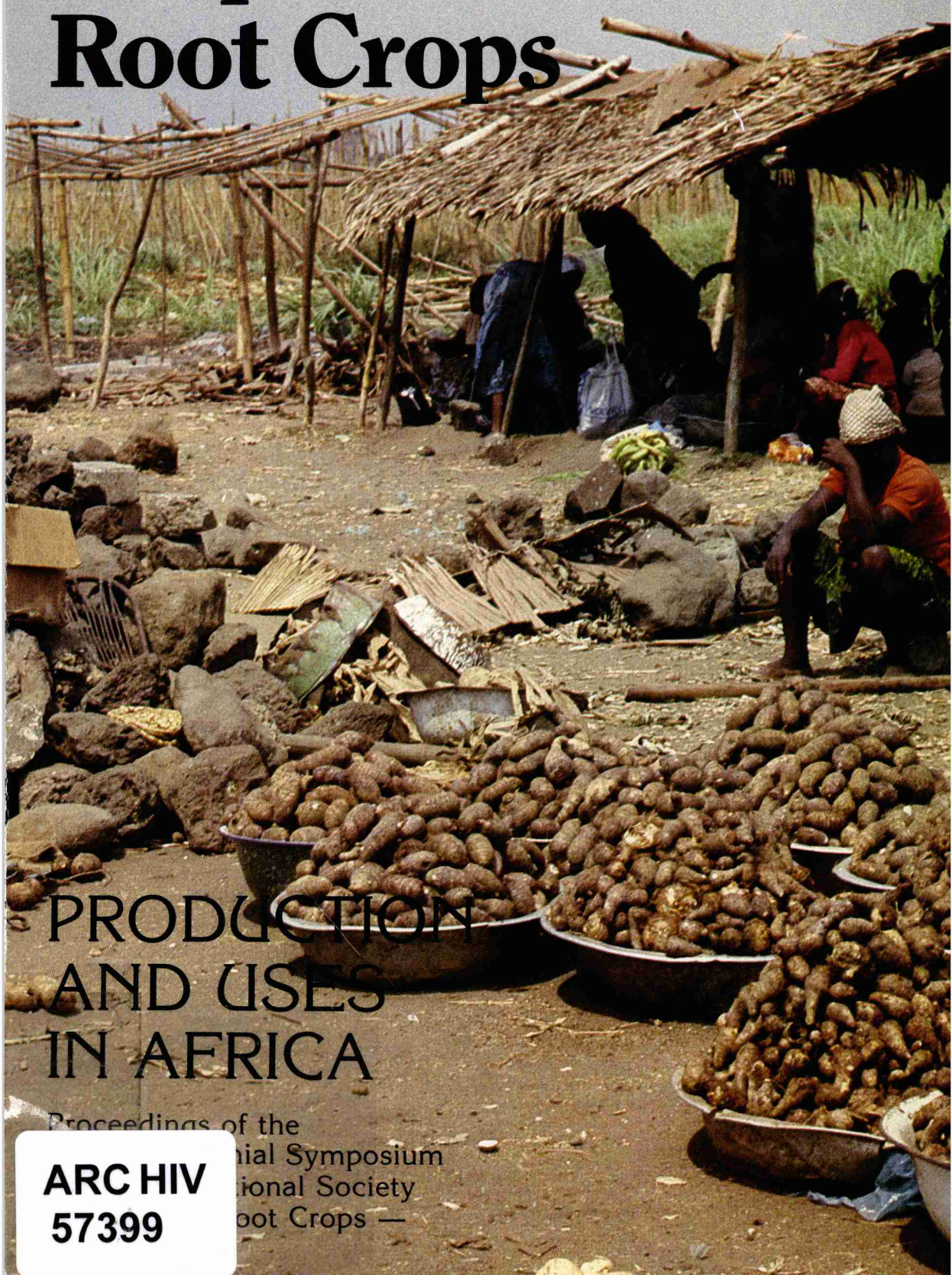


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Tropical Root Crops



PRODUCTION AND USES IN AFRICA

Proceedings of the
International Symposium
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The International Society for Tropical Root Crops — Africa Branch was created in 1978 to stimulate research, production, and utilization of root and tuber crops in Africa and the adjacent islands. The activities include encouragement of training and extension, organization of workshops and symposia, exchange of genetic materials, and facilitation of contacts between personnel working with root and tuber crops. The Society's headquarters are at the International Institute of Tropical Agriculture in Ibadan, Nigeria, but its executive council comprises eminent root and tuber researchers from national programs throughout the continent.

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ABSTRACT

A mixture of original research, updates on procedures, literature reviews, and survey reports, this document resulted from the second symposium of the International Society for Tropical Root Crops — Africa Branch, with 77 participants from 16 countries. The focus was cassava, yams, cocoyams, and sweet potatoes, from the perspectives of breeders, agronomists, soil specialists, plant pathologists, entomologists, nutritionists, food technologists, etc. Learning from past successes and failures, many of the researchers directed their efforts toward problems obstructing progress in reaching improved production and use of root crops and attempted to view, realistically, the context in which their results would be applied.

RÉSUMÉ

