Fusarium lateritium, Causal Agent of Sweetpotato Chlorotic Leaf Distortion

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ABSTRACT

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Symptoms and signs of chlorotic leaf distortion of sweetpotato (*Ipomoea batatas*) include a white superficial growth consisting of fungal hyphae and macroconidia of *Fusarium* on young leaves, followed by general chlorosis and, in some instances, twisting and distortion of leaves. *Fusarium lateritium* was isolated from all symptomatic plants and many symptomless field-grown plants, but not from symptomless plants derived from meristem-tip culture (mericlones). Healthy mericlones inoculated with *F. lateritium* isolated from chlorotic leaf distortion affected sweetpotato developed chlorotic leaf distortion symptoms after 3–7 wk in the greenhouse. Uninoculated control mericlones did not develop symptoms. Koch's postulates were fulfilled, confirming *F. lateritium* as the causal agent of chlorotic leaf distortion in sweetpotato.

During the early 1980s, a disorder was first observed on sweetpotato (*Ipomoea batatas* (L.) Lam.) vines in Louisiana and South Carolina (1–3,8). Symptoms developed on the youngest leaves after periods of sunny weather and included the appearance of white material on leaf surfaces, followed by a general chlorosis of young, expanding leaves and, in some instances, distortion of affected leaves. The disease appeared unique in that affected tissues and whole plants recovered from chlorosis. The disease has become distributed generally in Louisiana, affecting most, if not all, plants in all fields examined, and has been observed increasingly in South Carolina and North Carolina (3; C. W. Averre, P. D. Dukes, and J. W. Moyer, *personal communications*). To our knowledge, no similar disease of sweetpotato has been reported before our recent description of the symptoms (1,2).

This study was undertaken to determine the cause of chlorotic leaf distortion of sweetpotato, which also has been called vine tip chlorosis (3). Because the disease appeared to be systemic, caused chlorosis and distortion of young leaves, and was reduced by meristem-tip culture, initial efforts tested the hypothesis that it was caused by a virus or viroid (1,7,8). However, no such agent was consistently associated with the disease. This paper demonstrates that chlorotic leaf distortion is caused by *Fusarium lateritium* Nees.

MATERIALS AND METHODS

Symptoms and signs of chlorotic leaf distortion have been observed consistently on hundreds of sweetpotato genotypes grown in experimental field plots, commercial fields, and in the greenhouse since 1981.

Isolations. Plants were collected from four fields in East Baton Rouge, Evangeline, and Franklin parishes, LA, on four occasions and from a greenhouse in Baton Rouge on two occasions during the summer of 1989. On the same day or the day following collection, various aboveground parts of the plants were rinsed in running tap water, surface disinfested by immersion in 0.5% NaOCl for 5 min, rinsed in sterile distilled water, and dissected with flamed scalpels and forceps. Preliminary isolations were made on potato dextrose agar (PDA) acidified with one drop of 88% lactic acid per 15 ml of PDA. Because macroconidia typical of *Fusarium* consistently were observed on the surface of affected leaves, and a *Fusarium* sp. was the predominant organism isolated in the preliminary attempts, subsequent isolations were made on Komada's medium (5). Isolation plates were incubated in the dark at 28 C.

Isolations were made from true seed of sweetpotato on two occasions. The first seed lot was provided from the maternal parent W-238 in a 1986 polycross nursery (open pollinated) at the USDA Vegetable Laboratory, Charleston, SC. Seed were surface-disinfested without prior treatment by dipping them briefly in 95% ethanol and then immersing them in 0.5% NaOCl for 5 min. Seed were rinsed in sterile distilled water and placed on acidified PDA. A second seed lot was obtained from the maternal parent L85-237 in a 1989 polycross nursery in Baton Rouge. Half of this seed was scarified in 96.5% H_2SO_4 on a magnetic stirrer for 20 min, and the other half was not scarified. Both scarified and nonscarified seed then were placed in tap water, and those that floated or sank were surface-disinfested separately as described previously and plated on acidified PDA.

