A Seedling Screen for Phytophthora Root Rot of Pepper, *Capsicum annuum*

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ABSTRACT

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A new method to screen pepper seedlings that can help identify resistance and susceptibility of pepper cultivars to Phytophthora root rot, caused by *Phytophthora capsici*, is described. The technique was used to successfully screen peppers for PRR resistance at 14 days postemergence. Individual plants identified as resistant survived to maturity and, therefore, could be used directly in plant breeding schemes. Furthermore, the technique allows screening of large plant populations to segregate genetic material, permitting the plant breeder time- and space-efficient selection of resistant individuals.

Additional keywords: disease resistance, index

Pepper (*Capsicum annuum* L.) is an important cash crop in New Mexico and the most profitable for growers. Peppers had a value-added crop value of $230 million in 1989. Phytophthora root rot (PRR), caused by *Phytophthora capsici* Leonian, is a major disease that limits pepper production in New Mexico. Found worldwide, *P. capsici* causes root rot, stem blight, pod rot, and leaf blight on peppers (10). PRR is common in the furrow-irrigated fields of the southwestern United States. It was first described in 1918, and the type specimen came from southern New Mexico (6). A 1908 observation of chile wilt in New Mexico also may have been caused by *P. capsici* (6).

No pepper cultivars resistant to PRR are available. The lack of peppers resistant to PRR may be because there is no effective screening technique. Current options for the screening for PRR include inoculating mature plants or growing peppers in infested fields. Mycelial suspension poured onto soil (4,5), transplanting to infested soil (1,7), root-dip inoculation (7), and inoculation with a zoospore suspension (9) have all been reported in differentiating PRR resistance in pepper. However, none of these techniques combine the usefulness of a seedling screen and the prevention of susceptible escapes. For indexing, the application of zoospores to the plant base and the immersion of roots into a mycelial and zoospore suspension appeared to be the only effective inoculation techniques for eliminating all susceptible plants (9). Previous research indicates juvenile plants, less than 30 days old, were too young to identify as resistant (1,4,6–8). However, development of a seedling screen would permit time- and space-efficient selection of resistant individuals from large segregating pepper populations.

This investigation reveals a rapid, simple, and effective technique to screen for Phytophthora root rot when pepper plants are challenged at the seedling stage. It eliminates the escape of susceptible individuals, while effectively identifying resistant plant material. The screening technique described in this report allows for the differentiation of resistant plants at 14 days postemergence. It also has the advantage that resistant individuals survive to reproductive age.

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MATERIALS AND METHODS

Host preparation. Several preliminary experiments to establish an appropriate zoospore concentration and host preparation were conducted in cultivars known to be susceptible. Only the adopted methods are described.

Pepper lines representing a range of pepper types were used for this investigation (3). They include New Mexico 6-4, a New Mexican type; CalWonder, a bell type; TAM Jalapeño, a jalapeno type; and Criollo de Morelos #334, a piquin type. New Mexico 6-4, CalWonder, and TAM Jalapeño are susceptible to Phytophthora root rot, and Criollo de Morelos 334 is resistant. The first filial generation between the susceptible and resistant cultivar, and reciprocal F_1 generations between Criollo de Morelos 334 and the susceptible cultivar, New Mexico 6-4, were produced.

Seeds were sown in individual cells, $4 \times 3 \times 5.5$ cm deep, in 12-cell multipots (Compack D812, A.H. Hummert Seed Co., St. Louis, MO) containing Terra-Lite Redi-Earth Peat-Lite Mix, which consists of Canadian sphagnum peat moss and vermiculite (W. R. Grace Co., Cambridge, MA). After seeds were placed in each cell, eight of the 12-cell Compacks were placed in a $51.5 \times 25.5 \times 5.7$-cm plastic tray containing drainage holes. After sowing, the peat mixture was watered immediately and as often as needed, usually once a day. The trays were placed on a greenhouse bench, where the air temperature was maintained at 28 ± 1 C during the day and 15 ± 1 C at night. After the cotyledons were fully expanded, three to four beads of a controlled-release fertilizer (Osmocote 14-14-14) were placed on the soil surface of each cell.

