Nitrate Non-Utilizing Mutants of *Colletotrichum* and Their Use in Studies of Vegetative Compatibility and Genetic Relatedness

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ABSTRACT

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Seven strains from five different *Colletotrichum* species were tested for their ability to produce chlorate-resistant nitrate non-utilizing mutants when cultured on potato-dextrose agar (PDA) or on a minimal medium containing 1.5% $KClO_3$. Six of the seven strains produced mutants that could be placed into one of four phenotypic classes representing mutations at: the nitrate reductase structural locus (*nit1*), the global nitrogen regulatory locus (*nit2*), the nitrate-assimilation pathway-specific regulatory locus or the nitrite reductase structural locus (Nit3), and the loci that affect the assembly of a molybdenum-containing cofactor necessary for nitrate reductase activity (NitM). The seventh strain (*C. malvarum*) was sensitive to nitrite and the test to distinguish the *nit1* class from the Nit3 class could not be performed. Five of the strains examined were heterokaryon self-compatible since paired complementary *nit* mutant strains could form prototrophic heterokaryons. Although both intragenic and intergenic complementation occurred, some of the *nit* mutant sectors were unable to complement with any of the other *nit* mutants derived from the same culture. Strains of *C. destructivum* and *C. fragariae* were heterokaryon self-incompatible because none of the *nit* mutants were able to form a heterokaryon when paired on minimal medium containing nitrate as a sole nitrogen source. The five heterokaryon self-compatible strains were all in distinct vegetative compatibility groups since complementary *nit* mutants from different strains could not form a prototrophic heterokaryon when cultured on minimal medium with nitrate as the sole nitrogen source.

Isolates of *Colletotrichum* are known to vary greatly in morphology, host preference, pathogenicity, and physiology, making taxonomic studies difficult. Historically, phylogenetic relatedness has been based on host range, developmental and anamorphic characteristics. Increased knowledge of field isolates, however, has made it apparent that there are limitations associated with classification based solely on morphology, since critical morphological traits may vary greatly and be altered by cultural conditions (36). Taxonomic difficulties in *Colletotrichum* spp. may be accentuated by the recovery of isolates from plants grown in monoculture. The genetic isolation thus imposed on the fungal population may eventually lead to speciation, but identifying characters of significance at the species level can be extremely difficult under such conditions. These observations raise the question as to what constitutes a species in this genus and highlights the need to clarify both the status of the specific and subspecific groups within *Colletotrichum*.

Studies of vegetative compatibility are of particular interest in asexual fungi, such as *Colletotrichum* spp., since VCGs subdivide the population into groups that can exchange genetic information via heterokaryosis and the parasexual cycle. VCGs most commonly have been studied by using auxotrophic mutations that prevent the utilization of nitrate as a sole nitrogen source (7,32,34), although mutants with altered pigmentation (33) and inability to catabolize sulfate as a sole sulfur source (7,20) have also been used. VCGs have been shown to correlate with pathogenicity (4,9,21), although this correlation does not always hold (14).

Population studies of VCGs have frequently involved mutants that are unable to utilize nitrate as a sole nitrogen source (*nit* mutants), and are thus resistant to chlorate, a toxic analogue of nitrate. In many fungal species these mutants arise spontaneously as sectors at high frequency when the organism is cultured on a medium containing $KClO_3$ (6,7,29,39). Most of these sectors produce mutants that contain genetic lesions in either structural or regulatory loci in nitrate catabolism or related pathways (2,18,23,24,26). These *nit* mutants can be easily classified phenotypically by their growth on selective media (7,18,26).

Genetic instability in *Colletotrichum* is easily observed in the laboratory as a relatively high frequency of spontaneous sectoring. This phenomenon appears analogous to that observed in *Fusarium* spp. in which colonies may produce sectors differing in morphology, virulence, and other characteristics from the wild-type parent (28). It has been suggested that such instability may provide these primarily asexual fungi with a more rapid means to adapt to the environment (22). The observed genetic instability in *Colletotrichum* suggested that the *nit* mutants commonly used in VCG studies in other fungi might be readily recovered from field isolates of *Colletotrichum*.

Our objective in this study was to determine if *nit* mutants occurred in sufficient diversity and frequency with isolates of *Colletotrichum* in order to use these mutants as a useful tool in the characterization of VCGs and genetic relatedness. A preliminary report of portions of this work has been published (5).

