Vegetative Compatibility Groups within *Verticillium dahliae*

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ABSTRACT

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Ninety-six strains of *Verticillium dahliae* isolated from 38 different host-plant species in 15 countries were tested for ability to form heterokaryons with each other (vegetative compatibility). Heterokaryon formation was detected as a black line of microsclerotia that formed when albino and brown microsclerotial variants (induced by exposure to ultraviolet radiation) were paired on a complete agar medium. Of the strains tested, two had long conidia and may have been diploid. Among six other strains no heterokaryosis was detected. The remaining 86 strains were classified in 16 vegetative compatibility (v-c) groups. Strains within a v-c group formed heterokaryons with each other, but not with strains from a different group. The various v-c groups showed marked differences in frequency of occurrence and in geographical distribution. Evidence is given for genetic homogeneity within a group. Strains within a group may also be similar in virulence and host range. The v-c groups are viewed as genetically isolated populations within the species and should be taken into account in epidemiological studies.

Additional key words: genetic isolation, *Gossypium hirsutum*, melanin mutants, sanguinarine medium.

In an earlier study (8) 19 strains of *Verticillium dahliae* Kleb. were assayed for ability to form heterokaryons with one another (vegetative compatibility). Microsclerotial color mutants were used for this purpose. Microsclerotia were normally black, but both brown and colorless (albino) mutants could be induced with ultraviolet light. When a brown and an albino mutant were paired on agar medium, black microsclerotia developed along the line of contact, but only if the two mutants were vegetatively compatible. These pairing studies yielded four groups such that all strains within a group formed heterokaryons with each other, but not with strains from a different group. The ability of two strains to form heterokaryons was taken as evidence for a close genetic relatedness between them. There were indeed indications in the earlier study that strains within a group were genetically homogeneous and were similar in virulence and host ranges.

In this paper we have expanded the original study of vegetative compatibility in *V. dahliae* to include 94 strains. These strains were isolated from 38 different host-plant species in 15 countries. As a result, 16 vegetative compatibility (v-c) groups have been found. More evidence is presented for the genetic and pathogenic homogeneity of strains within a group.

MATERIALS AND METHODS

Fungal strains. The strains of *V. dahliae* and their sources are listed in Table 1. Those strains preceded by an asterisk were studied earlier (8).

Media. Potato-carrot-dextrose agar (PCDA) (7) and minimal agar medium with L-sorbose (MM) (8,11) were used routinely in this work. Sanguinarine medium (SM) consisted of commercial PDA (Difco Laboratories, Detroit, MI 48232) amended with 500 ppm sanguinarine nitrate (Aldrich Chemical Co., Milwaukee, WI 53233) before autoclaving.

Procedures. Cotton plants were inoculated by using the stem injection technique described earlier (8,10). Spontaneous nicotinamide-requiring mutants (*nic*) were recovered from several of the strains by using the techniques reported by Puhalla (9).

The methodology for the induction, identification, and purification of microsclerotial color mutants is given in the earlier report (8). Both brown (*brm*) and albino (*alm*) mutants were recovered in two or more strains of each of the v-c groups P1 through P7 (Table 1) and in a single strain in the remaining groups. Only *alm* mutants were recovered in the other strains. As in the previous study, pairings between *brm* and *alm* mutants were made on PCDA by placing mycelial blocks of the two mutants 8–10 mm apart and incubating them at 24 C in the dark. Formation of a line of black microsclerotia at the intersection zone of the two mutant colonies indicated heterokaryosis (8). Usually, one strain in each v-c group served as tester for that group, and all strains in this study were paired with the tester strains of the 16 v-c groups. However, additional intra- and intergroup pairings were made among mutants of the remaining strains.

Strains tested for sensitivity to sanguinarine were each inoculated to the center of a plate of SM and a plate of PDA and incubated at 24 C. The percentage of growth inhibition was calculated from the formula:

$$\{1 - [(\text{colony diameter on SM})/(\text{colony diameter on PDA})]\} \times 100.$$

RESULTS

The v-c groups. All 94 strains produced black microsclerotia on PCDA medium, although a few strains produced them belatedly or sparingly. All strains had a sensitivity to germicidal ultraviolet light of wave length 254 nm that is characteristic of *V. dahliae* (7). Two of the strains had long conidia and did not yield microsclerotial color mutants after UV treatment; they may be diploid. Six other strains failed to form heterokaryons when an *alm* and *brm* mutant of the same strain were paired. These eight strains are included at the end of Table 1.