Inoculum. The *P. capsici* strain used was isolated from a pepper plant grown in a New Mexico field that was infested with Phytophthora root rot. The pure culture of this *P. capsici* isolate, which belonged to mating type A2 (PWB-24), was grown at 25 C for 5–7 days on V8 juice agar media (12). Mycelial plugs, 10 mm in diameter, were cut from the colonized agar with a No. 5 cork borer. A maximum of 15 plugs were placed in

150 ml of sterile distilled water for 48–72 hr to induce formation of sporangia. The water plates containing the agar plugs were placed at 10 C for 45–90 min to induce zoospore release. After the cold treatment, the plates were returned to 25 C for 30 min, then checked for zoospore release. When the number of zoospores released appeared to be abundant, the agar plugs and water were washed through a double layer of cheesecloth with glass distilled water. Inoculum was adjusted with a hemacytometer to 2,000 zoospores per milliliter.

Inoculation. A plastic tray without drainage holes was filled with tap water. The plant tray with drainage holes containing the 14-day-old pepper seedlings was put into the water-filled tray to saturate plant roots. Each cell was infested with 5 ml of inoculum, giving a final concentration of 10,000 zoospores per cell. The roots were maintained in this flooded condition for 48 hr. The plant trays with drainage holes were removed from the water-filled tray and placed on a greenhouse bench until disease severity was scored 7–14 days later.

Plants were evaluated on a standardized 10-point interaction phenotype scale, where 0 = no response, vigorous, healthy (as in uninoculated control); 1 = slight root darkening, vigorous, healthy; 3 = brown roots, slight stunting, very small lesions on stems; 5 = brown roots, small lesions on stems, lower leaves wilted, stunted plants; 7 = brown roots, large lesions on stems, girdling, whole plant wilted, and stunted; and 9 = death. Even numbers were used for intermediate responses. A disease index value of 2 or less was considered resistant, and a value greater than 2 was susceptible.

RESULTS AND DISCUSSION

All susceptible cultivars (New Mexico 6-4, CalWonder, and TAM Jalapeño) inoculated by this technique using *P. capsici* zoospores died within 14 days after inoculation, but the resistant line, Criollo de Morales 334, was highly resistant (Table 1 and Fig 1.). Resistant plants lacked any lesions on stems or roots; the taproot was healthy, and vigorous secondary roots had emerged. No symptoms developed during the period of plant growth. All resistant plants matured and set fruit. Even after fruit maturity, the resistant plants lived another 6 mo until the experiment ended.

This inoculation technique allowed for differentiation between pepper seedlings resistant and susceptible to PRR. In addition, the zoospore technique provided an effective way to apply the inoculum to pepper seedlings. Screening peppers for PRR resistance at the seedling stage gives plant breeders the advantage of screening large populations of segregating genetic material. The standard pepper population in a field is usually 32,123 plants per hectare. Using the method mentioned earlier, 18.5 m^2 of greenhouse bench provides enough space to test 32,123 individuals. This allows the investigator to select for resistant individuals in a time- and space-efficient method.

The ability to screen peppers at 14 days postemergence for PRR resistance allows further refinement of inoculation techniques with other pepper pathogens. These techniques may lead to multiple-disease resistant screens. In contrast to indexing, where the challenged host does not survive to the reproductive age (2), this technique allows the resistant individuals to survive, flower, and set fruit. The investigator can also evaluate for horticultural traits, as well as disease resistance. Our ability to screen for PRR resistance while the plant is at an early age does not preclude screening later. Criollo de Morelos 334 was challenged at 6 wk of age and did not appear to be any more susceptible than when tested at seedling age. In fact, resistant plants were unaffected by *P. capsici* when transplanted into infested field soil (P. W. Bosland and D. L. Lindsey, *unpublished*).

It is important not to place more than 15 agar pieces into the water when preparing the zoospore inoculum. Sporangia will not form if agar pieces are too numerous. Further experimentation indicated that nutrients, which leached from the agar into the water, retarded development of sporangia (P. W. Bosland and D. L. Lindsey, *unpublished*). In addition, an automatic pipetting machine is helpful for inoculating large populations of seedlings.

