# Variation Among Isolates of *Fusarium lateritium* from Sweetpotato for Pathogenicity and Vegetative Compatibility

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## ABSTRACT

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Isolates (132) of *Fusarium lateritium* from sweetpotato and from other hosts (20) were compared for pathogenicity to sweetpotato vines and vegetative compatibility grouping (VCG) using complementation among nitrate nonutilizing (*nit*) mutants. Isolates from sweetpotato were either nonpathogenic or fell into one of two pathotypes: the chlorotic leaf distortion (CLD) pathotype, which induced all symptoms of CLD, or the epiphytic mycelial growth pathotype, which produced the typical epiphytic, systemic mycelial growth but did not induce chlorosis. With one possible exception, isolates from other hosts were nonpathogenic to sweetpotato. All isolates from sweetpotato produced *nit* mutants, and 44 VCGs were identified. Only 16 of 20 isolates from other hosts could be induced to produce *nit* mutants, only 10 of these were self-compatible, and none of these were compatible with any other isolate. Sweetpotato isolates from the United States appeared less diverse by VCG analysis than isolates from Africa.

Chlorotic leaf distortion (CLD) is a recently reported disease of sweetpotato (*Ipomoea batatas* (L.) Lam.) caused by the fungus *Fusarium lateritium* Nees:Fr. (10). Symptoms develop systemically on the vines and include chlorosis and distortion of very young leaves that recover from chlorosis as they mature (9,10). The fungus is an exopathogen on the shoot tips, where it grows on and in a mucilage-like material secreted by the plant and between halves of leaves that have not yet unfolded (7). It also can be isolated from within true seed of sweetpotato. Although CLD has been reported in the United States, Kenya, Brazil, and Peru (9,10,18,19,34), its origin and geographic distribution are largely unknown. A broader geographic distribution for the pathogen has been inferred from a study in which *F. lateritium* was isolated from true seed from many different countries (8). It is possible that the disease has been present but unrecognized in many places. The symptoms induced (10) and the nature of the association between fungus and plant (7) are distinct from any other *Fusarium*-plant pathosystem. Other strains of *F. lateritium* generally cause necroses on herbaceous hosts or bud blights and diebacks on woody perennials.

Isolates of *F. lateritium* associated with sweetpotato CLD have been placed in this species solely on the basis of morphological criteria (10,29). Although they share a common macroconidium morphology, there are morphological differences among isolates placed in the species *F. lateritium*. For example, sweetpotato CLD isolates generally do not produce chlamydospores but do produce abundant microconidia, which is at variance with some descriptions of other members of this species (4,28). In addition, sweetpotato isolates vary in production of monophialides versus polyphialides, whereas most other members of *F. lateritium* produce only monophialides.

Vegetative compatibility grouping (VCG) has been used frequently as a means of identifying isolates of a fungus that are closely related (25,26). This trend has been accelerated by the development of faster methods of identifying compatible isolates using nitrate nonutilizing (*nit*) mutants (13,15,20,22,32). These mutants produce thin growth on minimal media with nitrate as the nitrogen source. When complementary mutants are paired on minimal media, if they are vegetatively compatible and form heterokaryons, the heterokaryotic mycelium is easily distinguished as a line of robust growth where the colonies intersect. In *F. moniliforme*, 10 *vic* or *het* loci have been identified that control a homogenic vegetative compatibility system (25,33). To be compatible, isolates must share a common allele at each of these loci. Thus, isolates within a VCG are usually, but not necessarily, clonally related (26). This methodology has been applied to a long and growing list of fusaria. In some cases, a correlation has been found between VCG and pathogenicity characteristics, while in others no relationship has been observed (11,25,26).

In assessing the potential for distribution of the CLD pathogen via international exchange of true seed among breeding programs, it will be important to know as much as possible about the origin of the causal fungus and the relationship of sweetpotato CLD strains to strains of *F. lateritium* from different geographic regions and hosts. The primary objectives of this study were to determine the pathogenicity to sweetpotato of isolates from different hosts and geographic regions and to develop an initial assessment of genetic diversity among these isolates using vegetative compatibility analysis.

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## MATERIALS AND METHODS

Isolates of *F. lateritium* were collected from sweetpotato plants in the field in Louisiana (10), from true seed of sweetpotato (8), from sweetpotato from North Carolina (provided by C. W. Averre and G. Abad, Department of Plant Pathology, North Carolina State University, Raleigh), and from other hosts from the collection of P. E. Nelson. All the isolates used previously had been passed through a single-spore transfer and were stored on silica gel at approximately –20°C (30). Information on the source of each isolate is given in Tables 1 and 2.

