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Differentiation of *Verticillium dahliae* populations on the basis of vegetative compatibility and pathogenicity on cotton

Fouad Daayf, Michel Nicole and Jean-Paul Geiger*
Laboratoire de Phytopathologie Tropicale, ORSTOM, 911, Avenue Agropolis, 34032 Montpellier, France
* *Author for correspondence*

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Abstract

Complementary auxotrophic nitrate-nonutilizing (*nit*) mutants were used to investigate vegetative compatibility within 27 strains of *Verticillium dahliae* isolated from several hosts originating from Africa, Asia, Europe and the United States. Using about 500 *nit* mutants generated from these strains, three vegetative compatibility groups, 1, 2 and 4, were identified. Simultaneously, virulence of each strain was assessed on cultivars of *Gossypium hirsutum, G. barbadense* and *G. arboreum*, based upon Foliar Alteration Index (FAI) and Browning Index (BI) estimation. The strains in VCG1 were of both the cotton-defoliating pathotype and race 3 (on cotton) but were non pathogenic on tomato; those in VCG2 and VCG4 were of the nondefoliating pathotype and belonged to different races on cotton and on tomato. Hyaline mutants deriving from parental wild-type strain showed differences in pathogenicity but were always assigned to the parental VCG. A relationship was established between VCGs and the taxonomic position of host plants. Data from *nit* pairings indicated that the sub-populations of *V. dahliae* (VCGs) may not be completely isolated genetically.

Introduction

Verticillium dahliae Kleb. is a fungal pathogen causing vascular wilt diseases on more than 160 plant species [Schnathorst, 1981]. In general, most strains of *V. dahliae* infect many hosts although a few strains exhibit specialization. Characterization of populations of this pathogen with regard to pathogenicity is therefore rather complex. Usually strains are classified by reference to the host from which they were isolated and a given strain may be characterized independently on the basis of pathogenicity on different hosts. As a consequence, it is frequently impossible to establish any relationship between different population analyses of *V. dahliae*.

For example two races of *V. dahliae* have been identified on tomato plants: race 1, virulent only on cultivars lacking the *Ve* resistance gene and race 2 attacking all cultivars whether or not this gene is present [Alexander, 1962; Schaible et al., 1951] but for cotton cultivars (*Gossypium* sp.) three races were described in China [Gu et al., 1988; Qingji and Chiyi, 1990] and six in USSR [Kasyanenko and Ryabova, 1986; Koroleva and Kasyanenko, 1987; Portenko, 1990; Portenko and Kasyanenko, 1987]. These authors did not use the same cotton testor plants and so no relation between the two race-systems could be established. On cotton, strains of *V. dahliae* have also been classified into two groups on the basis of their ability to cause defoliation (D strains) or not (ND strains) [Schnathorst and Mathre, 1966]. Several additional characters were used to distinguish these pathotypes, including optimal growth temperature, germination rate of conidia, production rate of microconidia and microsclerotia [Willie and Devay, 1970], sanguinarine detoxifi-

cation [Presley, 1969] and physiological responses induced in cotton plants [Tzeng and Devay, 1985]. Subsequent investigations of vegetative compatibility showed that D strains all belong to one vegetative compatibility group (VCG) and were incompatible with ND strains [Joaquim and Rowe, 1990; Puhalla, 1979; Puhalla and Hummel, 1983; Qingji and Chiyi, 1990].

Recent studies have suggested that vegetative compatibility analysis is a powerful tool for investigating genetic diversity among natural populations of fungi [Bell, 1992; Correl et al., 1987, 1988; Elmer and Stephens, 1989, Jacobson and Gordon, 1988; Puhalla, 1985; Puhalla and Hummell, 1983; Puhalla and Mayfield, 1974; Tantaoui and Boisson, 1991; Fernandez et al., 1994]. In *V. dahliae*, the two most common types of mutants that have been used to study heterokaryosis are UV-induced microsclerotial color mutants and spontaneous nitrate-nonutilizing (*nit*) mutants. Using color mutants, Puhalla [1979] classified 19 strains of a *V. dahliae* collection into 4 VCGs. Sixteen VCGs were later identified in a collection of 94 strains [Puhalla and Hummel, 1983]. Using *nit* mutants, Joaquim and Rowe [1990] characterized only 4 VCGs among 22 strains that were formerly classified into 15 groups by Puhalla and Hummel [1983]. Recently, Strausbaugh et al. [1992] revising Joaquim and Rowe's classification, demonstrated the existence of sub-groups within two of the VCGs.