MATERIALS AND METHODS

Strains. Seven strains (field isolates) from different *Colletotrichum* species and subspecies were used in this study: *C. destructivum* 385 (host *Coronilla*), *C. gloeosporioides* 669 (host *Malus*), *C. gloeosporioides* 201 (host *Vicia*), *C. gloeosporioides* f. sp. *jussiaea*, *C. fragariae* 390 (host *Fragaria*), *C. malvarum* (host *Sida*), and *C. trifolii* (host *Medicago*). With the exception of *C. trifolii*, all strains were obtained from D. O. TeBeest, University of Arkansas. The seven *Colletotrichum* spp. were grown on complete medium (CM) for 7–14 days. Single conidia from these cultures were isolated on water agar and transferred to CM. All cultures were incubated at 25 C with a 12-h dark/12-h light cycle (two 40 W cool-white fluorescent lights). The resulting colonies were stored on sterile filter paper at −20 C (10).

Media. A basal medium was prepared as follows, per liter of distilled water: sucrose, 30 g; KH_2PO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5

g; KCl, 0.5 g; $FeSO_4 \cdot 7H_2O$, 10 mg; agar, 20 g; and trace element solution (35), 0.2 ml. Minimal medium (MM) was made by adding 3 g of $NaNO_3$ to 1 L of basal medium. CM was made by adding the following to 1 L of basal medium: $NaNO_3$ 2 g; N-Z amine, 2.5 g; yeast extract, 1 g; and 10 ml of a vitamin solution (7).

nit mutants were generated on minimal agar medium amended with chlorate (MMC) and potato-dextrose agar medium amended with chlorate (PDC). MMC was prepared by adding to 1 L of basal medium: L-asparagine, 1.6 g; $NaNO_3$, 2 g; and $KClO_3$, 15 g. PDC was prepared by adding the following to 1 L of distilled water: dehydrated potato-dextrose broth (Difco), 24 g; agar, 20 g; and $KClO_3$, 15 g.

Genetic terminology. Our terminology for *nit* mutants of *Colletotrichum* basically follows the terminology used in *Fusarium* (7,23), except as noted below, and is consistent with the guidelines proposed by Yoder et al (40) for plant pathogenic fungi. Two phenotypic classes will be designated as single locus mutations: *nit1*—nitrate reductase structural locus, and *nit2*—global nitrogen catabolism regulatory locus. Mutants in two other phenotypic classes may occur at more than one locus and will be designated as phenotypes. NitM mutants result from a mutation at one of several loci responsible for the assembly of a molybdenum-containing cofactor required for nitrate reductase and purine dehydrogenase activity. Mutants in the Nit3 class occur at the nitrate-catabolism pathway-specific regulatory locus, but cannot be distinguished from mutants at the nitrite reductase structural locus without additional tests. Mutants in the nitrite reductase structural locus are not commonly recovered when chlorate resistance is used as a screen, so all of our mutants are probably at the *nit3* locus.

Generation of *nit* mutants. Two-millimeter mycelial blocks of CM of each strain were transferred to the center of 20 glass petri dishes (10 cm in diameter) containing either PDC or MMC. The plates were incubated as described above and were examined weekly for the appearance of fast-growing sectors. The sectors grow at a visibly faster rate than the parent culture, and it has been proposed that wild-type growth is restricted due to the reduction of chlorate to toxic chlorite by nitrate reductase (1,27,35), although other mechanisms are also possible (10,12). Hence, *nit* mutants may reflect an inability to reduce chlorate to chlorite, thus making them chlorate resistant.

Twenty sectors from each fungal strain were selected on both PDC and MMC. These putative *nit* mutants were classified according to the medium of origin and the approximate time of sector appearance. All sectors were transferred to MM and those that grew as sparse colonies, having little aerial hyphal growth, were considered *nit* mutants. Only *nit* mutants that were resistant to chlorate and showed wild-type growth on CM were used.

***nit* mutant phenotypes.** The physiological phenotypes of *nit* mutants of *Colletotrichum* were identified (Table 1) based on the analysis of similar mutants from *Aspergillus nidulans* (10–12), *Neurospora crassa* (26), and *Fusarium oxysporum* (7). Growth on media containing different nitrogen sources was used to identify mutant phenotypes. The five *nit* mutant screening media were: 1) nitrate medium = NM described above; 2) nitrite medium = basal medium plus 0.5 g/L $NaNO_2$; 3) hypoxanthine medium = basal medium plus 0.2 g/L hypoxanthine; 4) ammonium medium = basal medium plus 1 g/L NH_4 tartrate, and 5) uric acid medium = basal medium plus 0.2 g/L uric acid.