**Pathogenicity tests.** Pathogenicity of each isolate was tested on sweetpotato vines during the summer, when conditions favor development of CLD symptoms, using previously described methods (10). Sweetpotato plants derived from meristem-tip culture were grown in the greenhouse in an insect-proof cage (covered with nylon mesh, 32 openings per 2.54 cm) as a source of CLD-free plants. These were increased in a separate greenhouse for use in pathogenicity tests. The sweetpotato clone NC-845 was used in all tests, and the cultivar Jewel also was used in most tests. Vine cuttings were removed from symptomless plants with a flamed scalpel, and unfolded leaves were removed by breaking the petiole from the stem.

Inoculum was prepared by rinsing the surface of a 5- to 7-day-old potato-dextrose agar (PDA) petri dish culture (90 mm in diameter) with 100 ml of sterile distilled water. The conidial suspension was filtered through cheesecloth, and the inoculum concentration was adjusted to $1.0 \times 10^6$ conidia per ml based on counts made with a hemacytometer. Cuttings were placed in a plastic bag containing the inoculum and manipulated to assure complete coverage of the cutting with inoculum. Each cutting was planted in river silt/sand/Jiffy Mix (Jiffy Products of America, Inc., West Chicago, IL) (1:1:1, vol/vol/vol) in a 10-cm-diameter clay pot and placed under a mister (8 s every 10 min, from 0600 to 2000 hours) for 3 days. Subsequently, pots were watered and fertilized as needed.

Plants were grown under high-intensity lights (Sylvania 1000-12, H34GV-1000) for 4 to 6 weeks, symptoms were recorded, plants were cut back with a flamed knife, and after another 4 to 6 weeks, symptoms were again assessed on the growth that proliferated from axillary buds after the plants were cut back. Symptoms were rated using a standardized scale, in which 0 = no symptoms: 1 = mycelia visible on youngest unfolded leaf, no chlorosis; 2 = mycelia visible on more than one leaf, no chlorosis; 3 = mycelia visible on more than one leaf, with chlorosis on youngest unfolded leaf; 4 = mycelia visible on more than one leaf, with chlorosis on more than one leaf; and 5 = mycelia visible on more than one leaf, with marginal necrosis on any chlorotic leaf. Because there were many isolates, the incubation period was long and the tests could only be conducted during the summer, it was necessary to conduct numerous tests over a 4-year period. In each test, each isolate was inoculated on at least four plants of each sweetpotato genotype, and isolate 91-27-2 was included as a standard. Controls were dipped in sterile distilled water. Each isolate was included in at least two separate tests.

**Vegetative compatibility.** Vegetative compatibility was determined by Puhalla's modification of Cove's method of complementation tests between *nit* mutants (15,32). Mycelia were transferred from PDA to potato-sucrose agar (KPS) or minimal salts medium (MM) plus L-asparagine at 1.6 g/liter (KMM), each supplemented with 1.5% $KClO_3$ (13). Some isolates had a uniform, rapid growth on media containing 1.5% $KClO_3$, and these were grown on the same media supplemented with 3.0 or 4.5% $KClO_3$. Transfers were made from the leading margin of any fast-growing sectors that appeared on KPS or KMM to MM. Those that had a thin, expansive growth on MM were considered *nit* mutants, and their phenotypes were determined by the method of Correll et al. (13). At least one *nit*M and one *nit1* or *nit3* mutant were obtained for each parent. The nonsweetpotato CLD isolates generally produced few *nit* mutants by the above approach, so we attempted to produce *nit* mutants for each of these recalcitrant isolates on 10 plates each of KPS, KMM, and KMM without asparagine, each with 1.5 and 6.0% $KClO_3$.

To test for complementation or heterokaryon formation between *nit* mutants, a mycelial transfer was made from PDA to MM with a *nit*M mutant in the center of a daisy configuration and several *nit1* and *nit3* mutants on the outer circle. The plates were incubated in darkness at 28°C for up to 21 days. If a continuous line of robust aerial mycelia developed within 7 to 14 days where the thin expansive growth of the *nit* mutants converged, heterokaryon formation was considered to have occurred, and the complementary *nit* mutants were assigned to the same VCG. For each VCG, a *nit*M, *nit1*, and *nit3* were selected as testers for pairing with new *nit* mutants.

As each group of new *nit* mutants was generated, they were paired against all testers for VCGs recognized at that time. In a few cases, *nit* mutants that formed heterokaryons when paired in dual culture failed to form heterokaryons when combined on the same plate with multiple *nit* mutants. Thus, to avoid the possibility that interference among some *nit* mutants might inhibit heterokaryon formation between other *nit* mutants, pairings that did not form heterokaryons were repeated by either transferring blocks of PDA from cultures of the two *nit* mutants to 6-cm-diameter petri dishes with MM or by transferring spore suspensions of the two *nit* mutants to MM in wells of 24-well Cell Culture Clusters (Costar, Cambridge, MA) (21). For each isolate, reciprocal pairings were made using a *nit*M and *nit1* mutant of both the VCG tester and the unknown isolates. For six of the nonsweetpotato CLD isolates, heterokaryons did not form between the original *nit*M and complementary *nit* mutants from the same isolate. Additional *nit*M mutants were generated for these isolates and paired with all available complementary *nit* mutants for the same parent. If no heterokaryon formed in any of these combinations, the isolate was considered heterokaryon self-incompatible (HSI) (14).