These results show that characterization of *V. dahliae* strains remains unclear. Attempts to correlate VCGs with geographical origin of the strains led to contradictory results [Puhalla, 1979; Puhalla and Hummel, 1983; Joaquim and Rowe, 1990].

In this paper, our first objective was to characterize pathogenicity of *V. dahliae* strains collected on cotton growing in different regions of the world. The second objective was to compare different methods for strain characterization and to correlate our results with those published elsewhere. Therefore, our collection of isolates included (a) reference strains (testors) from the VCGs identified by Joaquim and Rowe [1990], (b) reference strains of the russian race system [Koroleva and Kasyanenko, 1987] and (c) reference strains of the D and ND types. Some strains from hosts other than cotton were studied and progenies (hyaline variants) from two of our clones were used to assess intraclonal stability.

Materials and methods

Collection of V. dahliae *strains.* The 31 strains of *V. dahliae* used in this study were either black microsclerotial wild-type clones or hyaline variants (Table 1), the latter being progenies of wild-type clones that had lost the capacity to produce microsclerotia, thus exhibiting a white thallus [Boisson and Lahlou, 1982; Heale and Isaac, 1965; Pegg, 1957]. Strains were isolated from soil and from the following host plants grown in different regions of the world (Table 1) : tomato (*Lycopersicon esculentum*), sweet pepper (*Capsicum annuum*), cotton (*Gossypium* sp.), cocoa (*Theobroma cacao*) and eggplant (*Solanum melongenae*). Reference strains belonged to either race 1 or 2 on tomato, race 1, 2, 3 or 4 on cotton, pathotype D or ND on cotton and VCG 1 to 4.

Generation and selection of nit *mutants. Nit* mutants were used to assess vegetative compatibility relationships among strains of *V. dahliae*. The mutants were generated according to a procedure modified from that developed by Cove [1976] for *Aspergillus nidulans* and adapted for *Fusarium oxysporum* by Puhalla [1985] and for *V. dahliae* by Joaquim and Rowe [1990]. Agar plugs, approximately 15 × 2 mm, were cut from the colony margin of wild-type thallus growing on potato dextrose agar (Difco). These were placed on chlorate minimal medium (CMM : minimal medium (MM) amended with 30 $g.l^{-1}$ of potassium chlorate) in 9-cm-diameter Petri dishes. After ten days at 25 °C, a fragment (1 cm in diameter) was cut from the margin of the chlorate-resistant colony and transferred into a tube containing 8 ml of sterile water. The concentration of each conidial suspension was estimated using hemacytometer and then adjusted to a concentration of 1×10^4 conidia per ml. 50 µl of the conidial suspensions were pipetted onto Petri dishes containing CMM. After about 40 h, each individual germinated spore was transferred onto MM plates to obtain distinct chlorate-resistant monoconidial strains. 480 clones were selected from all 27 strains. Most of the resulting colonies displayed a specific colony mor-

Table 1. Characteristics of the strains of *Verticillium dahliae* and comparison betewen VCGs, pathotypes and races

Strain	Origin	Host	Source[a]	VCG[b]	Pathotype (D) or (ND)	Race/Cotton	Race/Tomato[c]
7	USA	Cotton	s2	1	D	3	0
V72[d]	USA	Cotton	s6	1	ND	0	-
V73[d]	USA	Cotton	s6	1	D	-	-
V74[d]	USA	Cotton	s6	1	D	-	-
V76[d]	USA	Cotton	s6	1	D	-	-
V77[d]	USA	Cotton	s6	1	D	-	-
11	USA	Cotton	s2	1	D	3	0
D3	CIN	Cotton	s4	1	D	3	-
E	CIN	(Soil)	s4	1	D	3	-
T9[e]	USA	Cotton	s7	1	D	-	-
1	France	Tomato	s1	2	ND	0	1
2	Spain	Sweet pepper	s1	2	ND	4	2
6	USA	Cotton	s2	2	ND	0	1
8	France	Cotton	s2	2	ND	0	1
V82[d]	France	Cotton	s6	2	ND	-	-
V85[d]	France	Cotton	s6	2	ND	-	-
10	USA	Cotton	s2	2	ND	-	1
A	CIN	Cotton	s4	2	ND	1	-
B	CIN	Cotton	s4	2	ND	2	-
C	CIN	Cotton	s4	2	ND	2	-
F	CIN	Cotton	s4	2	ND	4	-
WM[e]	USA	Cotton	s7	2	ND	-	-
3	Brazil	Tomato	s1	4	ND	0	2
4	Morocco	Tomato	s1	4	ND	4	2
BB[e]	USA	Potato	s7	4	ND	-	-
H	Guadaloupe	Eggplant	s6	4	ND	-	-
I	Reunion	Eggplant	s6	4	ND	2	-
S39[e]	USA	(Soil)	s7	4	ND	-	-
5	Morocco	Tomato	s1	-[f]	ND	-	2
9	Spain	Tomato	s3	-[g]	ND	-	2
G	Brazil	Cocoa	s5	-[g]	ND	-	-

