Resistance of Sweet Potato to Bacterial Root and Stem Rot Caused by *Erwinia chrysanthemi*

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ABSTRACT

Clark, C. A., Wilder-Ayers, J. A., and Duarte, V. 1989. Resistance of sweet potato to bacterial root and stem rot caused by *Erwinia chrysanthemi*. Plant Disease 73:984-987.

Greatest differences in resistance of sweet potato (*Ipomoea batatas*) storage roots to bacterial root rot were observed 6 or more days after a micropipette tip containing 50 μl of a 10^9 cfu/ml suspension of *Erwinia chrysanthemi* had been inserted 1 cm into the root. The frequency of disease was variable when the suspension contained less than 10^6 cfu/ml, but lesion size was consistent for each genotype at 10^7–10^9 cfu/ml. Differences among genotypes were related to the duration of active decay, whereas differences within genotypes were associated with location of the crop. Reaction among storage roots was not correlated with reaction of vines. Older "heirloom" cultivars were relatively resistant to root rot. Thus, susceptibility to bacterial root rot may have been introduced into breeding programs recently.

The first reported occurrence of bacterial stem and root rot of sweet potato (*Ipomoea batatas* (L.) Lam.), caused by *Erwinia chrysanthemi* Burkholder, McFadden, and Dimock, followed an epidemic in Georgia in 1974 (13,16). Although the disease can be found throughout the southeastern United States, it is not common and significant losses have been infrequent (4,13,16). In Louisiana, the disease has been observed most frequently on storage roots in plant beds. Economic loss to this disease may be associated with the use of sweet potato cultivars that are particularly susceptible (13,16). Initial evaluations for reaction to *E. chrysanthemi* were based on inoculation of vines. Significant differences in susceptibility and resistance to stem rot were observed among commonly used cultivars. However, sweet potato storage roots also are susceptible to *E. chrysanthemi* but may react differently than vines.

Recently, progress has been made in developing methodology for assessing and improving resistance in potato (*Solanum tuberosum* L.) tubers to *E. carotovora* subsp. *carotovora* (Jones) Bergey et al and *atroseptica* (van Hall) Dye (1,2,9,10,14,17). Below we report methods for screening sweet potato storage roots for reaction to bacterial root rot, data on the range of reaction in available sweet potato germ plasm, and a comparison of reactions of storage roots and vines of 55 cultivars. A preliminary account has been reported (5).

Approved for publication by the director of the Louisiana Agricultural Experiment Station as manuscript 89-38-3187.

Accepted for publication 19 July 1989 (submitted for electroic processing).

MATERIALS AND METHODS

Inoculum. Strain Ech-60 of *E. chrysanthemi* originally isolated from sweet potato and provided by J. W. Moyer, North Carolina State University, was used throughout this study. It is similar to other sweet potato strains in virulence to storage roots (Duarte, *unpublished*). Cells of the bacterium grown on slants of yeast extract-dextrose-calcium carbonate agar (10, 20, 20, and 15 g/L, respectively) at 32 C for 24–48 hr were suspended in sterile distilled water. The suspension was adjusted to an optical density of 1.0 at 620 nm (approximately 10^9 cfu/ml) unless otherwise specified.

Inoculation technique. In preliminary experiments, storage roots were sliced and inoculated by techniques modified from research with potato tubers (3,9,10,17). Cross sections approximately 5–7 mm thick were sliced from roots of either cv. Travis (intermediate to susceptible) or cv. Centennial (resistant) and placed on moistened filter paper in sterile petri dishes. A 10-μl aliquot of cell suspension from a 10-fold dilution series was deposited on the surface of each of four replicate slices. The slices were incubated at 16 or 28 C, and the extent of decay was observed at 1, 2, and 4 days after inoculation.

For whole storage roots, a micropipette tip containing 50 μl of inoculum was inserted to a depth of about 1 cm and left in position (12). The inoculated roots were placed in plastic vegetable baskets, which were stacked, covered with a black polyethylene bag, and incubated at 25–28 C for 6 days unless otherwise specified. The roots were then cut in cross section through the point of inoculation, and the depth and diameter of each lesion was measured. Ten replicate roots were inoculated for each genotype, and tests were conducted during the fall of 1986, 1987, and 1988. Centennial, Jewel, Travis, and Beauregard were included as standard cultivars in each test.

