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Morphological and molecular characterization of *Fusarium verticillioides* from rotten banana imported into Japan

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Seven strains of *Fusarium* were isolated from rotten fruit of banana (*Musa cavendishii*) imported into Japan from Mazanillo, Colima, Mexico. Morphological features of the isolates were described and illustrated, and their pathogenicity to banana was determined. Morphological and molecular phylogenetic analyses revealed that these isolates were assignable to *F. verticillioides*. However, constant production of septate aerial conidia in chains by the banana isolates distinguished these strains from previous descriptions of this species. Morphological examination of the isolates revealed that they are consistent with Wollenweber's original concept of *F. moniliforme* var. *minus*. The effect of black-light illumination on conidial production by these isolates was also tested. Black light stimulated production of sporodochial conidia of all banana isolates and had a positive effect on conidial length.

Key Words—*Fusarium moniliforme* var. *minus*; *Fusarium verticillioides*; imported Mexican banana; light reaction; pathogenicity.

Species concepts within the *Gibberella fujikuroi* (Sawada) Wollenw. species complex (hereafter referred to as the Gf complex), or sections *Liseola* and *Dlaminia*, have long been debated among *Fusarium* taxonomists. These concepts differ widely, because of the close morphological similarities among the species and the different characters emphasized for species delimitation. Wollenweber and Reinking (1935), Snyder and Hansen (1945), Booth (1971), Nirenberg (1976), Gerlach and Nirenberg (1982), Nelson et al. (1983) and Burgess et al. (1988) accepted 6, 1, 2, 10, 10, 4 and 4 species and varieties, respectively, within section *Liseola*. This section was erected to accommodate species that form whitish to purplish colonies on PDA, aerial and sporodochial conidia but lack chlamydospores (Wollenweber et al., 1925; Wollenweber and Reinking, 1935). Separately, eight biological species referred to as mating populations A to H have been described within the Gf complex (Hsieh et al. 1977; Kuhlman, 1982; Leslie, 1991; Klittich and Leslie, 1992; Klittich et al., 1997; Britz et al., 1999). Recently, O'Donnell et al. (1998), Nirenberg and O'Donnell (1998), Nirenberg et al. (1998) and O'Donnell et al. (2000) conducted molecular phylogenetic and morphological analyses of the Gf complex, and concluded that it was composed of at least 44 phylogenetically distinct species. Following Nirenberg (1976), these authors concluded that *F. moniliforme* Sheldon should be limited to *G. moniliformis* Wineland (i.e., mating population A) and that the epithet should also be abandoned because it is a later synonym of *F. verticillioides* (Sacc.) Nirenberg.

In Japan, *Fusarium* strains which cause *bakanae* disease of *Oryza sativa* L. and other diseases of various crops have been isolated and reported as *F. moniliforme* (Kishi, 1998). However, the *bakanae* disease is caused by *F. fujikuroi* Nirenberg (telomorph: *G. fujikuroi* (Sawada) Wollenw. = mating population C; Nirenberg, 1976; Kuhlman, 1982; Gerlach and Nirenberg, 1982; Domsch et al., 1993; Nirenberg and O'Donnell, 1998). *Fusarium proliferatum* (Matsushima) Nirenberg ex Gerlach and Nirenberg (mating population D) and *F. globosum* Rheeder et al. have also been reported from Japan (Nirenberg and O'Donnell, 1998; O'Donnell et al., 1998; Aoki and Nirenberg, 1999). *Fusarium verticillioides* (syn.: *F. moniliforme* s. str.) based on its most recent circumscription has not been reported from Japan. During a plant quarantine inspection of banana (*Musa cavendishii* Lamb. ex Paxt.=*M. acuminata* Colla) fruit imported from Mexico, *F. verticillioides* strains were isolated from rotten fruit. In a previous report, Hirata and Kimishima (1997) identified the fungus as *F. moniliforme* following Nelson et al. (1983). In the present study, morphological, molecular phylogenetic and pathogenicity data obtained

from analyses of these strains are presented. The species concept of *F. verticillioides* is also discussed.

Materials and Methods

Fungal isolation A total of 1,180 cartons (ca. 18 t) of green banana fruit (*M. cavendishii*), imported from Mexico to Japan in Nov. 1996 were inspected at the Kobe Plant Protection Station. In more than half of the fruits sampled (58.7%, w/w), many dark brownish spots were observed on the epidermis. Whitish mycelia were also frequently observed on the larger spots. Seven single-conidial isolates were obtained from aerial conidia from different spots on the fruit and were designated as MB (Mexican banana)-1 to 7.

Inoculation tests of the isolates to banana fruit and other plants Based on Koch's postulates, inoculation tests were carried out in duplicate to test the pathogenicity of the MB isolates to green banana fruit, corn and soybean plants. Healthy green banana fruit was obtained for the inoculation tests. Seven isolates (MB-1 to 7) from the rotten fruit cultured on potato dextrose agar (PDA, Difco Lab., Detroit, MI) at 25°C for 5 d were inoculated onto the healthy fruit. In addition, the surface of the fruit was first wounded with a bundle of 20 sewing needles (ca. 2 mm in total diam). Mycelial mats on PDA (ca. 5 × 5 mm) were placed onto wounded and non-wounded parts. As a negative control, uninoculated PDA blocks were also placed on the wounded and non-wounded fruit. The inoculated fruit was placed into plastic boxes and incubated at 25°C for 2 wk. Healthy young corn (*Zea mays* L.) and soybean (*Glycine max* Merrill) plants at the 10- to 20-leaf stage, growing in 9-cm diam pots, were inoculated with MB-1. Two methods of inoculation were used. In the first method, MB-1 was cultured on synthetic low nutrient agar (SNA) (Nirenberg, 1976) at 25°C for 2 wk to induce abundant conidia. Conidia were harvested and suspended in sterile distilled water (ca. 4×10^5 conidia/mL). Thirty ml of the suspension was applied onto the soil surface of each pot containing one 2-wk-old test plant. Inoculated plants were maintained in a greenhouse for 1 mo. In the second method, the stems and leaves of corn and the leaves and pods of soybean were wounded and mycelial mats of MB-1 were placed onto the wounded and non-wounded parts, as described above. Uninoculated PDA blocks were also placed onto these plants as negative controls. Inoculated plants were covered with polyethylene bags and incubated at ca. 25°C for 2 d, then the bags were removed and the plants were maintained in a greenhouse for 2 wk.