a : s1 INRA/Montfavet, s2 IRCT-CIRAD/Montpellier, s3 Vilmorin/Montpellier, s4 Dr Portenko/USSR, s5 Dr Flood/UK, s6 ORSTOM/Montpellier, s7 Pr Rowe/OHIO-OARDC-USA.
b : VCG sensu Joaquim & Rowe (1990).
c : races previously determined.
d : hyaline variants from strain 7 or 8.
e : nitM testors [references used for the characterization of the different VCGs sensu Joaquim & Rowe (1990)].
f : self-incompatible and non-compatible with any strain of the collection.
g : no nit mutant generated.
- : not determined.

phology which was characterized by thin and expansive mycelium on MM medium indicative of their inability to metabolize nitrate; these colonies were presumed to be *nit* mutants.

Characterization of nit *mutants.* A fragment of thallus for each chlorate-resistant mutant was transferred onto basal medium (MM without nitrogen) supplemented 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 $g.l^{-1}$). The *nit* mutant phenotype designations *nit*1, *nit*3 and *nit*M

corresponded to those used with *V. albo-atrum* [Correll et al., 1988], *F. oxysporum* [Correll et al., 1987] and *V. dahliae* [Joaquim and Rowe, 1990, 1991]. They refer respectively to a mutation at a structural locus of nitrate reductase (*nit*1), to a mutation at a specific regulatory locus of the nitrate assimilation pathway (*nit*3) and to a mutation at one of the loci that control the synthesis of the molybdenum-containing cofactor required for nitrate reductase and purine dehydrogenase activities (*nit*M).

Pairing of nit *mutants*. To determine VCG among the strains in our *V. dahliae* collection, 978 pairings were performed between *nit* mutants, by placing two plugs of mycelial fragments (approximately 1 mm^3), 7-to-8 mm from each other, in a 9-cm Petri dish containing MM. Four pairings were carried out on each plate without inter-pairing interactions. All pairings were performed at least twice.

Pathogenicity of V. dahliae *strains*. *G. hirsutum* cv. Isa-205 and *G. barbadense* cv. Ashmouni, selected for their susceptibility and tolerance to *V. dahliae* respectively, were used to characterize strains as defoliating (D) or non-defoliating (ND). *G. hirsutum* cv 108F and vc Tashkent1, and *G. arboreum* cv Xiao were used to identify races according to Koroleva and Kasyanenko [1987].

After a 5 min surface disinfection with a 5% sodium hypochlorite solution, seeds were placed for 24 h in Petri dishes containing moistened sterile filter paper; germinated seeds were then planted in potting mixture (mould and sand, 6:1, v:v).

Ten two-week-old cotton plants were carefully up-rooted and the roots immersed for 15 min in 100 ml of a conidial suspension containing 10^6 spores per ml. Ten control plants were immersed in sterile distilled water. All plants were then replanted, each in a plastic pot (9 cm in diameter) and grown under 12 h photoperiod, 24–29 °C and 70–90 % relative humidity.

Pathogenicity was determined on the basis of both external (plant stunting and foliar damage) and internal symptoms (vascular discoloration) which have developed 15 and 17 days after inoculation, respectively.

Stunting was quantified by measuring the epicotyl length (Epl) of each plant.

Foliar damage was evaluated by rating each cotyledon and each leaf (x_i) according to the following rating scale : 0 = no foliar symptoms; 1 = yellowing or partial necrosis of the cotyledon; 2 = cotyledon fall; 3 = yellowing of leaf; 4 = wilted or necrotic leaf; 5 = dead leaf. Foliar Alteration Index (FAI) (Hadisutrisno, 1987; Lahlou, 1983) was calculated for each inoculated plant : FAI = $100\ \Sigma x_i/(4 + 5n)$, where (4) is the maximum score for the cotyledons (maximum score for each cotyledon = 2), (5) the maximum score for each leaf and (n) the number of leaves of each plant.