Résultats de recherches récentes, mises à jour sur les méthodes de recherche, revues de publications et rapports de sondages sont contenus dans ce document issu du Deuxième symposium de la Société internationale pour les plantes-racines tropicales — Direction Afrique, qui a réuni 77 participants de 16 pays. Des communications sur le manioc, le taro, le yam et la patate douce ont été présentées par des phytosélectionneurs, des agronomes, des pédologues, des phytopathologistes, des entomologistes et des spécialistes de la nutrition et des aliments, entre autres. Tirant leçon de leurs succès et de leurs échecs, beaucoup de ces chercheurs ont dirigé leurs efforts vers la solution des problèmes qui entravent l'augmentation de la production et de la consommation des plantes-racines et ont tenté de considérer d'un œil réaliste le contexte qui sera celui de l'application de leurs recherches.

RESUMEN

Una mezcla de investigaciones originales, actualizaciones de procedimientos, reseñas de literatura e informes de encuestas, este documento es el resultado del segundo simposio de la Sociedad Internacional de Raíces Tropicales, Filial Africana, que contó con 77 participantes de 16 países. El simposio se centró en la yuca, el ñame, el cocoñame y las batatas, desde la perspectiva de los fitomejoradores, los agrónomos, los especialistas en suelos, los patólogos vegetales, los entomólogos, los nutricionistas, los tecnólogos alimenticios, etc. A partir de los éxitos y fracasos anteriores, muchos de los investigadores encaminaron sus esfuerzos hacia los problemas que obstaculizan el avance para lograr una producción y un uso mejorados de las raíces y trataron de obtener una visión realista del contexto en que los resultados pueden ser aplicados.

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TROPICAL ROOT CROPS: **PRODUCTION AND USES IN AFRICA**

EDITORS: E.R. TERRY, E.V. DOKU, O.B. ARENE, AND N.M. MAHUNGU

*PROCEEDINGS OF THE SECOND TRIENNIAL SYMPOSIUM OF THE INTERNATIONAL
SOCIETY FOR TROPICAL ROOT CROPS — AFRICA BRANCH HELD IN DOUALA,
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EFFECTS OF VESICULAR-ARBUSCULAR MYCORRHIZAE, TEMPERATURE, AND PHOSPHORUS ON *FUSARIUM* WILT OF SWEET POTATO

