EFFECT OF VARIED IRON, MANGANESE AND ZINC NUTRITION ON THE IN VITRO GROWTH OF RACE 2 FUSARIAOXYSPORUM F. SP. LYCOPERSICI AND UPON THE WILTING OF TOMATO CUTTINGS HELD IN FILTRATES FROM CULTURES OF THE FUNGUS

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ABSTRACT

Fusarium oxysporum f. sp. lycopersici race 2 was grown in stationary liquid cultures with varied amounts of iron, manganese and zinc. Iron deficiency (0.00, 0.06 and 0.13 ppm iron) severely limited growth in terms of dry weight of mycelial mats, but 0.25 ppm iron was adequate for maximal growth. Sporulation was limited when iron was omitted from the cultures. Omission of manganese slightly, but significantly, reduced mycelial growth. Sporulation was limited by low levels of manganese and was increased step-wise as manganese was increased from 0.06 to 1.0 ppm. Zinc deficiency severely limited growth and sporulation of the fungus. Sporulation increased step-wise as the zinc content was increased from 0.06 to 1.0 ppm. Germinability of spores was greater, however, for deficiencies and lower levels of the three micronutrients.

Unrooted cuttings of ‘Homestead 24’ and ‘Walter’ cultivars were placed for 24 hours in filtrates from all the cultures of Fusarium that had received varied iron, manganese, and zinc to bioassay for wilt toxins. Filtrates from cultures receiving higher levels of iron and zinc (0.5 to 2.0 ppm iron and 0.13 to 4.0 ppm zinc) were more effective in producing wilting than were the lower levels. Omission of manganese resulted in less wilting of ‘Homestead 24’ while both 0 and 0.06 ppm manganese were associated with less wilting of ‘Walter’. Higher levels of manganese generally were associated with severe wilting.

INTRODUCTION

There are many reports in the literature (2, 3, 4, 5, 6, 9) which implicate iron, manganese, and zinc in altering the virulences of Fusarium oxysporum Schlecht f. sp. lycopersici (Sacc.) Snyd. & Hans. Research (2, 3, 4, 9) has been underway for the past 5 years to develop cultural procedures that would permit the control of race 2 Fusarium in the field as well as to provide an understanding of the basic mechanisms involved in effective control procedures. This report presents results relative to the potential roles of iron, manganese, and zinc in the disease process.

Fusaric acid and lycomarasmin (1, 8) are produced by Fusarium oxysporum f. sp. lycopersici in liquid culture and act as toxins to tomato plants. There is, however, little agreement as to the in vivo roles of these toxins which are generally not considered to be important in the development of Fusarium wilt. The present report does not contribute to this debate but does present information as to the effects of iron, manganese and zinc on the production of unidentified wilt toxin(s) in cultural filtrates of F. oxysporum f. sp. lycopersici.

MATERIALS AND METHODS

F. oxysporum f. sp. lycopersici race 2 was grown from a single micro-conidial culture and was then tested for pathogenicity. Spores for inoculation of liquid cultures were produced on agar plates using galactose as the sole carbon source as a precautionary measure to inhibit growth of contaminating bacteria that might be present despite aseptic procedures that were used. Five ml of sterile deionized water was used to wash the spores from each petri plate. Each of the six 250 ml Erlenmeyer flasks containing 80 ml of nutrient solution was inoculated with 0.1 ml containing 370,000 microconidia. Following inoculation, all cultures were swirled to distribute spores throughout the liquid media.

Reagent grade chemicals used as sources of nutrient elements were purified in regard to heavy metal contaminants by dissolving ½ g sodium sulfide in each liter of stock solution, adding 20 g CaCO₃, and stirring for a reaction period of 5 min. Solutions were then filtered three times through the CaCO₃ layer that formed on the filter paper.
Care was taken not to disturb the CaCO₃ layer so that the removal of sulfide, hydroxide, and carbonate compounds of the heavy metals could be accomplished. All glassware used in the experiment including culture vessels was washed with 1:100 HCl and rinsed with deionized water. Iron, manganese and zinc sulfates were dissolved in hot water and cooled to purify these micronutrient compounds by recrystallization.