Comparison of root rot resistance by infectivity titration. Roots of Beauregard, Centennial, Jewel, and Travis were inoculated by the micropipette method with each of six 10-fold dilutions ranging from 10^4 to 10^9 cfu/ml in November 1986 and October 1988 (6–8). Each large root was inoculated with each concentration at randomly selected positions. Half the roots inoculated in the second test were wetted and wrapped in plastic wrap (Saran Wrap, Dow Chemical Company) to reduce oxygen concentration. The inoculum concentration that induced lesions in 50% of inoculations (ED_{50}) was estimated from plots of the $\log_{10}$ of inoculum concentration vs. probit response (converted from percentage of inoculations producing lesions) (7,8).

Root rot development over time. Centennial, Jewel (intermediate), and L81-10 (susceptible) roots were inoculated by the micropipette tip method with 50 μl of a 10^9 cfu/ml suspension and incubated at room temperature (±24 C). Lesion dimension was recorded for each of 10 roots per genotype at 1, 2, 3, 4, 6, 8, and 10 days after inoculation.

Effect of location on resistance of storage roots. Storage roots of the standard cultivars Beauregard, Centennial, Jewel, and Travis were collected from plots harvested on approximately the same date in the fall but in three different locations in Louisiana in 1987 and in 11 different locations in 1988. The roots used in 1987 came from a limed or a nonlimed plot in Baton Rouge or a limed plot in Chase that had soil calcium contents of 1,386, 341, and 1,147 mg/kg, respectively. These were inoculated by inserting micropipette tips containing 50 μl of a suspension of 10^9 cfu/ml, and lesion dimension was recorded after 6 days at about 24 C.

Resistance of genotypes to root rot and stem rot. Terminal cuttings about 25–30 cm long were taken from sweet potato vines growing in the field and trans-

planted (five per 15-cm-diameter clay pot) into sand:soil (1:1) in the greenhouse. They were inoculated 1–2 wk after transplanting by injecting approximately 0.1 ml of a 10^8 cfu/ml cell suspension into the pith of the vine at about 2–3 cm above the soil line (16). Each pot was either covered with a clear polyethylene bag or placed in a tent of clear polyethylene to maintain high humidity for 3 days. Stems were cut open longitudinally and severity of stem rot was recorded after 7 days using the following rating system: 0 = no symptoms, 1 = localized necrosis at point of inoculation, 2 = localized necrosis extending up to 1–2 cm from point of inoculation, 3 = stem collapsed at point of inoculation and decay extending 2–5 cm from point of inoculation, 4 = most of stem decayed but green tissue remaining at apex, and 5 = plant dead.

RESULTS

Inoculation technique. Bacterial soft rot developed very rapidly on storage root slices at 28 C. Although there was less decay on the resistant Centennial slices than on Travis, decay spread across most of the Centennial slice within 4 days, making it difficult to estimate differences. However, the lowest effective inoculum concentration in both cultivars was about 10^3–10^4 cfu/ml. At 16 C, soft rot developed slowly on both cultivars at inoculum concentrations of 10^8–10^9 cfu/ml.

Significant differences in lesion dimensions among cultivars were observed when whole storage roots were inoculated with micropipette tips containing cell suspensions of either 10^9 or 10^6 cfu/ml. At lower concentrations, however, measurements of lesion dimensions were more variable.

Qualitative differences in lesion appearance were observed at 6 days after inoculation. Lesions on the more resistant genotypes were small, desiccated in the center, and surrounded by a narrow black zone. Lesions on intermediate genotypes were intermediate in size, soft, moist, brown in the center, and surrounded by a black zone. The susceptible genotypes had the largest lesions, referred to as active lesions, which were tan in the center, watery, but not discolored at the margins.

