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RELATIONSHIP BETWEEN *VERTICILLIUM ALBOATRUM* STRAIN MORPHOGENESIS AND MOLECULAR FINGERPRINTS

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Abstract

Morphological characteristics of three different groups of isolates of *Verticillium alboatrum* which had previously been differentiated on the basis of rDNA polymorphism and mtDNA RFLPS, were studied. Nature and extent of variability differed between different groups of isolates. Statistically significant differences were observed in mean phialide count (MPC), mean phialide size (MPSz) and mean interverticil distance (MIVD), among groups of *V. alboatrum*. Highest MPC was noticed in group 1b (3.21) as compared to lowest in group 1a (2.10). The MPSz was largest in group 1a (47.40 μm) and lowest in group 2 (31.19 μm). Highest MIVD was observed in group 1a and group 2 (50.40 and 49.37 μm, respectively) with lowest in group 1b and group 3 (36.6 and 38.47 μm, respectively).

Introduction

Verticillium alboatrum Reinke & Berthold causes vascular wilt disease on over 300 crop plants in temperate regions of the world including hop, alfalfa, cotton, tomato, potato, olive and ornamental plants such as chrysanthemum and antirhinum (Heale, 1988). A mild form of wilt in hops (described as 'fluctuating') caused by *V. alboatrum* was first described in Kent during 1924 by Harris (1927). Isolates of *V. alboatrum*, have been considered as distinct since they are only pathogenic to alfalfa (Heale, 1985). Barasubiye *et al.* (1994) indicated that strains isolated from alfalfa and potato form pathologically and physiologically distinct groups and can be differentiated on the basis of optimum temperature for growth on medium. However, the taxonomy of this species is still uncertain and it needs further investigation.

Recently, a number of workers have applied various molecular techniques to investigate *Verticillium* species. Collins *et al.* (2003) studied diverse isolates of the soil-borne wilt fungi, *Verticillium alboatrum* and *V. dahliae* pathogenic to crucifers and found at least three distinct molecular types. Robb *et al.* (1994) developed a PCR based assay for the quantification of *V. alboatrum* and suggested that it should be divided into two subgroups. Similarly Griffen *et al.* (1996) analysed 47 *V. alboatrum* isolates and on the basis of rDNA polymorphism and mtDNA RFLPS divided the isolates into three distinct groups.

The purpose of this study was to investigate the morphological differences among the groups of *Verticillium alboatrum* strains as formulated by Griffen *et al.* (1996).

Table 1. Morphological characteristics* of *Verticillium alboatrum* strains.

Groups	Strains	MPSz (um)	MPC	MIVD (μm)
1A	Vaa10/1, 2011	47.40	2.10	50.40
1B	Vaa 220, 223, 235	32.13	3.21	36.60
2	Vaa 621, 690	30.19	2.65	49.37
3	Vaa Luc 2	36.11	2.57	38.47

*(Mean Phialide Count (MPC), Mean Phialide Size (MPSz) and Mean Interverticil Distance (MIVD)

Table 2. Level of significance (P values, t-test) for inter group differences.

Groups	G-3			G-2			G-1B		
	MPC	MPS	MIVD	MPC	MPS	MIVD	MPC	MPS	MIVD
G-1A	0.00	0.08	0.00	0.00	0.06	0.19	0.00	0.69	0.00
	(-)	ns	(-)	(-)	ns	(-)	ns	(-)	ns
G-1B	0.00	0.05	0.6	0.77	0.05	0.00	-	-	-
	(-)	ns	ns	ns	ns	(-)	-	-	-
G-2	0.09	0.55	0.00	-	-	-	-	-	-
	ns	ns	(-)	-	-	-	-	-	-

(-) indicates statistical significance at level of P value at least < 0.05

Materials and Methods

Morphological investigations of *V. alboatrum* were carried out by light-microscopy. Different strains used in this study were obtained from the culture collection of Microbial Physiology Laboratory, King's College, London. Ten days old *V. alboatrum* cultures, previously grown on Czapek-Dox agar medium (pH 7.3 at 21-23 °C) were used in this study (Fig. 1). Three different characteristics of each strain studied were mean phialide count (MPC), mean phialide size (MPSz) and mean interverticil distance (MIVD, Fig. 2A). Measurements of MPSz and MIVD were taken with the help of ocular and stage micrometer.