Flasks containing the nutrient solutions, adjusted to pH 6.5, six replicates per treatment, were autoclaved for 15 min at 121 C. The standard nutrient solution was prepared by adding the appropriate amounts of nutrient compounds for each liter of solution prepared with deionized water. Macronutrients (g/L) were NH₄NO₃, 1.14; NaH₂PO₄·H₂O, 0.45; KC1, 0.58; MgSO₄·7H₂O, 0.41; and CaCl₂, 0.15. Micronutrient compounds (mg/L), were CuSO₄·5H₂O, 0.38; FeSO₄·7H₂O, 2.5; MnSO₄·H₂O, 1.5; NaMoO₄·2H₂O, 0.05; and ZnSO₄·7H₂O, 4.3. Cultures were maintained in a controlled environment, 28 ± 0.5C and 500 ft-c of illumination from “cool white” fluorescent tubes. Cultures were held stationary during a one week incubation period. The contents of each of 3 culture flasks per treatment were macerated three times with three-second mincings in a microblendor and the contents used as sources of inoculum and spore suspensions for counting and germination studies. The remaining triplicate flasks were used for collection of mycelial mat yield. Contents of flasks were filtered, washed twice with deionized water, and dried at 70 C before weighing mats. Germinability of spores was determined by holding vials of race 2 Fusarium oxysporum f. sp. lycopersici on growth and nutritional requirements of the fungus. Deficient cultures developed little pigmentation. As the iron level was increased, pigment production (pink-purple) increased.

Zinc deficiency (0.00 ppm) severely reduced mycelial growth compared with all levels of added manganese but the effect was mild. Mycelial mats grown without added manganese or with 0.06 ppm had a different color and physical appearance than all other mats, in that they had a tannish color and the mats were convoluted and dense rather than fluffy in appearance. At 0.13 ppm manganese and above, the mats were a pink-purple color with slightly more pigmentation at the higher manganese levels.

Table 1. Effect of varied micronutrient supply in liquid cultures of race 2 Fusarium oxysporum f. sp. lycopersici on growth and pH of culture filtrates.

<table>
<thead>
<tr>
<th>Micronutrient</th>
<th>Fe</th>
<th>Mn</th>
<th>Zn</th>
<th>Fe</th>
<th>Mn</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content, ppm</td>
<td>δ%</td>
<td>δ%</td>
<td>δ%</td>
<td>δ%</td>
<td>δ%</td>
<td>δ%</td>
</tr>
<tr>
<td>0</td>
<td>139</td>
<td>508</td>
<td>119</td>
<td>3.8</td>
<td>5.1</td>
<td>4.8</td>
</tr>
<tr>
<td>0.06</td>
<td>253</td>
<td>596</td>
<td>186</td>
<td>4.0</td>
<td>5.5</td>
<td>4.7</td>
</tr>
<tr>
<td>0.13</td>
<td>320</td>
<td>610</td>
<td>287</td>
<td>4.0</td>
<td>5.5</td>
<td>4.7</td>
</tr>
<tr>
<td>0.25</td>
<td>368</td>
<td>615</td>
<td>391</td>
<td>4.9</td>
<td>5.5</td>
<td>4.7</td>
</tr>
<tr>
<td>0.3</td>
<td>528</td>
<td>378</td>
<td>382</td>
<td>4.9</td>
<td>4.9</td>
<td>3.1</td>
</tr>
<tr>
<td>1.0</td>
<td>622</td>
<td>618</td>
<td>578</td>
<td>5.4</td>
<td>5.4</td>
<td>6.9</td>
</tr>
<tr>
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<td>395</td>
<td>646</td>
<td>5.2</td>
<td>5.3</td>
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</tr>
<tr>
<td>4.0</td>
<td>-</td>
<td>410</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

LSD, 5% Level 71 0.4

Visual ratings of degree of wilt were made after a 24 hour period of filtrate imbibition of cuttings.

Results and Discussion

Iron deficiency (0.00 to 0.13 ppm) severely reduced the dry weight yield of mycelial mat (Table 1) whereas 0.25 ppm iron was adequate to meet nutritional requirements of the fungus. Deficient cultures developed little pigmentation. As the iron level was increased, pigment production (pink-purple) increased.

Fluorides from the 0.00 to 0.13 ppm iron and 4 ppm zinc cultures were distinctly lower in pH than all others, indicating a disruption in metabolism.
lism that probably caused greater organic acid production. All pH values of the filtrates were somewhat lower than the original 6.5 of the nutrient solutions at the outset of the experiment. In general, the effect of increasing iron levels was to increase pH while variations in manganese content had less effect on pH. Increasing zinc levels did not cause pH changes of great magnitude until the level of 4 ppm was reached which caused a very acid reaction of pH 4.1 indicating a possible disruption in metabolism that resulted in organic acid accumulation.

A deficiency of iron resulted in decreased sporulation (Table 2). Increasing iron contents caused increasing sporulation until levels of 0.5 ppm and higher were reached when there was a decrease in sporulation. Germinability of spores was greatest for the 0.00 ppm iron cultures, with somewhat greater germinability also at 0.06 ppm iron compared with higher levels of iron. Spore production increased going from 0 to 1 ppm and then decreased at 2 ppm manganese. Germinability was greater at 0 ppm manganese than at higher levels. Sporulation increased as zinc was increased from 0 to 1 ppm and then leveled off for 2 and 4 ppm zinc. Decreasing zinc content generally resulted in decreasing germinability going from 4 to 0.00 ppm. The density of spores in the original nutrient solution may have influenced germinability due to possible auto-inhibition at high spore concentrations. The data for manganese and iron do not indicate such an effect predominates while data for zinc appear to suggest this possibility. Future experiments will be conducted in a manner to separate the effects of the original environment and nutrient solution and spore density.