## RESULTS

**Pathogenicity.** Three distinct reactions were observed in NC-845 sweetpotato to 132 sweetpotato isolates of *F. lateritium*: i) 66 isolates (50%) induced the full CLD syndrome with epiphytic mycelia, chlorosis of varying severity, and distortion of young leaves (CLD pathotype); ii) 52 isolates (39%) grew systemically as epiphytic mycelia without inducing chlorosis or distortion (EMG pathotype); and iii) 14 isolates (11%) were nonpathogenic and did not induce symptoms or grow epiphytically (Table 1). Generally, a higher proportion of the isolates from the United States were of the CLD pathotype than were isolates from other sources. None of the isolates from other hosts from the United States induced chlorosis or distortion, and only one, an isolate from a white pine rust canker in Oregon, showed weak development as an EMG pathotype. Exotic isolates could not be included in pathogenicity tests because of permit restrictions.

**VCG.** Although there were differences among sweetpotato isolates in the ease of obtaining *nit* mutants and in the proportion of different phenotypes, *nit*M mutants were obtained for each of these isolates, except 91-69-2. For isolate 91-69-2, robust mycelia developed when *nit1* and *nit3* mutants were paired, and these were used for analysis. For a randomly selected group of six sweetpotato CLD isolates, the percentage of sectors that were *nit*M, *nit1*, or *nit3* mutants or that produced robust growth on MM was 8.6, 43.1, 4.1, and 44.2 for KPS, 8.3, 51.1, 6.4, and 34.2 for KMM, and 9.4, 54.0, 5.3, and 31.3 for KMM without asparagine, respectively. All of the isolates were self-compatible, although a small number of *nit* mutants did not form heterokaryons with other *nit* mutants from the same isolate. When paired on MM,

TABLE 1. Source of sweetpotato chlorotic leaf distortion and seed isolates of *Fusarium lateritium* and data on pathogenicity and vegetative compatibility group (VCG) of each isolate

