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Commercial Planting Media Effective in Screening for Verticillium Wilt of *Capsicum annuum*

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Abstract. **Three commercial planting media (Sunshine Mix #1, Sunshine Mix #5, and Metro Mix 360) were compared with the standard soil mixture prepared at New Mexico State Univ. for their effectiveness in differentiating Verticillium wilt–resistant and susceptible accessions of chile (*Capsicum annuum* L.) Each medium was infested with *Verticillium dahliae* Kleb. microsclerotia and planted with the resistant and susceptible accessions. When susceptible populations exhibited severe symptoms, individual plants were rated for disease severity (1 = no symptoms to 9 = death). Mean disease severities of populations differed among planting media, and, regardless of medium, resistant and susceptible populations were readily differentiated. Mean disease severities of plants grown in Sunshine Mix #1 and Sunshine Mix #5 differed from those grown in the standard University soil mix, but all media provided reliable screening tests. Mean disease severities of plants grown in Metro Mix 360 were most similar to those of plants grown in the University mix. Furthermore, greater differentiation was apparent between resistant and susceptible accessions in Metro Mix 360 than in accessions grown in the University medium.**

Verticillium wilt causes severe loss in chile in many areas of the world, including the Rio Grande Valley of New Mexico (Bosland, 1987; Bruehl, 1987; Lindsey, 1985). The causal agent of this disease is the soil-borne fungus *Verticillium dahliae* Kleb. (Bruehl, 1987; Mace et al., 1981). Control with crop rotation, chemicals, and biological methods has been unsatisfactory, whereas genetic resistance of chiles to *V. dahliae* offers the best approach for management of this disease (Goldberg, 1995). Reports on screening *Capsicum* accessions for disease resistance are numerous, as are the techniques used (Palloix et al., 1990; Vargus et al., 1992; Woolliams et al., 1962). Many published techniques are reproducible, with the exception of the planting media. Proper replication of the screening procedure is possible only if the planting medium is readily available to other programs. Most programs use a planting medium that is unique to their location (Christen, 1981; Garas et al., 1986; MacHardy et al., 1974; Spink and Rowe, 1989), such as the medium used by the Chile Breeding Program at New Mexico State Univ. This problem could be eliminated by using a commercially available planting medium that is consistent and reliable in screening for Verticillium wilt. The medium must allow for the differentiation of resistant and susceptible genotypes, and must do so with equal or greater distinction than the unique, local soil mix known to be reliable in these tests. The medium also should provide an environment favorable for plant growth and disease development. To date, no commercial medium has been cited for use in these screening tests. These experiments were performed in an effort to identify a standard planting medium to be used in screening chiles for Verticillium wilt resistance.

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Materials and Methods

Three brands of commercial planting media were compared with the standard University soil mix prepared and used at New Mexico State Univ. The standard mix consists of a steam-pasteurized (1 h at 71°C) blend of 1 peatmoss : 1 silica sand : 1 native sandy loam (by volume). The commercial brands tested were Sunshine Mix #1, Sunshine Mix #5 (Fison's Horticulture, Vancouver, B.C.), and Metro Mix 360 (Grace Sierra Horticultural Products Co., Milpitas, Calif.).

The *V. dahliae* inoculum was cultured on potato carrot dextrose agar (Johnson, 1988) in petri plates for 6 weeks in darkness at 24 °C. The contents of the plates were blended with distilled water at a rate of 25 plates per 500 mL water. Number of microsclerotia per mL of water was quantified using an eosinophil counter slide. Inoculum was added at the rate of 1622 microsclerotia per cm^3 of planting medium, modified from a standard rate of 2000 microsclerotia per g of planting medium used in previous screenings (González-Salán and Bosland, 1992). The medium, inoculum, and a slow release fertilizer (Osmocote 14–14–14; Scott's-Sierra Horticultural Products, Marysville, Ohio; .7 mg·cm^{-3} of medium) were placed together in a cement mixer and mixed for 1 h. For each medium, three planting trays (37 × 30 × 8 cm) of hard plastic with no drainage holes were filled and the trays placed in a fiberglass soil temperature tank, located within the New Mexico State Univ. Chile Breeding Program greenhouse at the Fabian Garcia Science Center, Las Cruces, N.M. The tank (1.25 × 1.00 × 0.75 m) was filled with water and a plastic bench placed in the water; heating and cooling elements regulated the water temperature. The trays were placed on the bench in contact with the water and temperature of the media was maintained at 25 ± 1°C throughout the experiment. Air temperature ranged from 24–35 °C (day) to 13–24 °C (night). To control photoperiod and light intensity, sunlight was blocked with bamboo shades around the tank and a shade cloth as a roof. A high-pressure sodium lamp was centered above the tank providing an 18-h photoperiod with a light intensity of 250 μmol·m^{-2}·s^{-1} at plant height. Treatments were arranged in a completely randomized design within the tank.