J.M. NGEVE¹ AND R.W. RONCADORI²

Three sweet-potato cultivars were inoculated with *Fusarium oxysporum* f. sp. *batatas* (FOB) alone, vesicular-arbuscular mycorrhizae (VAM) fungi alone, or a combination of the two and grown at two temperatures and in soil with different levels of phosphorus. At 32°C, *Fusarium* wilt prevalence and severity were highest when plants were inoculated with FOB alone but, at 21°C, were highest when plants were inoculated with FOB and VAM jointly. Wilt prevalence and severity increased with increasing P fertilization, and VAM conferred protection to plants at low-P fertility (5 : 10 : 15 N, P, and K, ppm/g soil being 10, 4.4, and 8.3, respectively). This is the first report of the interaction of VAM fungi, P fertility, and temperature on *Fusarium* wilt of sweet potato. The role of VAM fungi in protecting plants against wilt and possible explanations for the observed results are discussed.

Much is now known about the etiology, epidemiology, and control of *Fusarium* wilt. However, little is known about how environmental factors affect disease development. This study was conducted, therefore, to determine the combined and separate effects of ambient temperature, soil phosphorus, and vesicular-arbuscular mycorrhizae (VAM) on incidence and severity of *Fusarium* wilt.

MATERIALS AND METHODS

We used three sweet-potato cultivars—W160, Centennial, and Sunnyside—that were obtained from the Southern Region Vegetable Laboratory, at Charleston, South Carolina, USA, and rated, respectively, as highly resistant, moderately resistant, and highly susceptible to *Fusarium* wilt.

The cultivars were grown in a mix of loamy soil and sand (1 : 1, v/v) and fertilized every 21 days with a 10-4.4-8.3 NPK fertilizer at a rate of 400 µg/g soil. Tip cuttings, 15-20 cm long, were taken from each cultivar and used in the experiments. All but four or five fully expanded leaves were removed, and succulent as well as woody cuttings were discarded.

A mix of shredded and screened Dothan loamy sand, washed river sand, and vermiculite (4 : 1 : 1, v/v/v) was used. Raw soil was analyzed by the Soil Testing and Plant Analysis Laboratory, University of Georgia, USA, and the results indicated pH 5.2, NO₃-N 9 µg/g, P 3.5 µg/g, K 50 µg/g, Ca 599 µg/g, Mg 119 µg/g, Zn 2 µg/g, Mn 97 µg/g, B 0.2 µg/g, 2.5% organic matter, and 10 × 10⁻¹ mhos soluble salts. The pH of the soil was adjusted to 5.5-6.0 with lime. The soil was amended with NPK (5 : 10 : 15), 700 µg/g, and supplemented with various concentrations of CaHPO₄ to supply additional P at 0, 50, 100, 400, and 800 µg/g. The soil was fumigated with methyl bromide (Dowfume MC-2) at a minimum rate of 1.4 kg/800 L of soil mix for at least 48 h under polyethylene and was aired for a minimum 5 days. In all studies, soil was placed in 15-cm-diameter plastic pots and maintained on a greenhouse bench or in a plant-growth chamber.

After screening isolates of *Fusarium oxysporum* f. sp. *batatas* (FOB) from South Carolina and Louisiana, we chose two isolates, FOB 6 (highly virulent) and FOB 5 (moderately virulent), for all investigations. The isolates were determined to be free from bacteria, and inoculum was prepared from a single-spore culture maintained at 5°C on potato dextrose agar (PDA). Only one culture in a single plate was used to make all transfers for inoculum increase.

FOB was increased on PDA or potato dextrose broth (PDB). PDA was prepared with infusion from 200 g potato (*Solanum tuberosum*), 20 g glucose, 20 g agar, and water to make 1 L.