Lower levels of iron nutrition, 0.00 to 0.13 ppm (Table 3), generally resulted in culture filtrates that produced little or no wilt in 'Homestead 24' and 'Walter' cultivars. The higher levels (0.25 to 2 ppm) caused moderate to severe wilting in both cultivars. Variations in manganese per se had little effect in reducing the wilting found in either cultivar with adequate iron (0.5 ppm) and zinc (1 ppm). Variations in the zinc nutrition of the Fusarium did not have a clear-cut effect. A deficiency (0.00 ppm) resulted in little or no wilting of both cultivars. Also, little wilt occurred in 'Homestead 24' with 1 ppm zinc.

It thus appears that low levels of iron nutrition result in the failure of the filtrates to produce wilting. Variations in manganese nutritional status have little effect and acute zinc deficiency (0.00 ppm) causes a failure of filtrate to cause wilting of tomato cuttings.

Disease index ratings were made for Manapal seedlings inoculated with the race 2 Fusarium growth with varied iron, manganese and zinc concentrations. There was significant effect of varied zinc nutrition, pre-conditioning the fungus generally reduced virulence at the lower levels of zinc nutrition (0.06 and 0.13 ppm) and also at the highest level (4.0 ppm). Insufficient inoculum was recovered from the 0.00 ppm zinc cultures to permit the standardized inoculation. The disease rating indexes made at 10 days after inoculation did not show significant differences continuing. The effect of zinc nutrition pre-conditioning was probably overcome by the zinc content of the composted soil.

**LITERATURE CITED**

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EFFECT OF OFF-SEASON CULTURE ON WEEDS, NEMATODES, AND POTATO YIELDS ON MARL SOILS

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Abstract

Three off-season cultures were evaluated on a Perrine marl soil devoted to annual winter production of potatoes in Dade County, Florida: mechanical and chemical fallowing, mowing, and summer cover cropping with mixed hybrid sorghums (Sorghum vulgare Pers.). The effects of these management practices were measured on weed infestations, nematode dynamics, and yields of subsequent potato crops.

The total yield of ‘La Rouge’ potatoes was directly related to weed control whether by fallowing or mowing. Poor sorghum stands resulted in heavy weed infestations and low yields. Discing 5 times at 3 to 6-week intervals or use of 2 applications of dalapon (5.1 lb ai/A) and paraquat (0.5 lb ai/A) alternately at 4 to 5-week intervals prior to the potato crop increased yields of U.S. No. 1 size A potatoes.

Four nematode species infested the soil of the potato field: Criconemoides onoense Luc, Helicotylenchus pseudorobustus (Steiner) Golden, Meloidogyne sp., and Tylenchorhynchus martini Fielding. The total yield of potatoes was inversely related to the population density of C. onoense prior to plowing in preparation for the potato crop.

Introduction

Approximately 7700 acres of Perrine marl soil in Dade County, Florida are planted to Irish potatoes each year from October through December and mechanically harvested the following spring. Because heavy weed growth hinders the operation of the mechanical harvesters, the crop is cultivated 4 to 6 times before lay-by to control weed pests such as nutgrass (Cyperus esculentus L.), bermudagrass (Cynodon dactylon (L.) Pers.), goosegrass (Eleusine indica Gaertn.), and smartweed (Polygonum persicaria L.). In the off-season fields are either seeded to a cover crop of mixed hybrid sorghums (short, medium, and tall cultivars) or abandoned to a native weed cover.

Several herbicides have been evaluated for control of weed species prevalent in Florida potato fields. McCubbin (5) obtained control of ragweed, pigweed, and smartweed with CDEC and DNBP on sandy soil, but failed to control nutgrass or crabgrass. Noonan (6) found that EPTC (Eptam) at the rate of 6 lb ai/A suppressed nutgrass for a period of 6 to 7 weeks on marl soil. Dalapon (6 lb ai/A applied 4 to 6 weeks prior to planting) is registered (9) for control of grasses in fields of white potatoes.

Off-season observational trials on the marl soil indicated that mowing programs might also be beneficial in reducing weed infestations in the following potato crops.

Upchurch, et al. (8) noted that relative nematode densities in the soil of cultivated areas may be dependent on the weed species which occupy the field and that differential control of weed species in a mixed population may alter species dominance in the nematode community associated with the ensuing crop.

Two experiments were initiated in a potato field on the Perrine marl of Dade County to evaluate effects of off-season cultural practices on 1)