Ultraviolet Light–Induced Instability of Vegetative Compatibility Groups of *Cryphonectria parasitica*

R. Rizwana and W. A. Powell

Graduate research assistant and assistant professor, respectively, Department of Environmental and Forest Biology, State University of New York, College of Environmental Science and Forestry, Syracuse 13210-2788.

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ABSTRACT

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The main objective was to study the effect of ultraviolet (UV) light on vegetative compatibility in the fungus *Cryphonectria parasitica*. A procedure was developed to detect individual incompatible colonies from cultured lawns of compatible colonies. Incompatibility reactions were observed when protoplasts from two different vegetative compatibility (v-c) groups were mixed and plated on a suitable medium containing red food color. With this technique, incompatible colonies were observed in plated protoplasts prepared from UV light–treated mycelium of a single v-c group. UV–treated conidia originating from a single v-c group also produced incompatible colonies detected by this procedure. Single conidial isolates from incompatible colonies were tested for vegetative compatibility, confirming a change in v-c grouping. The isolates with changed v-c groups were unstable, reverting to their original v-c group or changing to different v-c groups. The unstable v-c group mutants changed throughout the course of this study. Because of this instability, the v-c groups of most of the changed isolates could not be identified. These observations suggest that UV light induces v-c group instability. This phenomenon may contribute to the large diversity of v-c groups in natural populations of *C. parasitica*.

Additional keywords: cellular incompatibility, chestnut blight, *Endothia parasitica*, heterokaryon incompatibility.

Cryphonectria parasitica (Murrill) Barr (formerly *Endothia parasitica* (Murrill) P. J. Anderson & H. W. Anderson) caused almost complete destruction of the American chestnut (*Castanea dentata* (Marsh.) Borkh.) during the early decades of this century (7,28,33). In 1938, chestnut blight was discovered in Europe (7), but the losses there have been limited, in part because of the presence of cytoplasmically transmitted hypovirulence factors (3,27,34,35). *C. parasitica* strains that contain these factors demonstrate blight-curative properties associated with the presence of double-stranded (ds) RNA (7,16,35). The dsRNA is associated with down-regulation of specific fungal genes (29,30), reduced sporulation and virulence, and changes in colony morphology (29,33,34). Transmission of the dsRNA is dependent upon the establishment of stable hyphal anastomosis bridges between colonies (28). A barrier to stable hyphal fusion, and therefore

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to transmission of hypovirulence factors, is vegetative incompatibility (1,19,35).

Vegetative incompatibility is the inability of genetically different strains of a fungal species to fuse and form heterokaryons (2,8,32), and it results when different alleles are present at one or more vegetative incompatibility loci in the fusing hyphae (8). Vegetative incompatibility has been reported in many genera of ascomycetes (13,18,24,31) and is a useful tool in distinguishing strains of fungi. The natural function of vegetative incompatibility is not clear, but it has been hypothesized that vegetative incompatibility may act as an effective barrier to cytoplasmic exchange, which reduces the spread of viral infections among incompatible strains (13). Therefore, the primary role of vegetative incompatibility is considered to be that of a cellular defense mechanism against cytoplasmic infection (13,14).

The mechanism of vegetative incompatibility is not well understood. In *Podospora anserina*, the incompatibility reaction results in cell death of the fused and adjacent hyphae caused by the synthesis of specific proteolytic enzymes and also results in the inhibition of protein and ribonucleic acid synthesis (10,11,24,25). In *Aspergillus nidulans*, incompatibility can be divided into two types—one can be overcome by protoplast fusion (producing morphologically normal heterokaryons), and the other cannot (15). These differences in the incompatibility reaction demonstrate that the mechanisms of vegetative incompatibility can vary within a species.