Each tray was planted with two rows each of Verticillium wilt–resistant (P.I. 215699) and Verticillium wilt–susceptible (B.G.1668) accessions of chile. The resistant accession is not completely resistant and has reached a threshold of 75% resistance (González-Salán, 1993). Twenty seeds were planted in each row and the four rows in each tray were randomized. Individual plants were rated for disease when the susceptible population exhibited a disease severity of 7 or greater, ≈70 d after planting, using the following disease severity scale: 1 = no symptoms; 3 = slight chlorosis of leaves; 5 = leaf chlorosis and some necrosis with defoliation of lower leaves; 7 = nearly complete defoliation; and 9 = death. Even numbers were used to assess intermediate symptoms. Data were analyzed by the SAS System using the GLM procedure for analysis of variance and Duncan's new multiple range test for mean separation (SAS Inst., 1990). Contrasts also were conducted with the GLM procedure to compare the amount of differentiation between resistant and susceptible populations in the University medium with that between accessions grown in each of the three commercial media. The experiment was performed twice and the mean disease severity from populations in each planting medium was averaged over both experiments.

Results

Verticillium wilt–resistant and susceptible chile seedlings were successfully differentiated within each of the four planting media (Table 1). Mean disease severities of the populations, however, differed with medium used.

Both resistant and susceptible populations grown in the commercial media had mean disease severities that were either equal to or greater than those in the University mix.

The greatest amount of differentiation in mean disease severity of resistant and susceptible populations (DIFF) was with Metro Mix 360, followed by the University mix, Sunshine Mix #5, and Sunshine Mix #1. The set of individual contrasts that compared the DIFF of the University mix with each of the three commercial media revealed that Sunshine #5 had the DIFF most similar to that of the University mix ($P = 0.96$). The DIFF of Metro Mix 360 was next ($P = 0.91$), followed by Sunshine #1 ($P = 0.77$). While Sunshine #5 had accessions with a DIFF closest to accessions in the University mix, accessions grown in Metro Mix 360 had a DIFF greater than exhibited by accessions in the University mix. Of the commercial planting media evaluated, Metro Mix 360 was the best for differentiating between resistant and susceptible chile populations.

Discussion

The mean disease severity of both resistant and susceptible chile populations grown in the commercial media was greater than that of plants grown in the University mix, resulting in a more robust screening for Verticillium wilt.

Differentiation between resistant and susceptible populations was achieved in each of the three commercial media. The relatively high disease severity of the resistant population is accounted for by the incomplete resistance found in this accession. Nevertheless, the resistant populations were easily distinguished from the susceptible populations by visual observation which was corroborated by the statistical analyses.

The amount of differentiation (DIFF) is also an important criterion in screening chiles for resistance to Verticillium wilt. The contrasts revealed whether the two accessions in a planting medium have a DIFF that deviates significantly from that in the standard University soil mix. In fact, DIFFs of populations in the three commercial media were very similar to those in the University mix. No commercial planting medium differed significantly from the University mix with respect to differentiation, but only accessions grown in Metro Mix 360 exhibited a DIFF that was greater than those of accessions grown in the University mix. The greater the DIFF, the better the screening, making Metro Mix 360 a more suitable medium than the University mix.

The amount of differentiation between resistant and susceptible populations in all three commercial media was close to or greater than that in the University mix. Therefore, any of the commercial media tested could be used for reliable screening of chiles for resistance to Verticillium wilt. Metro Mix 360, however, provided the greatest amount of differentiation in the experiment, and therefore was chosen as the planting medium for future screenings of *Capsicum* for Verticillium wilt resistance.

Table 1. Mean disease severity[z] (averaged over two experiments) of resistant (R) and susceptible (S) populations of *Capsicum* grown in four planting media (standard University = STD, Sunshine Mix #1 = S1, Sunshine Mix #5 = S5, and Metro Mix 360 = MM) and the amount of differentiation between the populations (DIFF).

Planting medium	Mean disease severity		
	R plants	S plants	DIFF
STD	3.27 c[y]	7.13 ab	3.86
MM	4.09 bc	8.19 a	4.10
S5	4.28 bc	8.05 a	3.77
S1	5.20 a–c	8.46 a	3.26

[z]Disease severity from 1 = no foliar symptoms to 9 = plant death.
[y]Mean separation within columns by Duncan's new multiple range test, $P \leq 0.05$.

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