Improved Methodology for Evaluating Resistance in Sweet Potato to Streptomyces ipomoea

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ABSTRACT


A method was developed for evaluating resistance in sweet potato to Streptomyces ipomoea. Resistance in 15 sweet potato clones was evaluated under field, greenhouse, and laboratory conditions. Before transplanting was done, field plots were artificially infested with an enriched sand medium colonized with S. ipomoea. Greenhouse trials were conducted in 15-cm clay pots containing infested sand diluted with autoclaved sand to provide an inoculum concentration of $10^5$ to $10^6$ propagules per cubic centimeter of sand.

Streptomyces ipomoea (Person & Martin) Waks. & Henrici causes a disease of fibrous and fleshy roots of sweet potato (Ipomoea batatas (L.) Lam.) (1,11). The disease is widespread, but its economic importance in North Carolina has been recognized only since 1978. Infected fibrous roots usually become necrotic, resulting in a significant suppression of stem growth and a lower yield of fleshy roots (1,5,9,10). Necrotic lesions may also occur on the fleshy roots, reducing their marketability.

Management of this disease has been limited to fumigating the soil and reducing the soil pH to 5.2 (9). Resistance to S. ipomoea has been reported in sweet potato breeding lines (10), but only two resistant cultivars are available, and they lack desirable horticultural traits and have not been widely accepted (6,7). The hexaploid genetics of sweet potato dictates evaluating large numbers of seedlings from every generation to ensure that selected clones do have the desired combination of horticultural traits and disease resistance. Efforts to incorporate resistance to S. ipomoea into new cultivars have been limited by the need to make evaluations in naturally infested fields, which are seldom large and uniform enough for such evaluations (10,12). Field tests are also adversely affected by excessive soil moisture due to abnormally high rainfall.

Several methods for evaluating sweet potato for resistance to S. ipomoea have been reported, including inoculating fibrous or fleshy root tissue with colonized agar media (9) and dipping root pieces in an inoculum suspension, then incubating them in moist vermiculite (3). These methods have not been adopted for general use, possibly because one study indicated that resistance to S. ipomoea in fibrous and fleshy sweet potato roots may be independently inherited (9).

The objective of our research was to develop reliable greenhouse or laboratory methods for evaluating resistance to S. ipomoea in sweet potato germ plasm.

MATERIALS AND METHODS

Pathogen. A strain of S. ipomoea was isolated from a lesion on a naturally infected sweet potato root collected in Johnston County, NC. This strain was pathogenic on sweet potato and had morphological and cultural characters consistent with those reported for S. ipomoea (4,11). The pathogen was maintained in sterile distilled water at 12-16 C and was increased from these stock cultures on a growth medium (4) for each experiment. Inoculum was prepared by transferring S. ipomoea from the growth medium to enriched sand (20 g of CaCO₃ and 20 g of dried and sieved [40 mesh] horse manure per liter of coarse sand that had been autoclaved for 1 hr), followed by incubation at 30 C for 5-7 wk. Containers were shaken at weekly intervals.

Infection of sweet potato roots by S. ipomoea was confirmed by isolating the organism from symptomatic tissue. Isolates were obtained by washing the root (fleshy or fibrous) in distilled water, crushing 1-mm³ pieces of necrotic tissue in 0.2 ml of sterile distilled water containing 0.85% NaCl and 2 mM mannitol, applying two loops of the macerate to the isolation medium (0.5 g of mannitol, 0.2 g of K₂HPO₄, 0.2 g of MgSO₄·7H₂O, 5.0 g of NaCl, 0.2 g of CaCO₃, 0.2 mg of CoCl₂, 0.05 g of yeast extract, and 20.0 g of agar in 1 L of distilled water; C. A. Clark, personal communication), then incubating the isolates at 32-36 C for 3-5 days in the dark.

Sweet potato germ plasm. Fifteen sweet potato cultivars and advanced breeding clones were selected for verification of methods and disease reaction assessment. These included Jasper and Travis, developed in part for their resistance to S. ipomoea at the Louisiana State Agricultural Experiment Station (6,7), and the susceptible cultivar Jewell. The cultivar Pope and I 1 advanced breeding clones, which had not been previously evaluated for S. ipomoea resistance, were included to further document the reproducibility of the disease reactions.