Examination of taxonomic characters All MB isolates were cultured at 20°C on PDA in plastic Petri dishes in total darkness for examination of color, odor and growth rates. Color codes cited are from the Methuen Handbook of Colour (Kornerup and Wanscher, 1978). For microscopic examination, the isolates were cultured on SNA with a ca. 1 × 2 cm strip of sterile filter paper on the agar surface (Nirenberg, 1976). Cultures were incubated for 10–14 d at 20°C either in complete darkness or under continuous black light (National FL 20S·BL-B 20W, peak wavelength: 352 nm; with the light source at a distance of 20 cm from the Petri dishes). Cultures were examined microscopically under low magnification (× 100-200) to study morphological features of the aerial mycelia. When sporulation was observed in the cultures, agar blocks containing conidial structures were mounted on a microscopic slide with a drop of sterile water and examined at × 400 or × 800. Diagnostic morphological features were recorded photographically and detailed measurements were taken. Measurement of 0-septate aerial conidia is based on 100 conidia selected randomly from individual isolates incubated under each set of culture conditions. At least 30 1- and 2-septate aerial conidia, aerial phialides and 3-septate sporodochial conidia were selected randomly for the measurements. Minimal and maximal sizes, arithmetic means and standard deviations (S.D.) were obtained for each character from each of the seven MB isolates. The MB isolates were deposited in BBA (Institut für Pflanzenvirologie, Mikrobiologie und biologische Sicherheit, Biologische Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem, Germany), NRRL (National Center for Agricultural Utilization Research, Agricultural Research Service, United States Department of Agriculture, Peoria, Illinois, USA), and MAFF (Genetic Resources Center, National Institute of Agrobiological Resources, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Ibaraki, Japan). For morphological comparison, two reference strains of *G. moniliformis* (i.e., mating population A) were obtained from the American Type Culture Collection (Rockville, Maryland, USA), ATCC 38932 (A+; ex corn, USA) and ATCC 38933 (A–; ex sorghum, USA) (Kuhlman, 1982) and two strains of *F. verticillioides* preserved at BBA, BBA 62264 (=NRRL 22172; ex corn, Germany) and BBA 65898 (ex corn, USA) (Nirenberg and O'Donnell, 1998; O'Donnell et al., 1998) were also examined.

Examination of optimal growth temperature of the banana isolates Growth rates of the strains were exa-

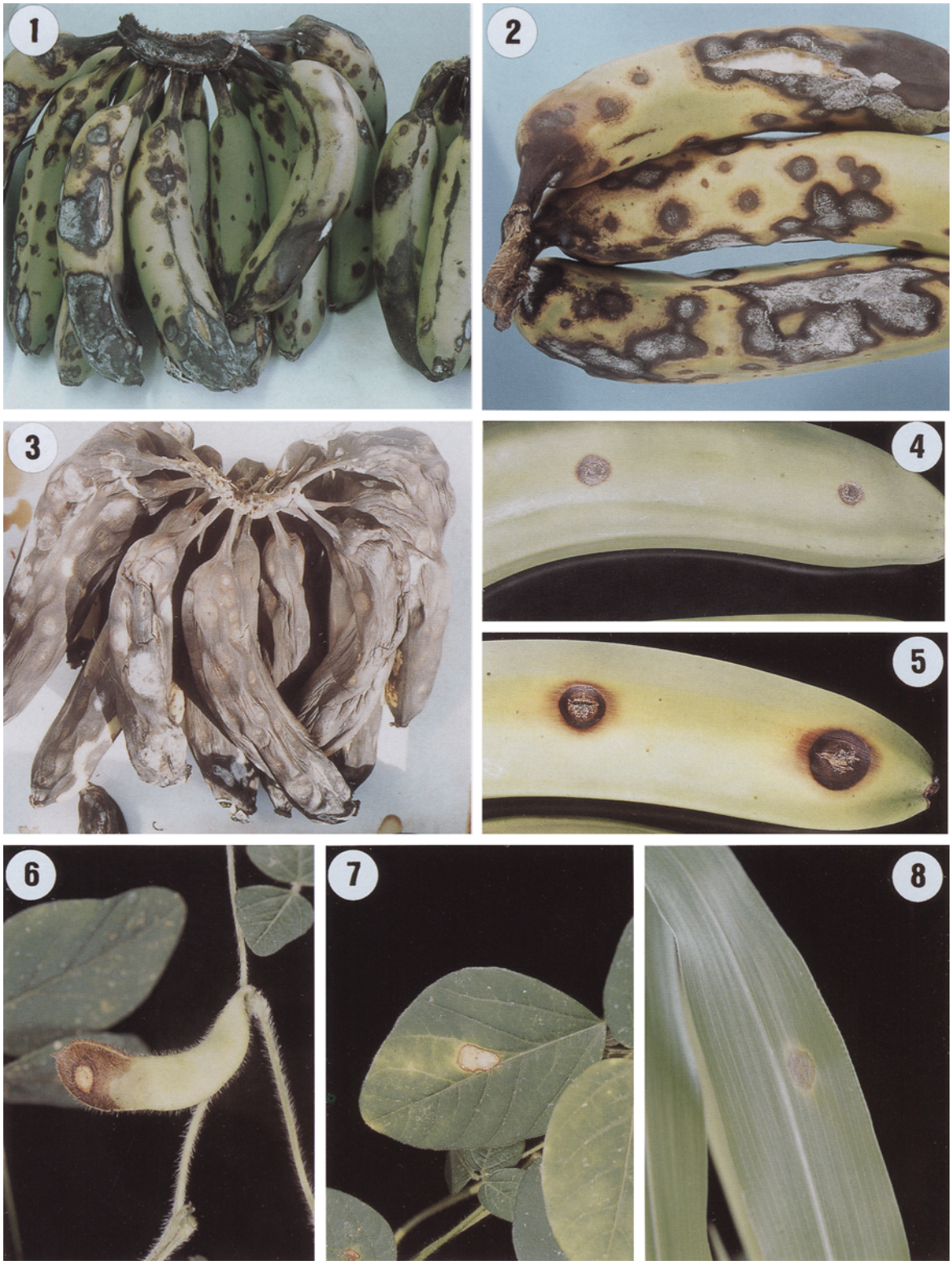
Figs. 1–5. Symptoms of *Fusarium* fruit rot of banana. 1, 2. Symptoms on rotten green banana imported into Japan from Mexico. Cut surfaces of crowns and pedicels blackened and often covered with whitish mycelia (Fig. 1). 3. Seriously damaged, blackened and shrunken Mexican banana fruit. 4, 5. Brownish spots formed 1 wk (Fig. 4) and 2 wk (Fig. 5) after inoculation of strain MB-1 on wounded fruit of green banana. Whitish hyphae and conidia of *Fusarium verticillioides* were observed on the surface of infected fruit.

Fig. 6. Light brownish to yellowish spot surrounded by a brownish discoloration on a soybean pod surface 2 wk after wound inoculation with *F. verticillioides* (MB-1).

Fig. 7. Light brownish to yellowish spot with a brownish margin developed on a soybean leaf surface 2 wk after wound inoculation with *F. verticillioides* (MB-1).

