Genetic Variation Among *Fusarium oxysporum* Isolates Associated with Root Rot of *Amaranthus hybridus* in South Africa

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ABSTRACT

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Thirty isolates of *Fusarium oxysporum* were recovered from six substrates: (i) tap roots of *Amaranthus hybridus* showing symptoms of root rot, (ii) side roots of *A. hybridus* showing symptoms of root rot, (iii) soil surrounding plants of *A. hybridus*, (iv) pigweed weevils (*Hypolixus haerens*) that feed on *A. hybridus*, (v) rhizosphere of maize plants growing adjacent to a field of amaranth, and (vi) rhizosphere of dry bean plants growing adjacent to a field of amaranth. The isolates were characterized by means of pathogenicity tests, isozyme analysis, and vegetative compatibility group (VCG) tests. In the pathogenicity tests, toothpick tips were infested with *F. oxysporum* and inserted into amaranth stems. All 30 isolates were pathogenic on *A. hybridus*, with significant differences in pathogenicity based on lesion length measured 4 weeks after inoculation. The isolates were grouped into nine VCGs by complementation tests using nitrate nonutilizing mutants. Self-incompatibility was not observed for any of the isolates. The most common VCG was VCG1, which comprised 20 of the 30 isolates tested. The second most common group was VCG3, which included three isolates, while the remaining seven VCGs each consisted of a single isolate. The results indicate that the population of the amaranth root rot pathogen examined in this study is relatively homogeneous. Results of the isozyme analysis supported the results of VCG tests. Three major groups were delineated within the 30 isolates of *F. oxysporum* following cluster analysis of electrophoretic phenotypic values for seven isozymes (EST, IDH, G6PDH, ACP, PEP1, PEP2, and PEP3) tested. No relationship was found between isozyme phenotype and the substrate from which the isolates were recovered.

Fusarium oxysporum Schlechtend. emend. W. C. Snyder & H. N. Hans. is an important and cosmopolitan fungal species commonly found in a wide range of soils (13). The different formae speciales of *F. oxysporum* are responsible for wilt and root rot of more than 100 economically-important plants (18). In South Africa, isolates of *F. oxysporum* have been associated with stem decay (1) and root rot (4) of *Amaranthus hybridus* L., a nutritious, rapidly growing vegetable produced on increasing acreage in semiarid regions of the country. The fungus was also shown to be the predominant fungal species vectored by the pigweed weevil *Hypolixus haerens*, the

main insect pest of *A. hybridus* in South Africa (15,19).

Genetic diversity within populations of *F. oxysporum* has been characterized extensively by means of vegetative compatibility group (VCG) tests (5,7-9,12,14,17,27). Detailed studies by Kistler et al. (18) showed that isolates belonging to the same VCG typically possess very similar or identical multilocus haplotypes and belong to the same clonal lineage. Isozyme analysis has also proved to be a useful tool for differentiating intraspecies variability among isolates of *F. oxysporum* recovered from diverse substrates (3,11,23,25).

The primary objective of this study was to examine the genetic relatedness of 30 isolates of *F. oxysporum* obtained from an isolated field planted to amaranth for three successive growing seasons, from pigweed weevils, and from an adjacent field of maize and dry bean, using VCG tests, isozyme analysis, and pathogenicity tests. The secondary objective was to assess possible relationships among the three methods used for differentiating the isolates of *F. oxysporum*.

MATERIALS AND METHODS

Sample collection, culture isolation, and identification. Forty amaranth plants displaying symptoms of root rot were collected at random from a 0.25-ha plot of 2-

month-old plants of *A. hybridus* on an experimental farm near Bloemfontein, South Africa. The predominant soil type in the area was a medium loam (10 to 20% clay) with a mean pH of 7.4. The site had been planted to *A. hybridus* for the preceding 3 years and commercial fertilizer had been applied periodically to meet a nitrogen requirement of 160 kg/ha. Isolates of *F. oxysporum* were recovered from (i) the tap roots of amaranth plants showing typical root rot symptoms, (ii) the lateral roots of amaranth plants showing typical root rot symptoms, (iii) debris from the rhizosphere of diseased amaranth plants, (iv) pigweed weevils captured in the amaranth plot, (v) debris from the rhizosphere of dry bean plants, and (vi) debris from the rhizosphere of maize plants growing in two fields adjacent to the amaranth plot. Each field had been planted to maize and dry beans, respectively, for the previous 4 years with the same rate of fertilizer application as for the *A. hybridus* plot.