Heterokaryon formation. Heterokaryons were formed by placing mycelia from different *nit* mutants 1 cm apart on NM. The plates were incubated as previously described for 7–14 days and then scored for complementation, i.e., dense aerial growth where the two sparsely growing *nit* mutant colonies come in contact. The 20 *nit* mutants from each of the 14 strain/media

TABLE 1. Identification of nitrate non-utilizing (*nit*) mutants from *Colletotrichum* spp. by growth on different nitrogen sources

Mutation[a]	Mutant designation	Growth on nitrogen sources[b]				
		Nitrate	Nitrite	Ammonium	Hypoxanthine	Uric acid
None	Wild-type	+	+	+	+	+
Nitrate reductase structural locus	*nit1*	−	+	+	+	+
Major nitrogen regulatory locus	*nit2*	−	−	+	−	−
Pathway-specific regulatory locus	Nit3	−	−	+	+	+
Molybdenum cofactor loci	NitM	−	+	+	−	+

[a]Compiled from Garrett and Amy (18), Marzluff (26), Correll et al (7), and Klittich and Leslie (23), on the basis of analysis of mutants from *Aspergillus*, *Fusarium*, and *Neurospora crassa*.
[b]Growth on basal medium with one of several nitrogen sources; + = typical wild-type growth; − = variable thin growth with little aerial mycelium.

TABLE 2. Frequency and phenotype of nitrate non-utilizing (*nit*) *Colletotrichum* spp. mutants recovered from two media

Colletotrichum species	Medium[a]	Sectors per colony[b]	% *nit* mutant - classes[c]			
			nit1	*nit2*	Nit3	NitM
C. destructivum	PDC	1.25	90	0	5	5
	MMC	1.18	30	15	15	40
C. gloeosporoides (Host *Malus*)	PDC	1.30	65	0	25	10
	MMC	1.18	65	0	35	0
C. gloeosporoides (Host *Vicia*)	PDC	1.10	90	0	10	0
	MMC	1.18	30	40	10	20
C. gloeosporoides f. sp. *jussiaea*	PDC	1.25	25	10	50	15
	MMC	1.25	20	0	60	20
C. fragariae	PDC	1.25	70	5	25	0
	MMC	1.10	10	20	65	5
C. malvarum[d]	PDC	1.10	(70)	20	(70)	10
	MMC	1.10	(50)	35	(50)	15
C. trifolii	PDC	1.10	20	0	30	50
	MMC	1.25	10	20	35	35

[a]PDC = Potato-dextrose agar + 1.5% $KClO_3$; MMC = minimal agar medium + 1.5% $KClO_3$.
[b]Mean frequency of chlorate-resistant sectors per colony.
[c]*nit* mutant phenotypes determined according to growth on basal medium amended with different nitrogen sources (see Table 1); 20 *nit* mutants were screened for each chlorate media.
[d]*C. malvarum* was sensitive to nitrite. Thus the frequencies for the *nit1* and Nit3 classes could not be determined and combined frequencies are presented in parentheses.

combinations were paired with the other mutants from the same strain/media combination (a total of 210 complementations, including selfed mutants) on NM to determine the number of complementation groups into which the different *nit* mutants could be placed. These pairings also permitted us to test for heterokaryon self-incompatibility (8).

Heterokaryon tests can also be used to determine if two strains are vegetatively compatible. If two strains are vegetatively compatible and carry complementary *nit* or other auxotrophic mutants, then they can form a prototrophic heterokaryon when cultured on NM. Strains that are not vegetatively compatible belong to different VCGs. To determine if the strains used in this study belonged to distinct VCGs, we paired four *nit* mutants (one from each phenotypic class) from each strain with one another in all possible pairwise combinations.