Vascular discoloration was evaluated according to a modified method of Erwin et al. [1976]; discoloration was scored (yi) for every internode using the following scale : 0 = no discoloration; 1 = two to five localized brown regions within the vascular tissue of the same internode; 2 = long brown stretches of the vascular tissue; 3 = browning of vessels but not of the adjacent tissues; 4 = browning of both vessels and adjacent tissues. The browning Index (BI) was calculated as follows; BI = $100\Sigma yi/4d$; where (d) is the total number of seedling internodes including hypocotyl and (4) is the maximum score for an internode.

Mean values of Epl, FAI, and BI were calculated based on ten replicates for both inoculated and control plants. Variance analysis was performed with p = 0.05. For each descriptor (roman numerals I to VI), multiple comparison of mean values was carried out using Newman-Keuls test [Sokal and Rohlf, 1981] in order to assign strains to different cluster (a, b, c, etc. . . .); within each cluster, mean values are not statistically different at p = 0.05.

Results

Selection and characterization of chlorate-resistant mutants. When sub-cultured on CMM, two kinds of colonies were observed. The most frequently encountered resembled wild-type strains, developing a dense mycelium all around the periphery of the colony. The second colony type was of dense aerial mycelium in fan-like sectors only, showing sparse growth elsewhere. Both

types could display morphological characteristics of *nit* mutants when cultivated on MM (containing nitrate as the only nitrogen source), grew as expansive colonies with this non-aerial mycelium. Except for strains 9 and G, *nit* mutants were recovered from all the strains of *V. dahliae* tested. Among 480 chlorate-resistant strains that were selected, 36.3 % were characterized as *nit*1, 17.9 % as *nit*M and only 2.9 % as *nit*3. About 30% grew like wild type colonies. The remaining chlorate-resistant mutants did not correspond to the usually cited *nit* mutants : *nit*1, *nit*3 or *nit*M, most showed wild type mycelium on CMM, on MM and on medium containing nitrite, and thin mycelium on medium containing hypoxanthin.

Pairing of nit *mutants and characterization of vegetative compatibility groups.* Aerial mycelium developed at the contact zone usually within 4–5 days when heterokaryosis occurred between complementary mutants. In this study we performed 186 '*nit*1 × *nit*1', 54 '*nit*1 × *nit*3', 23 '*nit*3 × *nit*3', 48 '*nit*3 × *nit*M', 204 '*nit*M × *nit*M', and 463 '*nit*1 × *nit*M' pairings.

At the contact-line between paired *nit* mutants, 5 reaction types were identified : (r1) formation of microsclerotia without aerial mycelium; (r2) visible reaction within the medium only; (r3) late formation of sparse aerial mycelium; (r4) strong reaction with differentiation of a dense aerial mycelium in localized areas; (r5) development of a dense aerial mycelium showing numerous microconidia-containing spherules and often microsclerotia production later; (r6) production of slimy mycelium, often forming microscelerotia. Types (r1), (r2), (r3) and (r4) were regarded as weak reactions and designated as (+/–). Reaction types (r5) and (r6) were strong reactions and designated as (+). When no visible reaction occurred, strains were considered as incompatible and the test was scored (–).

Results of pairings between *nit* mutants issued from 20 strains are presented in Table 2. Three VCGs were identified: the first contained two strains (7 and 11) and four hyaline variants from strain 7 (V72, V73, V74 and V77), the second included eight strains (1, 6, 8, 10, A, B, C and F) and two variants from strain 8 (V82 and V85), and the third included two strains (3 and 4). Strain 5 could not be assigned to a VCG because it was