Foliage and petioles of symptomatic amaranth plants were cut off at a height of 50 mm or more above the root crown with a sterilized clipper and discarded. Each denuded plant stem was then placed in a separate plastic bag. The tap roots and side roots associated with collected plants were unearthed and shaken to remove adhering soil before being placed in separate plastic bags. Five soil samples (100 g each) were collected from each of five plants per host (amaranth, maize, and dry bean; i.e., a total of 25 soil samples per host plant). The soil samples were collected directly from the rhizosphere of the amaranth plants and randomly from the rhizosphere of maize plants and dry bean plants. Eighty adult pigweed weevils were collected over a period of 3 months during the summer of 1999. Upon capture, individual weevils were placed in 1.5-ml Eppendorf tubes to minimize possible cross-contamination of insects by fungal spores carried on their bodies. Weevils were killed in the lab by exposure to -75°C for 2 h. Each weevil was then plated aseptically onto 2% malt-extract agar (MEA).

Tap root and side root samples from each of the 40 amaranth plants were trimmed, washed in running tap water to remove all soil, blotted dry, and cut into 10-mm segments. Root segments were soaked in 0.5% NaOCl for 5 min, rinsed with sterile distilled water, dipped in 95% ethanol, and flamed briefly over a bunsen

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burner to allow for evaporation of the ethanol. Each segment was cleaved longitudinally with a sterilized scalpel. Three tap root samples and three side root samples were removed from each of the 40 amaranth plants, yielding a total of 120 of both tap and side root samples. Isolations from symptomatic roots were made onto potato-dextrose agar (PDA) amended with streptomycin sulfate (0.33 g/liter of water). Each longitudinal section was placed on a separate petri dish containing PDA. No isolations were made from asymptomatic roots.

Soil isolations were conducted from three replicate subsamples taken from a pooled, homogenized mixture of each of the five soil samples from each host. The soil was air dried at room temperature for 20 min and then passed through a 200- μ m sieve. Three subsamples of 8 mg of soil from each sample were spread over Van Wyk's rose bengal-glycerine-urea selective medium for isolation of *Fusarium* spp. (28). Three plates were prepared for each replicate and incubated under daily schedules of 16 h fluorescent light and 8 h dark at room temperature for 6 days.

Fusarium colonies were observed microscopically. Those colonies identified as *F. oxysporum* were transferred to carnation-leaf agar (CLA) and PDA (22). Single spore isolations were made from each colony, and isolates were identified morphologically to species based on characteristics of the macroconidia, phialides, microconidia, chlamydospores, and colony growth traits (22).

Pathogenicity tests. Ten seeds of a single commercially planted cultivar (cv. 15/SA) of *A. hybridus* were planted in each of 30 4-cm-diameter plastic pots (500 ml) containing a soil mixture (vol/vol) of 50% sand-loam (10 to 15% clay) and 50% peat. Soils were steam sterilized twice for 1 h at 80°C before planting seeds 10 mm deep. Plants were left to grow for 2 weeks before being transferred to larger plastic pots (1 liter) also containing steam-sterilized soil. Plants grew for a further 4 weeks before they were inoculated with the tips of wooden toothpicks colonized by each of the 30 isolates of *F. oxysporum*. Wooden toothpick tips (6 to 7 mm long) were autoclaved for 20 min in distilled water, removed from the water, blotted dry, autoclaved a second time in water to remove substances that might inhibit fungal growth, then autoclaved for a third time in potato-dextrose broth (PDB). The toothpick tips were cooled in a sterile petri dish and transferred individually to the margins of colonies of *F. oxysporum* on PDA. After incubation under fluorescent light at room temperature for 48 h, the colonized toothpick tips were removed and used as an inoculum source. A single toothpick tip was inserted into a small hole made in the stem of an amaranth plant 5 to 7 cm above soil level using a dissecting needle. Each

hole was covered with parafilm after inoculation to prevent desiccation and contamination. Five replicate plants were inoculated with one each of the 30 isolates of *F. oxysporum*. Control inoculations were performed with toothpick tips prepared as described above but incubated on sterile PDA.