Results and Discussion

The results indicate statistically significant differences in mean phialide count (MPC), mean phialide size (MPSz) and mean interverticil distance (MIVD), among groups of *V. alboatrum* (Tables 1 & 2, Fig. 3). Highest MPC was observed in group 1b (3.21) and lowest in group 1a (2.1). The MPSz was largest in group 1a (47.40 μm) and lowest in group 2 (30.19 μm). Highest MIVD was observed in group 1a and group 2 (50.40 and 49.37 μm, respectively) and the lowest in group 1b and group 3 (36.6 and 38.47 μm, respectively).

No two groups were significantly different from each other in all three aspects (MPC, MPSz and MIVD), however group 1a and group 3 seem to be most different from each other as their MPC and mean MIVD was very significantly different and difference in MPSz was only marginally non significant (P=0.08). Morphological differences between group 1a-1b, 1a-3 and 1b-2 were significant for MPC and MIVD but not for MPSz. Group 1b and group 3 were significantly different in terms of MPSz only and group 2 and group 3 were significantly different in terms of MIVD. Similar trend is also evidenced by dendogram generated by heiarchal cluster analysis procedure (Fig. 4). Number 0-25 are rescaled distance coefficients, where 0 indicates 100 % similarity and 25 indicates 100 % dissimilarity. Group 1b and 3 thus were the most similar groups.

