Plant Pathology (1998) 47, 523–529 (n°4)

Hyaline mutants from *Verticillium dahliae*, an example of selection and characterization of strains for host–parasite interaction studies

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The objective of the present work was to select stable well-characterized strains of *Verticillium dahliae* that could be used as biological tools in genetic and plant–microbe interaction studies. Hyaline mutants, known for their stability in pathogenicity were chosen for the study. Diversity in pathogenicity was found among hyaline subclones obtained from a defoliating wild-type clone, but not within those from nondefoliating ones. Most subclones from the defoliating clone had parental pathotypes, but one (V7-2) exhibited weak pathogenicity. This subclone (V7-2), together with a highly virulent one (V7-7) deriving from the same defoliating parent clone (7), were selected for further characterization, because of their differences in pathogenicity. When studied on the basis of their growth requirements, the two subclones expressed marked differences. V7-7 grew better than V7-2 over a wider range of temperature conditions. Both subclones grew similarly in media supplemented with NH_4 as nitrogen source, but in those with NO_3, V7-7 grew more vigorously than V7-2 and only the former could grow when NO_2 was used. In spite of these differences, the two subclones were found to belong to the same vegetative compatibility group, confirming their genetic proximity. These results highlight the physiological and genetic complexity inherent in *V. dahliae*. In view of their characteristics, the clones obtained in this study should prove to be valuable tools in furthering the understanding of genetic and host–*V. dahliae* interactions.

Introduction

Diverse behaviour has been reported within the genus *Verticillium*, including parasitism of animals (Samson *et al.*, 1988; Jun *et al.*, 1991) and other fungi (Barron & Fletcher, 1970; Lim & Nik, 1983), although it is as a pathogen of higher plants that this genus is better known (Gubler *et al.*, 1978; Schnathorst, 1981b; Heale, 1985). Even within the same species, namely *V. albo-atrum* and *V. dahliae*, there may be a wide host range. The latter can infect over 160 plant species belonging to more than 40 different families (Schnathorst, 1981a), including economically important crops all over the world (Gubler *et al.*, 1978; Heale, 1985; Follin, 1986; Koroleva & Kasyanenko, 1987; Gu *et al.*, 1988; Bejarano-Alcazar *et al.*, 1995). In cotton crops, *V. dahliae* limits production in several countries (Tjamos & Kornaros, 1978; Follin, 1986; Koroleva & Kasyanenko, 1987; Bejarano-Alcazar *et al.*, 1990). Current procedures for management of this pathogen, such as the use of resistant cultivars and crop rotations, are often not durable, because of the lack of specificity of the parasite and its extreme variability. In addition, *V. dahliae* displays a wide spectrum of variation in morphology (Tolmsoff, 1972), ecology (Heale, 1985) and biochemistry (Heale & Isaac, 1963; Wheeler *et al.*, 1978), leading to interference with studies on genetic diversity and on plant–microbe interactions. The choice of fungal strains to be compared for the reactions they induce in host plants is often based solely on differences in virulence. Such variability has been shown to be high in wild-type strains of *V. dahliae*, even among conidial progeny of clones. However, this seems to be exclusive to microsclerotial wild-type strains (Hadisutrisno, 1987). On the other hand, hyaline variants appear to possess a certain stability in pathogenicity (Heale & Isaac, 1965; Boisson & Lahlou, 1982). The hyaline character is a result of loss of the capacity to produce microsclerotia (Brandt & Roth, 1965) or the melanin pigment (Typas & Heale, 1976). These mutants may be obtained spontaneously or by mutagenesis (Bell *et al.*, 1976; Typas & Heale, 1976, 1979; Wheeler *et al.*, 1978). The objectives of this study were: (i) to analyse intraclonal diversity within

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Accepted 9 February 1998.

V. dahliae, using spontaneous hyaline variants and (ii) to select hyaline subclones showing differences in pathogenicity and to characterize these according to pathogenicity on cotton, vegetative compatibility(VC) and growth on several nitrogen sources and under different temperatures. Fulfilment of these objectives should lead to the selection of well-characterized, stable subclones differing from one another in pathogenicity. Such biological material would help to circumvent some problems associated with *V. dahliae* variability.