In American strains of *C. parasitica* there are at least five known vegetative incompatibility (*VIC*) genes and more than 106 vegetative compatibility (v-c) groups (8). Incompatibility is recognized by the formation of a barrage line (1,12) (a line of dead cells, sometimes bordered by pycnidia) between merging colonies of *C. parasitica* that differ in alleles of at least one *VIC* gene (8). The strength of the incompatibility reaction (determined by the sharpness of the barrage line and the ability to inhibit the transfer of dsRNA) is dependent upon allelic differences in *VIC* genes and upon the total number of alleles that differ between the fusing strains (8). Vegetative incompatibility is not an absolute barrier to the transmission of dsRNA, but it reduces the chances of transfer in proportion to the strength of the incompatibility reaction (9,22,23). The presence of a large number of vegetative compatibility groups within small geographical areas in the United States has severely limited the natural spread of hypovirulence factors and therefore reduced their usefulness as biological control agents for chestnut blight (6,7,26,33,35). This paper describes UV light–induced changes in vegetative compatibility that could explain some of the present diversity in *C. parasitica* v-c groups. In this study of the effects of UV light on vegetative compatibility, emphasis was given to methods using protoplasts that are needed to lay the groundwork for research on the mechanisms of vegetative incompatibility.

MATERIALS AND METHODS

Strains and media. The wild-type tester strains of *C. parasitica* obtained from the American Type Culture Collection (ATCC) were v-c 39 (ATCC 38750), v-c 5 (ATCC 38751), v-c 8 (ATCC 22511), and v-c 16 (ATCC 38754). Auxotrophic strains obtained from ATCC were v-c 5, *met* (ATCC 38750) and v-c 8, *arg* (ATCC 22506). Other auxotrophic strains, v-c 39, *leu* and v-c 16, *arg*, were made from v-c 39 and v-c 16, respectively, by UV mutation (8) and selected by a filtration enrichment method (17). The v-c group genotypes of these strains are shown in Table 1. The 97 v-c tester strains used in some of the incompatibility tests were provided by S. L. Anagnostakis, Connecticut Agricultural Experimental Station, New Haven.

The media used were PDAmb (Difco potato-dextrose agar, methionine, and biotin) (4), MMLT (minimal media supplemented with lactic and tannic acids) (4), MMLT + leucine (100 mg/L), PDAmys (PDA with malt extract at 7.5 g/L, yeast extract at 2.5 g/L, methionine at 100 mg/L, biotin at 2 mg/L, and thiamine at 2 mg/L), PDAmbal (PDA with methionine at 100 mg/L, biotin at 2 mg/L, arginine at 100 mg/L, and leucine at 100 mg/L), and PDBmbal (Difco potato-dextrose broth with methionine at 100 mg/L, biotin at 2 mg/L, arginine at 100 mg/L, and leucine at 100 mg/L). Each medium was tested with and without red food color (20,21) (16 drops per 500 ml of medium) added to enhance detection of the incompatibility reactions. For protoplasts, the osmotic stabilizers examined were 0.5 M and 0.8 M sucrose and 0.5 M and 0.8 M glucose.