Preservation and identification of cultures. Single-conidium cultures were made of the predominant fungus either directly from isolation plates or from mass-transfer cultures made from the isolation plates. Single-conidium cultures were grown on PDA slants, and a suspension of conidia in skim milk and glycerol was transferred to sterile silica gel crystals for preservation (11).

Colony characteristics of the fungi were observed on PDA after 1 wk and after 1–2 mo. Observations on morphology and size of conidia and morphology of phialides were made on cultures grown on carnation leaf agar (CLA)(10). Attempts to induce chlamydospore formation were made by placing a 1-mm^2 piece of PDA with actively growing hyphae in a sterile petri dish containing sterile distilled water or by placing a 1-cm^2 piece of CLA from a 7-day-old culture on soil agar (4).

Mericlone regeneration. Storage roots were planted with the distal end in an autoclaved soil mix (1:1 [v/v], river silt:sand) in 15-cm-diameter clay pots and with the proximal half of the roots above the soil. Vines that grew from the roots were tied to stakes, and plants were carefully watered to avoid wetting the foliage. Shoot tips (3–5 cm long) were collected from these

vines and surface disinfested in 0.5% NaOCl for 10 min. After three rinses in sterile distilled water, meristem tips approximately 0.4–0.8 mm long, which included a pair of leaf primordia, were cut with flamed scalpels from up to the first four axillary buds and the apical meristem. Meristem tips were transferred aseptically to culture tubes (20 × 150 mm) containing 8 ml of shoot-initiation medium, which included the following per liter: 30 g of sucrose, 2 mg of thiamine-HCl, 100 mg of myo-inositol, 93 mg of Na_2EDTA, 69.5 mg of $FeSO_4 \cdot 7H_2O$, 0.2 mg of 6-benzyl-amino purine (BAP), 100 ml of L & S major salts (16.5 g of NH_4NO_3, 19.0 g of KNO_3, 1.7 g of KH_2PO_4, 4.4 g of $CaCl_2 \cdot 2H_2O$, and 3.7 g of $MgSO_4 \cdot 7H_2O$), 10 ml of L & S minor salts (620 mg of H_3BO_3, 1.68 g of $MnSO_4 \cdot H_2O$, 1.06 g of $ZnSO_4 \cdot 7H_2O$, 83 mg of KI, 25 mg of $Na_2MoO_4 \cdot 2H_2O$, 2.5 mg of $CuSO_4 \cdot 5H_2O$, and 2.5 mg of $CoCl_2 \cdot 6H_2O$), and 7 g of tissue culture agar; the pH was adjusted to 5.7 with 1 N KOH (6).

Meristem cultures were incubated under fluorescent lights at 27 ± 3 C until leaves appeared. Shoots were transferred to root-initiation medium that was the same as shoot-initiation medium, except that 1 mg/L of α-naphthalene acetic acid (NAA) was substituted for BAP. When shoots grew to the top of the tube, they were removed aseptically, cut into two-node pieces, and the pieces were planted on maintenance medium. This medium was the same as the above media, except that it did not contain NAA or BAP. Each mericlone (all plants clonally propagated from a single meristem) was maintained on maintenance medium in aseptic tissue culture. Each mericlone also was transplanted to a potting medium (1:1:1 [v/v/v]; river silt, sand, Jiffy Mix [Jiffy Products of America, Inc., West Chicago, IL]) in 10-cm-diameter clay pots. Plants were covered with a polyethylene bag to reduce transplant shock and placed inside an insect-proof cage (covered with 32 × 32 nylon mesh) in the greenhouse. Bags were removed after 1–2 wk. Mericlones maintained in the cage served as a nuclear stock of healthy mericlones for pathogenicity experiments.

Inoculations. Inoculum was prepared by washing the surface of a 5- to 7-day-old PDA petri dish culture (90 mm in diameter) with 100 ml of sterile distilled water. Vine cuttings (20–25 cm long) were cut from symptomless mericlones with a flamed scalpel, and all unfolded leaves were removed by breaking the petiole from the stem before inoculation and transplanting.