Materials and methods

Plant material

Gossypium hirsutum Isa-205, *G. barbadense* Ashmouni, *G. hirsutum* Tashkent1 and *G. arboreum* Xiao were used, because of their reported different levels of resistance to *Verticillium* wilt. *G. hirsutum* is more susceptible than *G. barbadense* and *G. arboreum* (Barrow, 1970, 1973; Wilhelm *et al.*, 1974; Bell & Mace, 1984). After a 5-min surface disinfection with 5% calcium hypochlorite, seeds were placed for 24 h at 25°C in Petri dishes containing sterile filter paper moistened with sterilized distilled water. Germinated seeds were then sown in a potting mixture (loam and sand, 6 : 1, v/v).

Fungal material and generation of subclones

The three microsclerotial wild-type clones used in this study originated from single spores obtained from pure cultures of isolates 6, 8 (nondefoliating) or 7 (defoliating), provided by IRCT-CIRAD, Montpellier, France. A drop of a conidial suspension from each clone was placed at the margin of a Petri dish containing potato dextrose agar (PDA) and spread to separate conidia. After 24 h, isolated germinated conidia were identified under a dissecting microscope, excised from the agar, deposited in the centre of a new Petri dish and grown for 2 weeks. Two hundred subclones were isolated from each clone.

Mode of infection

For each cotton cultivar to be inoculated, 25 2-week-old plants were carefully uprooted and the roots immersed for 15 min in 100 mL of a conidial suspension containing 10^6 spores/mL. Twenty-five plants were immersed in sterile distilled water to serve as controls. All plants were replanted in separate plastic pots (9 cm diameter) and placed in a growth chamber with a 12-h photoperiod, at 27 ± 2°C and 70–90% relative humidity.

Estimation of disease severity

Disease severity was estimated by observing plant stunting and foliar damage 23 days after inoculation and vascular discoloration 1 day later. Quantification of these symptoms was made as follows. To estimate stunting, the length of the epicotyl (Epl) was measured, or the stunting index (SI) was calculated from $SI = 100(Epl_c - Epl_x)/Epl_c$, where Epl_c is the mean Epls for control plants and Epl_x is the epicotyl length for each treated plant. To evaluate foliar damage on each plant, a score (*xi*) was attributed to each cotyledon and leaf according to the scale (0) no foliar symptoms; (1) yellowing or partial necrosis of cotyledon; (2) cotyledon scar; (3) yellowed leaf; (4) wilted or necrotic leaf; (5) leaf scar (Daayf *et al.*, 1995). A 'Foliar Alteration Index' (FAI) was then calculated for each inoculated plant as $FAI = (100 \times xi)/(4 + 5n)$, where4 is the maximum score for cotyledons (2×2), 5 is the maximum score for each leaf and *n* is the number of leaves of each plant. Vascular discoloration was evaluated on each plant according to a modified method of Erwin *et al.* (1976); discoloration was scored (*yi*) for each internode based on the scale (0) no discoloration, (1) 2–5 localized brown regions within the vascular tissue of the same internode; (2) browning of long stretches of the vascular tissue; (3) browning of all the vessels but not the adjacent tissues; (4) browning of both vessels and adjacent tissues. Browning Index (BI) was then calculated for each plant as $BI(\%) = (100 \times yi)/(4d)$, where *d* is the total number of seedling internodes including hypocotyl and 4 the maximum score for an internode. For each treatment, a mean was calculated for SI, FAI and BI.

Effect of temperature and nitrogen source on the growth of *V. dahliae*

Four replicates of culture pieces were deposited in separate Petri dishes containing potato dextrose agar (PDA) for temperature studies, or a minimal medium (Daayf *et al.*, 1995), where the nitrogen sources were: ammonium tartrate (0·8 g L^{-1}) (MM), sodium nitrate (2 g L^{-1}) (MM_2), ammonium tartrate (0·8 g L^{-1}) amended with calcium carbonate (0·5 g L^{-1}) (MM_3), or sodium nitrite (0·5 g L^{-1}) (MM_4). The Petri dishes were placed in incubators at 21 and27°C for temperature studies and 25°C for nitrogen effects studies. Growth was estimated 15 or 20 days later, by measuring two diameters of each colony.