Preparation of UV-treated protoplasts. A method was used to prepare protoplasts from UV-treated mycelium of the v-c groups to be tested. A single stock of protoplasts was produced from each strain, and aliquots from these stocks were used to perform all the experiments that used protoplasts. To produce these stocks, mycelial plugs of the auxotrophic strains from v-c groups 5, 39, 16, and 8 were inoculated on PDAmbal plates, treated with UV_{254nm} (for 70 s, at 40 cm from source, using an 8W Sylvania GTE germicidal G3T5 bulb), and left in the dark for 12 h. Mycelium on the leading edge of the colony was scraped off (unpigmented portion) and chopped into small fragments in 20 ml of PDBmbal in a sterile blender for 1–2 min. The mycelial suspension was then poured into 30 ml of PDBmbal and left on a shaker overnight. After checking samples for new growth under the microscope, the mycelium was centrifuged (1,900 *g*) and washed twice in O.6 M $MgSO_4$ and 10 mM NaH_2PO_4 buffer (pH 5.8). After washing, each gram of pelleted mycelium was suspended in 10 ml of 0.2% NovoZym 234 (Novo BioLabs, Danbury, CT), 200 μl of β-glucoronidase (100,000 U/ml) (Sigma Chemical Co, St Louis, MO), 0.06% bovine serum albumin (Sigma), 1.2 M $MgSO_4$, and 20 mM NaH_2PO_4 buffer (pH 5.8). The suspension was shaken at 80 rpm overnight and then filtered through two layers of sterile cheesecloth to remove the larger fragments of undigested mycelium. The protoplast suspension was poured into sterile 15-ml Falcon tubes and overlaid with 0.5 ml of 1.0 M sorbitol 10 mM $CaCl_2$ and 50 mM Tris (pH 7.0) buffer. The tubes were centrifuged for 30 min at 1,900 *g* to float the protoplasts to the buffer interface. The opaque band of protoplasts at the buffer interface was collected, and the protoplasts were washed twice by dilution in 10 ml of 1.0 M sorbitol, 10 mM $CaCl_2$, and 50 mM Tris (pH 7.0) and centrifuged at 1,900 *g* for 10 min. The pelleted protoplasts were suspended in 10 ml of the same buffer. A sample of the protoplasts were diluted and counted in a hemacytometer. The protoplasts were suspended at 10^8 per milliliter of 1.0 M sorbitol, 100 mM $CaCl_2$, and 50 mM Tris (pH 7.0). Dimethylsulfoxide (10 μl) and 200 μl of 40% polyethylene glycol (PEG 4000) was added to each milliliter of protoplasts. The protoplasts were prepared once from each strain and stored at −80 C until use. The non-UV-treated protoplasts were prepared in the same way and stored.

Preparation of UV-treated conidia. To determine whether UV treatment of conidia could affect vegetative incompatibility, freshly isolated conidia were UV-treated as previously described (8) to achieve 95% kill. After the UV-treated conidia were stored at room temperature in the dark for 12 h, they were immediately used in the screening procedure.

Screening procedure. A procedure was developed to detect incompatibility reactions in a lawn of colonies by mixing protoplasts from two different v-c groups in a 9:1 ratio. In one experiment, 10^6 v-c 39 protoplasts were mixed with 9×10^6 v-c 5

TABLE 1. *VIC* genotypes for vegetative compatibility (v-c) groups of *Cryphonectria parasitica* strains

C. parasitica strains[a]		
Wild-type	Auxotrophic	*VIC* gene alleles[b]
v-c 16	v-c 16, *arg*	*VIC1-1, VIC2-1, VIC3-2*
v-c 5	v-c 5, *met*	*VIC1-1, VIC2-1, VIC3-1*
v-c 39	v-c 39, *leu*	*VIC1-2, VIC2-1, VIC3-1*
v-c 8	v-c 8, *arg*	*VIC1-2, VIC2-2, VIC3-1*

[a]Strains were named by v-c group, followed by auxotrophic marker if applicable. Auxotrophs originated from wild-type strains listed.

[b]Between five and seven *VIC* genes have been reported. This table shows only the genes that differ among the strains used in this study; strains are homozygous for all other loci (9).

protoplasts; in another experiment, 10^6 v-c 5 protoplasts were mixed with 9×10^6 v-c 39 protoplasts. Serial dilutions from both experiments were plated on PDAmys with 0.5 M and 0.8 M sucrose, PDAmb with 0.5 M and 0.8 M sucrose, and PDAmys with 0.5 M and 0.8 M glucose. Incompatibility reactions were tested on each medium with and without red food color added to enhance detection of these reactions.

UV-induced changes in v-c groups. The optimized screening procedure was used to detect changes in vegetative incompatibility within a single v-c group. After thawing on ice, six serial 1:10 dilutions of protoplasts from UV-treated mycelium of the strains v-c 5, *met*; v-c 39, *leu*; v-c 8, *arg*; and v-c 16, *arg* were made to get approximately 50 protoplasts per plate. Ten replications of the dilution, producing approximately 50 colonies per plate, were plated alone on PDAmbal with 0.8 M sucrose and red food color. The procedure was repeated with non-UV-treated protoplasts as a control. To determine if the procedure would work with conidia, UV-treated and non-UV-treated conidia from wild-type strains v-c 39, v-c 5, v-c 8, and v-c 16 were plated alone on PDAmbal containing red food color.