The temperature in the greenhouse varied between 15 and 28°C. Plants were watered daily and fertilized weekly with a hydroponic nutrient solution (10 g/liter of water; 50 ml per pot) containing N (5.6%), P (4.6%), K (2.74%), Mg (3%), S (11.2%), Fe (0.069%), Mn (0.03%), Zn (0.027%), B (0.044%), Cu (0.014%), and Mo (0.009%). Plants were observed every other day for disease development. After incubation for 4 weeks in the greenhouse, the plants were harvested and the stems of all five replications of each isolate were cut longitudinally at the inoculation wound with a sterile knife. Length of the necrotic lesion extending either side of the inoculation wound was measured. A randomized complete block design was used and the experiment was conducted twice.

Analysis of variance (ANOVA) was conducted with ANOVA of NCSS 2000 (BMDP Statistical Software, Inc., Los Angeles). The experiment was treated as a block, the isolate was treated as an experimental factor, and the interaction of experiment-isolate was used as an error term to determine the significance of variance in lesion length among experiments and isolates. This error term was also used in multiple comparisons among isolates by DUNCAN comparison method after determination of insignificance between two experiments. To verify that necrosis was caused by the particular isolate of *F. oxysporum* that had been inoculated, one piece of stem tissue from each of the 150 inoculated plants was taken from each side of the toothpick insertion point, plated on 50% PDA, incubated for 4 to 5 days, and examined for the presence of *F. oxysporum*.

Vegetative compatibility tests. Mutants were generated for each isolate as described by Correll et al. (7) and Correll (5), using minimal medium or PDA containing 1.5% potassium chlorate. For isolates that failed to form mutants on either of these two media, chlorate concentrations were increased to 3% or higher, or isolates were transferred to a rose bengal-amended medium for selecting nitrate-metabolism mutants (10). Mutant sectors appearing on the chlorate media were transferred to minimal medium (MM) containing nitrate as the sole nitrogen source. Colony sectors with thin, expansive growth and no aerial mycelium were assumed to be nitrate nonutilizing mutants (*nits*). Subsequent transfer to minimal medium with nitrate, nitrite, or hypoxanthine was used to identify the physiological phenotypes. Sparse growth on nitrate, hypoxanthine, or nitrite-contain-

ing medium determined a *nit* 1, *nit* M, or *nit* 3 phenotype, respectively (6,7). At least one *nit* M and one *nit* 1 or *nit* 3 were obtained for each isolate.

Pairings between isolates were made on MM in 90-mm petri plates. All isolates were paired in all possible combinations and with a positive control. A mycelial plug of a *nit* M was placed in the center of the plate and surrounded at a distance of 10 mm by four *nit* 1s or *nit* 3s in a "daisy" configuration as described by Puhala (24). The plates were incubated in the dark at 28°C for up to 14 days. Wild-type growth in the contact zone between any two mutants indicated that the two isolates belonged to the same VCG. Sparse growth in the contact zone indicated that the isolates belonged to different VCGs. All pairings were performed twice.

Isozyme analysis. All isolates were grown on PDA in petri plates for 5 days at 25°C. Each isolate was prepared for electrophoresis by inoculating 100 ml of PDB in a 500-ml flask with a single mycelial plug taken from the edge of a growing colony. Inoculated flasks were shaken continuously at 90 rpm for 6 days at room temperature (24 $\pm$ 1°C). Mycelium was harvested from the broth by filtration through two layers of cheesecloth, washed three times with sterile distilled water, dried on filter paper, and ground in liquid nitrogen using a mortar and pestle. A 1.5-ml Eppendorf tube was filled halfway up the conical portion with ground mycelium. One milliliter of extraction buffer (1.21 g of Tris, 0.292 g of EDTA, 38 mg of NADP per liter of distilled water, 0.5% polyvinylpyrrolidone 360) was added to the tube, which was shaken vigorously for 10 min, then centrifuged for 5 min in a tabletop centrifuge at 9,000 rpm. The supernatant was collected and frozen at -75°C until assayed.