Fig. 8. Brownish spot with a yellowish margin developed on a corn leaf surface within 3 d after wound inoculation with *F. verticillioides* (MB-1).

mined at eight different temperatures from 5 to 40°C in intervals of 5°C. Mycelial mats of 3 × 3 mm were cut from the margins of actively growing colonies on SNA. They were transferred to the center of 9-cm PDA plates and incubated at the different temperatures in total darkness. After 1 and 4 d, the colony margin of each strain



Figs. 1–8

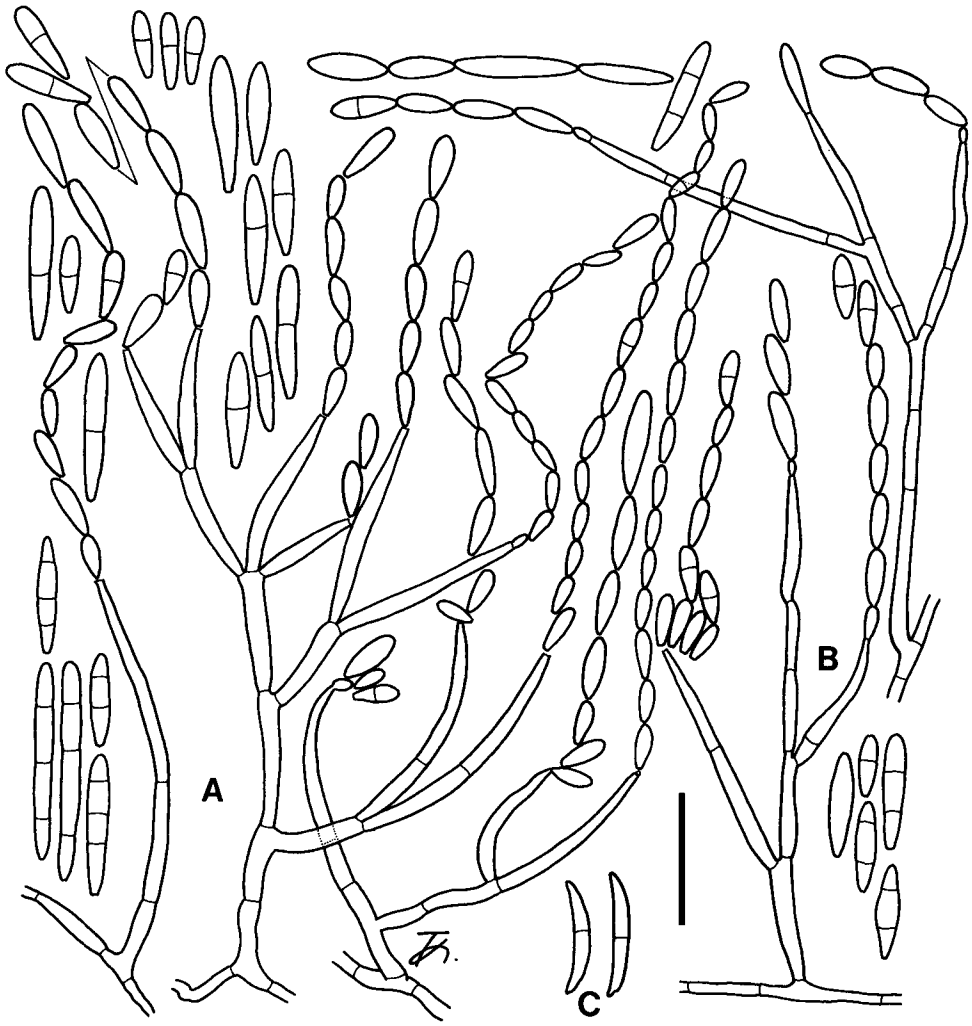


Fig. 9. Microscopic morphology of banana strains of *Fusarium verticillioides* cultured on SNA at 20°C in complete darkness. A, B. Branched and unbranched aerial conidiophores bearing exclusively monophialides. Clavate aerial conidia were formed from apical tips of monophialides in chains or in false heads, some of which were 1-3-septate. C. No sporodochia were formed, but a few 1-septate conidia with a distinct basal foot were observed in a few isolates. A, C from MB-4 (=MAFF 237856), and B from MB-5 (=MAFF 237857). Scale bar: 25 μm.

was marked at 16 different places with permanent ink on the reverse side of the Petri dishes under a dissecting microscope. These values were used to calculate the mean growth per d and to estimate the cardinal growth temperatures for the strains.

Molecular methods DNA extraction, polymerase chain reaction amplification and sequencing of portions of the mitochondrial small subunit (mtSSU) rDNA, and translation elongation factor (EF-1) introns and exons are described in O'Donnell et al. (2000). Sequencing reactions were purified by gel filtration through Sephadex G50 (Pharmacia, Piscataway, NJ) columns and were run on an Applied Biosystems 377 DNA sequencer (Foster City, CA). Sequences were aligned with Sequencher 4.0.5 (Gene Codes, Ann Arbor, MI) and have been deposited in the GenBank database under the following accession numbers: AF273305-AF273318. Individual and combined data sets were analyzed by maximum parsimony using PAUP* (Swofford, 1998). Clade stability was assessed by 1,000 parsimony bootstrap replications implemented in PAUP*. For comparison, *F. verticillioides* NRRL 22172 (=BBA 62264) was used as a reference strain of the species.

Results

Symptoms Dark brownish spots of various sizes were observed on the epidermis of the imported Mexican banana fruit (Figs. 1, 2). Occasionally they coalesced yielding much larger lesions. The spots were brown to dark brown with lacunae at the center and typically surrounded by light brown halos. Larger spots occasionally had cracks inside which exposed the pulp. Whitish fungal colonies were also frequently observed on these spots. In the early stages of decay, virtually no damage was observed on the fruit pulp as viewed in transverse