| VCG Isolate | FRC no.[a] | Source | Geographic origin | EMG[b] | CLD[c] |
|---|---|---|---|---|---|
| 01 | | | | | |
| F89-01 | L-256 | Leaf | Baton Rouge, LA | +[d] | +[d] |
| F89-02 | | Leaf | Baton Rouge, LA | + | ++ |
| F89-03 | | Petiole | Baton Rouge, LA | + | + |
| F89-07 | | Meristem | Baton Rouge, LA | + | + |
| 91-33-1 | | Seed | Charleston, SC | + | + |
| 02 | | | | | |
| 3647 | L-263 | Leaf | Chowan Co., NC | + | + |
| 3691 | L-264 | Leaf | Lenoir Co., NC | + | – |
| 4482-2 | L-266 | Stem? | Harnett Co., NC | + | + |
| 4608 | L-267 | Leaf | Columbus Co., NC | + | + |
| 4785-1 | | Stem? | Edgecomb Co., NC | + | – |
| 4785-2 | L-269 | Stem? | Edgecomb Co., NC | + | +? |
| 4785-3 | | Leaf | Edgecomb Co., NC | + | + |
| 4785-4 | L-305 | Stem? | Edgecomb Co., NC | + | + |
| 91-26-1 | L-278 | Seed | Mayaguez, Puerto Rico | + | – |
| 91-26-2 | L-279 | Seed | Mayaguez, Puerto Rico | + | + |
| 91-26-3 | L-280 | Seed | Mayaguez, Puerto Rico | + | – |
| 91-27-1 | L-281 | Seed | Mayaguez, Puerto Rico | + | – |
| 91-42-1 | | Seed | Charleston, SC | + | ++ |
| 91-73-2 | L-319 | Seed | Charleston, SC | + | +? |
| F89-08 | | Meristem | Baton Rouge, LA | + | + |
| F89-09 | | Meristem | Chase, LA | + | + |
| F89-15 | | Seed | Charleston, SC | + | + |
| F89-16 | L-260 | Seed | Charleston, SC | + | ++ |
| F89-19 | | *I. setosa* seed | Baton Rouge, LA | + | – |
| F89-20 | | *I. setosa* seed | Baton Rouge, LA | + | + |
| F89-21 | | *I. setosa* seed | Baton Rouge, LA | + | ++ |
| F89-29 | | Meristem | Washington, LA | + | + |
| F89-34 | L-262 | Floral bud | Baton Rouge, LA | + | – |
| 03 | | | | | |
| 3925 | L-265 | Leaf | Harnett Co., NC | | + |
| 4784 | L-268 | Stem? | Pender Co., NC | + | + |
| 91-35-1 | L-297 | Seed | Charleston, SC | + | +? |
| 91-40-1 | | Seed | Charleston, SC | + | +? |
| 91-41-1 | L-298 | Seed | Charleston, SC | + | + |
| 91-73-1 | | Seed | Charleston, SC | + | + |
| 91-73-3 | L-320 | Seed | Charleston, SC | + | +? |
| F89-10 | L-258 | Meristem | Chase, LA | + | + |
| F89-12 | | Meristem | Chase, LA | + | + |
| F89-14 | | Meristem | Wisner, LA | + | + |
| F89-17 | | Seed | Charleston, SC | + | + |
| F89-23 | | Seed | Charleston, SC | + | +? |
| F89-24 | | Seed | Charleston, SC | + | – |
| F89-30 | | Meristem | Washington, LA | + | + |
| 04 | | | | | |
| 91-27-2 | L-282 | Seed | Mayaguez, Puerto Rico | + | ++ |
| 91-31-1 | L-296 | Seed | Charleston, SC | + | +? |
| 91-36-1 | | Seed | Charleston, SC | + | + |
| 91-70-2 | L-313 | Seed | Kumasi, Ghana | + | – |
| 91-70-3 | L-314 | Seed | Kumasi, Ghana | + | +? |
| 91-71-1 | L-315 | Seed | Kumasi, Ghana | + | –? |
| 91-71-3 | L-316 | Seed | Kumasi, Ghana | + | +? |
| 93-150-3 | L-354 | Seed | Bogor, Indonesia | + | – |
| 93-151-1 | L-355 | Seed | Bogor, Indonesia | + | + |
| 93-151-2 | L-356 | Seed | Bogor, Indonesia | + | – |
| 93-157-1 | L-359 | Seed | Lembang, Indonesia | + | + |
| 93-157-2 | L-360 | Seed | Lembang, Indonesia | – | – |
| 93-166-2 | L-366 | Seed | Batac, Philippines | + | + |
| F89-04 | L-257 | Leaf | Baton Rouge, LA | + | + |
| F89-05 | | Meristem | Baton Rouge, LA | + | + |
| F89-22 | | *I. setosa* seed | Baton Rouge, LA | + | + |
| F89-26 | | Meristem | Baton Rouge, LA | + | + |
| F89-32 | | Meristem | Washington, LA | + | + |
| 05 | | | | | |
| F89-06 | | Meristem | Baton Rouge, LA | + | + |
| F89-13 | L-259 | Meristem | Chase, LA | + | + |
| 06 | | | | | |
| 91-34-1 | | Seed | Charleston, SC | + | + |
| F89-31 | L-294 | Meristem | Washington, LA | + | + |
| 07 | | | | | |
| 91-38-1 | | Seed | Charleston, SC | +? | – |
| 08 | | | | | |
| 91-55-1 | L-299 | Seed | Ibadan, Nigeria | +? | +? |
| 09 | | | | | |
| 91-58-1 | | Seed | Ibadan, Nigeria | + | – |
| 91-58-2 | L-301 | Seed | Ibadan, Nigeria | + | – |
| 91-58-3 | | Seed | Ibadan, Nigeria | + | – |
| 10 | | | | | |