Vegetative compatibility studies

Selection of mutants

Mutants affected in the nitrogen assimilation pathway, called nitrate-nonutilizing mutants or '*nit*' mutants, were used to assess VC relationships among strains of *V. dahliae*. They were produced using a modified procedure (Daayf *et al.*, 1995) of Cove (1976), adapted for *F. oxysporum* by Puhalla (1985) and for *V. dahliae* by Joaquim & Rowe (1990). Agar pieces (approximately 15 × 2 mm) were cut from the edge of wild-type colonies of *V. dahliae* clones growing on PDA and placed on MM amended with 30 g L^{-1} potassium chlorate in 9-cm Petri dishes. After incubation for 10 days at 25°C, a fragment (1 cm diameter) was cut from the advancing margin of a chlorate-resistant colony, placed in a tube containing 8 mL of sterile water and then shaken to release the spores. The spore suspension was adjusted to a concentration of 10^4 conidia/mL and 50 μL of this suspension was placed on MM. Each spore that germinated was transferred to a new MM plate.

Characterization of nit mutants

A piece of each chlorate-resistant mutant culture was transferred to basal medium (nitrogen-free) (Correll *et al.*, 1987) amended with one of the following nitrogen sources: sodium nitrate (0·2 g L^{-1}), sodium nitrite (0·4 g L^{-1}), hypoxanthine (0·5 g L^{-1}) or ammonium tartrate (0·8 g L^{-1}) buffered with calcium carbonate (0·5 g L^{-1}). Assignment of *nit* mutant phenotype designations, *nit*1, *nit*3 and *nit*M, corresponded to those used for *V. albo-atrum* (Correll *et al.*, 1988), *F. oxysporum* (Correll *et al.*, 1987) and *V. dahliae* (Joaquim & Rowe, 1990). *Nit*1 mutants originate from a mutation at the structural locus of nitrate reductase, *Nit*3 mutants from a mutation at a specific regulatory locus of the nitrate assimilation pathway and *Nit*M mutants from a mutation at one of the loci controlling synthesis of a molybdenum-cofactor necessary for nitrate reductase and purine dehydrogenase activities (Correll *et al.*, 1987).

Pairing of mutants

To determine VC grouping among the strains of *V. dahliae*, different *nit* mutants were paired by placing two agar culture pieces (1 mm^3) 7–8 mm apart on MM. Four separate pairings were carried out in the same plate without inter-pairing interactions. Aerial mycelium in the meeting zone was visible as early as 4–5 days when heterokaryosis occurred. The trial was concluded 10 days after the pairing of mutants.

Different growth types occurred at the mycelial interface as follows: r1, formation of microsclerotia