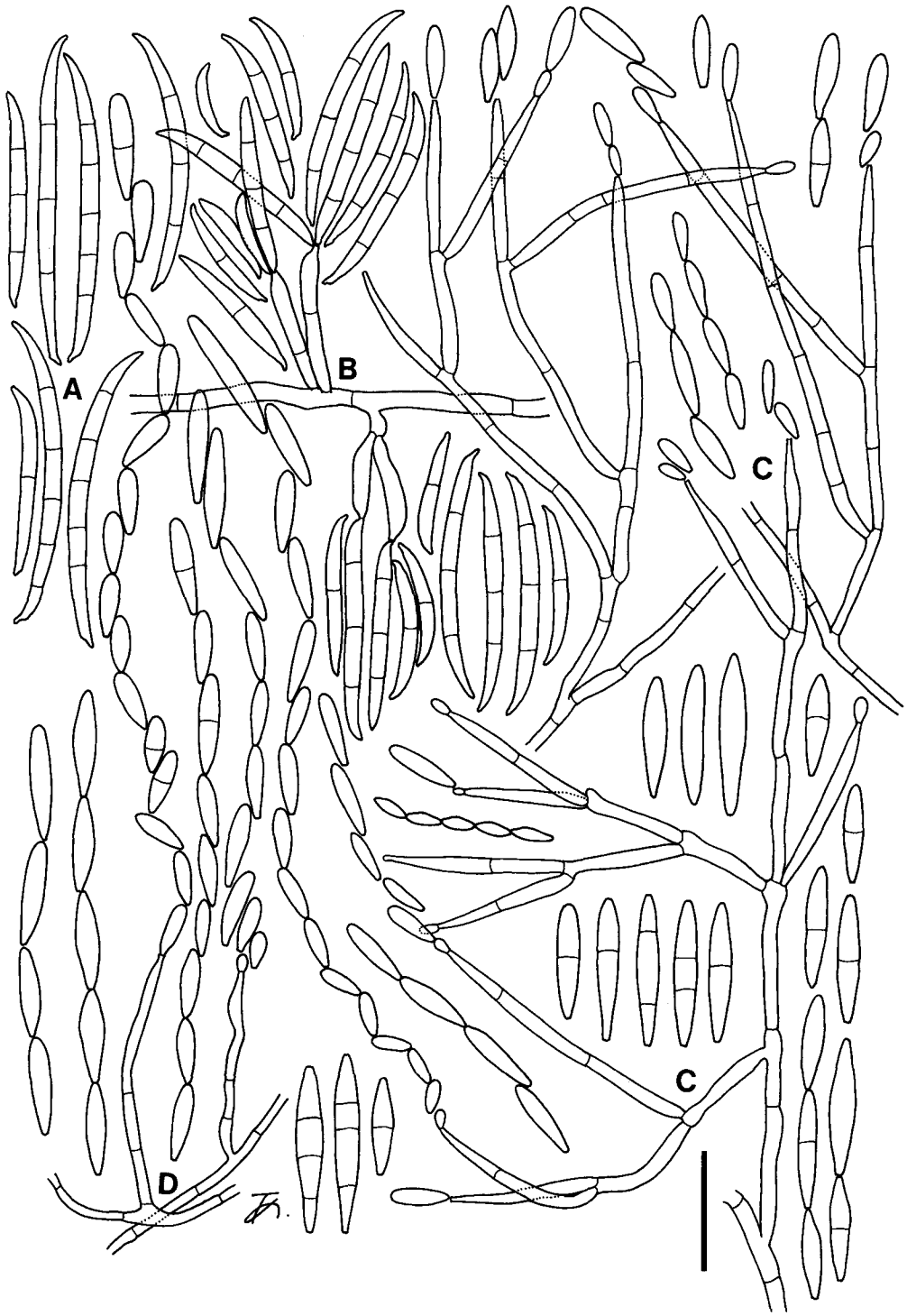
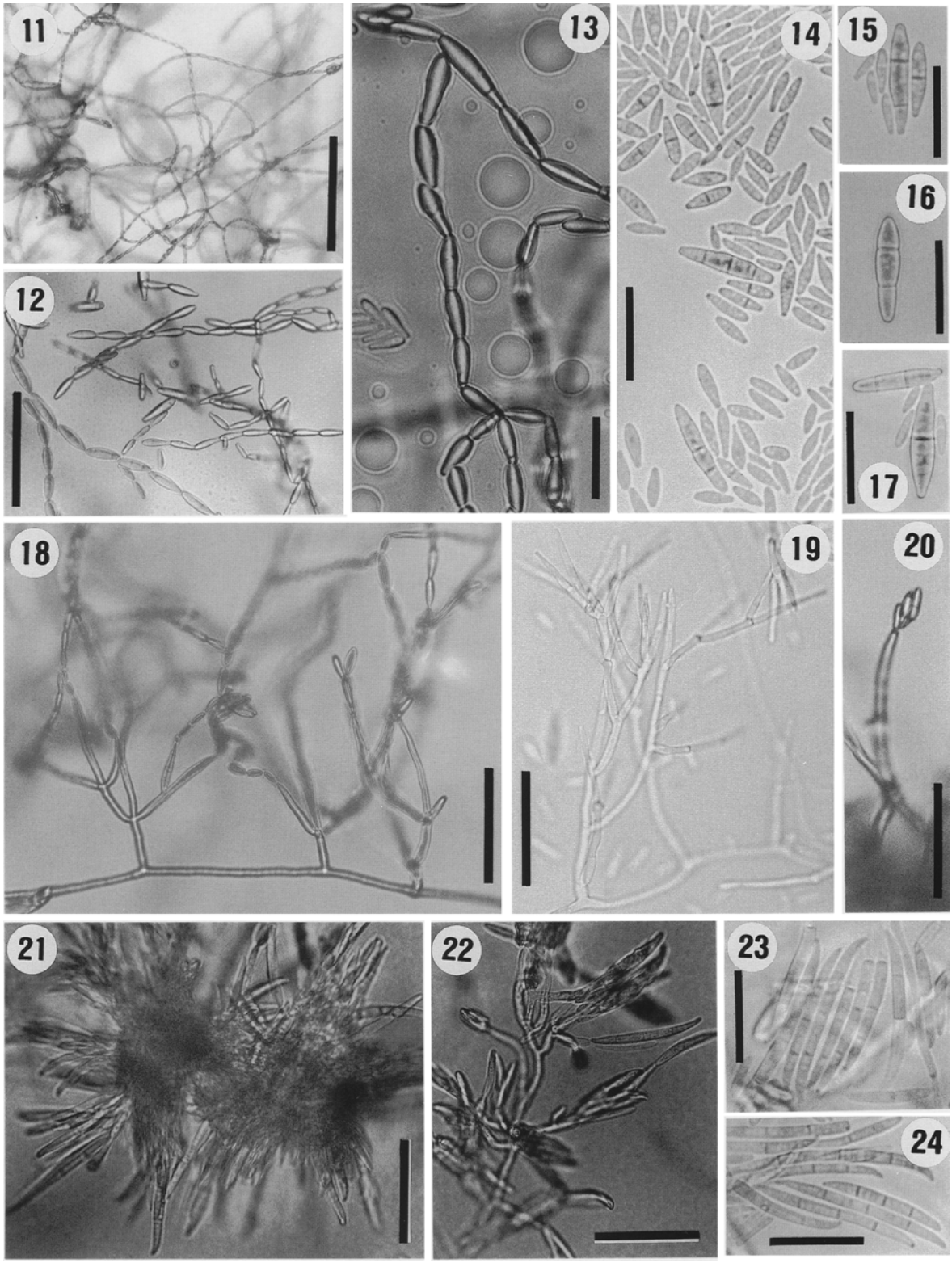


Fig. 10. Microscopic morphology of a banana strain of *Fusarium verticillioides* cultured on SNA at 20°C under continuous black light. A, B. 1- to 5-septate sporodochial conidia formed from phialides on the agar surface. C, D. Branched and unbranched aerial conidiophores bearing strictly monophialides from which clavate aerial conidia were formed in chains. A-D from MB-4 (=MAFF 237856). Scale bar: 25 μm.

section. Cut surfaces of crowns and pedicels sometimes were also blackened and often covered with whitish mycelia. As the disease progressed, the spots became covered by whitish fungal colonies that nearly covered the entire fruit (Fig. 3). At this stage, the fruit pulp also showed extensive decay.

Pathogenicity of *Fusarium verticillioides* strains to banana and other plants All MB isolates induced light to

dark brown spots on wounded and non-wounded parts of inoculated healthy green banana fruit. Spots on wounded parts, however, were larger than those on non-wounded parts. Some larger brown spots, especially on the wounded parts, had whitish mycelial colonies and were often surrounded by halos (Fig. 4). These spots