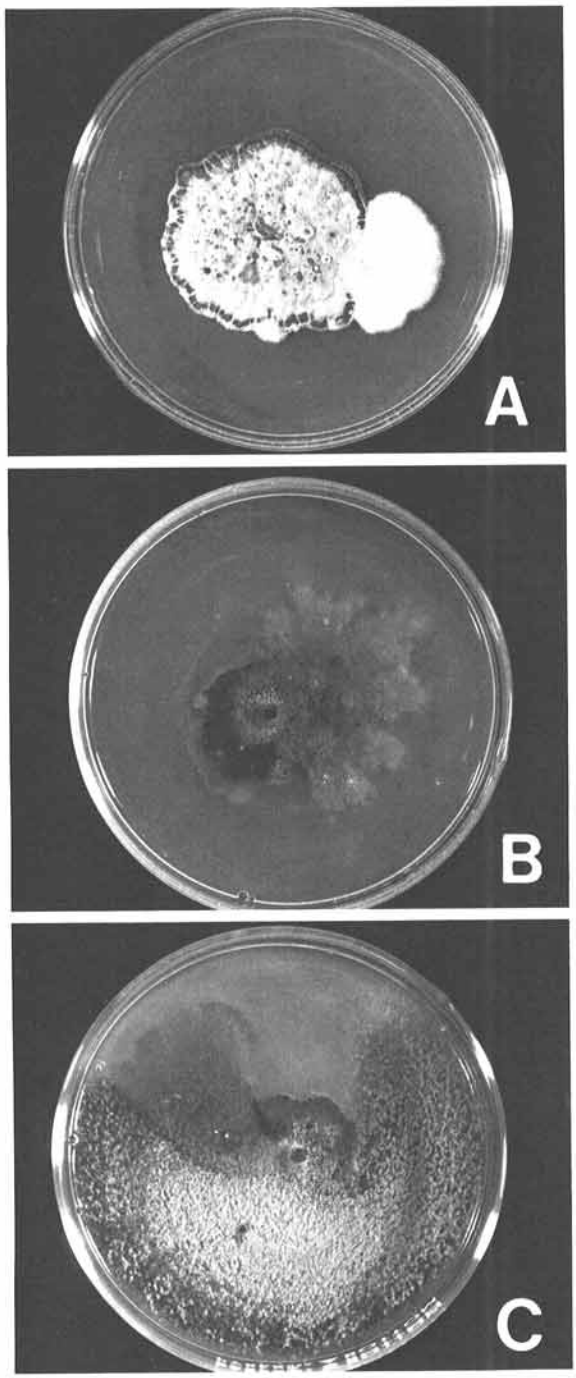

Fig. 1. Growth patterns of *Colletotrichum* species on media containing 120 mM $KClO_3$ in petri dishes 100 × 15 mm. **A,** *C. gloeosporioides* f. sp. *jussiae* with very low melanizing sector, no sporulation; **B,** *C. gloeosporioides* (host *Malus*) with feathery growth pattern, reduced melanization; and **C,** *C. gloeosporioides* (host *Malus*) with multiple growth patterned sectors, ranging from highly melanized to low melanized as well as variable mycelial density.

RESULTS

***nit* mutant isolation.** Forty chlorate-resistant sectors were isolated from each of the seven *Colletotrichum* strains, 20 from MMC, and 20 from PDC (Table 2). An exception was *C. gloeosporioides* 388 (host *Stylosanthes*), which was naturally resistant to chlorate. All of the chlorate-resistant sectors were unable to utilize nitrate as a sole nitrogen source, and the resulting growth of the mutants on MM was variably sparse in comparison with the wild type (Fig. 1). These sectors were designated *nit* mutants. A few sectors (≤5%) were recovered from each species that were chlorate resistant but were unable to grow well on any of the screening media, including CM. These strains were not included in any further tests. Thus, the Crn class reported in both *Aspergillus* (11) and *Fusarium* (24) appears to be either missing or substantially altered in *Colletotrichum*.

The rate at which sectoring occurred varied by strain. The mean frequency for chlorate resistant sectors was the same for both MMC and PDC, (ranging from 1.1 to 1.25 sectors per colony); however, individual strains showed differences in sectoring rates on the two chlorate media (Tables 2 and 3). Two weeks after inoculation *C. destructivum* and *C. gloeosporioides* (host *Malus*) were sectoring more rapidly on PDC, while *C. trifolii* and *C. malvarum* were sectoring more rapidly on MMC. In contrast, *C. gloeosporioides* (host *Vicia*), *C. fragariae*, and *C. g. jussiaea* (host *Jussiaea*) were sectoring at comparable rates on both MMC and PDC.