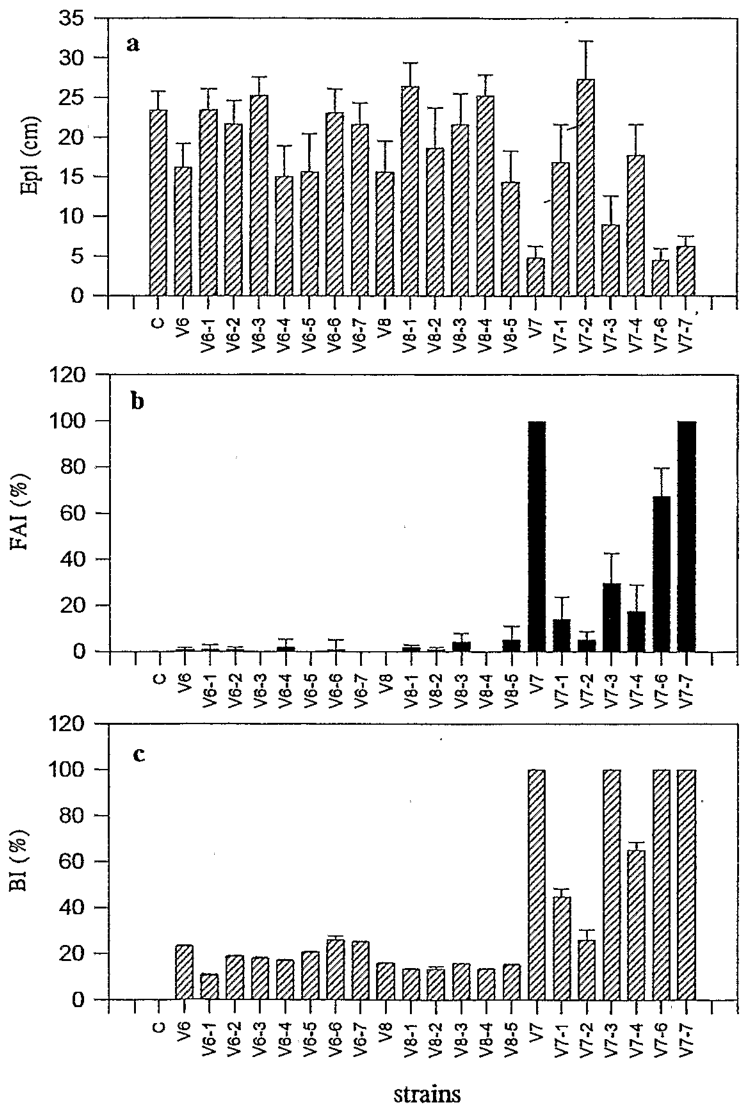

Figure 1 Epicotyl length(Epl)(a), Foliar alteration index(FAI)(b) and Browning index(BI)(c), estimated on *Gossypium hirsutum* cv. Isa-205, 23, 23 and 24 days, respectively, after inoculation with clones 6 (V6), 7 (V7) and 8 (V8) of *Verticillium dahliae and* their subclones.

without aerial mycelium; r2, visible reaction in the medium matrix only; r3, late formation of loose aerial mycelium in the contact line; r4, high reaction with differentiation of a dense aerial mycelium in localized areas at the contact line; r5, elaboration of a dense aerial mycelium in the confrontation line with differentiation of microconidia-containing spherules; and r6, production of mucoid thalli identical to those described with some wild-type strains and often forming microsclerotia. Types r1 to r4 were regarded as weak reactions and scored (±). Only r5 and r6 reactions were scored (+) (Daayf *et al.*, 1995). To determine whether our VCGs corresponded to those defined by Joaquim & Rowe (1990), pairings between their testers (T9 from VCG1, WM from VCG2 and BB and S39 from VCG4) and our strains were conducted.

Results

Intraclonal diversity in pathogenicity of hyaline mutants of *V. dahliae* on cotton

Among the 200 subclones isolated from each of the three microsclerotial wild-type single-spore clones, a few were hyaline variants (seven, seven and five from clones 6, 7 and 8, respectively). Most hyaline subclones produced a few microsclerotia after 5–6 weeks, but some (V6-1, V6-4, V7-2, V7-6, V7-7, V8-1 and V8-2) did not.

The three wild-type microsclerotial clones and18 of their hyaline subclones were tested for pathogenicity on *G. hirsutum* cv. Isa-205. Results recorded after 23 days are shown in Fig. 1. Only those plants inoculated with clones 6 and 8 and subclones V6-4, V6-5 and V8-5 showed a significant reduction of epicotyl length (Fig. 1a). Clone 7 and its subclones exhibited wide differences in reducing plant growth, from the least aggressive, V7-2, to the most aggressive, V7-6 and V7-7. Leaf alterations (FAI) caused by clones 6 and 8 and their subclones were quite low, never exceeding 10% (Fig. 1b); those caused by clone 7 and its subclones V7-3, V7-6 and V7-7 were severe, sometimes leading to death, but subclones V7-1, V7-2 and V7-4 caused relatively weak foliar symptoms. All tested strains caused browning of vessels and adjacent tissues (Fig. 1c), but the browning index (BI) showed a pattern of severity among clones and subclones that was variable, similar to that recorded by the FAI index. The lowest BI values were recorded for clones 6, 8 and their subclones, with very little differences among them (Fig. 1c). As noticed for Epls and FAIs, the BIs for clone 7 and its subclones were variable, 7, V7-3, V7-6 and V7-7 being the most aggressive. Subclone V7-2 caused the least severe internal symptoms, similar to those of nondefoliating strains. Among the subclones with which BI reached 100%, V7-6 and V7-7 caused plant death the most rapidly. Further experiments using most of these subclones (V6-1, V6-2, V6-4, V8-1, V8-2, V7-2, V7-3, V7-6 and V7-7) yielded the same results (data not shown). Thus, in general, the subclones obtained from clone 7 were the most diverse and appeared to be the most interesting for selection of hyaline subclones possessing wide differences in pathogenicity.