Figs. 11–24

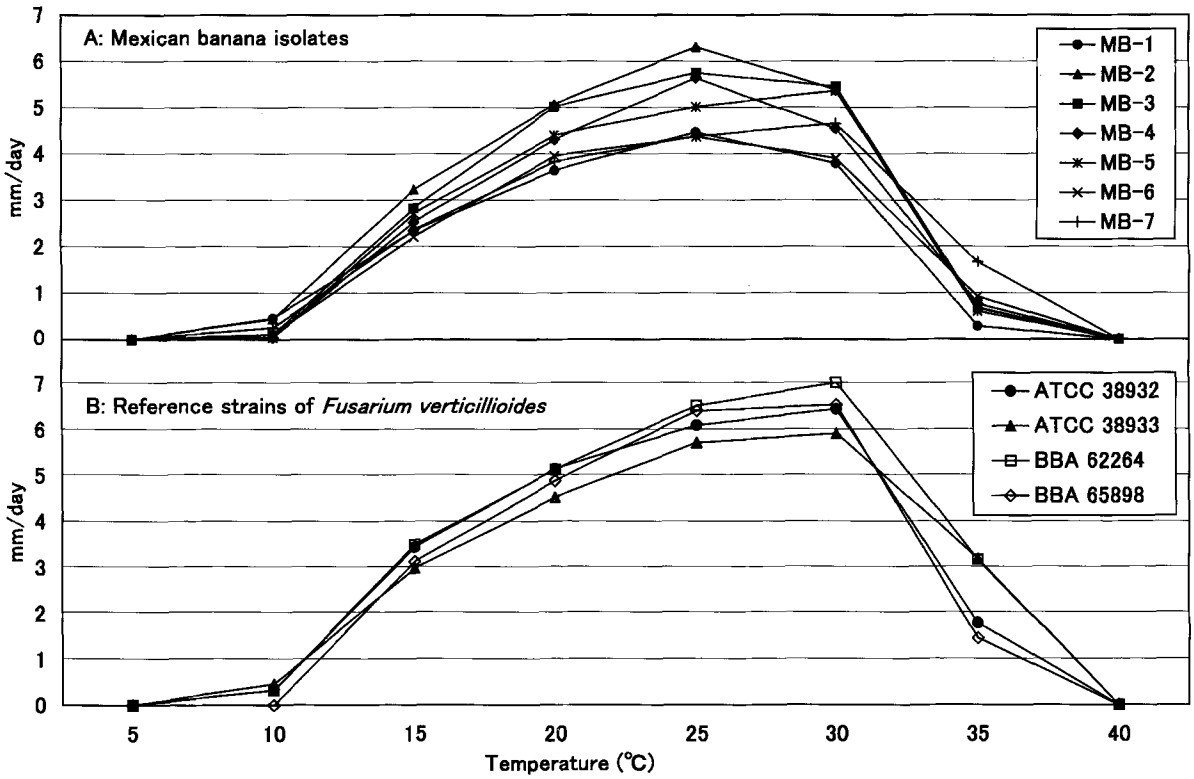


Fig. 25. Mean mycelial growth rates of strains grown on PDA in total darkness at eight different temperatures (°C). A. Seven isolates from rotten Mexican banana (MB-1 to 7). B. Four reference strains of *Fusarium verticillioides* preserved at ATCC and BBA.

extended over the fruit epidermis after 2 wk (Fig. 5), yielding symptoms similar to those of the diseased banana imported from Mexico. The inoculated fungus was re-isolated from diseased banana.

In contrast, no symptoms were observed on the corn and soybean plants in which the potting soil had been inoculated with a conidial suspension of MB-1. Although yellowish or light brownish spots occasionally developed on the wounded pods and leaves of soybean (Figs. 6, 7) and on the wounded leaves of corn (Fig. 8), no symptoms developed on the non-wounded inoculated parts or on the uninoculated negative control.

Fungal description Based on morphological examination of the isolates and comparison with published descriptions, the ATCC strains of *G. moniliformis* (referred to as mating population A of the Gf complex; anamorph=*F. verticillioides*) and the BBA strains of *F. verticillioides*, the banana isolates were identified as *F. verticillioides*. A description of these isolates follows.

Fusarium verticillioides (Sacc.) Nirenberg, Mitt. Biol. Bundesanst. Land- Forstwirtsch. Berlin-Dahlem **169**: 26. 1976/ Gerlach and Nirenberg, Mitt. Biol. Bundesanst. Land-Forstwirtsch. Berlin-Dahlem **209**: 301–304. 1982.

≡*Oospora verticillioides* Sacc., Fung. Ital. Fig. 789. 1881; Michelia **2**: 546. 1882.

=*Fusarium moniliforme* Sheldon s. str., Rep. Nebraska Agric. Exp. Stn. **17**: 23-32. 1904.

=*Fusarium moniliforme* Sheldon sensu Wollenweber pr. p., *Fusarium*-Monographie. Fungi parasitici et saprophytici. Z. Parasitenk. **3**: 391–393. 1931.

=*Fusarium moniliforme* var. *minus* Wollenweber s. str., *Fusarium*-Monographie. Fungi parasitici et saprophytici. Z. Parasitenk. **3**: 397. 1931. / Wollenweber et Reinking, Die Fusarien, p. 102, 1935. (pro parte)/ Wollenweber, Fusaria autographice delineata. Berlin. Nr. 969. 1930.

Colonies on PDA showing mycelial growth rate of

Figs. 11–24. Microscopic morphology of the banana strains of *Fusarium verticillioides* cultured on SNA at 20°C. 11, 12. Long chains of aerial conidia formed on colony surface. 13. Larger conidia formed successively in chains. 14. 1- to 3-septate aerial conidia within a mass of aseptate conidia. 15-17. 1- and 2-septate aerial conidia. 18. Verticillately branched conidiophores produced on aerial mycelium. 19. Densely branched aerial conidiophores, bearing only monophialides. 20. Aerial conidia formed from a monophialide in a false head. 21. Sporodochium and sporodochial conidia produced in the agar. 22. Sparsely branched sporodochial conidiophores and conidia. 23, 24. Sporodochial conidia formed on the agar surface. Figs. 11, 12, 15, 16, 18, 23 from MB-4 (=MAFF 237856), Figs. 13, 21, 22 from MB-1 (=MAFF 237853), Figs. 14, 20 from MB-7 (=MAFF 237859), Fig. 17 from MB-5 (=MAFF 237857), and Fig. 19 from MB-3 (=MAFF 237855). Figs. 11-14, 20 from cultures in complete darkness and Figs. 15–19, 21–24 under continuous black light. Scale bars: 100 μm in Fig. 11, 50 μm in Figs. 12, 18–22, and 20 μm in Figs. 13–17, 23, 24.