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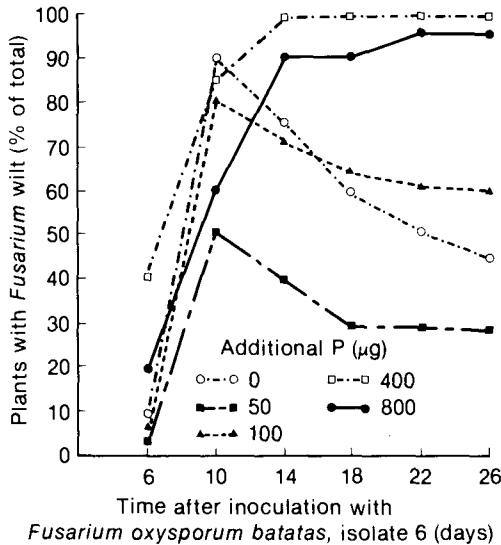


Fig. 1. Influence of increasing phosphorus fertility on prevalence of *Fusarium* wilt in Sunnyside (all treatments included a basic soil amendment of 700 µg 10-4-4-8.3 NPK/g soil).

The only difference in PDB was that the agar was not included.

Bud-cell inoculum was grown by modification of the method of French (1965). Bud cells were collected on cheesecloth; resuspended in sterile, distilled water; and adjusted to 1.5×10^6 cells/mL with a colorimeter at 600 nm wavelength.

Vesicular-arbuscular mycorrhizal (VAM) fungi were maintained on *Sorghum bicolor* in pot culture, and the spores were extracted by centrifugal flotation (Jenkins 1964). Of the several VAM fungi studied, *Glomus fasciculatum* and *G. mosseae*, which colonized sweet-potato roots well, were selected as test symbionts.

Two inoculation techniques were used. In studies of FOB inoculum alone, we used a modification of McClure's (1950) method, immersing the severed ends of vine cuttings in a beaker of bud-cell suspension for 5 minutes before planting. With this technique, wilt symptoms appeared within 7 days. In FOB-VAM interaction tests, simultaneous inoculations were done, along with sequential inoculations, mainly to confirm results of simultaneous inoculations. In simultaneous inoculations, 25 mL each of FOB, VAM, and spore filtrate were placed in the planting holes and the cutting planted 6 cm deep. The delayed inoculation consisted of adding VAM inoculum or filtrate (to standardize the internal mycoflora) at planting and FOB inoculum 21 days later, at which time the roots

were assayed for development of mycorrhizae. *Fusarium* wilt studies lasted 28 days, and all tests involving mycorrhizae lasted 42-84 days.

Two plant-growth chambers were set at 21° and 32°C for studies of the effects of ambient temperature on *Fusarium* wilt.

The treatments were arranged in a randomized block design, with 10 replications. Treatment means were compared, where appropriate, with Fisher's least-significant difference test ($P < 0.05$).

Fusarium wilt development was scored subjectively, with 0 being no symptoms; 1, one-third of foliage showing interveinal yellowing; 2, two-thirds of foliage with interveinal yellowing; 3, all leaves showing interveinal yellowing, beginning of wilting; and 4, plants wilted and dead. Prevalence was calculated as the number of infected plants divided by the total number of plants in the treatment and was expressed as a percentage. Percent severity was also computed: the sum of scores for a treatment was divided by the product of the maximum score and the total number of plants in that treatment $\times 100$.

RESULTS

Fusarium wilt was generally influenced by phosphorus concentration. Prevalence was maximum in all treatments 10-14 days after inoculation (Fig. 1). However, after this period, it declined to 60% or less in plants grown in soils

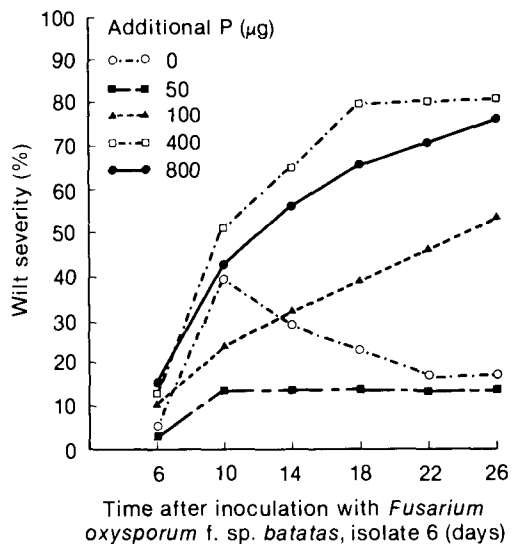


Fig. 2. Influence of phosphorus fertility on severity of *Fusarium* wilt in Sunnyside.