The remaining 86 strains of *V. dahliae* were assigned to one of 16 v-c groups. Within each group, pairing of the *alm* and *brm* mutants of the tester formed the black microsclerotia which indicate the heterokaryon reaction. Each of the 86 strains reacted with the tester of only one group. Among the numerous other intra- and intergroup pairings made, only the intragroup ones yielded a positive heterokaryon reaction. The v-c groups were labeled P1 through P16; groups P1 through P4 were defined in the earlier

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study (8). The most frequently recovered groups were P1, P2, P3, and P5. V-c groups P1 and P4 showed limited geographical distributions, and most Australian strains were in P6. In contrast, group P3 was found in widely separated geographical areas.

Recovery of spontaneous *nic* mutants. In the present sample strains from 15 v-c groups produced a high frequency of *nic* mutants (9). The remaining v-c group P4 had nine strains, and all but one of them failed to produce *nic* mutants in repeated attempts. The exception, strain MG, produced a few *nic* mutants.

Sensitivity to sanguinarine. There were large differences in sanguinarine sensitivity among the 92 strains tested, but those within a group usually showed the same sensitivity (Table 1). Strains in P1 and P5 were very resistant, whereas those in P2, P6, and P7 were uniformly sensitive. On the other hand, although most strains of P3 were resistant to sanguinarine, two strains, PM6 and CP, were quite sensitive.

TABLE 1. Strains of *Verticillium dahliae* grouped according to ability to form heterokaryons

Strain	Host	Country	Instability at *nic-B*[c]	Growth inhibition by sanguinarine[d] (%)
V-c group P1[a]				
*T9[=T1 (14)][b]	Cotton	Cal., USA	Yes	32
*V44 (10)	Cotton	Tx., USA	Yes	35
*138 (10)	Cotton	Mo., USA	Yes	27
*V76	Cotton	Mexico	Yes	31
*PT-1 (12)	Cotton	Peru	Yes	43
*AR	Cotton	Ark., USA	Yes	36
*BA	Cotton	Cal., USA	Yes	25
*OL	Olive	Cal., USA	Yes	35
RN	Rose	NH., USA	Yes	27
AI	American elm	Ill., USA	Yes	31
MI	Maple	Ind., USA	Yes	24
PC	Peanut	OK., USA	nt	32
SS	Sesame	Cal., USA	nt	23
VC	Velvet leaf	Canada	nt	20
CM	Cotton	Miss., USA	Yes	36
CIH[=V102 (13)]	Cotton	Iran	Yes	19
V-c group P2				
*106[=SS4 (14)]	Cotton	Cal., USA	Yes	57
*PH	Pistachio	Cal., USA	Yes	48
*WM	Cotton	Tx., USA	Yes	55
*GR	Grape	Cal., USA	Yes	57
*TS-1	Tomato	Cal., USA	Yes	58
*TS-2	Tomato	Cal., USA	Yes	57
TG	Tomato	Greece	Yes	65
TI	Tomato	Italy	nt	52
TN-1	Tomato	Netherlands	nt	50
TGC	Tomato	Jordan	nt	69
TF	Tomato	France	nt	65
AC	Almond	Italy	nt	57
AIC	Apricot	Italy	nt	59
EI	Eggplant	Italy	nt	57
WC	Watermelon	Canada	nt	61
OC	Olive	Cal., USA	nt	57
V-c group P3				
*115[=V107 (13)]	Cotton	Syria	Yes	40
*207	Potato	S. Australia	Yes	33
PU	Potato	U.K.	Yes	26
PB	Potato	Canada	nt	31
PK	Potato	U.K.	nt	19
PL	Potato	Canada	nt	26
SW	Spearmint	Wash., USA	Yes	34
PM6[=S-6 (4)]	Potato	Ind., USA	nt	59
CP	Cotton	Peru	Yes	64
SI	Sugar maple	Ill., USA	Yes	14
SB	Sumac	Canada	nt	28
ST	Strawflower	Canada	nt	34
PUK	Pea	U.K.	nt	16
CK	Mum	U.K.	nt	20
SG	Strawberry	Germany	nt	27
GG	Gerbera	Germany	nt	23
PN-1	Sweet pepper	Netherlands	nt	36
EF	Eggplant	France	nt	42
BF	Benincasa	France	nt	35
CFW	Cantaloupe	France	nt	36
V-c group P4				
*277	Sugarbeet	Wash., USA	No	45
*TA	Potato	Ida., USA	No	45
*BB	Potato	Ida., USA	No	45
HW	Horseradish	Wis., USA	No	66

(continued on next page)

Host range and virulence patterns. Only a few pathogenicity tests were made on these strains. The first eight strains listed under group P1 were earlier shown to cause severe disease and defoliation of cotton (8). Two other strains, AI and MI, were subsequently found also to defoliate cotton plants.