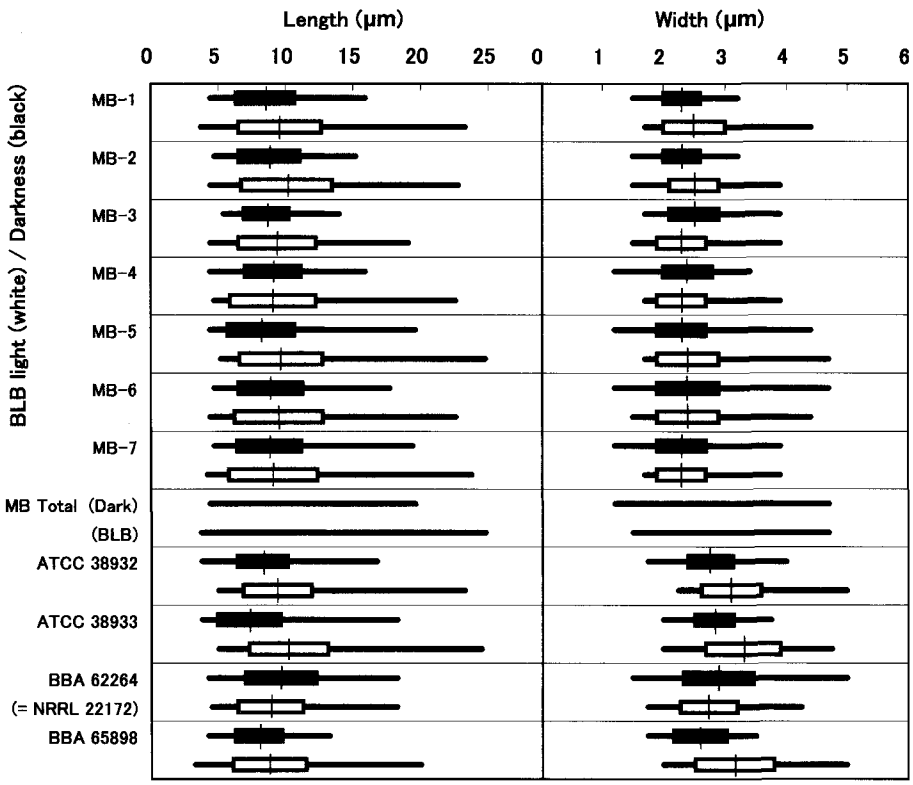


Fig. 26. Comparison of length and width of 0-septate conidia of seven Mexican banana isolates (MB-1 to 7) and four reference strains of *Fusarium verticillioides* cultured on SNA with filter paper at 20°C. Ranges (bars) and means±S.D. (rectangles) for individual strains are presented. Black rectangle=in complete darkness, white rectangle=under continuous black light. Total ranges for all MB strains are shown separately.

3.6–5.1 mm per d at 20°C. Colony margin entire. Aerial mycelium white (1A1) to purplish white (14A2) to lilac-grey (15B2), loosely to densely floccose, sometimes funiculose. Pigmentation in the reverse white (1A1), purplish grey (14D2), lilac-grey (15B2), violet-grey (17D2), lilac (15B4), dull lilac (15C3), dull violet (17D3), greyish violet (17D5), greyish magenta (13D5), dark magenta (13F8) to dark purple (14F8) to sometimes reddish grey (10B2) to birch-grey (5C2). Sclerotial bodies absent. Odor absent or, if present, sweet. Sporulation on SNA starting early in the aerial mycelium. Sporodochia not observed in complete darkness but formed under continuous black light on or in the agar, branched sparsely to densely. Aerial conidiophores at first unbranched, later branched sparsely, often alternately or oppositely, terminating with up to 3 verticillate phialides. Some sympodially proliferating conidiophores were also observed. Conidiogenous cells on the aerial conidiophores strictly monophialidic; phialides almost cylindrical, up to 45.9 μm long and 1.7–3.9 μm wide. Conidia borne in the aerial mycelium arranged mostly in long linear chains and sometimes in false heads, 0–1(-3)-septate, mostly clavate, sometimes fusiform when septate, 0-septate: measuring 4.4–19.6×1.2–4.7 μm, 8.5–9.1×2.3–2.5 μm on average in complete darkness, 3.7–24.8×1.5–4.7 μm, 9.1–10.1×2.3–2.5 μm on average under black light; 1-septate: 5.2–22.1×2.0–4.9 μm, 9.7–13.8×2.4–3.3 μm on average in complete darkness, 9.3–24.5×2.5–4.9 μm, 13.9–16.4×3.0–3.5 μm on average under black light. Sporodochial conidia rarely formed in complete darkness, but formed frequently under black light, (1–)3(–7)-septate, long, fusiform to falcate, slightly curved with an acuate apical cell and a foot-like basal cell; 3-septate: 24.0–54.0×2.2-4.7 μm, 32.7–35.5×2.2–2.5 μm on average under black light. Chlamydospores absent.

Strains examined: MB-1 (deposited as MAFF 237853 =NRRL 28893), MB-2 (as MAFF 237854=NRRL 28894), MB-3 (as MAFF 237855=NRRL 28895), MB-4 (as MAFF 237856=NRRL 28896), MB-5 (as MAFF 237857=BBA 70914=NRRL 28897), MB-6 (as MAFF 237858=NRRL 28898), MB-7 (as MAFF 237859=BBA 70915=NRRL 28899), isolated from rotten fruit of *Musa cavendishii* Lamb. ex Paxt. imported from Mazanillo, Colima, Mexico, A. Ishikawa, Nov. 1996.

Optimal growth temperatures of the banana isolates

Mean mycelial growth rates of the strains examined at eight different temperatures ranging from 5 to 40°C are shown in Fig. 25. Graphs for the data of the MB isolates and those of the four reference strains from ATCC and BBA are presented separately (Fig. 25 A, B). Growth rates of the MB isolates increased with temperature from 10 to 25°C. At 30°C, two of the banana isolates, MB-5 and MB-7, still showed an increase in their growth rates, but those of the other strains decreased to nearly those observed at 20°C. Growth rates of all MB isolates

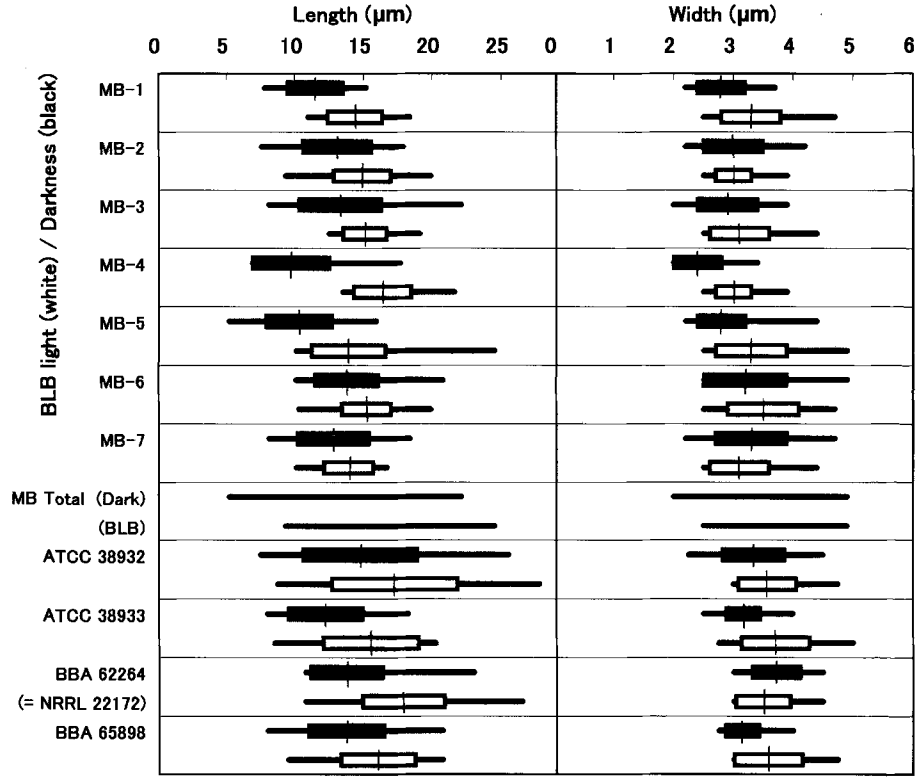


Fig. 27. Comparison of length and width of 1-septate conidia of seven Mexican banana isolates (MB-1 to 7) and four reference strains of *Fusarium verticillioides* cultured on SNA at 20°C. Ranges (bars) and means±S.D. (rectangles) for individual strains are presented. Black rectangle=in complete darkness, white rectangle=under continuous black light. Total ranges for all MB strains are shown separately.

dropped significantly at 35°C. The four reference strains of *F. verticillioides* showed a maximal growth rate at 30°C. No growth was observed at 5 and 40°C for any of the strains examined. In general, isolates from the rotten banana fruit seemed to exhibit a slightly lower optimal growth temperature than the four reference strains of *F. verticillioides*.