Root system testing and evaluations. Several preliminary studies were conducted with known resistant and susceptible cultivars to establish appropriate greenhouse conditions for evaluating sweet potato root systems for resistance to S. ipomoea. Only the adopted methods are described. In all trials, stem cuttings of each clone were grown for 10 wk in clay pots (15 cm diam) containing infested enriched sand adjusted with autoclaved sand to give a final inoculum concentration of $10^5$ to $10^6$ propagules per cubic centimeter of sand.

For each test, three pots of each clone were placed on a greenhouse bench in a completely randomized design. Each pot contained two cuttings of a single clone. Plants were watered twice
a day and fertilized once a week for 3 wk. During the remaining 7 wk, two methods were used to provide sufficient water for plant growth with minimal interference to disease development. The first method was to apply water by subirrigation as previously described for common potato scab (2). The second method, which was ultimately adopted, consisted of watering only after the plant had wilted. During winter months, the water temperature was equilibrated to the ambient greenhouse temperature for at least 24 hr in 113-L containers. The average ambient maximum/minimum greenhouse temperatures (C) for the three runs were 32/20.6, 30.7/21.6, and 27.9/21.6, respectively. Disease reactions were assessed separately for fibrous and fleshy roots. The number and the weight of fleshy roots, with and without surface lesions, were recorded for each plant. The proportion of necrotic fibrous roots (Fig. 1A) was visually estimated for each plant and analyzed by means of a modified Horsfall-Barrett scale (8): 0 = 0%, 1 = trace-6%, 2 = 7-12%, 3 = 13-25%, 4 = 26-50%, 5 = 51-75%, 6 = 76-87%, 7 = 88-94%, 8 = 95-99%, 9 = 100%.

**Fleshy root evaluations in the laboratory.** Disease development on fleshy roots was measured directly on No. 1 fleshy roots as a potential method of detecting differences between fleshy roots and fibrous roots in resistance to S. ipomoea. The freshly cut surfaces of 2-cm-thick transverse root slices were placed on infested sand adjusted with autoclaved sand to an initial inoculum density of $10^5-10^6$ propagules per cubic centimeter of sand. Propagule concentration was determined by dilution plating on the isolation medium previously described. The slices were arranged in a completely randomized design in plastic trays and incubated for 7-14 days at 30 C in the dark. Sand and surface growth of S. ipomoea were then removed by gently rubbing the slices with steel wool to expose necrotic tissue resulting from infection. The proportion of the surface area that was necrotic (Fig. 1B) was estimated by means of a pictorial guide (Fig. 2) based on the Horsfall-Barrett rating scale (8).

**Field evaluations.** The sweet potato cultivars and clones were

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**Fig. 1.** Symptoms caused on sweet potato roots by *Streptomyces ipomoea* after artificial inoculation: A, Fibrous root systems of Travis (resistant) and Jewel (susceptible) after 10 wk of growth in infested sand under greenhouse conditions. B, Travis and Jewel root slices after incubation on infested sand for 7-14 days at 30 C.

**Fig. 2.** Pictorial guide for estimating the percentage of sweet potato root slice surface area infected by *Streptomyces ipomoea*. Dark areas represent the observed pattern of necrosis after infection. Percentage classes were based on a modified Horsfall-Barrett scale.
evaluated in an artificially infested field plot of Wagner loamy sand. Inoculum (infested enriched sand) at the rate of 0.45 L of infested sand per meter of row was incorporated into the row by rototilling immediately before planting. The rows were on 1.07-m centers and the plants were on 0.3-m centers within rows. The experiment was arranged in a randomized complete block design consisting of five blocks, each containing 25 plants of each clone. Sprouts were transplanted from plant beds 3 June 1981 and harvested 23 September 1981. Fleshy roots were classified as diseased or healthy based on the presence or absence of lesions. Each class was further graded by size into U.S. market grades (No. 1 = 4.8 - 8.6 cm x 5.0 - 17.8 cm, canner = 2.5 - 4.8 cm x 5.0 - 17.8 cm, jumbo = 2.5 - 4.8 cm x 5.0 - 17.8 cm), and the weight of each grade was determined. Rainfall distribution during the test was 6.86 cm in June, 12.27 cm in July, 18.73 cm in August, and 1.27 cm in September.