| 91-60-1 | | Seed | Kodeira, Kenya | + | +? |
| 91-60-2 | L-303 | Seed | Kodeira, Kenya | + | – |
| 91-60-a | L-304 | Seed | Kodeira, Kenya | + | +? |
| 91-60-b | | Seed | Kodeira, Kenya | + | +? |
| L-306 | L-306 | Vine | Kenya | NT | NT |
| L-309 | L-309 | Vine | S. Nyanza ,Kenya | NT | NT |
| 11 | | | | | |
| 91-57-1 | | Seed | Ibadan, Nigeria | +? | – |
| 12 | | | | | |
| 91-57-3 | | Seed | Ibadan, Nigeria | +[d] | –[d] |
| 13 | | | | | |
| 91-59-1 | | Seed | Ibadan, Nigeria | + | – |
| 91-59-2 | L-302 | Seed | Ibadan, Nigeria | + | + |
| 91-59-3 | | Seed | Ibadan, Nigeria | + | – |
| 14 | | | | | |
| 91-69-2 | L-311 | Seed | Kumasi, Ghana | + | – |
| 91-69-3 | L-312 | Seed | Kumasi, Ghana | + | – |
| 15 | | | | | |
| 91-67-1 | L-310 | Seed | S. Nyanza, Kenya | + | – |
| L-307 | L-307 | Vine | S. Nyanza, Kenya | NT | NT |
| 16 | | | | | |
| 91-57-2 | L-300 | Seed | Ibadan, Nigeria | – | – |
| 17 | | | | | |
| 91-79-1 | L-321 | Seed | Mansa, Zambia | + | –? |
| 91-79-2 | L-322 | Seed | Mansa, Zambia | + | – |
| 91-79-3 | L-323 | Seed | Mansa, Zambia | + | + |
| 91-81-1 | L-324 | Seed | Mansa, Zambia | + | – |
| 18 | | | | | |
| 91-82-1 | L-325 | Seed | Mansa, Zambia | + | ++ |
| 91-82-2 | L-326 | Seed | Mansa, Zambia | + | +? |
| 19 | | | | | |
| 91-83-1 | L-327 | Seed | Rwanda | + | –? |
| 91-83-3 | L-328 | Seed | Rwanda | + | – |
| 91-83-4 | L-329 | Seed | Rwanda | + | – |
| 20 | | | | | |
| 91-90-1 | L-330 | Seed | Rwanda | +? | – |
| 21 | | | | | |
| 92-129-2 | L-336 | Seed | Porto Alegre, Brazil | – | – |
| 92-129-3 | L-337 | Seed | Porto Alegre, Brazil | + | – |
| 22 | | | | | |
| 92-129-4 | L-338 | Seed | Porto Alegre, Brazil | – | – |
| 23 | | | | | |
| 92-129-5 | L-339 | Seed | Porto Alegre, Brazil | – | – |
| 24 | | | | | |
| 91-29-1 | L-283 | Seed | Philippines | + | – |
| 92-136-1 | L-340 | Seed | Moso, Burundi | + | – |
| 92-139-1 | L-343 | Seed | Moso, Burundi | + | – |
| 93-098-1 | L-350 | Seed | Rwanda | + | – |
| 93-098-2 | L-369 | Seed | Rwanda | –? | – |
| 25 | | | | | |
| 92-138-1 | L-342 | Seed | Moso, Burundi | – | – |
| 26 | | | | | |
| 92-136-2 | L-341 | Seed | Moso, Burundi | +? | – |
| 27 | | | | | |
| 91-23-1 | L-274 | Seed | Papua New Guinea | + | – |
| 28 | | | | | |
| 91-60-3 | | Seed | Kodeira, Kenya | + | – |
| 29 | | | | | |
| 92-107-1 | L-335 | Seed | Taiwan | +? | – |
| 30 | | | | | |
| 91-25-1 | L-275 | Seed | Brasilia, Brazil | + | – |
| 91-25-2 | L-276 | Seed | Brasilia, Brazil | + | – |
| 91-25-3 | L-277 | Seed | Brasilia, Brazil | + | – |
| 31 | | | | | |
| 93-145-1 | L-352 | Seed | Brasilia, Brazil | + | +? |
| 32 | | | | | |
| 91-08-1 | L-286 | Seed | Tacna, Peru | + | – |
| 33 | | | | | |
| 93-153-1 | L-370 | Seed | Bogor, Indonesia | +? | – |
| 34 | | | | | |
| 93-162-1 | L-361 | Seed | Taba-ao, Philippines | + | – |
| 35 | | | | | |
| 93-165-1 | L-362 | Seed | Batac, Philippines | + | – |
| 93-165-2 | L-363 | Seed | Batac, Philippines | + | – |
| 93-165-3 | L-364 | Seed | Batac, Philippines | –? | – |
| 93-166-1 | L-365 | Seed | Batac, Philippines | + | – |
| 93-166-3 | L-367 | Seed | Batac, Philippines | –? | – |
| 36 | | | | | |
| 91-02-1 | L-273 | Seed | Tacna, Peru | + | – |
| 37 | | | | | |
| 91-29-2 | L-284 | Seed | Philippines | + | –? |
| 91-29-3 | L-285 | Seed | Philippines | + | – |
| 93-154-1 | L-357 | Seed | Lembang, Indonesia | –? | – |
| 38 | | | | | |
| 93-102-1 | L-351 | Seed | Rwanda | – | – |
| 39 | | | | | |
| 93-148-1 | L-353 | Seed | Brasilia, Brazil | + | + |
| 40 | | | | | |
| 93-153-2 | L-368 | Seed | Bogor, Indonesia | – | – |
| 41 | | | | | |
| 93-155-1 | L-358 | Seed | Lembang, Indonesia | + | – |
| 42 | | | | | |
| 94-170 | | Seed | Papua New Guinea | + | – |
| 43 | | | | | |
| 94-171 | | Seed | Papua New Guinea | + | – |
| 44 | | | | | |
| 94-172 | | Seed | Papua New Guinea | + | +? |