Effect of black light illumination on the size of aerial conidia of the banana isolates A distinctive morphological feature of the MB isolates was the production of sporodochia and typical sporodochial conidia only under black-light illumination (Figs. 10A, B, 21–24). No sporodochia were formed in complete darkness. In a few MB isolates, 1-septate conidia with a distinct basal foot were observed occasionally (Fig. 9C), but no typical multiseptate sporodochial conidia were found in the culture plates. Under continuous black light, sporodochial conidia were constantly formed by the banana isolates and by ATCC 38932, BBA 62264 and BBA 65898. For ATCC 38933 cultured under the same condition, however, only a single falcate conidium with a distinct foot-shaped basal cell was found but no sporodochia were observed. Sporodochial conidia were also formed by ATCC 38932 in complete darkness.

In addition to the production of typical sporodochial conidia only under black light, black-light illumination also affected the size of aerial conidia of the MB isolates. Comparisons were made of the length and width of 0- and 1-septate aerial conidia formed in complete darkness and under continuous black light for each strain (Figs. 26, 27). Arithmetic means and their standard deviations (S.D.) calculated for individual strains and light conditions are shown with their ranges. Most of the MB isolates formed longer 0- and 1-septate conidia under black light than in complete darkness. This effect was also observed in the four reference strains of *F. verticillioides*, except for the 0-septate conidia in BBA 62264. Furthermore, the 1-septate conidia also tended to increase in width under black light, but some exceptions were observed (Fig. 27). No significant difference between the two different light conditions was observed in the width of 0-septate conidia (Fig. 26). The MB isolates showed slightly narrower 0-septate conidia than the four reference strains of *F. verticillioides* under both light conditions.

Molecular phylogeny of the banana strains Unweighted maximum parsimony analysis of the individual (data not shown) and combined mtSSU rDNA and EF-1α data sets resolved *F. verticillioides* NRRL 22172 (=BBA 62264) and strains MB 1–7 from banana as a highly supported (bootstrap=100%) exclusive group in the molecular phylogeny (Fig. 28).

Discussion

All seven banana strains were consistent with morpho-

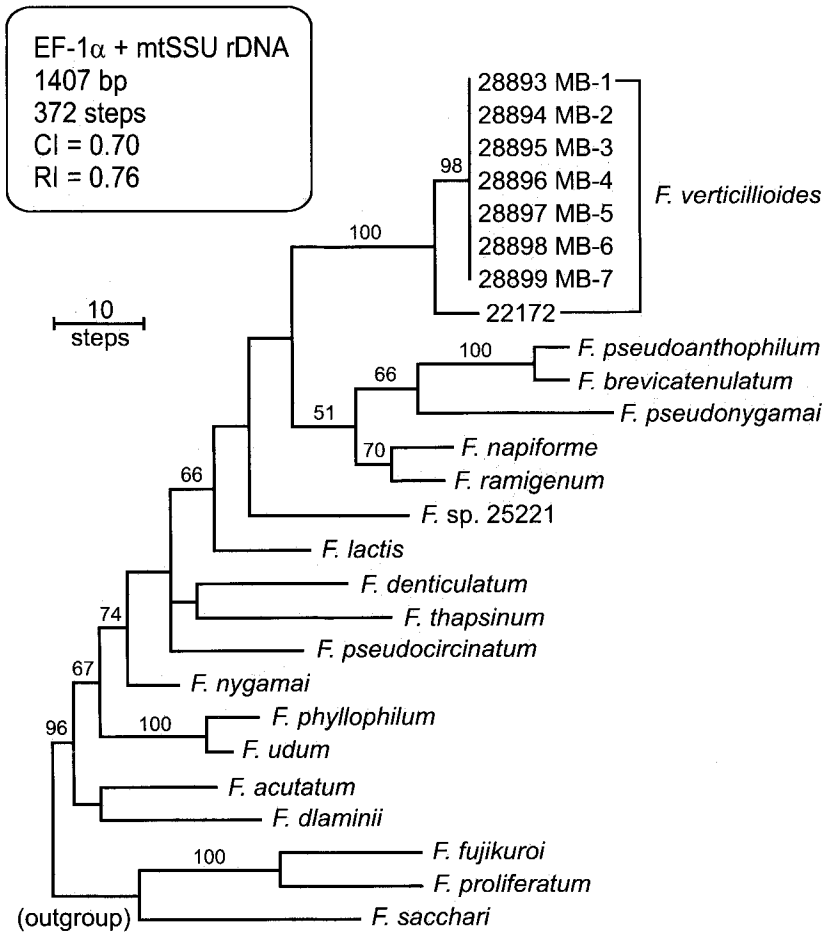


Fig. 28. One of six most-parsimonious phylograms inferred from the combined mtSSU rDNA and EF-1α data. The tree was rooted by the outgroup method using sequences of three Asian species. Of the 1407 aligned nucleotide characters, 718 are mtSSU rDNA and 689 are EF-1α. Of the variable characters, 104 are synapomorphic and 110 are autapomorphic. Bootstrap intervals from 1,000 replicates are indicated by nodes. Strain numbers with 5 digits indicate those of NRRL.

logical descriptions of *F. verticillioides* given by Nirenberg (1976) and Gerlach and Nirenberg (1982), but they exhibited some minor differences. A distinctive morphological feature of the banana isolates was the production of 1(-3)-septate conidia, which were often found in long chains formed on aerial conidiophores. Although most of the aerial conidia in chains were aseptate, 1- and 2-septate aerial conidia were constantly observed in all banana isolates cultured on SNA either in complete darkness or under black light. Even so, the proportion of septate conidia in chains represented ca. 1% or less of all conidia produced. Nelson et al. (1983) characterized *F. moniliforme* as producing primarily aseptate microconidia (=aerial conidia) in long chains. Burgess et al. (1994) also reported that *F. moniliforme* typically produces unicellular microconidia in chains. However, these authors did not report on the proportion of septate aerial conidia in this species. Nirenberg (1976) and Gerlach and Nirenberg (1982), however, stated that *F. verticillioides* also forms 1- and 2-septate conidia. Wollenweber and Reinking (1935) characterized *F. moniliforme* (var. *moniliforme*) as producing 1- or 2-celled microconidia. Reference strains of *G. moniliformis*, ATCC 38932 (A+) and ATCC 38933 (A–) (Kuhlman, 1982) and those of *F. verticillioides*, BBA 62264 (=NRRL 22172) and BBA 65898 (Nirenberg and O'Donnell, 1998; O'Donnell et al., 1998) cultured under identical conditions were examined microscopically for comparison. All four reference strains also formed 1- and 2-septate aerial conidia in complete darkness and under black light, although in lower frequency (less than 0.5% of all conidia) than the banana isolates. Their dimensions were very similar to those of the banana isolates, except that the arithmetic means of 0-septate conidia width were somewhat smaller in the latter (Figs. 26, 27).