Table 1 lists the host plants from which the strains were isolated. Four of the six strains from pepper are in group P5, seven of the nine strains from tomato are in P2, nine of the 10 strains from potato are in P3 and P4. Note that strains from the same host species were from widely different locations. Conversely the existence of two different v-c groups in the same location was not determined in this study.

DISCUSSION

Strains of *V. dahliae* can exchange genetic information through heterokaryosis and subsequent parasexuality (11). Because the

TABLE 1 (continued)

Strain	Host	Country	Instability at *nic-B*[c]	Growth inhibition by sanguinarine[d] (%)
PW	Potato	Wis., USA	No	47
VW	Velvet leaf	Wis., USA	No	37
GW	Grape	Wash., USA	No	50
CY	Cantaloupe	Wash., USA	No	40
MG[=M24 (4)]	Mint	Ind., USA	Low level	64
V-c group P5				
PG	Pepper	Greece	Yes	35
PJ (3)	Pepper	Canada	Yes	28
PHI	Pepper	Italy	nt	39
PIC	Pepper	Italy	nt	36
OI	Olive	Italy	nt	34
EC	Eggplant	Canada	nt	33
V-c group P6				
SN	Soybean	N.S. Wales	nt	69
FN	Flax	N.S. Wales	nt	65
197	Cotton	N.S. Wales	Yes	68
PS	Pelargonium	S. Australia	nt	62
MT	Maple	Canada	nt	58
OIC	Olive	Italy	nt	57
EN	Eggplant	Netherlands	nt	55
V-c group P7				
CS-1	Cotton	Swaziland	Yes	65
CS-2	Cotton	Swaziland	nt	61
CA	Cotton	Argentina	nt	61
POC	Potato	Canada	nt	62
V-c group P8				
CF	Cotton	France	Yes	68
V-c group P9				
RI	Redbud	Ill., USA	Yes	65
V-c group P10				
CU	Catalpa	Ill., USA	Yes	68
V-c group P11				
CW	Cherry	Wash., USA	Yes	63
V-c group P12				
HY	Hops	Wash., USA	Yes	54
V-c group P13				
TC	Tomato	Canada	Yes	52
V-c group P14				
MC	Mum	Cal., USA	Yes	64
V-c group P15				
TO	Tomato	Canada	Yes	29
V-c group P16				
PCW	Pepper	Cal., USA	Yes	18
No heterokaryon formation[e]				
SU	Strawberry	U.K.	nt	33
PA	Pear	Australia	nt	50
PR	Potato	Russia	nt	32
CRC	Cotton	Russia	nt	42
ARW	Asclepias	Russia	nt	25
SOS-2	Rape	Sweden	nt	57
Strains with long conidia				
SBS-1	Sugarbeet	Sweden	nt	nt
SOS-1	Rape	Sweden	nt	nt

[a] All strains within a v-c (vegetative compatibility) group formed heterokaryons with the tester strain for that group and/or with each other.

[b] Strains preceded by an asterisk were studied earlier (8). Numbers in parentheses after some strain designations refer to publications cited here in which the strains were researched or described. The remaining strain designations are those of our laboratory.

[c] Detected as low levels of nicotinamide requirers among untreated conidia; nt = not tested.

[d] Computed by the formula:

$$\{1-[(\text{colony diameter on PDA} + \text{sanguinarine})/(\text{colony diameter on PDA})]\} \times 100.$$

[e] No line of black microsclerotia formed when a *brm* and an *alm* mutant of the same strain were paired.

fungus has no known sexual cycle, these processes may be its only means of gene exchange. Barriers to heterokaryosis should therefore result in genetic isolation. Because only strains within a v-c group can form heterokaryons with each other, these v-c groups can be viewed as genetically isolated populations within *V. dahliae*.

Not all v-c groups were equally frequent among the 86 strains in our sample. Groups P1, P2, and P3 had 16, 16, and 20 representatives, respectively; whereas groups P8 through P16 contained only one strain each. This unequal distribution of v-c groups may reflect the situation in nature.