Table 2. Results of vegetative compatibility pairings between *nit* mutants

	11	V72	7	V73	V74	V77	1	8	10	V85	V82	C	A	2	B	F	6	3	4	5
11	+	+	+	+	+	+	(+/–)	–	–				–			–	–	–	–	–
V72	+		+	+	+		–	–	–	–		–				–	–	–	–	–
7	+	+	+				–	–	–					–			(+/–)	–	–	–
V73	+	+					–	–	–								–	–	–	–
V74	+	+					–	–	–								(+/–)	–	–	–
V77	+						–					–						–		–
1	(+/–)	–	–	–	–	–	+	+	+	+		–	–	(+/–)	–	+	+	–	–	–
8	–	–	–	–	–		+	+	+	+							+	–	–	–
10	–	–	–	–	–		+	+	+								+	–	–	–
V85		–					+	+									+		–	–
V82																	+			
C		–					–						–		+	+	+	–	–	
A	–						–					–	+	+	–		(+/–)	–		–
2			–				(+/–)						+	+	+	+	–	–		–
B							–					+	–	+	+	+	(+/–)	–		–
F	–	–					+					+		+	+	+	+	–	–	
6	–	–	(+/–)	–	(+/–)		+	+	+	+	+	+	(+/–)	–	(+/–)	+	+	–	(+/–)	–
3	–	–	–	–	–	–	–	–	–			–	–	–	–	–	–	+	+	–
4	–	–	–	–	–		–	–	–	–		–				–	(+/–)	+	+	–
5	–	–	–	–	–	–	–	–	–	–			–	–	–		–	–	–	–

Every (+, -, or +/-) represents the interpretation of all reactions observed resulting from pairing 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. Strain 5 was self-incompatible and incompatible with other strains.

self-incompatible and incompatible with the other *nit* mutants tested. Vegetative compatibility grouping was more difficult to establish within the second group than within the others, since some paired strains were incompatible and others exhibited only weak reactions, *i.e.* 1 × 2, A × 6, and B × 6. Weak responses were also obtained between strains belonging to distinct VCGs, *i.e.* 1 × 11, 6 × 7, 6 × V74, 6 × 4, 2 × T9, 1 × BB. Positive reactions were obtained at a rate of 68%, 27% and 5%, respectively when using '*nit*1 × *nit*M', '*nit*M × *nit*M', and '*nit*3 × *nit*M' pairings. '*nit*1 × *nit*1', '*nit*1 × nit3', and '*nit*3 × *nit*3' pairings never resulted in a positive reaction.

Self-compatibility within V85 and V73 could not be verified because they failed to produce *nit*M mutants.

In order to correlate our three groups with those previously described by Joaquim and Rowe (1990), we paired representatives (*nit*3 and *nit*M mutants) of our groups (V72 and 11; 1, 2, A, B and C; 3 and 4) with the following four *nit*M testors : T9 (VCG1), WM (VCG2), S39 (VCG4A) and BB (VCG4B). This experiment also included four additional strains (D3, E, H and I) from our collection which had not been tested earlier. Results are presented in Table 3. Strain I was

Table 3. Correlation of our vegetative compatibility groups with those of Joaquim and Rowe (1990)

		VCGs *			
		VCG1	VCG2	VCG4A	VCG4B
		T9	WM	S39	BB
VCG 1**	V72	+		-	-
	11	+	-	-	-
	D3	+			
	E	+		-	-
VCG 2**	1	-	+	-	(+/-)
	2	(+/-)	(+/-)	(+/-)	(+/-)
	A	-	+	-	(+/-)
	B	-	(+/-)	-	(+/-)
	C		+	-	-
VCG 4**	3	-	-	+	(+/-)
	4	-			-
	H		-	+	(+/-)
	I	-		+	+

* : VCGs 1, 2, 4A and 4B as identified by Joaquim & Rowe (1990); Strains T9, WM, S39 and BB refer to the nitM testors of the corresponding VCGs.

** : VCGs identified among our strains. V72 and 11; 1, 2, A, B and C; 3 and 4 are representative of the three groups characterized in Table 2. (+) : strong reaction; (+/-) : weak reaction; (-) : no reaction; ( ) : not tested.

vegetatively compatible with both S39 (VCG-4A testor) and BB (VCG-4B testor) so we assume that S39 and BB belong to a same VCG; consequently, we disregarded the former subdivision of VCG4 into two subgroups, and our three groups corresponded to VCG1, VCG2 and VCG4 respectively.

Characterization of D and ND pathotypes to cotton. Virulence of *V. dahliae* was estimated on basis of the three criteria : Epicotyl length (Epl), Foliar alteration (FAI) and Browning (BI) indexes. Data from artificial infections are reported in Table 4 as are the statistical classes resulting from Newman-Keuls analysis. Two groups were identified : the first (D3, 7, 11, and E) exhibited high virulence especially on *G. hirsutum* cv Isa 205 (Epl < 25 mm, FAI > 39%, BI > 67%), the second group induced symptoms of a much lower severity : (Epl > 34 mm, FAI < 10%, BI < 37%). The first group included the two D reference strains (7 and 11), whereas the second included the three ND reference strains (6, 8 and 10). Therefore strains belonging to the first group were assigned to D strains, while those in the second group were assigned to ND strains.