Starch gel electrophoresis and staining procedures were conducted according to the procedures of Bonde et al. (2) and Michales et al. (20,21). Fifteen enzymes were tested for activity in four buffer systems; the Ridgway buffer (pH 8.5, 75A, 4h) was used throughout this study because it provided the best results. Each of the isolates of *F. oxysporum* were screened for isozyme activity at least twice. Thirty isolates of *F. oxysporum* from all six substrates were characterized using the seven selected enzymes: esterase (EST, EC no. 3.1.1.1); isocitrate dehydrogenase (IDH, EC no. 1.1.1.42); glucose-6-phosphate dehydrogenase (G6PDH, EC no. 1.1.1.49); acid phosphatase (ACP, EC no. 3.1.3.2); and peptidase (PEP, EC no. 3.4.11) using glycyl-leucine (GL), leucyl-tyrosine (LT), and leucyl-glycyl-glycine (LGG) as substrates. Isozyme bands were recorded as 0 if absent or 1 if present in the data matrix for each enzyme. Each isolate of *F. oxysporum* was assigned to a phenotype class based on the banding pattern. To determine

relatedness of the fungal isolates, cluster analysis was performed for similarity coefficients by using the unweighted pair-group method with arithmetic averages (UPGMA) in the statistical program NCSS 2000 (BMDP Statistical Software, Inc.).

RESULTS

More than 200 isolates of *F. oxysporum* were recovered from the six different substrates. Thirty isolates were chosen based on the frequency that they were recovered from each of the six substrates for characterization of their genetic relatedness.

Pathogenicity tests. All 30 isolates were pathogenic on *A. hybridus* plants, producing significantly longer ($P < 0.05$) necrotic lesions on stems (mean = 18 mm, range 13 to 28 mm; Table 1) than on non-inoculated control treatments (mean = 8 mm). ANOVA demonstrated that there was no significant difference in lesion length ($P = 0.8459$) between the two experiments, while there was a significant difference among isolates ($P < 0.001$). No relationship was observed between VCGs and

pathogenicity. *F. oxysporum* was reisolated from each of the inoculated plants but not from any of the noninoculated plants.

Vegetative compatibility tests. A total of 765 chlorate-resistance mutants were obtained from the 30 isolates. Between 3 and 25 *nits* were obtained from each isolate of *F. oxysporum*. Isolates differed in the number of mutants recovered and the phenotypic ratio of *nits* recovered, although sectoring frequency per isolate was not recorded. The *nit* 1 phenotype was recovered most frequently (49.5% of all mutants), followed by *nit* M (26.7%) and *nit* 3 (6.3%). The most evident pairing (regeneration of wide type growth) was the combination of *nit* 1 and *nit* M. For each isolate of *F. oxysporum*, the *nits* and the tester strains formed heterokaryons evident by robust growth at the interface of the two colonies. Self-incompatibility was not observed between complementary *nits* and the original isolates of *F. oxysporum*.

The 30 isolates of *F. oxysporum* fell into nine VCGs. Of these, the largest group (VCG 1) included 20 of the isolates. Seven

of the VCGs consisted of a single isolate, and one VCG (VCG3) consisted of three isolates (Table 1). Isolates within a VCG were not compatible with isolates from another VCG. Self-incompatibility was not observed between complementary *nits* recovered from single isolates of *F. oxysporum*.

Isozyme analysis. Of the seven enzymes evaluated, EST, ACP, and G6PDH produced the most intensive bands. Bands of IDH and PEP3 were weaker, and their intensity decreased after extraction samples had been stored for more than 2 weeks. A total of 126 electrophoretic phenotypes was detected among the 30 isolates of *F. oxysporum* examined. The number of electrophoretic phenotypes varied among substrates from which the isolates were recovered. Three major groups of *F. oxysporum*, designated I, II, and III, were delineated by the cluster analysis (Fig. 1). No relationship was found between isozyme groups and the substrates from which the isolates were recovered; for example, group II included three isolates recovered from the maize rhizosphere, one isolate from dry bean rhizosphere, and one isolate from a pigweed weevil.