There is also evidence from our sample for an unequal geographical distribution of v-c groups. With one exception, all strains of P1 were from the western hemisphere. All strains of P4 were from the northern USA; most of the Australian strains belonged in P6.

If the v-c groups are isolated populations, they may have become genetically divergent. Moreover, strains within a v-c group should be more similar to each other than to strains from another group. Our findings with the unstable *nic-B* locus (9), with sanguinarine sensitivity, and with host source do indeed indicate such homogeneity within a v-c group.

Most natural isolates of *V. dahliae* are prototrophic. However, in an earlier study (9) many strains had a high frequency ($1–5 \times 10^{-4}$) of nicotinamide requirers among their conidia. These nicotinamide requirers were all true mutations at one nuclear gene locus designated *nic-B* (9). Strains from all 16 v-c groups were tested for instability at *nic-B*, and all of them except P4 had the unstable locus. In spite of repeated attempts, no *nic-B* mutants could be recovered from eight of the nine strains in P4. The exception was MG; its placement in P4 is somewhat questionable, however, because it did not form heterokaryons with all the other strains in this group. The basis for the instability at *nic-B* is not known, but these findings do suggest that instability is under genetic control. The fact that nearly all strains of group P4 lack this instability is evidence for genetic homogeneity within P4.

The alkaloid sanguinarine was first shown by Presley (6) and later by Howell (5) to inhibit the growth of certain strains of *V. dahliae*. They found that, without exception, all defoliating strains tested were relatively resistant to sanguinarine. Our data corroborate their findings. Moreover, there is a tendency for strains within a v-c group to show the same degree of resistance. For example, all strains in group P1 were quite resistant. This group contains all known defoliating strains. A genetic basis for resistance to sanguinarine has not yet been established, but resistance in fungi to other antibiotics is often under genetic control (2).

Information on the host-range of a particular strain comes from two sources in our study: the plant host from which the strain was originally isolated and controlled pathogenicity tests on selected hosts. There was often a tendency for strains from the same host to fall in the same v-c group. Thus, four of the six strains isolated from pepper were in group P5; most strains from tomato were in group P2; and nearly all the strains from potato were either in P3 or P4. Most hosts of P1 strains were woody plants. Information about the original host plant is, however, of limited usefulness. We do not know, for example, if the strain was the only one present in the host or how severely diseased the plant host was. More accurate data on host range require pathogenicity tests. We have only made a few such tests. Ten of the 16 P1 strains were inoculated into cotton plants and all caused defoliation. Two of these 10 strains were assigned to P1 before pathogenicity tests were made. At least in the case of P1 strains we may be able to predict the disease pattern caused by them even before pathogenicity tests are carried out.

The existence and distribution of v-c groups within the species *V. dahliae* closely parallel the findings in an earlier study of *Aspergillus nidulans*, in which 19 v-c (= h-c) groups were defined among 100 strains collected throughout Great Britain (1). Some groups occurred much more frequently than others, and some showed a limited geographical range. Strains within a given group had similar growth rates, fruiting ability, and antibiotic production. *A. nidulans* apparently does not produce its sexual state in nature, but it can be induced to do so in the laboratory. Because vegetative incompatibility did not prevent sexual union in this fungus, crosses between groups could be made. Progeny showed segregation for many gene loci, but also were decidedly less vigorous than the parents. It was concluded that strains within a group were genetically much more similar to each other than to strains from a different group. Moreover, the groups already showed significant genetic divergence from one another.

V. dahliae is a widespread plant pathogen that attacks a large number of plant hosts. We can view this species in at least two ways: it may be a single population of strains that can readily alter their virulence patterns so that they can attack any host plants present, or it may be a collection of morphologically similar strains that are partitioned into a finite number of genetically isolated groups, each with specific virulence capabilities. Our data favor the latter view. These isolated groups are probably the v-c groups defined in the present study. However, more extensive pathogenicity tests are required before the breadth of host range and pathotype within a v-c group can be defined.

The concept of v-c groups allows more exact identification and characterization of individual strains of *V. dahliae*. Such refinements are necessary if we are to understand the epidemiology of specific Verticillium wilt diseases and to estimate their potential for spreading. We believe that not every strain of *V. dahliae* has the same potential for virulence and host range. Moreover, the geographical distribution of certain important strains appears still to be restricted. Such strains can be identified through their v-c grouping and thereby monitored and quarantined.

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