***nit* mutant phenotype identification.** *nit* mutant phenotypes were identified after growth on media that contained one of five different nitrogen sources. Four phenotypic *nit* mutant classes could be identified by using this screening regime. These classes represent mutations at: the nitrate reductase structural locus (*nit1*), the global nitrogen regulatory locus (*nit2*), the nitrate-assimilation pathway-specific regulatory locus or the nitrite reductase structural locus (Nit3), and the loci that affect the assembly of a molybdenum-containing cofactor necessary for nitrate reductase activity (NitM). Nitrite excretion studies were not conducted to distinguish nitrite reductase mutants from pathway specific regulatory mutants. These findings are similar to those for *Fusarium* (7,23), with the exception of the *nit2* class. In *Neurospora crassa* (16,31), the *nit2* locus encodes the global nitrogen regulatory

TABLE 3. Rate and phenotype of nitrate non-utilizing (*nit*) *Colletotrichum* spp. mutants formed on two media

Species	Medium[a]	% Sectors[b] Incubation time (wk) 1	2	3	4	5
C. destructivum	PDC	0	100	0	0	0
	MMC	35	0	50	15	0
C. gloeosporoides (Host *Malus*)	PDC	90	10	0	0	0
	MMC	25	15	35	25	0
C. gloeosporoides (Host *Vicia*)	PDC	15	70	5	10	0
	MMC	40	45	15	0	0
C. gloeosporoides f. sp. *jussiaea*	PDC	35	15	15	35	0
	MMC	0	35	65	0	0
C. fragariae	PDC	35	55	10	0	0
	MMC	60	35	5	0	0
C. malvarum	PDC	20	20	20	0	40
	MMC	80	10	10	0	0
C. trifolii	PDC	40	15	10	35	0
	MMC	95	5	0	0	0

[a] PDC = Potato-dextrose agar + 1.5% $KClO_3$; MMC = minimal medium + 1.5% $KClO_3$.

[b] Sector collection stopped once 20 sectors were obtained. Numbers represent the percentage of the sectors (out of 20) which appeared at that incubation time (weeks).

protein. Under conditions of nitrogen limitation, this protein turns on the expression of numerous unlinked structural genes that specify nitrogen-catabolic enzymes. Thus, *nit2* mutants can grow on only a very limited number of nitrogen sources. Our *nit2* mutants have been named by analogy with these *Neurospora* mutants, since mutants in the corresponding locus of *Fusarium* are not commonly found as chlorate-resistant sectors (7,13,25).

Strains differed in mutant frequencies on the two chlorate media (Table 2). In three strains, *C. destructivum*, *C. gloeosporioides* (host *Vicia*), and *C. fragariae*, the majority of mutants recovered from PDC were *nit1* (70–90%). In contrast, on MMC, *C. destructivum* produced primarily NitM mutants (40%), *C. gloeosporioides* (host *Vicia*) produced primarily *nit2* mutants (40%), and *C. fragariae* produced a majority of Nit3 mutants (65%). Each of these strains, when cultured on MMC, produced all four mutant phenotypes. In contrast, when these strains were cultured on PDC, a majority of the sectors were *nit1* mutants, and mutants representative of the four different phenotypic classes were not recovered. In three other strains, the mutant spectra were similar regardless of the type of chlorate medium on which they were cultured. *C. g. jussiaea* produced a majority of Nit3 mutants on both PDC and MMC (50–60%). *C. trifolii* had a majority of NitM mutants (35–50%); and *C. gloeosporoides* (host *Malus*) produced a majority of *nit1* mutants on both chlorate media (65%). Results with the *C. malvarum* strain cannot be compared directly with the other six strains since this strain was sensitive to nitrite. Consequently, we could not distinguish between the *nit1* and Nit3 phenotypic classes, although we know both classes are represented, and the combined frequencies are noted in both columns in Table 2 in parentheses.

Complementation tests. Complementation occurred between *nit* mutants derived from five of the seven strains that we used. Complementation was usually evident after 7–9 days, with the three *C. gloeosporioides* strains (Fig. 2). The *C. trifolii* and *C. malvarum* strains grow considerably more slowly than the other strains, and complementation reactions required 2–3 wk of incubation before they could be scored. Mutants derived from one of these five strains were able to complement mutants in other phenotypic classes that had been derived from the same strain. These strains were all termed heterokaryon self-compatible (8).

Heterokaryon self-incompatibility. No complementation was observed between any pair of *nit* mutants derived from the *C. destructivum* or the *C. fragariae* strains. A total of 420 pairings were attempted for each of these strains, and representative mutants from each phenotypic class were present. Therefore, these strains were designated heterokaryon self-incompatible (8).