A similar experiment was carried out on hyaline sub-clones (progenies) from two clones : 7 (D type) and 8 (ND type) (date not shown). In spite of a very high intraclonal diversity in virulence (Fig. 1) the progenies appeared to belong to their parental pathotypes, except for variant V72.

Characterization of races to cotton. Strains were inoculated to *G. hirsutum* cv 108F and cv Tashkent 1 and to *G. arboreum* cv Xiao and virulence estimated on basis of the criteria described above (Epl, FAI, and BI). A comparison was made with the race-reference strains A (race 1), B and C (race 2), D and E (race 3) and F (race 4) thus allowing the strains to be classified according to the race system of Koroleva and Kasyanenko [1987]. Strains 1, 3, 6, 8 and the variant V72 were of race 0 (not virulent on any of the three cultivars); strains I and S were of race 2; strain 7 and its hyaline variant V77 were of race 3; strains 2 and 4 were of race 4. None of the strains tested were of race 1 (except the reference strain). Variant V72 was identified as race 0 despite the parental clone 7 and variant V77 being race 3.

Table 4. Pathogenicity (D or ND) of *Verticillium dahliae* strains based on Epicotyl length (Epl), Foliar Index (FAI), and Browning Index (BI) on two cotton species

	G. hirsutum		*G. barbadense*		*G. hirsutum*		*G. barbadense*		*G. hirsutum*		*G. barbadense*		Pathogenicity
Strain designation	Epl (mm)	SC (I)	Epl (mm)	SC (II)	FAI (%)	SC (III)	FAI (%)	SC (IV)	BI (%)	SC (V)	BI (%)	SC (VI)	D or ND
7	24.2	I a	57.5	II abc	71.3	III b	17	IV c	93.5	V b	81	VI b	D
11	23	I a	62.5	II bc	67.4	III b	16.9	IV b	92.2	V b	83.4	VI b	D
D3	20	I a	61	II abc	39	III c	4.7	IV c	96.7	V ab	67.2	VI c	D
E	15.5	I a	34.5	II a	100	III a	37.4	IV a	100	V a	100	VI a	D
1	71	I d	70.5	II c	0	III d	1.4	IV c	13.9	V de	17.8	VI ef	ND
2	50	I bc	61	II abc	0	III d	0.4	IV c	13.5	V de	15.5	VI fg	ND
3	103	I e	63.5	II bc	0	III d	0.3	IV c	0	V f	21.1	VI ef	ND
4	48	I bc	40.5	II ab	0	III d	8.7	IV c	15	V de	30	VI de	ND
5	75	I d	61	II abc	0	III d	3.3	IV c	3.6	V f	19	VI ef	ND
6	65.5	I d	58.5	II abc	0	III d	2.6	IV c	12.6	V de	12.1	VI fgh	ND
8	57.5	I d	72	II c	0	III d	0	IV c	14.3	V de	12.1	VI fgh	ND
9	65.5	I d	123	II e	0	III d	0	IV c	0	V f	0	VI i	ND
10	59.5	I d	97	II d	2.1	III d	3.5	IV c	13	V de	12.2	VI fgh	ND
A	37	I b	67.5	II bc	9.5	III d	4.2	IV c	19.5	V d	37.8	VI d	ND
B	35	I a	85.5	II cd	9.2	III d	0	IV c	47.3	V c	24.2	VI de	ND
C	46	I bc	97.5	II d	5	III d	0	IV c	11.1	V e	18.7	VI ef	ND
F	52	I bc	85	II c	3.5	III d	0	IV c	11.6	V e	3	VI ghi	ND

Data were analysed by Neuwman-Keuls multiple comparison test of mean values leading to the assignation of strains to statistical classes (SC) identified by letters (a, b, ...). Isolates identified by the same letter for a given character (identified with roman numbers) are not different at p = 0.05 for this character. Severity is maximum within SC = a. Strains 7 and 11, and strains 6, 8 and 10 are references of (D) and (ND) pathotypes, respectively.

Discussion

Table 1 summarizes the information about the *V. dahliae* strains used in this study in order to facilitate a comparison between the different groupings.