Based on these results, the subclones V7-2 and V7-7 were selected as the least and most aggressive, respectively. The observation of stunting and foliar symptoms showed, from the first week following inoculation, that V7-2 had a weak pathogenicity potential (SI = 0%, FAI = 0%), when compared with V7-7 (SI = 5%, FAI = 9%). This observation was confirmed 13 days (SI = −5% for V7-2 vs. 43% for V7-7; FAI = 0% for V7-2 vs. 36% for V7-7) and 22 days (SI = 5% for V7-2 vs. 77% for V7-7; FAI = 4% for V7-2 vs. 100% for V7-7) after inoculation. Studies on differences in pathogenicity on cotton between subclones V7-2 and V7-7 were extended to other cotton cultivars. The degree of stunting caused by the two subclones on the four tested cultivars varied. *G. hirsutum* cultivars Tashkent-1 and108F and *G. arboreum* cv. Xiao were more stunted by V7-7 than was *G. barbadense* cv. Ashmouni. In general, V7-7 caused more foliar damage than V7-2 (Table 1). Such alterations were lowest on *G. arboreum* cv. Xiao for both V7-2 and V7-7, but, as for SI values, cv. 108F was most affected by V7-7. All cultivars infected by V7-7 exhibited a BI > 85%, 23 days after infection, while this index did not exceed 33% in cultivars infected by V7-2 (Table 1).

Intraclonal diversity in vegetative compatibility

Results of pairings between *nit*-mutants selected from the hyaline subclones are presented in Table 2. Mutants obtained from clone 8 and its subclones were compatible

Table 1 Comparison of pathogenicity of subclones V7-2 and V7-7 of *Verticillium dahliae* estimated on four cotton cultivars 23 days after inoculation

	SI(%)		FAI(%)		BI(%)	
	V7-2	V7-7	V7-2	V7-7	V7-2	V7-7
G. hirsutum 108F	6 ± 1	84 ± 15	3 ± 4	90 ± 10	4 ± 8	100 ± 0
G. hirsutum Tashkent-1	12 ± 2	72 ± 35	11 ± 13	83 ± 16	33 ± 23	98 ± 6
G. barbadense Ashmouni	15 ± 5	67 ± 8	14 ± 14	57 ± 28	25 ± 9	98 ± 4
G. arboreum Xiao	3 ± 3	81 ± 11	1 ± 2	37 ± 13	18 ± 15	86 ± 11

SI, stunting index; FAI, foliar alteration index; BI, browning index; ± represents standard deviation.

Table 2 Vegetative compatibility pairings between *nit* mutants of *Verticillium dahliae*

	6	V6-1	V6-4	8	V8-1	V8-2	7	V7-2	V7-4	V7-6	V7-7	T9	WM	BB	S39
6	+	+	+	+	+	+	−	−	−	−	−	−	+	±	−
V6-1		+	+	+	+	+	−	−	−	−	−	−	+	−	−
V6-4			+	+	+	+	−	−	−	−	−	−	+	−	−
8				+	+	+	−	−	−	−	−	−	+	−	−
V8-1					.	+	−	−	−	−	−	−	+	±	−
V8-2						.	−	−	−	−	−	−	+	−	−
7							+	+	+	+	+	+	−	−	−
V7-2								.	+	+	+	+	−	−	−
V7-4									.	+	+	+	−	−	−
V7-6										.	+	+	−	−	−
V7-7											.	+	−	−	−

Every (+, − or ±) represents the interpretation of all reactions observed resulting from pairings of *nit1*, *nit3* and *nitM* mutants of one strain with complementary *nit* mutants of another strain; (+), strong reaction; (±), weak reaction; (−), no visual reaction observed; (.), not paired.

with each other and also with *nit*-mutants deriving from clone 6 and its subclones and with *nit*M from WM (VCG2). Mutants from clone 7 and its subclones were also compatible with each other and with *nit*M of T9 (VCG1), but were never compatible with *nit* mutants from clones 6 and8 or their subclones. Tester S39 of VCG4 did not show compatibility with any mutants from tested subclones. These results confirmed that clones 6 and8 and their subclones belong to the same VCG and that they are not compatible with strains from VCG1. They also confirmed that V7-2 and V7-7, produced from the same defoliating clone 7, are genetically close, although they differ in pathogenicity on cotton.