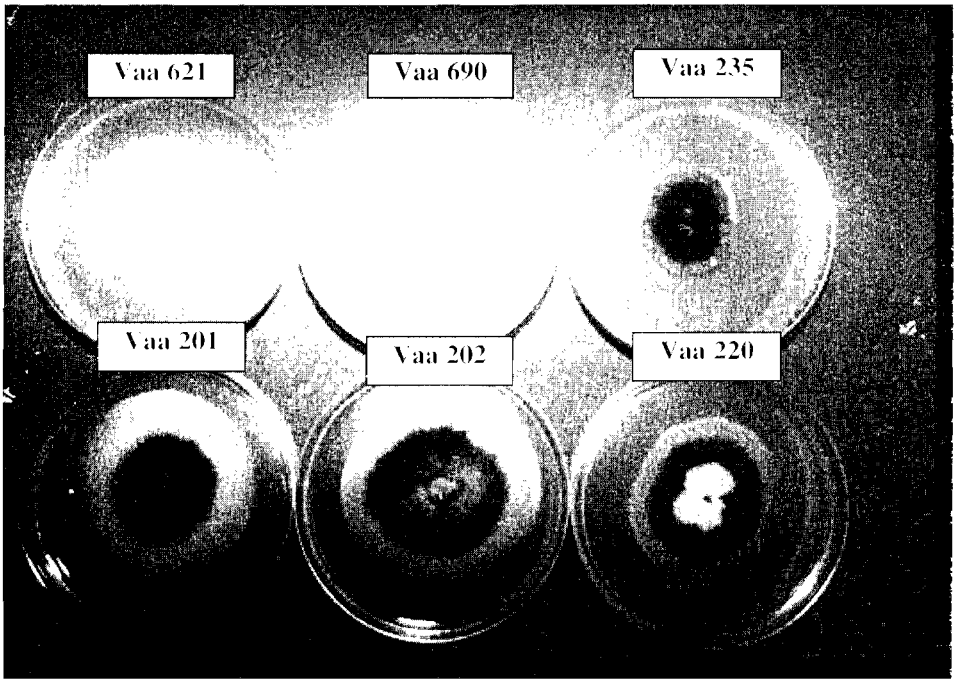

Fig. 1. Few selected isolates of *V. alboatrum*

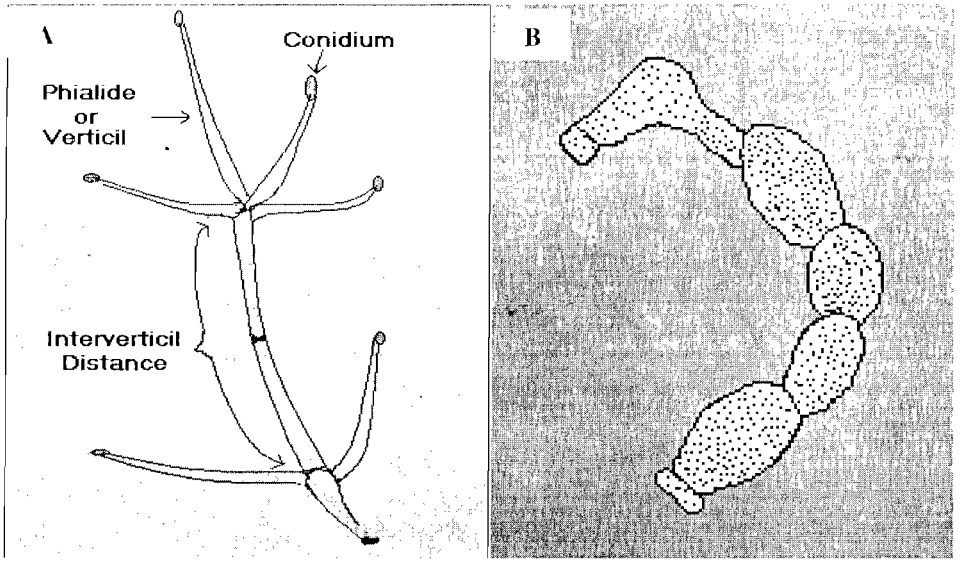

Fig. 2. Morphology of *Verticillium alboatrum*.
A. Phialide & Conidia, B. Resting Mycelium