The first experiment was begun on 25 July 1989. Inoculum from four isolates of *F. lateritium* (F89-1 to 4) from chlorotic leaf distortion affected sweetpotato was combined in equal volumes and adjusted to 5×10^6 propagules per milliliter based on counts made with the aid of a hemacytometer. Both terminal and subterminal cuttings were used, and one cutting from each mericlone was inoculated by dipping the entire cutting in the inoculum. A control treatment consisted of one cutting from each mericlone dipped in thiabendazole (8.4 ml/L Folatec [42.28% a.i., The Nitragin Company, Inc., Milwaukee, WI]) to prevent contamination during transplanting. Cuttings were planted in an autoclaved soil mix in 10-cm-diameter clay pots, watered, and covered with a double layer of cheesecloth for 4–5 days. Inoculated and control plants were kept in separate blocks on the same greenhouse bench. A total of 133 different mericlones were used representing the following sweetpotato cultivars or breeding lines: 13 of Beauregard, 34 of Jewel, 14 of Porto Rico, 25 of Travis, 41 of L82-66, two of L82-527, three of L85-237, and one of NC-845. Symptoms were recorded on 17 and 28 August, and 4 September (23, 34, and 41 days postinoculation, respectively). After the 4 September observations, vines were cut back to 2–5 cm above the soil line with a flamed scalpel and axillary buds were left to proliferate. Symptoms were recorded on regrowth on 20 and 29 September (57 and 66 days postinoculation, respectively).

Two additional experiments were initiated on 18 and 28 August 1989. Eight isolates (F89-1 to -8) and 19 isolates (F89-9 to -10, -12 to -17, -19 to -24, -26, and -29-32) in the second and third experiments, respectively, of *F. lateritium* were inoculated separately as described previously on mericlones of cultivars Jewel and Beauregard. Inoculum concentration was not adjusted in these experiments. Two controls were included in each experiment: a nontreated control and a treatment in which cuttings were dipped in thiabendazole as above. Five cuttings for each treatment for each cultivar were transplanted into soil mix in a 15-cm-diameter pot. The pots were placed in a completely randomized design in a greenhouse under intermittent mist (8 sec every 10 min, 600–2,000 hours) for 1 wk and, subsequently, watered as needed without misting. Symptoms were recorded on 11 and 22 September (24 and 35 days postinoculation) and 22 September and 9 October (25 and 42 days postinoculation) for the second and third experiments, respectively.

RESULTS

Symptoms and signs. A white material, visible to the unaided eye, appeared on the surface of the youngest leaves on affected plants before the development of other symptoms. On leaves that had not yet unfolded, this material was most pronounced along the margin. On leaves that had recently unfolded, it appeared as a nearly continuous, waxlike covering of the adaxial surface (Fig. 1). On progressively older leaves that had expanded, the white material was distributed as small (<1 mm) scattered clumps that macroscopically resembled salt crystals and that often were more concentrated near the margin of the leaf (Fig. 1). Microscopic examination of this material revealed that it consisted of hyphae (2–5 μm in diameter) and macroconidia typical of *Fusarium*.

Immature leaves were slower to unfold on chlorotic leaf distortion-affected plants, and often the opposite margins did not separate as fast as on symptomless plants. During sunny weather, leaves that opened subsequent to the appearance of the white fungal growth were entirely chlorotic (Fig. 2). Affected leaves were pink on genotypes that normally have purple leaves. As individual chlorotic leaves expanded and matured during sunny weather, they gradually became green, with lobes turning green before the center of the leaf. These leaves often were stunted. Fully expanded leaves eventually appeared normal despite the continuing development of young chlorotic leaves. When sunny weather persisted, symptoms became progressively more severe and often involved development of a dry, brown necrosis at the margins or tips of chlorotic leaves. Distortion, including twisting, cupping, or failure of the leaf to expand at the tip, developed on some genotypes (Fig. 3).