Effects of temperature and nitrogen source on the growth of V7-2 and V7-7

Twenty days after incubation of clones V7-2 and V7-7 at two different temperatures (21 and 27°C), growth reduction at 27°C was greater in clone V7-2 than in clone V7-7 (Table 3). Of the nitrogen sources, NH_4 plus $CaCO_3$ was the most favourable for growth of both clones. A difference in growth was found between V7-2 and V7-7 on NO_3- and NO_2-supplemented media, both more favourable to V7-7 (Table 3).

Table 3 Effects of temperature and nitrogen source on the growth of *Verticillium dahliae* subclones V7-2 and V7-7

		Colony diameter (mm)	
		V7-2	V7-7
Temperature (°C)	21 C	85 ± 2	78 ± 2
	27 C	74 ± 4	74 ± 4
Nitrogen source	NH_4	34 ± 2	27 ± 2
	NH_4+CO_3	71 ± 3	70 ± 1
	NO_3	24 ± 1	57 ± 3
	NO_2	4 ± 4	32 ± 3

± represents standard deviation.

Discussion

This study has characterized two subclones of *V. dahliae* that showed large differences in pathogenicity and growth. Intraclonal diversity in pathogenicity was particularly common in subclones from defoliating clone 7. Strains from nondefoliating clones 6 and8 also differed pathogenically, but they always remained nondefoliating as previously shown for V8-2 and V8-5 (Daayf *et al.*, 1995). Within the progeny of defoliating clone 7, where differences in pathogenicity were most noticeable, subclone V7-2 produced weak symptoms. Results obtained from vegetative compatibility studies, a powerful tool for genetic proximity determination (Leslie, 1993), confirmed that all tested subclones from clone 7, including V7-2, belonged to the same group.

Comparison of these results with those previously obtained with V7-2, V7-4, V7-6, V7-7, V8-2 and V8-5 (Daayf *et al.*, 1995) confirmed the stability in pathogenicity of these hyaline mutants. Because of the wide range in pathogenicity among subclones from clone 7, V7-2 (the least aggressive) and V7-7 (the most) were chosen for further characterization studies.

Differences in pathogenicity among strains of *V. dahliae* may be partly related to their different adaptation to high temperatures, as reported by Bell (1992) and other workers (Heale, 1985) and may be true for distribution of defoliating and nondefoliating strains of *V. dahliae* throughout the world. Results confirmed the tendency of the most virulent strains to grow better than the less virulent ones over a wider range of temperature conditions. Temperature may also affect parasitism of the fungus in the plant as observed in V7-2-infected cotton plants, which expressed more symptoms when they were first left 3 days in a cooler growth chamber (21°C) before being transferred to 24°C (data not shown).

Growth of V7-2 and V7-7 was similar on media containing buffered or unbuffered NH_4. On the other hand, V7-7 grew more vigorously than V7-2 on NO_3. V7-2 failed to grow on NO_2 whereas V7-7 grew well. This correlates well with differences in pathogenicity of such subclones and supports the statement by Bell (1992) that pathogenicity may be related to the ability of *V. dahliae* to disturb hormonal and nitrogen metabolism. The same author has also shown a relation between pathotype and capacity to use ammonium and nitrate nitrogen in diseased plants (Bell, 1991).

The accumulation of fungitoxic compounds in cotton plants in response to inoculation with *V. dahliae* subclone V7-2 was greater than in those inoculated with V7-7 (data not shown). This indicates that in spite of their genetic proximity, the two subclones either elicit different responses in the host plant or have different capacities for phytoalexin detoxification.

In conclusion, using hyaline mutants, it was possible to show intraclonal diversity in pathogenicity of *V. dahliae* and to characterize two subclones differing in their behaviour, although they were obtained from the same clone. Comparable hyaline mutants are in use by some breeders for selection of tomato cultivars resistant to *Verticillium* wilt (C. Boisson, personal communication, ORSTOM, France, retired) and their stability is in great demand for studying molecular genetic diversity and host–parasite interactions. The use of such strains may be of great interest for assessment of interactions of *V. dahliae* with other hosts, since this pathogen can attack plants belonging to many species.