Table 1. Isolate number, substrate from which isolates were recovered, lesion length resulting from inoculation of *Amaranthus hybridus*, and vegetative compatibility group (VCG) of 30 isolates of *Fusarium oxysporum*^a

Isolate	Designation ^w	Substrate for isolation ^x	Lesion length (mm) ^y	VCG ^z
28	Db6	Dry bean rhizosphere	28 a	9
26	M3	Maize rhizosphere	26 ab	1
6	106	Amaranth soil	22 bc	1
12	78	Amaranth tap root	22 bc	1
24	169.1	Weevil	22 bc	1
4	104	Amaranth soil	22 bc	1
9	48	Amaranth tap root	22 bc	3
13	79	Amaranth tap root	21 cd	1
2	102	Amaranth soil	20 cde	1
1	101	Amaranth soil	18 cdef	1
20	139.1	Weevil	18 cdef	1
5	105	Amaranth soil	18 cdef	1
25	M1	Maize rhizosphere	18 cdef	1
18	23.2.3	Weevil	17 defg	5
3	103	Amaranth soil	17 defg	1
7	109	Amaranth soil	17 defg	2
27	M5	Maize rhizosphere	17 defg	8
21	138.1	Weevil	17 defg	6
15	Srr10	Amaranth side root	17 defg	1
23	148.1	Weevil	16 efg	1
17	Srr14	Amaranth side root	16 efg	1
30	Db2	Dry bean rhizosphere	16 efg	1
10	63	Amaranth tap root	16 efg	3
11	58	Amaranth tap root	15 fg	3
22	145.1	Weevil	15 fg	7
14	Srr1	Amaranth side root	15 fg	1
29	Db1	Dry bean rhizosphere	14 fg	1
19	69.2	Weevil	14 fg	1
8	35	Amaranth tap root	13 g	1
16	Srr12	Amaranth side root	13 g	4

^a Means followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

^w Isolates of *F. oxysporum* recovered from symptomatic roots were made on potato-dextrose agar amended with streptomycin sulfate after surface sterilization. Isolates recovered from soil debris were made on Van Wyk's selective medium (28). Isolates recovered from weevils were made by plating dead weevils onto 2% malt-extract agar.

^x Tap root and side root samples were all from *A. hybridus* plants, and from roots showing symptoms of root rot. Weevil = pigweed weevil (*Hypolixus haerens*).

^y Mean length of necrotic lesion produced on amaranth stems following inoculation with *F. oxysporum*-infested toothpicks (average of 10 plants from five replications and two experiments per isolate from two trials).

^z Isolates from different VCGs were not compatible.

DISCUSSION

Based on the biological and genetic characteristics evaluated in this study, the population of *F. oxysporum* colonizing amaranth roots appears to be relatively homogeneous and genetically indistinct from isolates obtained from associated pigweed weevils and from an adjacent field of maize and dry bean. These results are similar to those of Bosland et al. (3), who classified isolates of *F. oxysporum* from crucifers into subspecific taxa according to their pathogenicity, isozyme polymorphism, and vegetative compatibility. They found little variation in isozyme patterns among isolates of *F. oxysporum* for 15 of 18 enzymes examined. This uniformity shows that *F. oxysporum* is consistent with respect to enzyme profile; therefore, not much polymorphism should be expected. However, only seven enzymes were evaluated in this study, and greater variation in isozyme patterns may have been found if a greater number of enzymes were evaluated.

Our results are relatively consistent with a number of studies on *Fusarium* VCGs that demonstrate a lack of genetic diversity. For example, a collection of 44 isolates of *F. oxysporum* Schlechtend.:Fr. f. sp. *albedinis* (Killian & Maire) Gordon, causing Bayoud disease of date palm, all belonged to a single VCG, and no polymorphisms were observed in restriction fragment length polymorphism studies from mitochondrial DNA and random amplified polymorphic DNA analysis (26). All 47 isolates of *F. oxysporum* f. sp. *apii* collected from celery showing wilt symptoms belonged to a single VCG (7). Iso-

lates of *F. oxysporum* f. sp. *melonis* collected from a single field in California also were represented by a single VCG (16). Nine VCGs were recovered in the current study, but two-thirds of the isolates assayed belonged to a single VCG and included isolates recovered from all six substrates. The second largest VCG was only represented by three isolates, and the remaining seven VCGs by only one isolate each. Isolates of *F. oxysporum* from the single-member VCGs clustered into the same group as isolates assigned to VCG1 by isozyme analysis (Fig. 1). Elias and Schneider (11) similarly recovered a number of single-member VCGs of *F. oxysporum* f. sp. *lycopersici*, whose electrophoretic phenotypes clustered with the multiple-member VCGs, providing evidence for their recent development from the multiple-member VCGs. The isolates of single-member VCGs grouped into the same clade with members in VCG1 by isozyme analysis (Fig. 1), suggesting that they may have been members of major VCGs that underwent a simple mutation at one or more of the vegetative compatibility loci, such that they can no longer form heterokaryons with the other members.