Another unique morphological feature of the banana isolates was the production of sporodochia and typical multiseptate sporodochial conidia only under black-light illumination (Figs. 10, 21–24). No sporodochia were formed in complete darkness and only a few 1-septate conidia with a basal foot cell were formed under this growth condition. Nirenberg (1976) and Gerlach and Nirenberg (1982) stated that sporodochia of this species were produced very rarely and then late. Similarly, Nelson et al. (1983) and Burgess et al. (1994) also reported that sporodochia of *F. moniliforme*, a later synonym of *F.*

verticillioides, may be present or absent. In the seven banana isolates, sporodochial conidia were constantly formed only under continuous black light. Under continuous black light, strains ATCC 38932, BBA 62264 and BBA 65898 also formed sporodochial conidia. Only a single sporodochial conidium was observed in ATCC 38933 cultured under the same condition. ATCC 38932, however, also formed sporodochia in complete darkness.

Wollenweber (1931) described *F. moniliforme* var. *minus* as a variety based on isolates from banana fruit (*Musa sapientum* L.) imported from tropical America and from tomato fruit (*Lycopersicon esculentum* Mill.) grown in Germany. This variety was characterized as forming sparse macroconidia (= sporodochial conidia) but no sporodochia or pionnotes. However, Wollenweber did not use black-light illumination for morphological studies of *Fusarium*. Later, Wollenweber and Reinking (1935) added similar isolates from *Sansevieria* spp. and from soil to this variety because they did not produce sporodochia in culture. Nirenberg (1976) re-evaluated Wollenweber's concept of this variety and concluded that the isolates from *Sansevieria* should be classified as *F. proliferatum* (Matsushima) Nirenberg ex Gerlach & Nirenberg var. *minus* Nirenberg (=*F. phyllophilum* Nirenberg & O'Donnell) (Nirenberg and O'Donnell, 1998). Strains of *F. phyllophilum* form many polyphialides and short conidial chains on aerial conidiophores. Nirenberg (1976) also concluded that Wollenweber's isolates of *F. moniliforme* var. *minus* from *Musa* spp., which formed long conidial chains in culture (Wollenweber, 1931), should be classified as *F. verticillioides* or *F. proliferatum* (Nirenberg, 1976; Nirenberg and O'Donnell, 1998), because both species are commonly isolated from *Musa* spp. Comparison of Wollenweber's (1931) original description of this variety as well as his illustration and notes (Nr. 969; Wollenweber, 1930) with our data on the banana isolates suggests they are conspecific, in part, because of the production of septate microconidia (=aerial conidia). Wollenweber (1931) stated that *F. moniliforme* var. *minus* formed 0–1-septate microconidia in long chains. Based on the morphological and physiological data, we have identified the seven isolates from rotten banana as *F. verticillioides*, although they possess a slight morphological difference from typical reference strains of this species from corn and sorghum. Molecular phylogenetic analyses based on mtSSU rDNA and EF-1α sequence data also demonstrated that the MB isolates form a monophyletic group with *F. verticillioides*. This result suggests that Wollenweber's (1931) original concept of *F. moniliforme* var. *minus*, based exclusively on isolates from *Musa*, is conspecific with the current circumscription of *F. verticillioides*.

The 0- and 1-septate conidia of most of the Mexican banana strains and the reference strains from ATCC and BBA were longer under black light than in complete darkness in their mean values and often in their size ranges (Figs. 26, 27). Black light was also reported to affect the conidial morphology of *F. globosum* (Aoki and Nirenberg, 1999): clavate conidia of two strains were longer and slightly wider under black light than those cultivated in total darkness; and an increase in the production of sporodochial conidia was also observed under black-light illumination. These observations are in agreement with results of the present study. In addition to the induction effect, production of globose conidia in *F. globosum* appeared to be suppressed by black light. As discussed by Aoki and Nirenberg (1999), these features may relate to the ecology of the species and their responses to environmental factors. As also shown by the present study, cultural conditions that employ continuous black light and complete darkness are essential to obtain reliable data for accurate identification of fusaria using morphology.

Because *F. verticillioides* (syn.: *F. moniliforme* s. str.) has been reported as the cause of stalk or cob rot of corn in warmer climates (Gerlach and Nirenberg, 1982) and Fusarium blight of soybean caused by *F. moniliforme* has been reported in Japan (Kishi, 1998), we conducted inoculation tests of corn and soybean plants with one of the banana isolates. This isolate, however, did not induce stalk rot on corn or Fusarium blight symptoms on soybean. By wound inoculation tests, it only produced light brownish to yellowish spots on corn leaves and on leaves and pods of soybean. In Mexico, Guatemala, Honduras and other countries in Central America, a similar leaf spot disease of corn caused by a fungus identified as *F. moniliforme* has been reported (Schieber and Muller, 1968), but *F. verticillioides* has not been implicated in any significant disease of soybean.

Crown rot (crown mold or pedicel rot), a common post-harvest disease of banana, is prevalent in tropical countries where bananas are cultured intensively as an important cash crop. This disease is often found during storage and shipment after their harvest (Wollenweber and Reinking, 1935; Greene and Goos, 1963; Stover, 1981; Ploetz et al., 1994). Several phytopathogenic fungi including *F. verticillioides* (=*F. moniliforme*), *F. incarnatum* (Rob.) Sacc. (=*F. pallidoroseum* (Cooke) Sacc.=*F. semitectum* Berk. & Ravenel var. *majus* Wollenw.), *Colletotrichum musae* (Berk. & Curt.) v. Arx, among others, have been reported as causal agents of the disease. In this disease, infection typically occurs at cut surfaces of banana crowns either during handing with contaminated knives or during passage through contaminated wash water. Symptoms begin as a softening and blackening of tissues at the cut crown surfaces where mold growth may be seen (i.e., crown mold). Subsequently, the pathogen penetrates into the pedicel of individual fingers, where it spoils the flesh of ripening banana fruit. Individual fingers may fall from the weakened crown (i.e., pedicel rot) when the rot becomes widespread. Although rot is usually restricted to the crown, in severe cases the decay reaches the pulp of individual fingers and the entire fruit is lost (Greene and Goos, 1963; Ploetz et al., 1994; Jones, 2000). In the present imported bananas, disease symptoms were found mainly on the surface of fruit, where many scattered dark brownish spots were observed. Cut surfaces of crowns and pedicels were also blackened occasionally

and covered with whitish mycelia, but lesions on the crowns and pedicels appear to be independent of the spots on the fruit surface. When damaged severely, inner parts of the fingers and the entire fruit were rotten. Although several common features indicated that the present disease could be classified as an extensive case of crown rot, symptoms on the bananas indicated that the disease was caused primarily by secondary infection of the pathogen. Many scattered dark brownish spots on the epidermis bearing lacunae and whitish fungal colonies of the pathogen represented distinctive features of the disease. Therefore, we consider it to be different epidemiologically from crown rot. Results of our study suggest that crown rot caused by *F. verticillioides* was most likely initiated at the banana plantation in Mexico. In contrast, our results suggest that the disease symptoms characterized by brownish spots and destruction of flesh should be recognized as an independent post-harvest disease called "Fusarium fruit rot" of banana (Hirata and Kimishima, 1997).

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