Table 1. Effect of ambient temperature on severity of *Fusarium* wilt.

| | Inoculum ^a | Severity (%) | | | | | | Mortality ^b (%) |
|--------------------------------------|-----------------------|------------------------|-----|-----|-----|-----|-----|----------------------------|
| | | Days after inoculation | | | | | | |
| | | 8 | 12 | 16 | 20 | 24 | 28 | |
| 21°C | | | | | | | | |
| Sunnyside | FOB-5 | 5 | 15 | 18 | 10 | 18 | 20 | 0 |
| | FOB-6 | 10 | 30 | 30 | 38 | 38 | 48 | 30 |
| Centennial | FOB-5 | 18 | 20 | 23 | 20 | 20 | 20 | 0 |
| | FOB-6 | 28 | 38 | 28 | 30 | 33 | 30 | 10 |
| W160 | FOB-5 | 5 | 3 | 3 | 3 | 0 | 0 | 0 |
| | FOB-6 | 25 | 25 | 18 | 13 | 10 | 3 | 0 |
| 32°C | | | | | | | | |
| Sunnyside | FOB-5 | 8 | 30 | 53 | 55 | 55 | 63 | 50 |
| | FOB-6 | 15 | 38 | 53 | 65 | 70 | 68 | 60 |
| Centennial | FOB-5 | 13 | 33 | 33 | 25 | 25 | 20 | 0 |
| | FOB-6 | 13 | 15 | 23 | 23 | 30 | 25 | 10 |
| W160 | FOB-5 | 0 | 0 | 3 | 3 | 3 | 15 | 10 |
| | FOB-6 | 0 | 13 | 13 | 13 | 18 | 23 | 0 |
| LSD (P < 0.05)^c | | 1.9 | 3.2 | 4.3 | 4.5 | 5.1 | 4.7 | — |

^aModerately virulent isolate 5 or the highly virulent isolate 6 of *Fusarium oxysporum* f. sp.

^bPlants killed at the end of trial expressed as a percentage of the total number of plants in the treatment.

^cFisher's least-significant difference.

containing 100 µg P or less. More than 90% of the plants in the 400 and 800 µg P soils had wilt symptoms 26 days after inoculation.

Disease symptoms, also, were increased by higher concentrations of P in the soil. Plants grown in soil supplemented with 400 µg or 800 µg P did not differ significantly ($P < 0.05$) overall from the others, but disease severity among them progressed rapidly from 10–20% 6 days after inoculation to 80% at the end of the study (Fig. 2). The plants in the other treatments were equally affected eventually; however, symptom severity peaked 10 days after inoculation and, in the soils that either were unsupplemented or had only 50 µg additional P, was significantly ($P < 0.05$) less than at P concentrations of 400 µg and 800 µg. Plants grown in the soil supplemented with 100 µg P expressed a medium level of symptom severity and were significantly different from plants grown at other P rates.

The effect of ambient temperature on *Fusarium* wilt varied with the plant cultivar and isolate of FOB. *Fusarium* wilt developed more rapidly at 32°C than at 21°C in Sunnyside irrespective of the pathogen isolate (Table 1). Likewise, plant mortality of Sunnyside was greater at the higher temperature and increased at both temperatures during incubation.

The W160 cultivar reacted similarly, with the temperature effect more evident at the end of the study (Table 1). Resistant plants inoculated

with either isolate were more severely affected by wilt at the higher temperature than those at low temperature during the early portion of the study. Plant mortality was 10% or less at both temperatures.