[a] Accession number in the Fusarium Research Center Collection, The Pennsylvania State University, University Park.
[b] EMG = white epiphytic mycelial growth visible on young leaves at 4 to 6 wk postinoculation.
[c] CLD = chlorotic leaf distortion symptoms produced on sweetpotato clone NC-845 at 4 to 6 wk postinoculation.
[d] NT = not tested because of quarantine restrictions; + = consisitently positive; ++ = consistently severe; +? = mostly positive; – = consistently negative; and –? = mostly negative.

compatible isolates formed lines of robust aerial mycelia within 9 to 14 days after pairing. In some instances, reactions occurred between *nit* mutants of incompatible isolates: after about 12 to 20 days, a broken line of tufts of robust aerial mycelia developed (Fig. 1). These tufts of mycelia generally grew more slowly than the robust aerial mycelia produced as a result of heterokaryon formation between compatible isolates.

The 132 sweetpotato isolates were grouped into 44 VCGs, including 25 single-member VCGs (Table 1). The 56 isolates from the United States fell into seven VCGs; however, 50 (91% of the United States isolates) fell into four VCGs, and each of these VCGs contained isolates from more than one state. The 42 isolates from Africa were grouped into 19 VCGs, most of which contained isolates from the same location. One group, VCG 04, included isolates from three continents, North America, Africa, and Asia.

The nonsweetpotato isolates of *F. lateritium* were problematic: A few were uninhibited on media containing $KClO_3$; some did not grow on KMM, with or without asparagine, and did not generate chlorate-resistant sectors; most produced a high proportion of sectors that developed robust growth on MM, similar to *crn* mutants (25); and a few produced *nit* mutants. In some cases, sectors transferred directly from KPS or KMM to MM did not grow. Subsequently, they were transferred to PDA and then to MM. Approximately half of the sectors transferred to PDA did not grow. Thus, we were unable to obtain *nit* mutants or determine vegetative compatibility for four of these isolates. Of the 16 isolates for which *nit* mutants were obtained, six were HSI, and 10 were self-compatible, but none was compatible with any of the other isolates included in this study (Table 2).

## DISCUSSION

Isolates of *F. lateritium* from sweetpotato seed or vines with symptoms of CLD varied in the symptoms produced on sweetpotato vines. Most of the isolates (89%) were capable of systemically colonizing the surface of the growing shoot. However, only a portion of these was capable of inducing chlorosis over an extended incubation period in the greenhouse. Interpretation of CLD pathogenicity tests is complicated by the facts that the incubation period for symptom development is long and plants may go in and out of symptom remission (10). Thus, while many of the isolates clearly induced strong chlorosis symptoms, some produced a transient or less definitive response, and some consistently colonized the plant surface without inducing chlorosis. Some of the plants from these tests were planted to the field and continued to display symptoms similar to those observed in the greenhouse (C. A. Clark, *unpublished data*).

The proportion of isolates of the CLD pathotype was greater among isolates from the United States than from other sources. This may partially explain why CLD has not been reported from some regions where the fungus occurs in seed, since the EMG pathotype is less conspicuous in the field. It also suggests less diversity among the United States isolates than among isolates from Africa.

Since the pathogen does not enter the symptomatic plant tissue (7), it is likely that it elaborates a toxin or similar chemical that induces chlorosis in the leaves. Some isolates of *F. lateritium* from sweetpotato produce a novel trichothecene (S. McCormick, *personal communication*), and preliminary data suggest a possible correlation between production of this trichothecene by isolates in vitro and induction of chlorosis on sweetpotato leaves (C. A. Clark and S. McCormick, *unpublished data*). Future research should be

TABLE 2. Source of isolates of *Fusarium lateritium*, data on pathogenicity, and comments on attempts to induce nitrate nonutilizing (*nit*) mutants for vegetative compatibility determination

| Isolate[a] | Source | Geographic origin | EMG[b] | CLD[c] | Vegetative compatibility[d] |
|---|---|---|---|---|---|
| L-052 | Spanish moss | Florida | –[e] | –[e] | SC (weak), cross– |
| L-055 | Elm canker | Louisiana | – | – | SC (weak), cross– |
| L-056 | Mulberry | Australia | NT | NT | SC, cross– |
| L-067 | Loblolly pine | Missouri | – | – | SC, cross– |
| L-068 | Albizia twig | Florida | – | – | SC, cross– |
| L-080 | Pine seed | Georgia | –? | – | SC (weak), cross– |
| L-081 | Orange twig | New Caledonia | NT | NT | SC, cross– |
| L-087 | Coffee berry | New Caledonia | NT | NT | SC, cross– |
| L-105 | *Anoda cristata* | Mississippi | – | – | No *nits* recovered |
| L-106 | White pine rust | Oregon | +? | – | No *nits* recovered |
| L-111 | Coffee twig | New Guinea | NT | NT | HSI |
| L-116 | *A. cristata* | Mississippi | – | – | HSI |
| L-125 | *Coffea arabica* | Ethiopia | NT | NT | HSI |
| L-223 | Juniper scales | Florida | NT | NT | SC, cross– |
| L-233 | Soil | Transkei, S. Africa | NT | NT | HSI |
| L-240 | Lucerne tree | Tasmania, Australia | NT | NT | No *nits* recovered |
| L-248 | Sweetpotato[f] | Papua New Guinea | NT | NT | SC (weak), cross– |
| L-251 | Sweetpotato[f] | Papua New Guinea | NT | NT | HSI |
| L-255 | Tomato | Chile | NT | NT | No *nits* recovered |
| L-270 | Sicklepod | Mississippi | – | – | HSI |

[a] Accession number in the Fusarium Research Center Collection, The Pennsylvania State University, University Park.