There was no association between VCGs and the different sources from which the isolates of *F. oxysporum* were recovered. For example, VCG3 included three isolates

recovered from diseased tap roots, but three other isolates from the same source were assigned to VCG1. Isolates of *F. oxysporum* recovered from phytophagous weevils, and shown to be pathogenic in artificial inoculations of potted amaranth plants, were distributed among four VCGs, of which the majority were from VCG1 and three were specific to weevils (Table 1). This suggests that pigweed weevils carry spores of *F. oxysporum* which they inadvertently acquire from a wide range of niches and localities. The recovery of isolates in VCG1 from different sources also suggests that this genetically homogeneous population is widespread among various ecological niches.

Commercial cultivation of amaranth is at a very early stage of development in South Africa, suggesting that a more diverse pathogen population within *F. oxysporum* may develop in future. Additional work is needed on *F. oxysporum* populations pathogenic to amaranth, including evaluation of isolates from more diverse locations. The host range of isolates of *F. oxysporum* pathogenic to *A. hybridus* should also be examined to determine whether they are specific to this host. These results could provide valuable information on the origin and spread of this soil inhabitant and might suggest novel methods, such as crop rotation, for controlling

root rot of *A. hybridus*. It also follows that, when selecting for resistance in *A. hybridus* to *F. oxysporum*, strict attention should be paid to the level of variation that exists within the local population of the pathogen. An integrated pest management program involving cultural practices, selection for disease resistance, and biological or chemical control of pigweed weevils should therefore go a long way to reduce losses associated with *F. oxysporum* in amaranth plantings.

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LITERATURE CITED

- Blodgett, J. T., Swart, W. J., and Louw, S. vdM. 1998. First report of *Fusarium sambucinum*, *F. oxysporum*, and *F. subglutinans* associated with stem decay of *Amaranthus hybridus* in South Africa. *Plant Dis.* 82:1062.
- Bonde, M. R., Micales, J. A., and Peterson G. L. 1993. The use of isozyme analysis for identification of plant-pathogenic fungi. *Plant Dis.* 77:961-968.
- Bosland, P. W., and Williams, P. H. 1987. An evaluation of *Fusarium oxysporum* from crucifers based on pathogenicity, isozyme polymorphism, vegetative compatibility and geographic origin. *Can. J. Bot.* 65:2067-2073.
- Chen, W. Q., and Swart, W. J. 2000. *Fusarium oxysporum* and *F. sambucinum* associated with root rot of *Amaranthus hybridus* in South Africa. *Plant Dis.* 84:101.
- Correll, J. C. 1991. The relationship between formae speciales, races, and vegetative compatibility groups in *Fusarium oxysporum*. *Phytopathology* 81:1061-1064.
- Correll, J. C. 1992. Genetic, biochemical and molecular techniques for the identification and detection of soil borne plant-pathogenic fungi. Pages 1-10 in: *Methods for Research on Soilborne Phytopathogenic Fungi*. L. L. Singleton, J. D. Mihail, and C. M. Rush, eds. American Phytopathological Society Press, St. Paul, MN.
- Correll, J. C., Klittich, C. J. R., and Leslie, J. F. 1987. Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. *Phytopathology* 77:1640-1646.
- Correll, J. C., Puhalla, J. E., and Schneider, R. W. 1986. Identification of *Fusarium oxysporum* f. sp. *apii* on the basis of colony size, virulence, and vegetative compatibility. *Phytopathology* 76:396-400.
- Elena, K., and Tjamos E. C. 1995. Vegetative compatibility groups of *Fusarium oxysporum* f. sp. *dianthi* from plants and the rhizosphere of carnation in Greece. *Plant Pathol.* 44:148-152.
- Elias, K. S., and Cotty, P. J. 1995. A rose bengal amended medium for selecting nitrate-metabolism mutants from fungi. *Can. J. Bot.* 73:680-682.
- Elias, K. S., and Schneider, R. W. 1992. Genetic diversity within and among races and vegetative compatibility groups of *Fusarium oxysporum* f. sp. *lycopersici* as determined by isozyme analysis. *Phytopathology* 82:1421-1427.
- Elmer, W. H., and Stephens, C. T. 1989. Classification of *Fusarium oxysporum* f. sp. *asparagi* into vegetatively compatible groups. *Phytopathology* 79:88-93.
- Gerlach, W., and Nirenberg, H. 1982. The genus *Fusarium*—a Pictorial Atlas. Mitt. Biol. Bundesanst. Land-Fortwirtsch. Berl.-Dahl.