As indicated in the Introduction, a large number of VCGs were identified when using UV-induced color mutants to determine vegetative compatibility between *V. dahliae* strains [Puhalla, 1979]. The characterization of *nit* mutants [Cove, 1976, 1993] and their use for studying fungal populations allowed Joaquim and Rowe [1990] to identify only four VCGs, among 22 *V. dahliae* isolates previously assigned to 15 VCGs by Puhalla and Hummel [1983]. Subsequent work [Strausbaugh et al., 1992] has also revealed only four VCGs (1, 2, 4, and 5) in *V. dahliae*. Additional experiments also revealed the occurrence of only few VCGs among a *V. dahliae* population [Joaquim and Rowe, 1991; Qingji and Chiyi, 1990; Strausbaugh et al., 1992]. The differences in findings seen when using either color mutants or *nit* mutants may be related to confusion between albino-microscelerotia (alm) mutants and hyaline (hyl-) variants that are known to be mutations at the mitochondrial level [Typas and Heale, 1976, 1978, 1979]. Thus, when UV-induced color mutants are paired, the occurrence of black microsclerotia in the mycelial meeting zone may

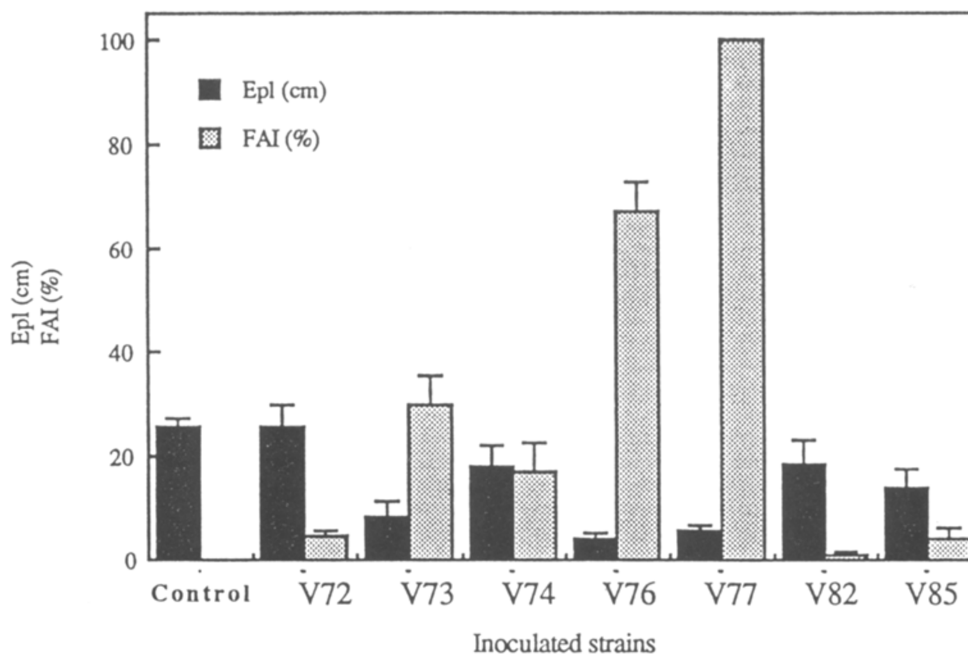

Fig. 1. Pathogenic variations among hyaline variants of *verticillium dahliae* originating from a defoliating and a nondefoliating strain. Pathogenicity is assessed by FAI(%) and Epl(cm), 23 days afer inoculaton of *G. hirsutum* Isa-205 plants.

result from merging between cytoplasms rather than between nuclei, as hypothesized by Joaquim and Rowe [1990].

Using the *nit*-mutant technique, the strains investigated in our study were shown to belong to either VCG 1, 2 or 4 [*sensu* Joaquim and Rowe, 1990], thus confirming the occurrence of only few VCGs within this *V. dahliae* population. The progenies from a clone (hyaline variants) were always assigned to the parental VCG. This observation was confirmed by additional experiments on 7 variants from clone 6 (data not shown), which indicate that the (hyl-) mutation does not affect vegetative compatibility.

Previous studies using color mutants of *V. dahliae* demonstrated a relationship between geographical origin of strains and VCGs [Puhalla, 1979; Puhalla and Hummell, 1983]. Such a relationship was not observed in our study, as exemplified by VCG4, the most geographically diverse group. These results are in agreement with data obtained by Joaquim and Rowe [1990]. Similarly, the VCGs identified within a *V. albo-atrum* population [Correll et al., 1988] were not correlated with geographical origin.