Vegetative compatibility tests. Mycelial plugs (5 mm in diameter) from the tester strain and the unknown isolate were placed together, with the mycelial side up, on PDAmbal supplemented with red food color to enhance detection of barrage lines (5,12, 20,21). The cultures were incubated in the dark at 25 C for 5 days. Merging of the colonies indicated compatible strains (Fig. 1A). Barrage lines indicated the strains were incompatible with each other (Fig. 1B). V-c tests were repeated three or more times for each unknown isolate to confirm the results. Each group of v-c tests included one known compatible and one incompatible pairing as controls.

Single conidial isolates. Colonies were grown from uninucleate conidia to produce isogenic isolates for further v-c group testing in order to verify isolates with changed v-c groups. The isolates were grown on PDAmbal plates at room temperature with a 16-h light and 8-h dark cycle for 4 wk. The conidia were collected by washing the surface of the colony with sterile water and filtering out the mycelium fragments with sterile cheesecloth. The conidia were counted with a hemacytometer. After one to 10 serial dilutions of the conidia were plated on PDAmbal and grown at room temperature, well isolated, single colonies were collected. The single conidial isolates were grown on separate plates and then tested for vegetative compatibility with v-c tester strains to confirm the change in v-c group. A total of 282 single conidial isolates from the 3 v-c 39, *leu*-changed isolates and a total of 198 single conidial isolates from the 3 v-c 8, *arg*-changed isolates were tested against v-c 39 and v-c 8 tester strains, respectively.

Unstable v-c groups. To follow changes in unstable v-c groups, single conidial isolates were plated in the center of PDAmbal plates with red food color. Development of barrage lines within the single conidial isolates divided the colonies into sectors. Mycelial isolates were taken from these sectors and v-c tested against their original v-c group tester strain and other sectors on the plate. Some of the changed sectors were further examined by repeating this plating procedure.

RESULTS

Screening procedure. Using 9:1 mixtures of incompatible protoplasts, PDAmys with 0.8 M sucrose and PDAmb with 0.8 M sucrose gave the most distinct red, incompatible colonies. Red food color was necessary for easy detection of the incompatible colonies. The red colonies could be best seen by looking through the bottom of the plates. Typical barrage lines containing pycnidia were not detected when examining the plates from the top. Whether the minority v-c group was v-c 39 or v-c 5, the v-c group in the lower concentration produced the red colonies on a background of lighter-colored colonies of the majority v-c group. The optimum concentration of colonies was approximately 50 colonies per 80-mm plate. Isolation of the red colonies and subsequent v-c tests confirmed their v-c group.

UV-induced changes in v-c group. This procedure was designed to detect changes from compatible to incompatible isolates within a single UV-treated strain. UV-treated mycelium from v-c 8, *arg*; v-c 39, *leu*; v-c 16, *arg*; and v-c 5, *met* were used to form protoplasts. These protoplasts were plated at the optimum concentration of approximately 50 colony-forming units per plate. An examination of the bottom of the plates revealed dark red colonies among the lighter colonies, similar to the incompatible colonies seen in the control procedure above. These dark colonies were considered incompatible with the surrounding colonies (Fig. 2). These incompatible colonies were detected only in the v-c 8, *arg* and v-c 39, *leu* plates. The v-c 16, *arg*, v-c 5, *met*, and the non-UV-treated v-c 8, *arg*; v-c 39, *leu*; v-c 16, *arg*; and v-c 5, *met* controls did not produce any detectable dark colonies.