Reciprocal crosses between the susceptible parent, New Mexico 6-4, and the resistant parent, Criollo de Morales 334, displayed no maternal effect on PRR resistance. The F_1 generation was highly resistant when Criollo de Morales 334

Table 1. Disease severity of Phytophthora root rot on pepper cultivars 14 days after inoculation by the zoospore inoculation method

Cultivar[a]	Number of individuals	Disease index[b]
CalWonder	118	9.0 + 0.0
TAM Jalapeño	197	9.0 + 0.0
New Mexico 6-4	217	9.0 + 0.0
CdM 334	255	1.0 + 0.0
F_1 (NM 6-4 × CdM 334)	111	1.3 + 0.5
F_1 (CdM 334 × NM6-4)	98	1.5 + 0.6
F_1 (CW × CdM 334)	74	1.4 + 0.5
F_1 (TAMJ × CdM 334)	181	1.3 + 0.4

[a] CdM334 = Criollo de Morales 334, NM6-4 = New Mexico 6-4, CW = CalWonder, and TAMJ = TAM Jalapeño.

[b] Disease index and standard deviation; mean value of the number of individuals listed using a scale of 0–9, where 1 is highly resistant and 9 is very susceptible.

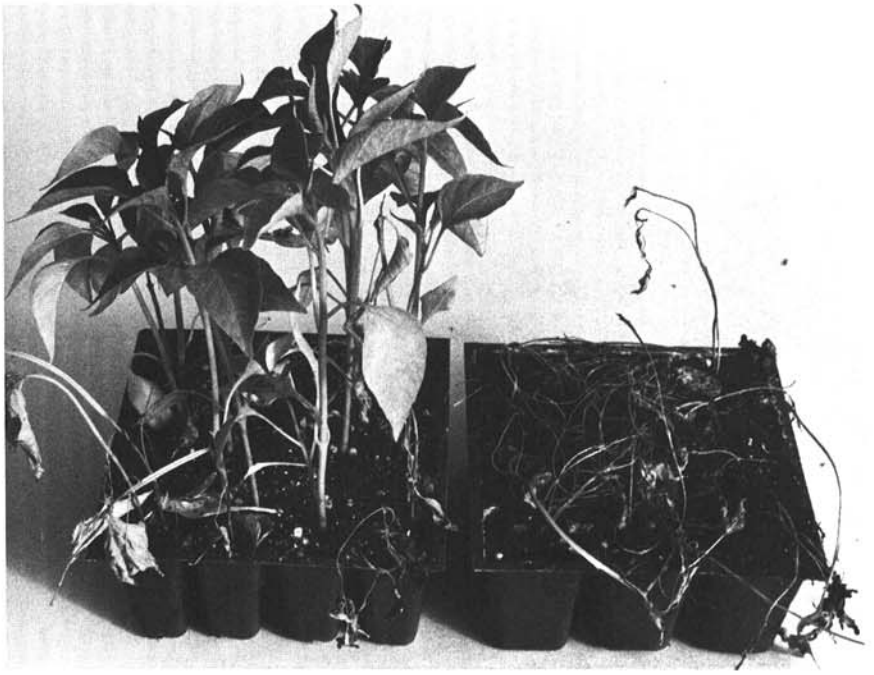

Fig. 1. Seedlings of the resistant F_1 of New Mexico 6-4 by Criollo de Morelos **(left)** and the susceptible New Mexico 6-4 **(right)** after being inoculated with zoospores of *Phytophthora capsici*.

was either the pollen source or the egg source (Table 1). Both F_1 populations had some susceptible individuals (Fig. 1). An earlier investigation indicates resistance to *P. capsici* is dominant, and our investigation supports this conclusion (11). An explanation for susceptible plants in the F_1 is that the resistant parent was heterozygous for resistance. Because resistance was reported to be dominant, the heterozygote parent would have a resistant phenotype, even though it contained an allele(s) for susceptibility.

This method has been used at New Mexico State University for the past 4 yr and is the only one used in our PRR testing program. Standard susceptible and resistant genotypes have reacted consistently from one test to another throughout this period. During this period of time, it has been observed that partial resistance can be seen in breeding material. This observation can be made by reducing the amount of time the roots are exposed to the saturated environment from 48 to 24 hr. In addition, the inoculum concentration can be easily manipulated to give a wide range of dosage responses.

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