References

Barron GL, Fletcher JT, 1970. *Verticillium albo-atrum* and *V. dahliae* as mycoparasites. *Canadian Journal of Botany* 48, 1137–9.

Barrow JR, 1970. Critical requirements for genetic expression of *Verticillium* wilt tolerance in Acala cotton. *Phytopathology* 60, 559–60.

Barrow JR, 1973. Genetics of *Verticillium* tolerance in cotton. In: *Verticillium Wilt of Cotton. USDA ARS-S-19*, Washington, USA. 89–97.

Bejarano-Alcázar J, Jiménez-Diaz RM, Melero-Vara M, Blanco-Lopez MA, 1990. Characterization and distribution of pathotypes of *Verticillium dahliae* infecting cotton in southern Spain. In: *5th International* Verticillium *Symposium, 25–30th June, Leningrad, USSR* 3.

Bejarano-Alcázar J, Melero-Vara M, Blanco-Lopez MA, Jiménez-Diaz RM, 1995. Influence of inoculum density of defoliating and nondefoliating pathotypes of *Verticillium dahliae* on epidemics of *Verticillium* wilt of cotton in southern Spain. *Phytopathology* 85, 1474–81.

Bell AA, 1991. Accumulation of ammonium ions in *Verticillium*-infected cotton and its relation to strain virulence, cultivar resistance and symptoms. In: *Proceedings of the Beltwide Cotton Production Research Conference, Memphis, TN, USA*. 186.

Bell AA, 1992. *Verticillium* diseases. In: Hillocks RJ, ed. *Cotton Diseases.* CAB International, Wallingford, UK: 87–126.

Bell AA, Mace ME, 1984. Physiology of *Verticillium* wilt of cotton. In: *Proceedings of the Beltwide Cotton Production Research Conference, Atlanta, GA, USA*. 43–47.

Bell AA, Puhalla JE, Tolmsoff WJ, Stipanovic RD, 1976. Use of mutants to establish (+)-scytalone as an intermediate in melanin biosynthesis by *Verticillium dahliae. Canadian Journal of Microbiology* 22, 787–99.

Boisson C, Lahlou H, 1982. Etude du polymorphisme intraclonal chez le *Verticillium albo-atrum*, forme à microsclérotes. II. Morphologie et morphogénèse comparées de deux isolats de phénotype sauvage et de leurs principaux variants. *Canadian Journal of Botany* **61**, 188–96.

Brandt WH, Roth JN, 1965. Loss of melanin-containing structures by *Verticillium* cultures. *Phytopathology* 55, 1200–2.

Correll JC, Gordon TR, McCain AH, 1988. Vegetative compatibility and pathogenicity of *Verticillium albo-atrum. Phytopathology* 78, 1017–21.

Correll JC, Klittich CJR, Leslie JF, 1987. Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. *Phytopathology* 77, 1640–6.

Cove DJ, 1976. Chlorate toxicity in *Aspergillus nidulans*: the selection and characterization of chlorate resistant mutants. *Heredity* 36, 191–203.

Daayf F, Nicole M, Geiger JP, 1995. Differentiation of *Verticillium dahliae* on the basis of vegetative compatibility and pathogenicity on cotton. *European Journal of Plant Pathology* **101**, 69–79.

Erwin DC, Tsai SD, Khan RA, 1976. Reduction of the severity of *Verticillium* wilt of cotton by the growth retardant, Tributyl (5 chloro-2-thienyl methyl) phosphonium chloride. *Phytopathology* 66, 106–10.

Follin JC, 1986. La sélection du cotonnier (*Gossypium hirsutum* L.) pour la résistance aux maladies présentes en Afrique au sud du Sahara. *Coton et Fibres Tropicales* (Suppl.).

Gu BK, Li JY, Lu X, Gu P, 1988. Studies of biology and pathogenicity of *Verticillium dahliae* Kleb. of cotton in Jiangsu province. *Journal of Jiangsu Agricultural Science* 4, 27–34.

Gubler WD, Grogan RG, Greathead AS, 1978. Wilt of cucumber caused by *Verticillium albo-atrum* in California. *Plant Disease Reporter* 62, 786–8.