Symptoms developed on plants in the field at all locations observed and in the greenhouse from approximately May through October. However, when cloudy weather occurred for more than 2–3 days, plants recovered and symptoms were no longer evident. When sunny weather returned, symptoms gradually reappeared on new leaves. Symptoms usually were not observed in the greenhouse from November through April, except occasionally during unusually long periods of sunny weather.

Chlorotic leaf distortion has not been observed on mericlones

Fig. 1. Cultivar Beauregard sweetpotato leaves with white superficial fungal growth on the adaxial surface of young leaves. The youngest expanded leaf has a waxlike continuous covering, whereas the next-oldest leaf has clumps of white fungal growth that resemble salt deposits.

maintained in the greenhouse insect cage. White fungal growth appeared on <1% of these mericlones, and these were discarded immediately.

Isolations and identifications. One type of fungal colony predominated on both PDA and Komada's medium in isolations from plants with chlorotic leaf distortion. The fungus was isolated from some part of all 40 symptomatic plants from commercial fields, from all 25 symptomless plants from the same fields, from all five mericlones that developed chlorotic leaf distortion after 3 mo in the field, and from all six symptomatic plants from the greenhouse. It was isolated from two of six symptomless plants from the greenhouse, but not from any of five symptomless mericlones from a field plot. The fungus was isolated from leaves that had not unfolded on all symptomatic plants. It was isolated less frequently from cross-sections of nodes 2–10 cm below the shoot apex. It was isolated from apical meristems of 38 of 40 symptomatic plants from commercial fields, but from only 14 of 25 symptomless plants from these fields.

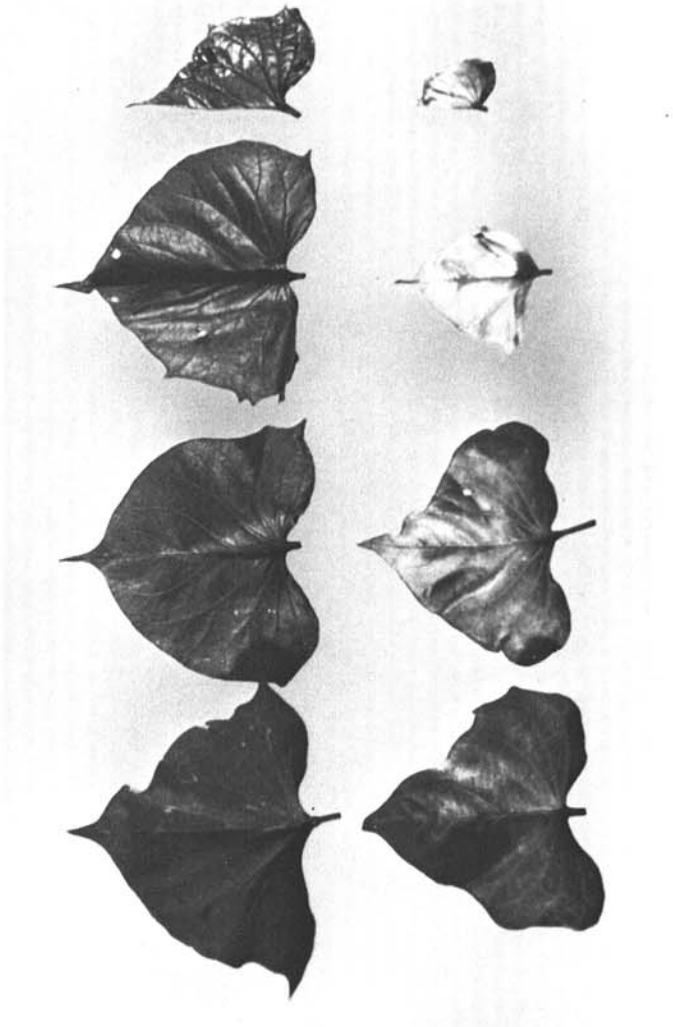

Fig. 2. Detached leaves showing the sequence in development of and recovery from chlorotic leaf distortion from apical (top) through sequentially older leaves. Column on left = leaves from symptomless plant, column on right = leaves from plant with chlorotic leaf distortion.