Comparison of root rot resistance by infectivity titration. There was no difference in lesion dimension between roots that were wrapped and unwrapped in infectivity titrations. Consequently, these data were pooled. A small proportion of the lesions in the wrapped Beauregard roots appeared stickier than all other lesions. Although the frequency of disease was variable, especially at inoculum concentrations less than 10^6 cfu/ml, the lesion dimensions differed among genotypes and were relatively constant for each genotype. Mean lesion dimensions (± standard deviation) for the 10^9 cfu/ml inoculations were: 38 (±8.0) and 11 (±7.9) in the first test for Beauregard and Jewel, respectively, and 28 (±13.8), 11 (±7.9), 18 (±10.4), and 5 (±5.0) in the second test for Beauregard, Jewel, Travis, and Centennial, respectively. For the first test, $\log_{10}$ ED_{50} values were 5.86 and 5.61 and slopes of the plots were 0.80 and 0.58 for Beauregard and Jewel, respectively. For the second test, ED_{50} values were 7.08, 7.38, 8.28, and 9.33 and slopes were 0.51, 0.51, 0.58, and 0.55 for Beauregard, Jewel, Travis, and Centennial, respectively. Each regression gave a straight line.

Root rot development over time. Enlargement of lesions ceased after 2–3 days in Centennial, 3–4 days in Jewel, and 8–10 days in L81-10 (Fig. 1). However, the initial appearance of symptoms and the rate of lesion expansion were similar for all three genotypes during the first 2 days. As each lesion stopped expanding, a black zone appeared at the margin of the lesion in roots of each genotype, and subsequently the center of the lesion dried out.

Effect of location on resistance of storage roots. The relative reaction of cultivars for root rot resistance was the same for each location at which they were produced. However, the size of lesions varied with location for each genotype. Mean lesion dimensions (±standard deviation) on storage roots inoculated in 1987 were 3 (±2.4), 4 (±3.9), and 9 (±6.5) mm for Centennial and 15 (±2.9), 7 (±5.6), and 31 (±6.8) mm for Travis for roots grown in nonlimed and limed plots in Baton Rouge and limed plots at Chase, respectively. Trends were similar in 1988, but the overall lesion dimension was less and differences were not significant.

Resistance of genotypes to root rot and stem rot. The majority of genotypes screened appeared to be resistant to root rot. In 1987 and 1988, 76% of 33 and 74% of 122 sweet potato genotypes screened, respectively, appeared resistant. Results for breeding lines (data not included) were similar to results for cultivars in both years. Data for cultivars screened in 1988 are given in Table 1. Lesions were no longer active 6 days after inoculation in 1988 on roots of 93 of 122 genotypes. The other genotypes had 10–30% of roots with active lesions, except Beauregard, which had 50%. The severity of stem rot symptoms varied more among the cultivars (Table 1), and there was no overall correlation with the apparent resistance of roots ($r = -0.06$, $P = 0.56$). Several genotypes, including Beauregard, were susceptible to root rot but resistant to stem rot, whereas the reverse was true for other genotypes, including Vardaman.

DISCUSSION

Sweet potato genotypes compared in this study varied considerably for resistance of storage roots to *E. chrysanthemi*. Most of the cultivars that appeared susceptible were released after 1970, whereas most of the older, "heirloom" cultivars appeared resistant. Thus, susceptibility to bacterial root rot appears to have been introduced into U.S. sweet potato breeding programs relatively recently.

Differences in resistance were not easily discerned when evaluated with a root slice inoculation similar to the one used with potato tubers (2,9,10,17). However, lesion dimensions after inoculation of whole roots with 5×10^7 cfu (50 µl of 10^9 cfu/ml) were consistent for the cultivars Beauregard (susceptible), Centennial (resistant), Jewel (intermediate), and Travis (intermediate to suscep-