The procedure was repeated with conidia instead of protoplasts. The conidia were isolated from wild type v-c 8, v-c 39, v-c 16, and v-c 5 strains. In this procedure, the osmotic stabilizer was left out of the screening medium. Dark-colored incompatible colonies were detected in all the UV-treated strains but not in any of the non-UV-treated strains. These colonies produced distinct barrage lines with pycnidia around the incompatible

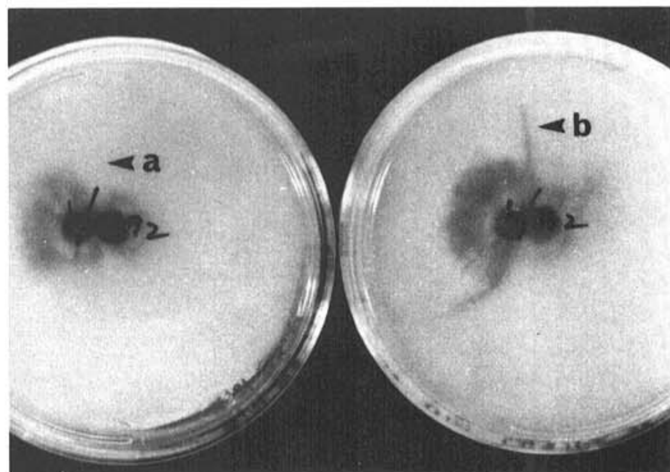

Fig. 1. Vegetative compatibility tests, viewed from the bottom of the plate. **A,** Colonies merged between compatible strains on tester plates. **B,** Incompatible strains formed distinct barrage lines (area of dead cells, detection enhanced by red food color) between paired colonies.

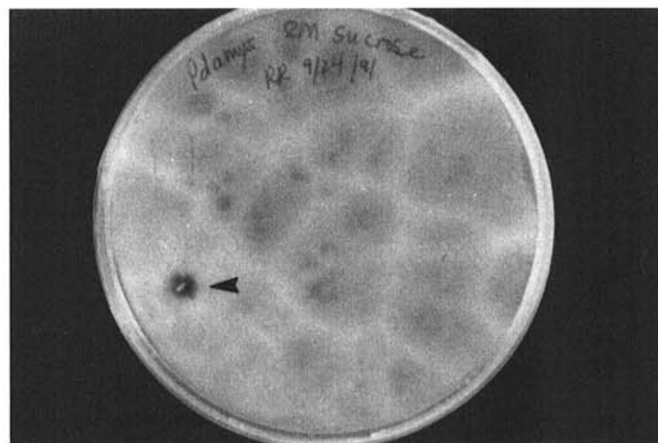

Fig. 2. Detection of incompatible colonies originating from protoplasts, viewed from the bottom of the plate. The protoplasts were made from UV-treated mycelium of a single vegetative compatibility group. Approximately 50 protoplasts were plated from a single strain. The arrow points to a dark (incompatible) colony among the lighter background colonies, which have merged to form a mycelial lawn. The hole in the center of the dark colony is where a mycelial plug was removed. The slightly darker spots on the lawn are the older parts of the merged colonies.

colony (Fig. 3), differing from incompatible colonies produced on the sucrose-containing media, which were wet and dark red without the formation of pycnidia (Fig. 2).

Isolation of incompatible isolates. From the UV-treated v-c 39, *leu* strain, 26 dark red colonies were detected. Fourteen of these colonies were chosen randomly and prescreened by testing for vegetative compatibility with a v-c 39 tester strain and, as an incompatible control, a v-c 5 tester strain. Four of these colonies were incompatible with both v-c 39 and v-c 5 in at least one of the v-c test replications. The other 10 were compatible with the v-c 39 tester strain and incompatible with the v-c 5 control strain. Since these colonies were taken from very crowded plates, there was a chance that the isolates contained mixed cultures. Therefore, three of the four changed isolates mentioned above were selected and grown to harvest conidia.