**TABLE I.** Reactions of sweet potato root systems evaluated under nonfield conditions for resistance to Streptomyces ipomoeae

<table>
<thead>
<tr>
<th>Clone</th>
<th>Percent fibrous roots* necrotic</th>
<th>Percent fleshy roots* infected</th>
<th>Percent root slice† surface necrotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jewel</td>
<td>34.6 a'</td>
<td>74.0 a</td>
<td>47.7 a</td>
</tr>
<tr>
<td>835</td>
<td>23.6 b</td>
<td>73.0 a</td>
<td>19.6 cdef</td>
</tr>
<tr>
<td>848</td>
<td>19.4 bc</td>
<td>59.4 ab</td>
<td>41.0 bcd</td>
</tr>
<tr>
<td>727</td>
<td>15.3 bcd</td>
<td>59.4 ab</td>
<td>30.9 bc</td>
</tr>
<tr>
<td>718</td>
<td>15.3 bcd</td>
<td>64.8 ab</td>
<td>25.8 bcde</td>
</tr>
<tr>
<td>Pope</td>
<td>13.1 cde</td>
<td>60.8 ab</td>
<td>16.8 cdef</td>
</tr>
<tr>
<td>925</td>
<td>16.8 bc</td>
<td>60.8 ab</td>
<td>16.8 cdef</td>
</tr>
<tr>
<td>847</td>
<td>11.9 cde</td>
<td>84.6 bc</td>
<td>11.0 def</td>
</tr>
<tr>
<td>845</td>
<td>11.3 cde</td>
<td>53.2 ab</td>
<td>25.4 bcde</td>
</tr>
<tr>
<td>928</td>
<td>11.1 de</td>
<td>60.0 ab</td>
<td>6.0 f</td>
</tr>
<tr>
<td>L166</td>
<td>8.0 de</td>
<td>38.2 ab</td>
<td>8.5 ef</td>
</tr>
<tr>
<td>927</td>
<td>3.2 de</td>
<td>26.2 f</td>
<td>3.3 f</td>
</tr>
<tr>
<td>Jasper</td>
<td>3.0 e</td>
<td>19.3 c</td>
<td>...†</td>
</tr>
</tbody>
</table>

*Plants were grown for 10 wk in infested sand under greenhouse conditions.

Freshly cut root slices were placed on infested sand (10⁶ - 10⁷ propagules per cubic centimeter of sand) and incubated at 30°C for 7 - 14 days in a moist chamber.

†Means in the same column not followed by the same letter are significantly different (P < 0.05).

**RESULTS**

Levels of infection by *S. ipomoeae* were most consistent when the enriched sand substrate was colonized by *S. ipomoeae* before the sweet potato cuttings were planted. The colonized sand is henceforth referred to as the inoculum. Infection resulted in severe necrosis of the fibrous roots and lesions on the surface of fleshy roots of both susceptible and resistant cultivars when plants were grown in undiluted inoculum. The concentration of *S. ipomoeae* in the undiluted inoculum was consistently greater than 5 x 10⁶ propagules per cubic centimeter of sand. However, necrosis developed on less than 5% of the fibrous root system of resistant plants and on over 30% of the fibrous root system of the susceptible cultivar Jewel (Table 1) grown in inoculum diluted to an initial density of between 10⁶ and 10⁷ propagules per cubic centimeter of sand. In general, the percentage of necrotic fleshy roots was greater for each clone than the percentage of necrotic fibrous roots in the greenhouse experiments. The amount of necrosis also was more variable on fleshy roots than on fibrous roots. The percentage of necrotic fleshy roots produced by Jewel, however, was still significantly greater than that produced by the resistant lines (Table 1).

The resistant clones were also detected by incubating freshly cut root slices on the infested sand medium. Preliminary studies indicated that the assay was most reliable at 30°C. Resistance was overcome when the incubation temperature exceeded 32 - 34°C, and no necrosis was visible on the susceptible cultivar Jewel below 20°C. Approximately 50% of the surface area of Jewel root slices was necrotic after incubation at 30°C for 7 - 14 days, but necrosis developed on less than 10% of the surface area of root slices from resistant cultivars (Table 1).

The percentage of necrosis on the fibrous root system in greenhouse trials and the percentage of surface area necrosis on the root slice assay correlated most closely with reactions of sweet potato clones to *S. ipomoeae* under field conditions (Tables 2 and 3). These two criteria gave similar results (r = 0.782; P < 0.01) and were significantly correlated with total yield of all clones and of No. 1 roots produced in field trials (Table 3). The percentage of necrotic surface areas of root slices was negatively correlated with both the amount of fleshy roots produced and the proportion that was marketable (Table 3). Fibrous root necrosis, however, was not significantly (negatively) correlated with the percentage of marketable (symptomless) fleshy roots in the field.