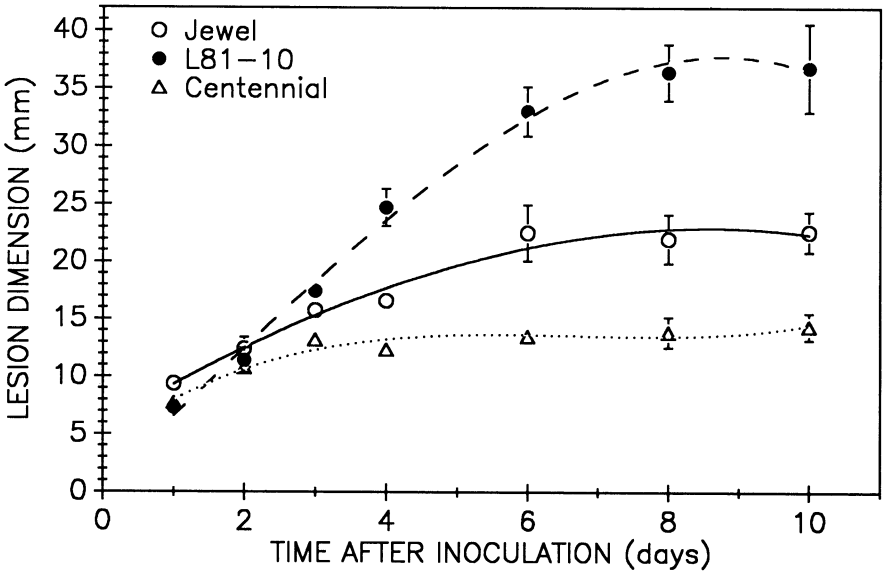

Fig. 1. Comparison of lesion dimension on storage roots of Centennial (resistant), Jewel (intermediate), and L81-10 (susceptible) as a function of time after inoculation with *Erwinia chrysanthemi*. Bars represent the standard errors of the means.

tible) over a 3-yr period. Similar measurements of lesion size are useful in evaluating resistance of potato tubers to Erwinia soft rot (6). The results also were consistent with limited observations of natural occurrence of bacterial stem and root rot on Centennial, Jewel, and Travis in the field (16; Clark, *unpublished*). The natural incidence of disease in Beauregard has not been determined, as the cultivar has only recently been grown commercially.

Table 1. Evaluations for 1988 of vine and storage root reaction of cultivars of sweet potato to bacterial stem and root rot, caused by *Erwinia chrysanthemi*

Cultivar	Stem rot index[x,z]	Storage root lesion dimension (mm)[y,z]
Beauregard	2.7 e–p	34.6 a
Jewel	2.3 h–p	16.7 b–e
Georgia Jet	NI	16.3 b–f
White Bunch	4.4 a–e	15.6 b–g
Kandee	2.4 g–p	15.4 b–h
Southern Delite	3.4 a–n	14.3 c–j
Nancy Hall	3.6 a–l	13.6 c–l
Heartogold	3.4 a–n	12.4 d–n
Topaz	2.2 i–p	12.4 d–n
Travis	NI	12.2 d–o
Shore Gold	2.2 i–p	11.5 d–p
Cordner	2.2 i–p	11.3 d–q
Excel	3.7 a–k	11.3 d–q
Jasper	2.5 f–p	11.1 d–r
Sweet Red	4.7 ab	10.8 d–s
Caromex	2.1 j–p	10.8 d–s
Georgia Red	3.4 a–n	10.5 d–t
Gold Jewel	2.1 j–p	10.2 d–v
Red Jewel	2.2 i–p	10.1 d–w
Allgold	1.3 p	9.9 d–w
Coastal Sweet	4.1 a–g	9.1 d–w
Hayman White	3.5 a–m	9.0 d–w
Scarlet	2.6 f–p	8.9 d–w
Porto Rico	NI	8.7 d–w
Eureka	3.1 b–o	8.3 d–w
Pelican Processor	3.3 a–n	8.2 e–w
Rojo Blanco	3.1 b–o	8.1 e–w
Cherokee	2.1 j–p	7.5 e–w
Red Resisto	2.6 f–p	7.4 e–w
White Jewel	2.9 c–p	7.2 e–w
Apache	3.3 a–n	7.0 e–w
Banana Yam	4.4 a–e	6.9 e–w
Buster Haynes	2.6 f–p	6.7 e–w
Copper Resisto	3.9 a–i	6.4 f–w
Pope	3.6 a–l	6.3 f–w
Creole	4.2 a–f	6.2 g–w
Goldrush	NI	6.0 g–w
Triumph	3.6 a–l	5.8 g–w
Oklamar	2.8 d–p	5.5 h–w
Hopi	3.2 a–n	5.3 h–w
Dooley	2.7 e–p	4.3 j–w
Regal	3.5 a–m	3.8 l–w
Gem	3.7 a–k	2.9 m–w
Hayman	2.1 j–p	2.9 m–w
Haynes	1.8 m–p	2.6 n–w
Painter	2.8 d–p	2.6 n–w
Centennial	2.5 f–p	2.5 n–w
Resisto	3.3 a–n	2.2 o–w
Carolina Nugget	1.8 m–p	2.2 o–w
Vardaman	4.9 a	1.6 p–w
Whitestar	2.4 g–p	1.3 q–w
Sumor	3.8 a–j	0.9 s–w
HyDri	3.3 a–n	0.9 s–w
Southern Queen	3.7 a–k	0.6 t–w
Nemagold	3.9 a–i	0.6 t–w

[x]Rated on a scale of 0–5, where 0 = no symptoms and 5 = plants dead. Values are the means for 10 plants. NI = not included in 1988 test.