A total of 282 single conidial isolates from these three isolates were tested against the v-c 39 tester strain to identify isolates with changed v-c groups. Single conidial isolates with confirmed v-c group changes are designated with an asterisk next to their original v-c group to indicate the origins of the changed isolates. From these, only three single conidial isolates (v-c 39*, *leu*) were identified that were incompatible with v-c 39 in three or more v-c tests. Vegetative compatibility tests of these changed isolates against each other indicated that two of the three v-c 39*, *leu* isolates were in the same v-c group, but the third isolate was in a different v-c group. To eliminate the possibility that the changed isolates could have resulted from contaminants, the above three v-c 39*, *leu* isolates were further characterized by testing for auxotrophy on minimal media, MMLT, and for viability on MMLT with leucine. Two of the isolates, which were not compatible with each other or v-c 39, retained their auxotrophic marker, indicating that these two changed isolates did not result from a contaminant in the screening plates. Isolation of two different v-c groups indicated that the original v-c 39, *leu* strain could change to more than one different v-c group. The other isolate that could grow on MMLT appeared to revert to prototrophy and therefore was not used in any further studies. The two v-c 39*, *leu* isolates that were confirmed auxotrophs were further tested against 97 v-c group tester strains.

After v-c tests with the 97 v-c group tester strains (obtained from S. L. Anagnostakis), one v-c 39*, *leu* isolate did not give consistent results, but the other isolate was compatible with v-c 5 in three v-c tests. But after several transfers, this new v-c 5–compatible isolate reverted to its original v-c 39 group. A repeat of the v-c test with a sample taken from the original plate on which this isolate was stored showed the original isolate still incompatible with v-c 39 but no longer compatible with v-c 5. These results indicated that the v-c groups of these changed isolates were unstable.

The same procedure used for v-c 39, *leu* protoplasts was repeated for v-c 8, *arg* protoplasts. Eight red colonies were isolated and tested for vegetative compatibility against a v-c 8 tester strain and a v-c 39 tester strain, used as an incompatible control. As with v-c 39, *leu*, these isolates gave inconsistent results in the v-c tests. Some of the isolates were compatible with v-c 8 but not with v-c 39; some were incompatible with both; and two of the isolates were incompatible with v-c 8 but compatible with v-c 39 on at least one of the replicated v-c tests. Sectoring of the isolated colonies to different v-c groups was observed in these v-c 8*, *arg* isolates. As described for v-c 39*, *leu*, single conidial isolates from three of the red colony isolates were made to eliminate the possibility of mixed cultures and tested for vegetative compatibility with a v-c 8 tester strain. A total of 198 single conidial isolates were tested, and three v-c 8*, *arg* isolates were identified that were incompatible with the v-c 8 tester strain. These strains were less stable than the v-c 39–changed isolates and soon reverted back to v-c 8. Therefore, none of these isolates were tested against the 97 v-c group tester strains.

Unstable v-c group mutants. Because of this instability of v-c groups, the sectoring to different v-c groups in the v-c 8, *arg* red colony isolates, and because the majority of the single conidial isolates from all the red colonies tested were compatible with their original v-c group, the stability of these compatible, single conidial isolates was investigated. To check the stability of the v-c groups, four additional single-conidial isolates from a single v-c 39, *leu* red colony isolate were selected for further testing. Even though these four isolates came from a dark red, incompatible colony, v-c tests indicated they were initially compatible with v-c 39. When these four isolates were grown alone on PDAmbal with red food color, they would often sector (Fig. 4). The margins of these sectors resembled barrage lines. V-c tests of isolates from these sectors indicated that they were no longer compatible with v-c 39. After subculturing, or further sectoring, the v-c group of the isolates would change again, often reverting back to v-c 39 compatibility but sometimes changing to a different v-c group.

From each of the four single conidial isolates designated A, B, C, and D, the number of sector isolates incompatible with v-c 39 ranged from four to nine. In single conidial isolates A, B, and D, v-c 39 and one other v-c group was observed. In the single conidial isolate C, v-c 39 and two other v-c groups were observed, indicating more than a simple switch of two alleles in a single *VIC* gene was occurring. The isolates that changed v-c groups were too unstable to be tested against the 97 available v-c tester strains with enough repeats to reliably determine the v-c group to which the sectored isolates had changed.