[b] EMG = white epiphytic mycelia growth visible on young sweetpotato leaves at 4 to 6 wk postinoculation.

[c] CLD = chlorotic leaf distortion symptoms produced on sweetpotato clone NC-845 at 4 to 6 wk postinoculation.

[d] SC = compatible reaction among *nit* mutants from the same isolate; HSI = no reaction among *nit* mutants from the same isolate; cross– = no reaction when *nit* mutants were paired with *nit* mutants of any other isolate.

[e] NT = not tested because of quarantine restrictions; – = consistently negative; –? = mostly negative; and +? = mostly positive.

[f] Described as from sweetpotato with stem rot.

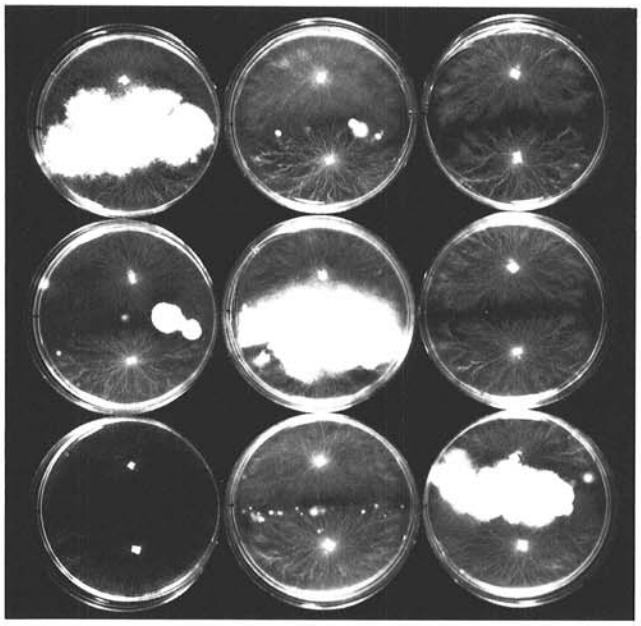

**Fig. 1.** Pairings on minimal salts medium of nitrate nonutilizing (*nit*) mutants of sweetpotato chlorotic leaf distortion isolates of *Fusarium lateritium* for vegetative compatibility group (VCG) determination. On each plate, a *nit*M mutant was plated on the upper side and a *nit1* mutant on the lower side of the plate. *nit*M mutants were from: top row = isolate F89-24 (VCG 03), middle row = F89-08 (VCG 02), and bottom row = F89-01 (VCG 01). *nit1* mutants were from: left column = isolate F89-24 (VCG 03), middle column = F89-08 (VCG 02), and right column = F89-01 (VCG 01). Plates were photographed after 15 days of incubation. Lines of robust mycelial growth between *nit* mutants of the same isolate indicate compatible reactions. "Bridge" or weak reactions between isolates from different VCGs are evident as clumps of robust mycelia in a broken line between *nit* mutants in the top and bottom rows of the middle column and the middle row of the left column.

designed to determine what substance the CLD pathotype produces that induces chlorosis and to determine what relationship, if any, trichothecenes have to induction of chlorosis.

The sweetpotato CLD isolates and the other isolates of *F. lateritium* behaved quite differently in our efforts to obtain *nit* mutants. Four of the nonsweetpotato CLD isolates did not produce *nit* mutants. On the other hand, the sweetpotato CLD isolates readily produced *nit* mutants, and the proportion of different *nit* phenotypes was similar regardless of the medium used to generate them. Of the 132 sweetpotato isolates analyzed, 44 VCGs were recognized. There did not appear to be any relationship between VCG and pathotype. However, there was an apparent relationship between VCG and geographic origin of the isolates. Most of the 56 United States isolates fell into four VCGs, each of which was represented in more than one location, whereas the 42 African isolates were grouped in 19 VCGs, most of which were found at only one location. VCGs 05 and 06 each had two members from different locations, and VCG 24 had two isolates each from the neighboring countries of Rwanda and Burundi. VCG 04 was the most geographically diverse, with isolates from four United States locations, Ghana, Indonesia, and the Philippines. However, each of the remaining VCGs was found at only one location. Thus, it appears that the isolates from Africa, which were isolated exclusively from seed, show greater VCG diversity than those from the United States, which were derived from various plant parts from several locations.