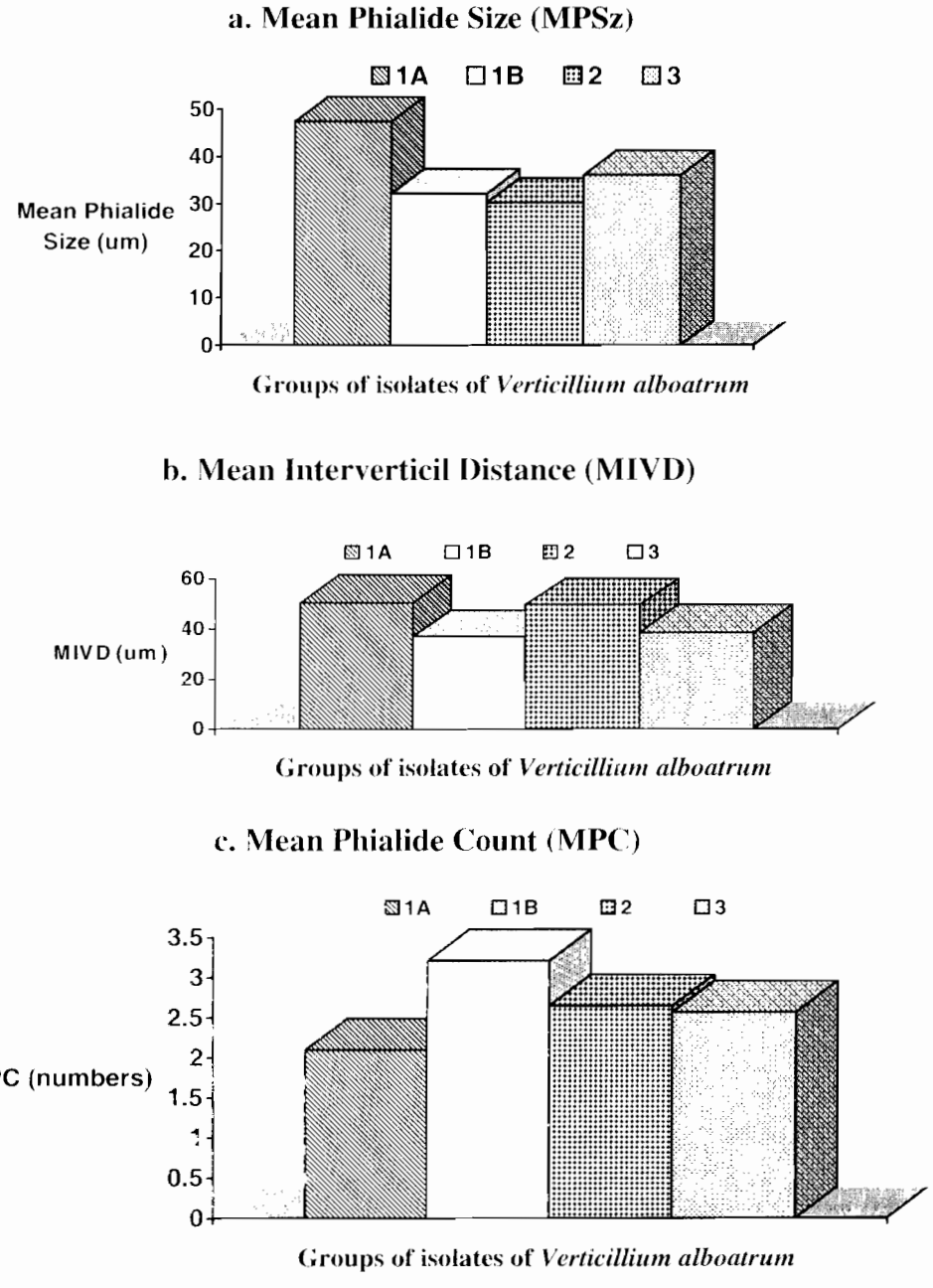

Fig. 3. Morphological differences (a, b & c) in different groups of *V. alboatrum*.

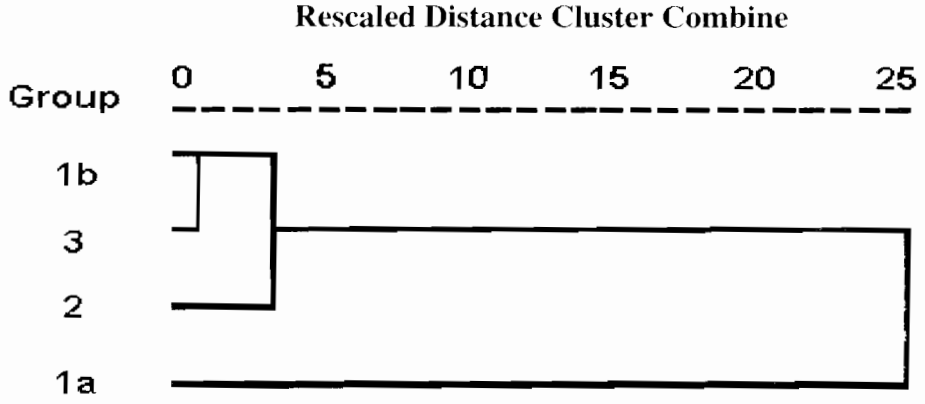

Fig. 4. Dendogram using average linkages between groups.

The above mentioned observations indicate that different groups of *Verticillium alboatrum* differ in their morphological characteristics and extent of variability differs between different groups of isolate. Previously it had been noticed that different isolates differ in terms of extensive bundling of the dark resting mycelium (Robb *et al.*, 1994). Steventon *et al.* (1996) differentiated 36 isolates of *Verticillium alboatrum* on the basis of conidial length. Current observations provide evidence that the various groups of isolates differ in terms of other morphological characteristics like MPSz, MPC and MIVD also. These observation indicate the feasibility of using morphological assessment to identify virulent and avirulent isolates of *Verticillium alboatrum* as compared to DNA finger printing technique which is more expensive and not feasible.

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