Wilt development was not as clearcut in the moderately resistant Centennial. Symptom expression was not increased with time at low temperature (Table 1) and was erratic at the higher temperature with FOB 5 but greater at the end of the study with FOB 6. Likewise, symptom severity was not clearly affected by temperature in plants inoculated with FOB 5 but was greater in plants infected with FOB 6 at low temperature.

Both phosphorus and mycorrhizae influenced expression of wilt in studies of mycorrhizal plants grown in NPK-fertilized soils with (100 µg/g) and without phosphorus supplements. For example, plants, irrespective of cultivar, generally had a higher prevalence of wilt in P-supplemented than in unsupplemented soils (Table 2). In the latter, mycorrhizae reduced the prevalence of wilt in Sunnyside but tended to increase it in Centennial and W160. *Glomus fasciculatum* suppressed wilt in Sunnyside, increased it in W160 grown in unsupplemented soils, but had no effect on Centennial. Mortality of plants with mycorrhizae was greater in the high-P soil (Table 2).

Glomus fasciculatum formed mycorrhizae

better than *G. mosseae* in all cultivars and synthesized mycorrhizae better in Sunnyside (92%) and W160 (90%) than in Centennial (72%) in plants grown in low-P soil.

Wilt severity was also affected by cultivar, temperature, and VAM development. Symptoms were greater in Sunnyside than in W160 irrespective of treatment (Table 3). Temperature did not affect wilt development noticeably in nonmycorrhizal Sunnyside, but, in W160, disease severity was greater at 21°C than at 32°C. Symptoms continued to develop at both temperatures in Sunnyside, but W160 plants generally recovered.

Temperature affected the VAM-FOB interaction in Sunnyside (Table 3). Disease severity at 21°C was delayed somewhat in mycorrhizal plants but was significantly different from that in nonmycorrhizal plants at the end of study. At 32°C, symptoms were suppressed markedly by both endophytes. Severity rating for nonmycorrhizal plants was 67.5% after 26 days but only 15% and 20% for plants inoculated with *G. fasciculatum* and *G. mosseae*, respectively. Root colonization by *G. fasciculatum* and *G. mosseae*

was 35% and 30%, respectively, at low temperature and 75% and 42% at the high temperature. The FOB-VAM interaction in resistant (W160) plants was similar.

DISCUSSION AND CONCLUSIONS

Host nutrition and environmental factors had a marked effect on the reaction of sweet-potato cultivars to *Fusarium* wilt. Some investigators (Harter and Whitney 1927; Hemmi and Watanabe 1933) have reported that FOB is a high-temperature pathogen mainly infecting sweet-potato plants at temperatures of 32–35°C. Hemmi and Watanabe (1933), reporting results from greenhouse studies, indicated that the fungus had a short incubation of 6 h in the host at 32°C. Harter and Whitney (1927) indicated that, for field infection, the fungus required a temperature of 30°C. In this investigation, the temperature at which maximum infection occurred was influenced by VAM. *Fusarium* wilt was highly prevalent and more severe at 32°C than at

Table 2. Interaction of vesicular-arbuscular mycorrhizae, soil phosphorus, and *Fusarium* wilt in sweet potato.

