylem width (relative units)					
	P			K			P			K		
	0	2	4	0	2	4	0	2	4	0	2	4
	5.8	6.0	6.8	5.4	5.2	5.3	9.0	9.67	8.67	7.45	10.11	9.33
											5.55	
		NS			NS			NS			0.025	
											0.597	

P = phosphorus. K = potassium. 0,2,4 = relative concentrations.
F = degrees of freedom. P = probability. LSD = least significant difference.

TABLE II

Effect of predisposition on vascular discolouration in stem inoculated plants

Treatment	Mean length of discolouration (mm)	F	P	LSD
0P	14.8			
2P	15.2	—	NS	—
4P	22.0			
0K	25.0			
2K	11.0	41.3	0.005	0.33
4K	10.4			

F = Degrees of freedom. P = probability. LSD = least significant difference.

and the leaf margins were again rolled under. At 2x concentration, potassium caused some leaf necrosis.

The addition of phosphorus at each concentration resulted in vigorous growth, but plants raised in 2x and 4x potassium or without phosphorus were reduced in size.

Root length decreased slightly with increasing concentration of phosphorus but no significant differences were found in the length of roots in different potassium concentrations. The mean number of lateral roots was unaffected by different levels of potassium or phosphorus.

Measurements of xylem diameter indicated a small though significant reduction in the absence of potassium, but no effects were recorded at different levels of phosphorus.

Vascular discolouration in stem-inoculated plants

The length of discoloured vascular tissue in plants raised without potassium was more than double that present in plants raised in 2x or 4x potassium. No significant difference was found between vascular discolouration of plants raised in different concentrations of phosphorus although results suggest a possible decrease with decreasing concentrations of this mineral. Of all the treatments (Table II), high potassium levels would seem to favour greatest resistance in terms of vascular discolouration.

Chitin assay

The mean weight of glucosamine in plants inoculated either *via* the stem or the root following each preinoculation treatment is shown in Table III.

Different levels of potassium had little effect on the degree of invasion of inoculated stems which was high at each concentration. Invasion was considerably enhanced, however, in plants raised in the absence of phosphorus, and greatly reduced in the presence of 2x and 4x phosphorus. Root inoculated plants were particularly resistant when raised in nutrients totally lacking in potassium and with 2x potassium.

As with stem inoculated plants, inoculation resulted in greatest tissue invasion when phosphorus was absent from the nutrient solution. However, the high figure obtained in root inoculated plants pretreated with 4x phosphorus is somewhat enigmatic.

Perhaps the most important result here, from the aspect of practical application is the clear indication that natural infection of the root system by this soil-borne pathogen is greatly reduced as long as potassium is absent or kept to a minimum in the medium in which these plants are raised.

Results shown in Tables I, II and III were subjected to analysis of variance and least significant differences are quoted.

TABLE III

Effects of predisposition on weight of glucosamine in plant tissue, two weeks after inoculation

	Treatment	µg glucosamine/mg plant tissue	F	P	LSD
	0K	0.8			
Root inoculation	2K	1.85	10104.7	0.005	9.24
	4K	15.27			
	0K	13.8			
Stem inoculation	2K	14.48	2.3	0.25	0.77
	4K	15.53			
	0P	21.5	202.44	0.005	2.715
Root inoculation	2P	6.36			
	4P	19.33			
	0P	28.06			
Stem inoculation	2P	8.56	10.02	0.025	0.228
	4P	9.68			

0P, 2P, 4P = relative levels of phosphorus
0K, 2K, 4K = relative levels of potassium
F = degrees of freedom; P = probability; LSD = least significant difference

DISCUSSION

Providing that all the conditions, apart from the varying potassium and phosphorus concentrations, remained constant, it can be assumed that predisposition of tomato plants to *Verticillium dahliae* was affected by these nutrients only. It can also be assumed that the conversion factor of chitin to glucosamine (Sharma, Fisher and Webster, 1971) also remained constant for the duration of the analyses.

Inorganic nutrients may change host structure, chemical composition and metabolism. These changes may affect the host as a medium, supporting growth and reproduction of the pathogen and the formation of its toxins and enzymes, or they may reflect the host reponse to pathogen invasion and to the action of its toxins. The correlation between these phenomena and susceptibility, however, varies with different diseases.

Considering root infection in the present work, it is very evident that excess potassium results in a marked increase in the degree of root invasion. It is well known that *Verticillium* does not normally invade the root system extensively causing significant decay as would a root-rot pathogen, but traverses the cortex in order to invade the xylem. Excess potassium may have the effect of increasing the availability of pectic acids to fungal polygalacturonase so that increased root invasion may result. This is known to occur in other host–parasite relationships (Bateman, 1964). Lack of potassium would have the opposite effect which is also reflected in the present results and it may be that the absence of potassium has its major effect in preventing the initial penetration of the intact root. The actual amount of root available for inoculation was not significantly affected by the different concentrations of either potassium or phosphorus.

Although there was increased stem invasion with increased potassium following stem inoculation the same order of difference was not apparent here as in the roots. It is possible that the somewhat narrower xylem vessels formed in the absence of potassium may have an effect on the degree of fungal invasion.

Other workers have conducted similar types of experiment but using modified rather than soilless compost. Burge and Isaac (1977), for example, obtained similar results with *Phialophora* wilt of *Aster*; observations of symptoms over a period of 81 days indicated that plants raised in the absence of potassium were relatively slow to produce symptoms, but when disease was established it progressed more rapidly. Foster and Walker (1947) also found a lowering of susceptibility of tomato to *Fusarium* wilt when the host was raised in a medium with lower than normal concentrations of potassium prior to inoculation.

High levels of phosphate have been demonstrated as factors which increase susceptibility of tomato to *Fusarium* wilt (Foster and Walker, 1947) and *Aster* to *Phialophora* wilt (Burge and Isaac, 1977). In the present work root-inoculated plants were infected to a high degree both in the absence and in the presence of 4x phosphorus, whereas plants grown in 2x phosphorus were relatively resistant. This result, which had a high level of significance ($P = 0.005$) is puzzling and requires further investigation. Differences in root length were too minor to be considered as contributory factors.

Chitin analysis showed that the greatest amount of fungal tissue was present in stem inoculated plants raised in medium with no phosphorus. These plants were generally in poor health prior to inoculation and it is not surprising that little resistance could be afforded to pathogenic invasion. Plants grown with 2x and 4x phosphorus were much less invaded by the pathogen, and values for chitin analysis at these concentrations were similar. Vessel diameters at all these concentrations were not significantly different.

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