Fig. 3. Distortion (twisting) of cultivar Porto Rico sweetpotato leaves with chlorotic leaf distortion.

The same fungus also was isolated from seven of 51 true seed from the first lot of nonscarified seed. Many of the seed from which it was not isolated failed to swell or crack. It also was isolated from 15 of 44 nonscarified sinkers, 21 of 30 nonscarified floaters, seven of 44 scarified sinkers, and 25 of 30 scarified floaters from the second seed lot.

Colonies initially were white, and later the center became light pink to buff or salmon. Abundant aerial mycelia were produced, which gave the colonies a feltlike appearance. Similar colonies developed on meristem culture medium from meristems collected from plants with severe chlorotic leaf distortion symptoms. These meristems died before shoots developed.

Twenty-nine single-spore cultures of fungi isolated from chlorotic leaf distortion-affected plants were examined, and 28 were identified as *F. lateritium* (10). The remaining isolate, from a sweetpotato anther, was identified as *F. moniliforme* Sheldon. Chlorotic leaf distortion isolates of *F. lateritium* produced abundant one-celled (2.3-[2.5]-2.9 × 7.2-[7.9]-9.2 μm) microconidia, some one-septate (2.5-[3.0]-3.3 × 14.4-[20.6]-27.5 μm) conidia, and abundant, mostly three-septate (3.1-[3.3]-3.4 × 31.7-[38.6]-47.5 μm) macroconidia. Conidia were borne in 'false heads' on simple, unbranched phialides. Macroconidia had a distinctive nipple or beak on the apical cell. Chlamydospores were absent. A brown diffusible pigment was produced in older (>3 wk) cultures of some isolates. Seven isolates have been deposited at the Fusarium Research Center (Accession Nos. L-256 to L-262) and the American Type Culture Collection (ATCC Nos. 66434 to 66440).

Inoculations and reisolations. Chlorotic leaf distortion, white fungal growth, and dieback developed on mericlones of each sweetpotato genotype and results were combined. In the first experiment, chlorotic leaf distortion developed on 16 and 18 of 133 inoculated mericlones and 1 and 1 of 133 control mericlones by 4 September (41 days postinoculation, first growth) and 29 September (67 days postinoculation, regrowth), respectively. White fungal growth without chlorosis was observed on an additional 53 and 91 of 133 inoculated and zero and five of 133 control mericlones by 4 and 29 September, respectively. In each case, Chi-square analysis indicated that the incidence of chlorotic leaf distortion or white fungal growth was greater ($P = 0.005$) on the inoculated than on the uninoculated mericlones. Nine inoculated mericlones and one control mericlone died back to the soil line within 1 wk after inoculation.

Reisolations attempted from cross-sections of nodes approximately 20–25 cm below the apex of the vines (the terminal cutting was used for propagation) on 4 September yielded *F. lateritium* from four of 10 inoculated and zero of 10 control mericlones. Reisolation attempts from shoot tips (1 cm long) on 12 October yielded *F. lateritium* from 20 of 20 inoculated and one of 17 control mericlones.

In the second and third experiments, only one isolate, F89-9, failed to induce either chlorotic leaf distortion or development of white fungal growth. Overall, chlorotic leaf distortion developed on 26 plants, white fungal growth on an additional 73, and another 47 of the 216 inoculated plants died back. One of the thiabendazole-treated mericlones died back, but no other symptoms were observed on any of the 16 nontreated or 16 thiabendazole-treated controls. Incidence of chlorotic leaf distortion and white fungal growth was greater on inoculated than on uninoculated plants (Chi-square analysis, $P = 0.1$ and 0.005, respectively). *F. lateritium* was reisolated from nine of nine

inoculated mericlones, but was not isolated from any of five nontreated controls or five thiabendazole-treated controls.