By contrast, a relationship seems to exist between VCGs and the taxonomic position of the host from which strains were isolated. Except for strain E collected from a cotton field, all strains of VCG1 and most of VCG2 were from cotton plants (Malvaceae), while strains of VCG4 were isolated from Solanaceous plants. A similar relationship has already been pointed out for *V. dahliae* populations isolated from both Malvaceae (cotton) and Solanaceae (potato, sweet pepper, tomato) [Joaquim and Rowe, 1990; Puhalla, 1979; Puhalla and Hummell, 1983], as well as for *V. albo-atrum* collected from hops (Cannabinaceae) or *Pelargonium* species (Geraniaceae) [Correll et al, 1988]. These observations may indicate that vegetative compatibility is related to the existence of preferential hosts for most *V. dahliae* strains however such a relationship was not found within a collection of *V. dahliae* strains isolated from diverse ornamental woody plants [Chen, 1994]. In light of these observations, it seems necessary to analyse a broader collection of strains before concluding wether or not a correlation may exist between VCGs and the taxonomic position of host-plants.

Pathogenicity of *V. dahliae* was characterized on basis of (a) the ability of defoliate cotton plants and (b) their behaviour on a range of cotton cultivars, according to the race-system determined by Koroleva and Kasyanenko [1987]. Except for V72, the strains grouped into VCG1 and those which displayed the highest virulence on cotton; they are of pathotype D and of race 3 (i.e. virulent on the three cotton cultivar host range). The other strains belong to VCG2 or VCG4 indicating that a relationship may exist between VCGs and pathogenicity on cotton. Similar observation has been evidenced by Corsini et al. [1985] and Joaquim and Rowe [1991] who identified two pathotypes in *V. dahliae* strains on the basis of their virulence to potato plants; these strains were grouped into three subgroups of VCG4 (VCG4A : virulent strains, subgroups 4A/B and 4B : less virulent strains).

As regards *V. dahliae* races on tomato (Table 1), the ND reference strain SS4 was previously identified as race 1, while D strains T1, T9 [Schnathorst and Mathre, 1966] and V798 [Hadisutrisno, 1987] did not attack tomato plants. According to this race-system, cotton-ND strains belonging to VCGs 2 and 4 may correspond to race 1 and race 2, respectively, whereas D strains (VCG 1) are not virulent on tomato plants. These results perhaps indicate (a) a certain degree of specialization among *V. dahliae* strains, with preferential or facultative hosts for each population and (b) the existence of a relationship between pathogenicity (pathotypes) and VCGs. By contrast, data reported by Ashworth [1983] support the idea that the virulence of soilborne *V. dahliae* occurs as a continuum form weakly virulent to highly virulent strains. This hypothesis was confirmed by Strausbaugh's [1992] findings on *V. dahliae* isolated from potatoes. Recent studies on molecular variation and sub-specific grouping within *V. dahlaie* [Okoli et al., 1993] also demonstrated that a few isolates of this pathogen were 'intermediate' and did not fall consistently into the two formerly identified groups. Thus, it appears that *V. dahliae* groupings seems to be less strictly delimited than within populations of other pathogens such as *V. albo-atrum* [Carder and Barbara, 1991; Correll et al., 1988; Okoli et al., 1993].

Occasional formation of a slight aerial mycelium between paired *nit* mutants of different VCGs suggests that merging of complementary-mutant fungal cells allows synthesis of functional nitrate reductase before cytoplasmic incompatibility really occurs [Joaquim and Rowe, 1990]. Our work (Tables 2 and 3) also shows the occurrence of weak reactions between some strains classified into distinct VCGs, demonstrating a transitory functional heterokaryosis before incompatibility occurs. This indicates that sub-populations of *V. dahliae* may not be completely genetically isolated. Anagnostakis et al. [1986] reported that weak reactions could also occur between strains of *Cryphonectria parasitica*, slightly different with regard to compatibility genes. It is likely that genetic variation results in strains of a given VCG that could show weak compatibility with strains of other VCGs. Variation already has been evidenced in pathogenicity of *Verticillium* strains [Ashworth, 1983; Boisson et al., 1991; Heale, 1988], resulting in modification of their specificity status [Fordyce and Green, 1962; Green, 1977].

In conclusion, our study showed that *V. dahliae* is characterized by a high genetic and pathogenic flexibility; but whether variations in pathogenicity within sub-populations is related to variation in vegetative compatibility remains to be elucidated.

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