DISCUSSION

Vegetative incompatibility leads to failure of heterokaryon formation between nonisogenic strains in fungi caused by

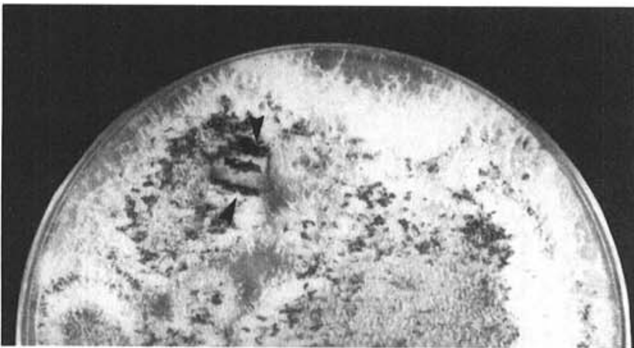

Fig. 3. Detection of incompatible colonies originating from UV-treated conidia, viewed from the top of the plate so that the pycnidia could be seen on the barrage lines. The conidia from a single vegetative compatibility group were UV-treated and plated at approximately 50 conidia per plate. The arrows point to barrage lines surrounding the incompatible (dark) colony. A hole is visible in the center of the colony, where a mycelial plug was removed to sample the colony.

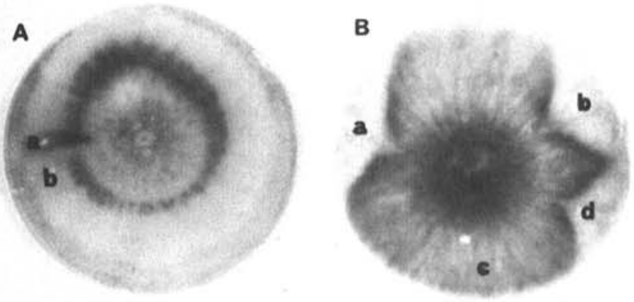

Fig. 4. Production of new vegetative compatibility groups by sectoring of single conidial isolates, viewed from the bottoms of the plates. **A,** Single conidial isolate in which one sector (a) was isolated along with a sample of the normal colony growth (b). **B,** Another isolate, in which three sectors (a, b, and d) were isolated along with a sample of the normal colony growth (c).

protoplasmic disintegration following hyphal fusions (1,10). The main objective of this research was to study the effect of ultraviolet light on the *VIC* genes in *C. parasitica*. This research indicated that UV light could induce changes in vegetative compatibility in this fungus. This instability induced by UV light could contribute to the diversity of v-c groups found in natural populations of *C. parasitica*.

To determine the effect of UV light on vegetative compatibility, a procedure was developed that would allow screening large numbers of fungal colonies for changes in v-c groups. With this procedure, incompatibility reactions could be easily detected when two incompatible strains, differing in the alleles of a single *VIC* gene, were mixed together and plated on the screening medium. The red food color added to the media was necessary to enhance the detection of incompatibility as incompatible colonies turned dark red. Red food color has been shown to enhance detection of incompatibility reactions in v-c tests of other fungi (20,21), but this is the first report of its use in mass cultures of *C. parasitica*. The advantage of this procedure was a great reduction in the number of cultures and transfers needed to screen the large numbers of isolates we examined. This procedure allowed up to 50 colony-forming units of conidia or protoplasts to be easily inoculated on a single 80-mm plate. For the application described in this paper, this procedure saved time and labor in identifying incompatible isolates from mycelial masses of compatible colonies. Because of the instability of our changed isolates during subculturing, conventional v-c tests could have missed the changed isolates detected by this procedure.