[y]Roots were cut in cross section through the center of the lesion, and the depth and diameter of the lesion were measured. The lesion dimension is the mean of these measurements for 10 roots.

[z]Means in the same column followed by a common letter are not significantly different (Duncan's multiple range test, $P = 0.05$).

Although the initial rate of lesion expansion did not differ among genotypes, differences in root lesion dimension appear related to the period between inoculation and host defense reactions that restrict lesion expansion. This relationship is similar to one reported on potato tubers inoculated with *E. c.* subsp. *atroseptica* (2). In both systems, lesions became surrounded by a dark zone (1). Except in Beauregard, lesion development was restricted in most roots of even the most susceptible genotypes.

A complete evaluation of the resistance of sweet potato to *E. chrysanthemi* requires assessment of reaction of both the stem and the root. The relative importance of each to development of the disease under field and postharvest conditions remains to be determined, however. Root and stem rot has not been observed in the field on many heirloom cultivars that are resistant to root rot but susceptible to stem rot. This may indicate the importance of root rot resistance. The greater natural occurrence of root and stem rot on the cultivars Georgia Jet and Georgia Red, on the other hand, has been associated with stem rot susceptibility (13,16). To fully assess the relative importance of stem rot and root rot, and to determine if resistance to only one is sufficient for disease control in the field, more information is needed about how and when infection by *E. chrysanthemi* occurs in the cycle of sweet potato production.

Because recently released cultivars, such as Beauregard, are susceptible to bacterial root rot, disease frequency may increase in the future. It is generally recommended that roots be cured in a relatively warm (28–30 C), humid (90% RH) environment, similar to the conditions used in this study, for 5–10 days immediately after harvest. This promotes wound healing and reduces infection by fungi that cause postharvest rots (4). Subsequent storage of roots is best at 16 C. However, it is often difficult to reduce the temperature to 16 C during the harvest period in regions where sweet potatoes are grown. Although the influence of temperature on root rot has not been reported, *E. chrysanthemi* causes greater stem rot at 32 than at 22 or 27 C (16). Thus, conditions in commercial storages are commonly conducive to disease development by *E. chrysanthemi.*

Growing conditions for sweet potatoes in the field affected the resistance of roots to *E. chrysanthemi.* Thus, comparisons should only be made among roots produced under the same conditions. However, more research is needed on the effect of location and cultural practices on resistance of storage roots after harvest. The increased availability of sweet potato cultivars that are resistant to soil rot, a disease caused by *Streptomyces ipomoea* (Person and W. J. Martin) Waksman and Henrici and favored by soil pH above 5.2, has led to increased interest among growers in liming soils. Increased tuber calcium content is associated with increased resistance of potatoes to soft rot (15). In this study, sweet potatoes grown in limed plots did not appear more resistant to root rot. However, root calcium content was not determined and only one source of calcium was provided.

Some lesions on roots of the cultivar Beauregard were similar to the "sticky rot" caused on other plants by pectolytic *Clostridium* spp. (3,11). However, wrapping roots in plastic did not affect lesion size. Sweet potato has not been reported as susceptible to *Clostridium* spp., but a rapid decay of storage roots, known as souring, develops when soil becomes saturated with water (4). The possible role of bacteria in this syndrome and the sticky rot of Beauregard should be investigated.

ACKNOWLEDGMENTS

We gratefully acknowledge the support provided under USDA Specific Cooperative Agreement 58-43YK-7-0018 and by the Louisiana Sweet Potato Advertising and Development Commission. We thank Cheryl S. Pace for technical assistance and J. W. Moyer, Department of Plant Pathology, North Carolina State University, Raleigh, for providing the culture of *Erwinia chrysanthemi.*

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