| Fertility ^a | Inoculum ^b | Prevalence (%) | Severity (%) | Mortality (%) | Mycorrhizae (%) |
|--------------------------------------|-----------------------------|----------------|--------------|---------------|-----------------|
| Sunnyside | | | | | |
| Low | FOB | 50 | 85 | 60 | — |
| Low | FOB, <i>G. fasciculatum</i> | 40 | 65 | 50 | 02 |
| Low | FOB, <i>G. mosseae</i> | 42 | 60 | 40 | 28 |
| High | FOB | 60 | 90 | 75 | — |
| High | FOB, <i>G. fasciculatum</i> | 50 | 70 | 60 | 62 |
| High | FOB, <i>G. mosseae</i> | 48 | 75 | 65 | 22 |
| Centennial | | | | | |
| Low | FOB | 40 | 60 | 55 | — |
| Low | FOB, <i>G. fasciculatum</i> | 45 | 40 | 40 | 72 |
| Low | FOB, <i>G. mosseae</i> | 55 | 35 | 30 | 20 |
| High | FOB | 55 | 65 | 62 | — |
| High | FOB, <i>G. fasciculatum</i> | 45 | 50 | 60 | 42 |
| High | FOB, <i>G. mosseae</i> | 50 | 50 | 85 | 38 |
| W160 | | | | | |
| Low | FOB | 20 | 40 | 40 | — |
| Low | FOB, <i>G. fasciculatum</i> | 30 | 20 | 20 | 90 |
| Low | FOB, <i>G. mosseae</i> | 38 | 25 | 45 | 28 |
| High | FOB | 35 | 50 | 45 | — |
| High | FOB, <i>G. fasciculatum</i> | 20 | 40 | 40 | 30 |
| High | FOB, <i>G. mosseae</i> | 35 | 45 | 45 | 32 |
| LSD (P < 0.05)^c | | 8 | 13 | 14 | — |

^aLow = 700 µg 10, 4.4, 8.3 ppm NPK/g soil; High = low plus 100 µg P/g soil (P as CaHPO₄).

^bFOB = *Fusarium oxysporum* f. sp. *batatas* (isolate 6); all inoculum applied at planting.

^cFisher's least-significant difference.

Table 3. The interaction of vesicular-arbuscular mycorrhizae and ambient temperature on severity of *Fusarium* wilt in Sunnyside (susceptible) and W160 (resistant).

| | Inoculum ^a | Severity (%) | | | | | |
|-----------------------------|-----------------------------|------------------------|------|------|------|------|------|
| | | Days after inoculation | | | | | |
| | | 6 | 10 | 14 | 18 | 22 | 26 |
| Sunnyside | | | | | | | |
| 21°C | FOB | 12.5 | 42.5 | 50.0 | 55.0 | 62.5 | 72.5 |
| | FOB, <i>G. fasciculatum</i> | 2.5 | 37.5 | 50.0 | 60.0 | 67.5 | 72.5 |
| | FOB, <i>G. mosseae</i> | 15.0 | 27.5 | 37.5 | 45.0 | 45.0 | 50.0 |
| 32°C | FOB | 35.0 | 47.5 | 42.5 | 57.5 | 57.5 | 67.5 |
| | FOB, <i>G. fasciculatum</i> | 0 | 2.5 | 2.5 | 7.5 | 7.5 | 15.0 |
| | FOB, <i>G. mosseae</i> | 5.0 | 17.5 | 22.5 | 32.5 | 27.5 | 20.0 |
| LSD (P < 0.05) ^b | | 19.2 | NS | 17.9 | 21.2 | 22.8 | 25.6 |
| W160 | | | | | | | |
| 21°C | FOB | 10.0 | 7.5 | 15.0 | 12.5 | 15.0 | 12.5 |
| | FOB, <i>G. fasciculatum</i> | 17.5 | 15.0 | 22.5 | 15.0 | 22.5 | 17.5 |
| | FOB, <i>G. mosseae</i> | 10.0 | 7.5 | 12.5 | 15.0 | 20.0 | 20.0 |
| 32°C | FOB | 5.0 | 2.5 | 2.5 | 7.5 | 5.0 | 2.5 |
| | FOB, <i>G. fasciculatum</i> | 15.0 | 0 | 0 | 5.0 | 5.0 | 0 |
| | FOB, <i>G. mosseae</i> | 20.0 | 2.5 | 5.0 | 2.5 | 0 | 0 |
| LSD (P < 0.05) ^b | | 6.4 | 2.0 | 3.7 | 3.7 | 2.8 | 2.5 |

^aPlants inoculated with isolate 6 of *Fusarium oxysporum* f. sp. *batatas* (FOB) alone, or simultaneously with either *Glomus fasciculatum* or *Glomus mosseae*.