Using this screening procedure, UV-treated strains from four v-c groups were examined. Results from these experiments demonstrated UV treatments of mycelium-derived protoplasts in two of the four v-c groups and UV treatments of conidia in all four v-c groups could induce changes in vegetative compatibility. Differences between the mycelium and conidia were most likely due to the inability to quantify the extent of UV treatment received by the mycelium. For the conidia, a kill-curve was determined, and consistent UV exposures resulting in 95% kill was applied to each sample. For the mycelium, only the time of exposure and the distance of the UV source could be duplicated. A uniform exposure could not be performed, because of shadowing effects and differential pigment content of mycelium of different ages on the plate. The two v-c groups that were never changed by the UV treatments might require higher doses to affect a genetic change.

The incompatible colonies resulting from the plated protoplasts (Fig. 2) appeared slightly different when compared to those of the plated conidia (Fig. 3). Well-developed barrage lines with pycnidia were observed around the incompatible colonies from the plated conidia, but not when the incompatible colonies from protoplasts were plated. This difference may be due to the addition of sucrose in the media used to stabilize the protoplasts. Formation of distinct barrage lines and development of pycnidia may be inhibited in the presence of high concentrations of sucrose.

When changed isolates from protoplasts (made from UV-treated mycelium in v-c groups 8 and 39) were studied in detail, isolates from mycelial masses of the changed colonies appeared to have unstable v-c groups. Since this could be explained by possible isolations of mixed cultures, single (uninucleate) conidial isolates were made. Ten of these single conidial isolates, which were studied in detail, were also found to be unstable and continued to change v-c groups during the course of this study. Conidia from *C. parasitica* typically are uninucleate; therefore, the instability in v-c groups of these isolates was due to changes within a single genome.

One of the single conidial isolates from v-c 39*, *leu* transiently changed from v-c 39 to v-c 5. Since v-c 39 and v-c 5 differ in the alleles of a single *VIC* gene (8), this change must have affected the alleles of *VIC1* (Table 1). This new v-c 5 isolate reverted back to v-c 39 upon subsequent transfers, indicating it was not a stable change. Subsequent v-c tests of this isolate from the original storage plate confirmed its original incompatibility with v-c 39 but not its change to v-c 5, suggesting that the isolate continued to change. A mycelial isolate from v-c 8, *arg* appeared to have transiently changed to v-c 39. This change would involve a change in *VIC2* (Table 1), since these two v-c groups differ in the alleles of this single *VIC* gene (8).

Because of the initial screening procedure that detected changes in any of the *VIC* genes and the subsequent instability of the changed isolates studied, it was not possible to accurately identify the transient v-c group changes in v-c 39*, *leu* and v-c 8*, *arg* isolates. Yet one unstable v-c mutant originating from v-c 39, *leu* was determined to have changed to at least two different v-c groups other than v-c 39 during sectoring of the culture. This suggested that either more than one *VIC* gene or more than two alleles of a single *VIC* gene could have been involved in these changes. All the changed isolates from these experiments would also frequently revert back to their original v-c group upon transfer, but many would subsequently produce sectors of a different v-c group, indicating continued instability during the course of this study.

These results raise many pertinent questions concerning the stability of v-c groups in *C. parasitica* strains. We have shown that changes in v-c groups can be induced by exposure to UV light, and it will be interesting to find out if analogous changes occur in the field. If so, it might help explain the present diversity of v-c groups of *C. parasitica* and should be taken into consideration when studying the biological control of chestnut blight with hypovirulence factors (3,19,26). The possibility of using this instability to our advantage for the biological control of chestnut blight by producing hypovirulent donor strains that continually change v-c groups could be significant. This could help the dispersal of hypovirulence in the field, overcoming the problems of the diverse v-c groups of *C. parasitica*.

Finally, it would be helpful to find out the mechanism behind these changes in vegetative compatibility. This is part of a larger project to study the mechanisms of vegetative compatibility in *C. parasitica*. The results reported here indicate that vegetative compatibility can be modified by UV light exposure. The next step is to clone several *VIC* genes to determine whether these changes in vegetative compatibility are due to simple gene mutations or a gene conversion mechanism, possibly analogous to mating-type–switching in *Saccharomyces cerevisiae* (17).

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