Although relative disease severity was similar on fibrous and fleshy roots for most clones, relative resistance in fibrous and fleshy root systems was less consistent than in fleshy root systems.
TABLE 3: Correlation coefficients and level of significance between responses of sweet potato to Streptomyces ipomoea under field and nonfield methods

<table>
<thead>
<tr>
<th>Nonfield methods</th>
<th>Field methods*</th>
<th>All grades</th>
<th>No. 1</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>Percent marketable</td>
<td>Total</td>
<td>Percent marketable</td>
</tr>
<tr>
<td>Fibrous roots(^1)</td>
<td></td>
<td>-0.614</td>
<td>-0.360</td>
<td>-0.608</td>
<td>-0.369</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.02)</td>
<td>(0.21)</td>
<td>(0.02)</td>
<td>(0.19)</td>
</tr>
<tr>
<td>Fleshy roots(^2)</td>
<td></td>
<td>-0.545</td>
<td>-0.385</td>
<td>-0.472</td>
<td>-0.39</td>
</tr>
<tr>
<td>(wt)</td>
<td></td>
<td>(0.04)</td>
<td>(0.17)</td>
<td>(0.08)</td>
<td>(0.16)</td>
</tr>
<tr>
<td>Root slices(^3)</td>
<td></td>
<td>-0.590</td>
<td>-0.606</td>
<td>-0.599</td>
<td>-0.621</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.03)</td>
<td>(0.03)</td>
<td>(0.03)</td>
<td>(0.02)</td>
</tr>
</tbody>
</table>

*Evaluations conducted in artificially infested field plots.

\(^1\) Roots free from lesions.

\(^2\) Experiments conducted under greenhouse conditions by growing vine cuttings (14 clones) in infested sand for 10 wk.

\(^3\) Selections evaluated by incubating fleshy root slices (13 selections) on infested sand for 1-2 wk at 30 C.

Roots varied in some, such as 835. Under greenhouse conditions, clone 835 had relatively susceptible fibrous roots. The root slice assay indicated that fleshy roots were somewhat more resistant than would be expected from the reaction of fleshy roots of other clones with similar fibrous root reaction (Table 1). These observations were consistent with the relatively low yield of clone 835 in the field trial, while over 90% of the fleshy roots remained symptomless (Table 2).

DISCUSSION

We have used these methods for the past 2 yr to evaluate the responses of sweet potato clones to infection by *S.* *ipomoea*. With other methods (3,9), artificial inoculation procedures frequently overcame resistance, large numbers of clones could not be handled, and resistance differences in fibrous and fleshy roots were not detected. We have not encountered these problems.

Management of the tests was critical. For example, greenhouse plants were watered either sparingly by subirrigation or when they had wilted. Although the effect of water temperature on disease development was not specifically determined, results of winter trials were more consistent when water was equilibrated to the ambient greenhouse temperature.

Sweet potato clones expressing extreme levels of susceptibility or resistance could be distinguished by means of the greenhouse and laboratory methods. Clones showing a range of intermediate responses were also observed. The level of disease development among the intermediate clones did not differ significantly, but their relative ranking according to disease development in laboratory and greenhouse trials correlated significantly with the yield of marketable roots in the field test (Table 3). We have also observed Pope, identified here as one of the intermediate clones, to produce marketable roots in naturally infested field plots in amounts between those produced by the susceptible (Jewel) and the resistant (Travis) clones.

The significant correlation between clonal responses in the root slice assay and production of marketable roots in the field trial suggests that the root slice assay alone is sufficient to detect resistant clones (Table 3). This assay requires less space and inoculum and is much quicker. Thus, it is more desirable for preliminary evaluation of large numbers of genotypes.

The range of responses by sweet potato clones to infection by *S.* *ipomoea* observed in our investigation was similar to that measured previously in naturally infested fields (9,10). Highly susceptible clones yielded few or no marketable roots, whereas other clones produced higher yields of fleshy roots with varying degrees of resistance to lesion development. Our investigations support the hypothesis that separate forms of resistance to *S.* *ipomoea* may occur in fibrous and fleshy roots and that they may occur independently (eg, clone 835).

LITERATURE CITED

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