Hadisutrisno B, 1987. Etude de la variabilité intraclonale du pouvoir pathogène du Verticillium dahliae Kleb. vis à vis de la tomate et du cotonnier. Montpellier, France: ENSA of Montpellier, PhD thesis.

Heale JB, 1985. *Verticillium* wilt of alfalfa, background and current research. *Canadian Journal of Plant Pathology* 7, 191–8.

Heale JB, Isaac I, 1963. Wilt of lucerne caused by species of *Verticillium*. IV. Pathogenicity of *V. albo-atrum* and *V. dahliae* to lucerne and other crops. Spread and survival of *V. albo-atrum* in soil and in weeds. Effect upon lucerne production. *Annals of Applied Biology* 52, 439–51.

Heale JB, Isaac I, 1965. Environmental factors in the production of dark resting structures in *Verticillium albo-atrum, V. dahliae*, and *V. tricorpus*. *Transactions of the British Mycological Society* 48, 39–50.

Joaquim RR, Rowe RC, 1990. Reassessment of vegetative compatibility relationships among strains of *Verticillium dahliae* using nitrate-nonutilizing mutants. *Phytopathology* 80, 1160–6.

Jun Y, Bridge PD, Evans HC, 1991. An integrated approach to the taxonomy of the genus *Verticillium*. *Journal of General Microbiology* 137, 1437–44.

Koroleva NS, Kasyanenko AG, 1987. Studies on the ecology, population and evolution of microfungi of the genus *Verticillium*. II. Dynamics of the racial composition of a population of *Verticillium dahliae*. *Mikologiya y Fitopatologiya* 21, 164–8.

Leslie JF, 1993. Fungal vegetative compatibility. *Annual Review of Phytopathology* 31, 127–51.

Lim TK, Nik WZ, 1983. Mycoparasitism of the coffee rust pathogen, *Hemileia vastatrix*, by *Verticillium psalliotae* in Malaysia. *Pertanika* 6, 23–5.

Puhalla JE, 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. *Canadian Journal of Botany* 63, 179–83.

Samson RA, Evans HC, Latge JP, 1988. *Atlas of Entomopathogenic Fungi*. Berlin, Germany: Springer-Verlag.

Schnathorst WC, 1981a. *Verticillium* wilt. In: The American Phytopathological Society, ed. *Compendium of Cotton Diseases*, St. Paul, MN, USA. 41–44.

Schnathorst WC, 1981b. Life cycle and epidemiology of *Verticillium*. In: Mace ME, Bell AA, Beckman CH, eds. *Fungal Wilt Diseases of Plants*. New York, USA: Academic Press, 81–111.

Tjamos EC, Kornaros E, 1978. Virulence of Greek *Verticillium dahliae* isolates on susceptible and tolerant cotton cultivars. *Plant Disease Reporter* 62, 456–8.

Tolmsoff WJ, 1972. Diploidization and heritable gene repression–derepression as major sources for variability in morphology, metabolism and pathogenicity of *Verticillium* species. *Phytopathology* 62, 407–13.

Typas MA, Heale JB, 1976. Acriflavine-induced hyaline variants of *Verticillium albo-atrum* and *V. dahliae*. *Transactions of the British Mycological Society* 66, 15–25.

Typas MA, Heale JB, 1979. Transfer of a cytoplasmic factor by micro-injection in *Verticillium*. *Journal of General Microbiology* 111, 375–86.

Wheeler MH, Tolmsoff WJ, Bell AA, Mollenhauer HH, 1978. Ultrastructural and chemical distinction of melanins formed by *Verticillium dahliae* from (+)-scytalone, 1,8-dihydroxynaphthalene, cathecol, and L-3,4–dihydroxyphenylalanine. *Canadian Journal of Microbiology* 24, 289–97.

Wilhem S, Sagen JE, Tietz H, 1974. Resistance of *Verticillium* within cotton: source, techniques of identification, inheritance trends and the resistance of potential multiline cultivars. *Phytopathology* 64, 924–31.

Volume 47, Number 4, August 1998

Plant Pathology

An International Journal edited by the
British Society for Plant Pathology

BSPP

Senior Editor R. Johnson

ICPP98

7th International Congress of Plant Pathology

Edinburgh, UK
9–16 August
1998

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ISSN 0032-0862

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