DISCUSSION

Koch's postulates have been fulfilled to demonstrate that sweetpotato chlorotic leaf distortion is caused by *F. lateritium*. The fungus produces an unusually prolific superficial growth on affected parts of the shoot tip. The relatively low incidence of chlorotic leaf distortion that developed in inoculated mericlones may be attributed to a long and variable latent period (3–6 wk) between inoculation and first development of white fungal growth and an even longer and more variable period until chlorosis appeared. Symptoms might have developed on additional mericlones following a longer incubation period. It also is likely that the high frequency of isolation of *F. lateritium* from symptomless plants collected from commercial fields is attributable to this long latent period. In fact, virtually all plants in these fields developed symptoms of chlorotic leaf distortion 1–2 wk after the samples were collected.

Although *F. lateritium* was isolated from surface-disinfested tissues and chlorotic leaf distortion appears to develop systemically, we prefer to refer to plants as chronically affected rather than systemically infected. Research is in progress to determine if the fungus actually enters the shoot tip or whether it is entirely superficial. The latter possibility is suggested by the fact that host tissue showing marked chlorosis recovered even during apparently conducive (sunny) weather, and whole plants recovered when weather was not conducive (cloudy).

Research is needed on several other aspects of chlorotic leaf distortion, especially to determine disease effects on growth, yield, and quality of the crop. We also need information on how and when the pathogen is spread during sweetpotato production; the host range of the pathogen; and how to control the disease. Since chlorotic leaf distortion has become widespread in commercial sweetpotato production and is apparently chronically associated with its vegetatively propagated host, it may be necessary to use methods of producing pathogen-tested propagating material to control the disease, as has been suggested for virus control in sweetpotatoes (8). Differences have been observed in the duration and severity of chlorotic leaf distortion on different sweetpotato genotypes; thus, resistance may be available (Clark, *unpublished data*; P. D. Dukes, *personal communication*).

Plant diseases caused by species of *Fusarium* generally are categorized as either vascular wilts or cortical rots (9). However, chlorotic leaf distortion clearly represents another type of disease syndrome induced by *Fusarium*. The origin of chlorotic leaf distortion is not clear, but the fact that *F. lateritium* was isolated from true seed warrants special attention, because true seed is preferred for international exchange of sweetpotato germ plasm (7).

LITERATURE CITED

1. Clark, C. A. 1988. New disease record. Int. Work. Group Sweet Potato Viruses, Newsl. 1:3-4.
2. Clark, C. A., and Moyer, J. W. 1988. Compendium of Sweet Potato Diseases. APS Press, St. Paul, MN. 74 pp.
3. Jones, A., Dukes, P. D., Schalk, J. M., Hamilton, M. G., and Baumgardner, R. A. 1987. 'Southern Delite' sweet potato. HortScience 22:329-330.
4. Klotz, L. V., Nelson, P. E., and Toussoun, T. A. 1988. A medium for enhancement of chlamydospore formation in *Fusarium* species. Mycologia 80:108-109.
5. Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Prot. Res. 8:114-125.
6. Love, S. L., Rhodes, B. B., and Moyer, J. W. 1987. Meristem-tip culture and virus indexing of sweet potatoes. Practical Manual for Handling Crop Germplasm In Vitro 1. Int. Board Plant Genetic Resources, Rome. 46 pp.
7. Moyer, J. W., Jackson, G. V. H., and Frison, E. A., eds. 1989. FAO/IBPGR Technical Guidelines for the Safe Movement of Sweet Potato Germplasm. FAO, Rome/Int. Board Plant Genetic Resources, Rome. 29 pp.
8. Moyer, J. W., and Salazar, L. F. 1989. Viruses and viruslike diseases of sweet potato. Plant Dis. 73:451-455.
9. Nelson, P. E., Toussoun, T. A., and Cook, R. J., eds. 1981. Fusarium: Diseases, Biology, and Taxonomy. The Pennsylvania State University Press, University Park. 457 pp.
10. Nelson, P. E., Toussoun, T. A., and Marasas, W. F. O. 1983. *Fusarium* Species: An Illustrated Manual for Identification. The Pennsylvania State University Press, University Park. 193 pp.
11. Windels, C. E., Burnes, P. M., and Kommedahl, T. 1988. Five-year preservation of *Fusarium* species on silica gel and soil. Phytopathology 78:107-109.