Fig. 1. Dendrogram based on isozyme analysis of 30 isolates of *Fusarium oxysporum*, recovered from amaranth plants and other substrates (maize and dry bean rhizosphere, weevils), using the unweighted pair-group method with arithmetic averages (NCSS 2000; BMDP Statistical Software Inc., Los Angeles).

- 209:1-406.
14. Gordon, T. R., and Okamoto, D. 1991. Vegetative compatibility groupings in a local population of *Fusarium oxysporum*. Can. J. Bot. 69:168-172.
 15. Honiball, S. J., Louw, S. vdM., Swart, W. J., and Chen, W. Q. 1999. Fungi associated with phytophagous weevils on *Amaranthus hybridus* in the central Free State, South Africa. Page 58 in: Proc. 12th S. Afr. Entomol. Congr. Potchefstroom, South Africa.
 16. Jacobson, D. J., and Gordon, T. R. 1990. Further investigation of vegetative compatibility within *Fusarium oxysporum* f. sp. *melonis*. Can. J. Bot. 68:1245-1248.
 17. Katan, T., and Katan, J. 1988. Vegetative compatibility grouping of *Fusarium oxysporum* f. sp. *vasinfectum* from tissue and the rhizosphere of cotton plants. Phytopathology 78:852-855.
 18. Kistler, H. C., Alabouvette, C., Baayen, R. P., Bentley, S., Brayford, D., Coddington, A., Correll, J., Daboussi, M.-J., Elias, K., Fernandez, D., Gordon, T. R., Katan, T., Kim, H. G., Leslie, J. F., Martyn, R. D., Migheli, Q., Moore, N. Y., O'Donnell, K., Ploetz, R. C., Rutherford, M. A., Summerell, B., Waalwijk, C., and Woo, S. 1998. Systematic numbering of vegetative compatibility groups in the plant pathogenic fungus *Fusarium oxysporum*. Phytopathology 88:30-32.
 19. Louw, S. vdM., van Eeden, C. F., and Weeks, W. J. 1995. Curculionidae (Coleoptera) associated with wild and cultivated *Amaranthus* spp. (Amaranthaceae) in South Africa. Afr. Crop Sci. J. 3(1): 93-98.
 20. Micales, J., Bonde, M., and Peterson, G. 1986. The use of isozyme analysis in fungal taxonomy and genetics. Mycotaxon 27:405-449.
 21. Micales, J., Bonde, M., and Peterson, G. 1992. Isozyme analysis in fungal taxonomy and molecular genetics. Pages 57-79 in: Handbook of Applied Mycology, Fungal Biotechnology, Vol. 4. D. Arora, R. Elander., and K. Mukerji, eds. Marcel-Dekker, Inc., New York.
 22. 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.
 23. Paavananen-Huhtala, S., Hyvonen, J., Bulat, S. A., and Yli-Mattila. 1999. RAPD-PCR, isozyme, rDNA RFLP and rDNA sequence analysis in identification of Finnish *Fusarium oxysporum* isolates. Mycol. Res. 103:625-634.
 24. Puhalla, J. E. 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. Can. J. Bot. 63:179-183.
 25. Skovgaard, K., and Rosendahl, S. 1998. Comparison of intra- and extracellular isozyme banding patterns of *Fusarium oxysporum*. Mycol. Res. 102:1077-1084.
 26. Tantaoui, A., Ouinten, M., Geiger, J. P., and Fernandez, D. 1996. Characterization of a single clonal lineage of *Fusarium oxysporum* f. sp. *albedinis* causing bayoud disease of date palm in Morocco. Phytopathology 86:787-792.
 27. Vakalounakis, D. J., and Fragkiadakis, G. A. 1999. Genetic diversity of *Fusarium oxysporum* isolates from cucumber: Differentiation by pathogenicity, vegetative compatibility, and RAPD fingerprinting. Phytopathology 89:161-168.
 28. Van Wyk, P. S., Scholtz, D. J., and Los, O. 1986. A selective medium for the isolation of *Fusarium* spp. from soil debris. Phytophylactica 18:67-69.