Vegetative compatibility tests. *nit* mutants from each strain, representing each of the physiological classes, were paired in all possible pairwise combinations to determine if any of these strains were vegetatively compatible with one another. No complementation was detected in any of these pairings, suggesting that all of these strains are in different vegetative compatibility groups.

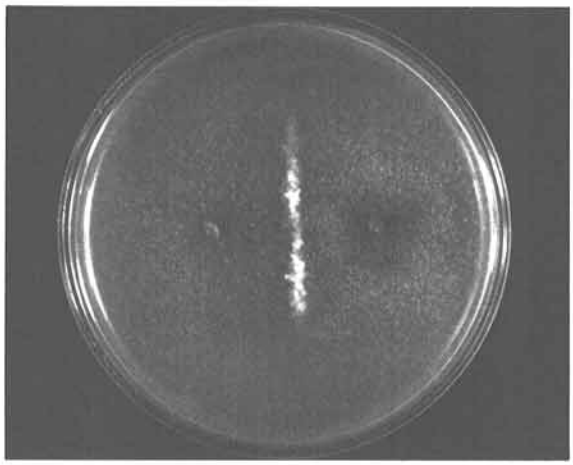

Fig. 2. Complementation of *nit* mutant isolates of *C. gloeosporioides* (host *Malus*) grown on MM. Note the line of dense prototrophic growth where the two colonies come in contact and anastomose.

DISCUSSION

Colletotrichum spp. are known anecdotally to be genetically unstable, but careful studies of such instability, in spite of its relevance to pathogenic characters, have been minimal. In this study, the genetic stability of seven different isolates of *Colletotrichum* was examined when the strains were cultured on media containing chlorate. As with several other filamentous fungi we can recover specific mutants that affect nitrate assimilation. The cause of this instability is presently unknown not only in *Colletotrichum*, but also in the other filamentous fungi in which the phenomenon has been reported, although the frequency at which sectors occur and the genes that are altered can vary widely within strains of a single species (7,22,23).

Nitrate non-utilizing, chlorate-resistant mutants were recovered from seven *Colletotrichum* strains that belong to five different species. These mutants could be divided into four phenotypic classes that are associated with the nitrate assimilation pathway. These phenotypic classes correspond to those assigned in previous studies to mutants of *Fusarium* (*nit1*, Nit 3, and NitM) and *Neurospora* (*nit2*). When the *nit* mutants we recovered were placed into these four different phenotypic classes, six of the seven *Colletotrichum* strains had representative mutants in each of the four classes; however, no single phenotypic class was predominant in every strain. The mutant frequencies varied both with the strain and with the type of chlorate media. This finding is in contrast to studies of *Fusarium* spp. in which *nit1* mutants usually predominate, regardless of either the chlorate medium or the strain employed (7,23). In addition, as in *Neurospora* and *Aspergillus*, mutants that affect the major nitrogen regulatory locus were readily recovered as chlorate-resistant sectors in all of the *Colletotrichum* strains that we examined except *C. gloeosporoides* (host *Malus*). Thus, the genetic control of nitrate assimilation in particular, and nitrogen catabolism in general appears to be similar in *Colletotrichum, Aspergillus, Neurospora,* and *Fusarium*.

Unlike studies with *Fusarium* spp. (7,23), no consistent differences in sectoring frequencies were associated with either of the two media types (Table 2). We also examined the rate of sectoring, however, and, for some strains, media type was associated with the rate at which the culture sectored (Table 3). Additionally, a few sectors were recovered from each species that were chlorate resistant but were unable to grow well on any of the screening media, including CM. These mutants may have mutations both at a *nit* locus and at one or more loci that are unrelated to nitrate assimilation. Alternatively, these mutants could be at loci such as those described by Cove (10–12) in *Aspergillus* where chlorate resistance may result in a general cessation of nitrogen metabolism rather than a simple inactivation of the nitrate assimilation machinery. Such an explanation has the advantage of requiring only a single mutation (rather than multiple mutations). It also can explain the overall poor growth of these mutants on any of the media used (including CM), which is inconsistent with the mutation affecting only one or a few metabolic pathways. Another important difference between *Colletotrichum* and either *Fusarium* (24) or *Aspergillus* (11) is the lack of a chlorate-resistant nitrate-utilizing class of mutants. In this sense, *Colletotrichum* more closely resembles *Neurospora* than it does either *Fusarium* or *Aspergillus*.