Many explanations could be postulated to account for the occurrence of both pathotypes in multiple VCGs. It has been assumed that vegetatively incompatible isolates with similar pathogenicity are not clonally related but that their pathogenicity characteristics developed independently (26). However, both pathotypes of *F. lateritium* colonize sweetpotato vines similarly—they differ in their ability to induce chlorosis. In this case, it also could be speculated that isolates have lost the ability to induce chlorosis independently of the evolution of VCGs. This could conceivably result from mutation of genes for chlorosis induction or if these genes were located on a dispensable chromosome.

In most cases, the interaction between compatible and incompatible *nit* mutants of *F. lateritium* was similar to that described in most reports on vegetative compatibility in *Fusarium* (12,13, 25,26). However, there were some pairings between *nit* mutants from different VCGs that resulted in limited development of presumably heterokaryotic mycelia. These were similar to the weakly positive reactions between certain isolates of *F. oxysporum* reported by Gordon and Okamoto (17) that bridged VCGs. In *F. oxysporum* f. sp. *cubense*, successful heterokaryon formation was observed between some isolates from two VCGs (31). Vegetative incompatibility is not considered by some to be an absolute barrier to parasexual recombination between isolates of *F. oxysporum* (27). *Cryphonectria parasitica* (1,2,23) and *Ophiostoma ulmi* (5) strains can vary in their degree of compatibility. In *C. parasitica*, the more *vc* loci that differ between two strains, the less likely they are to be able to transmit hypovirulence, which is associated with dsRNA. It is not yet possible to conclude whether the weak interactions between *F. lateritium* isolates from different VCGs are a result of a system similar to that described for *C. parasitica* and *O. ulmi* or whether suppressor mutations, analogous to those recently observed in *Neurospora crassa* (3), have partially suppressed the heterokaryon incompatibility in some of the sweetpotato CLD isolates.

Some of the isolates used in this study contain dsRNA (C. A. Clark, *unpublished data*). It would be of interest to determine in future studies if the dsRNA could be used as a marker to study the nature of cytoplasmic exchange between *nit* mutants that produce the typical compatible, incompatible, or weak interactions. Transmission of dsRNA could be compared to mitochondrial DNA as cytoplasmic markers and various nuclear markers to learn the extent and nature of exchange in each type of interaction.

VCG analysis did not provide definitive information on the relationship between sweetpotato isolates and those from other hosts. None of the nonsweetpotato CLD isolates was vegetatively compatible with any other isolates, sweetpotato or nonsweetpotato, used in this study. Leslie (26) pointed out that although VCGs are useful in demonstrating relatedness between closely or clonally related isolates, they are not useful in indicating relationships for isolates that are not compatible.

The taxonomic relationship between the sweetpotato strains and other strains of *F. lateritium* could be further clarified by determining whether or not they are sexually compatible. *Gibberella baccata* (Wallr.) Sacc. is the teleomorph of *F. lateritium*, and several isolates from hosts other than sweetpotato have been induced to produce perithecia in culture (24). Preliminary efforts to induce production of fertile perithecia with sweetpotato isolates of *F. lateritium* from the United States have failed (C. A. Clark, *unpublished data*; A. Desjardins, *personal communication*). It has been suggested that sexually reproducing populations of *Fusarium* will have many VCGs (25) and that compatible mating types are often geographically separated (35). Thus, the probability of finding sexually compatible combinations might be increased by testing isolates from other countries. On the other hand, Gordon (16) has argued that diversity in *F. oxysporum* does not necessarily indicate involvement of a sexual stage, past or present. Furthermore, Correll et al. (12) could not demonstrate sexual fertility in any isolates of *F. subglutinans* f. sp. *pini* from a genetically diverse population from Florida or a relatively narrow population from California. A systematic attempt should be made to determine if sweetpotato CLD isolates can produce a sexual stage and if they are sexually compatible with isolates from other hosts. In addition, other approaches, such as electrophoretic karyotyping, isozyme analysis, and random amplified polymorphic DNA and restriction fragment length polymorphism procedures, also should be applied to this problem.

The sweetpotato CLD-*F. lateritium* system presents an interesting model for study of pathogen dissemination on at least two levels. Since the pathogen systemically colonizes the shoot tip of the plant (7,10), which is routinely used in commercial propagation of the crop, it is likely that it is distributed as clones of the fungus in close association with clonal propagation of the host. However, it also potentially can be disseminated internationally in seed. It will be of interest to see if VCG or molecular markers could be used to investigate migration of the fungus. The presence of small VCGs in more than one location and VCG diversity within fields led Campbell et al. (6) to suggest the possible movement of *F. moniliforme* within seed. Little is known about diversity of sweetpotato CLD strains of *F. lateritium* on an individual plant much less within a field, but we speculate that the presence of VCG 04 in four geographically separated locations may be a result of movement in seed.

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