^bFisher's least-significant difference.

21°C. However, in another study in which mycorrhizal plants were challenged with FOB and grown at 21° and 32°C, the temperature effect was reversed, wilt prevalence and severity being higher at 21°C. Our results suggest that, at 32°C, VAM formed a lot faster and provided the plant with protection from infection by FOB. In contrast, Schenck and Kellam (1978) observed a growth depression of soybean caused by mycorrhizae at low temperatures, the mycorrhizae probably being so inefficient that they act as a sink for carbohydrates and thus aid in disease development.

The influence of soil P on *Fusarium* wilt is poorly understood. Dubey (1959) reported an increase in mortality caused by wilt in sweet potato with increased N and P fertilization in a field trial. He also found no effect with K or with the interaction of N, P, and K. The threshold at which mortality was increased was not demonstrated. In our investigation, prevalence, severity, and mortality of wilt-infected plants were higher with increasing P fertility. However, at a certain threshold of P (approximately 400 µg/g soil), wilt severity appeared to remain stable despite additional P. The exact role of P in increasing wilt in this crop is not known. Probably, the P-mediated increase in shoot growth makes

plants succulent and, thus, more susceptible, or increase in transpiration in high-P plants may increase their susceptibility.

VA mycorrhizae have been reported to increase P uptake, principally in P-deficient soils (Gerdemann 1968). In our study, we found that mycorrhizae reduced wilt in plants grown in low-P soil. Mycorrhizal plants grown in low-P soil were expected (according to Gerdemann 1968) to contain high concentrations of mycorrhizae-mediated P and, therefore, to show high wilt severity because earlier studies had established that wilt increased with high concentrations of P.

The findings, however, were not what we expected: wilt was reduced in mycorrhizal plants grown in low-P soil. Thus, we feel that other factors, modifying the physiology of the host plants, are responsible for the protection that mycorrhizae conferred against wilt in low-P soil. For instance, Gianinazzi-Pearson et al. (1978) reported that each VAM fungus produces specific phosphates. This may mean differences in the way they affect phosphorus metabolism, which in turn might be related to wilt severity. Dehne and Schonbeck (1979) reported that mycorrhizae influenced phenol metabolism and thus promoted lignification of tomato and cucumber roots. As phenols have been reported

(Matta et al. 1969) as the main substances responsible for wilt resistance, mycorrhizae may increase the amount of available phenols in sweet potato, thereby increasing wilt resistance by mycorrhizal plants. Baltruschat and Schonbeck (1972) showed that extracts from mycorrhizal roots added to malt agar cultures of *Thielaviopsis basicola* inhibited chlamydospore production by 80–100%. They later (1975) reported that the inhibition of chlamydospore production reflected the high content of arginine in mycorrhizal root extracts. Their findings lend support to the idea that changes in host physiology may be responsible for the antagonistic reaction of VAM to FOB.

Investigators have reported that VAM increases (Ross 1972), decreases (Baltruschat and Schonbeck 1972, 1975; Chou and Schmitthener 1974; Schonbeck and Dehne 1977; Schenck and Kellam 1978), or has no effect (Schenck and Kellam 1978) on plant diseases. Our investigation suggests that the exact outcome of mycorrhiza–FOB interaction in sweet potato is dependent on environmental conditions such as

temperature. Other variables such as host genotype and type of VAM play a significant role in the interaction.

The next logical step is to progress from growth chamber and greenhouse studies to the field to assess the importance of mycorrhizae in commercial disease control. Studies on techniques for practical increase and introduction of VAM on agricultural soils may become urgent in increasing yield and in controlling disease especially in developing countries. The effects of other environmental factors such as pH, photoperiod, and salinity on FOB–VAM interaction need to be studied so that the optimal conditions for the use of mycorrhizae in biological control may be better understood.

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