The *C. malvarum* strain that we used in this study was sensitive to nitrite, which prevented us from distinguishing the *nit1* mutant class from the Nit3 class. In such cases the existence of the two genotypic classes can still be demonstrated by complementation; however, the assignment of a name to either class can be made only following assays for nitrite reductase activity or following sexual crosses with a strains carrying identified *nit1* and *nit3* mutants. For screening populations for VCGs, it is sufficient to use a single mutant strain from the combined *nit1*/Nit3 class

in heterokaryon tests. For studies of nitrate assimilation or its regulation, however, the genetic basis of the nitrite sensitivity will need to be ascertained before the nitrate assimilation pathway can be properly analyzed.

Physiological complementation between paired *nit* mutants occurred in five of the seven strains of *Colletotrichum* used in this study. Although morphological and virulence traits are often used to study relatedness, *nit* mutant markers will allow isolates from the same or different species to be tested for their ability to complement each other via heterokaryosis. If two strains cannot form a viable heterokaryon in an asexual fungus, the difficulty in transfer of traits, e.g., pathogenicity, from one strain to another would provide both genetic isolation and a type of biological containment. The relative ease of generating mutants provides a useful alternative method for transformation of these fungi with the nitrate reductase gene from *Neurospora* (17) or *Aspergillus* (37,38), the nitrate-assimilation pathway-specific control gene from *Neurospora* (15), or the major nitrogen regulatory control gene from *Neurospora* (16) or *Fusarium* (13) as the selectable marker in the cloning vector.

Both intragenic and intergenic complementation were observed in all four phenotypic class mutants derived from the same heterokaryon self-compatible strain. Each strain also sectored mutants that were unable to complement with any of the other mutants derived from that same strain. In addition, some mutants could complement with only some of the other compatible strains. These findings will complicate analysis of populations of *Colletotrichum* using *nit* mutants since an extra set of complementation tests will be required. This problem will have two practical effects. First, it will make the development of tester strain sets, such as those proposed by Correll et al (1987) for studies with *Fusarium* more difficult since all members of the set must be capable of pairing with the widest range of sectors possible, and since more than one tester strain with the same phenotype may be necessary to meet this goal. Second, it will be necessary to perform complementation tests and phenotypic characterization on several mutant sectors derived from each of the strains in a population study. This additional effort may be sufficient to make large-scale screening of VCGs with this technique impractical for *Colletotrichum*.

The *C. destructivum* and *C. fragariae* strains used in this study were heterokaryon self-incompatibile. This phenomenon is not unique to *Colletotrichum* and has also been reported in *Fusarium oxysporum* (20), *F. moniliforme* (8), *Rhizoctonia solani* (19), and *Verticillium albo-atrum* (6), and probably in *Aspergillus flavus* (30), *Venturia inaequalis* (3), and *Verticillium dahliae* (33). This trait has been attributed to a mutant at a single locus that cosegregates with female sterility in *Fusarium* (8), and thus may affect aspects of the life cycle beyond the ability to make a vegetative heterokaryon. The occurrence of two heterokaryon self-incompatibile isolates within a sample of seven strains is somewhat surprising because the frequency of such strains in other studies has usually been no more than a few percent (8,20). It is not known whether other isolates of these two *Colletotrichum* species are heterokaryon self-compatible. Heterokaryon self-incompatible strains complicate VCG analyses of populations since it is usually difficult or impossible to assign such strains to a VCG. These strains are potentially very useful in recombinant DNA or biological control work where it is undesirable for the released strain to be able to form a heterokaryon with any other strain in the population. Thus, studies of heterokaryon self-incompatibility in *Colletotrichum* are potentially important from both a basic and an applied standpoint.

Among the heterokaryon self-compatible strains there was evidence of vegetative (heterokaryon) incompatibility. The five strains examined were all in distinct VCGs, suggesting that the exchange of genetic information within and between species is subject to significant limitations. There are genetic differences between these strains that are phenotypically distinct from the morphological and pathological characters that have been used to describe specific and subspecific groups within this genus. The *nit* mutants and VCGs we have described for *Colletotrichum* will be most useful in providing a framework in which the possibilities of asexual genetic exchange between different strains can be explored. The genetic differences within *Colletotrichum* provides additional evidence for the emerging complexity within this genus.

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