COMPARISON OF THE EFFECTIVENESS OF DIFFERENT METHODS OF SCREENING FOR BACTERIAL SOFT ROT RESISTANCE OF POTATO TUBERS

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Abstract

Tubers from eight potato cultivars (cvs) grown at two different locations in Wisconsin were tested for bacterial soft rot resistance using different inoculation techniques. The procedures included 1) point inoculations of tubers with different inoculum levels followed by incubation in ambient or low oxygen condtions, 2) inoculation of mechanically bruised tubers followed by incubation in a mist chamber, and 3) a standard slice inoculation method.

The point titration test followed by incubation in dew chamber and the mist chamber-bruise test showed similar patterns of resistance for cultivars that were used in these experiments. These two methods are considered to be effective for screening potato tubers for bacterial soft rot resistance. Point titration methods are very useful if only limited numbers of tubers are available. The mist chamber-bruise test is simpler than the other procedures; however, to obtain reproducible results large numbers of tubers are required. Because of the great variability of the results obtained in inoculation of slices, the reliability of this approach can be questioned as a standardized method for evaluation of resistance. Tubers of somatic hybrids of *S. brevidens* and *S. tuberosum* and their sexual progeny were significantly more resistant to bacterial soft rot than tubers of moderately resistant cultivars when evaluated by each of the assay procedures.

Compendio

Los tubérculos de ocho cultivares (cvs) de papa, producidos en dos diferentes localidades en Wisconsin, fueron probados para resistencia a la pudrición blanda utilizando diferentes técnicas de inoculación. Los procedimientos incluyeron 1) inoculación de ciertas zonas de los tubérculos con diferentes niveles de inóculo seguida por incubación bajo condiciones del ambiente o condiciones de baja oxigenación, 2) inoculación de tubér-

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culos magullados mecánicamente seguida por incubación en cámara de nebulización, y 3) un método estándar para inocular rebanadas.

La prueba de dosificación de zonas seguida por incubación en cámara de formación de rocío y la prueba de las magulladuras en cámara de nebulización mostraron formas similares de resistencia para los cultivares utilizados en estos experimentos. Se considera que estos dos métodos son efectivos para la evaluación y selección de tubérculos de papa para resistencia a la pudrición blanda bacteriana. Los métodos de dosificación de zonas son muy útiles si se tiene un limitado número de tubérculos disponibles. La prueba de las magulladuras en cámara de nebulización es más simple que los otros procedimientos. Sin embargo, para obtener resultados reproducibles, se requieren numerosos tubérculos. Debido a la gran variación en los resultados obtenidos en la inoculación de rebanadas, la confianza en este método puede ser cuestionada como un método estandarizado para la evaluación de resistencia. Tubérculos de híbridos somáticos de *S. brevidens* y *S. tuberosum* y su progenie sexual fueron significativamente más resistentes a la pudrición blanda bacteriana que los tubérculos de cultivares moderadamente resistentes cuando se les evaluó por cada uno de los métodos ensayados.

Introduction

Post harvest losses of stored potato tubers are caused by the bacterial soft rot pathogens, *Erwinia carotovora* subsp. *atroseptica* (Eca), *Erwinia carotovora* subsp. *carotovora* (Ecc) and *Erwinia chrysanthemi* (Echr). Among the factors affecting resistance of potato tubers to bacterial soft rot are: source and quality of seed tubers (11, 39); physiological status (24) and inoculum level of soft rot bacteria as well as other bacteria or fungal pathogens (39); weather (temperature and humidity) during growth and harvesting (33); chemicals (nutrients and herbicides) applied during growth period (17, 25); degree of bruising during harvest and transportation (5); conditions for wound healing in initial storage or curing period and general conditions during storage period (temperature, oxygen, humidity, air flow, light and chemicals accelerating or inhibiting wound healing) (8, 13, 19, 31); procedures involved in grading as well as washing and drying procedures prior to shipment (4); length of the storage period and conditions during shipping (16).

A number of genetically controlled factors are also important in potato resistance to soft rot (29). However, the expression of the inherent resistance of a sample of tubers of a given cultivar under evaluation also is influenced by the large number of environmental factors noted above and cultural practices during growth, harvesting, storage and shipping (18, 26, 37). Furthermore, it is generally accepted that high levels of tuber resistance to soft rot erwinias did not exist in commercial cultivars. However, potato

cultivars do vary in their relative susceptibility to soft rot; this has been shown in many studies in Europe (3, 6, 21, 23, 38). Relatively few studies have been published on resistance of American cultivars to the soft rot erwinias (10, 36).

Because of the large number of environmental, cultural and genetic factors that influence development and severity of soft rot, it is difficult to evaluate results obtained by different investigators in attempts to develop standardized procedures for evaluation of soft rot resistance of tubers. An important problem that impedes the breeding for soft rot resistance is the lack of a generally accepted method for the inoculation and selection of resistant clones (3, 18, 37). Severity of disease may vary widely when different screening methods are used because each method may examine different components of general resistance. Thus, the process of selection of lines for soft rot resistance in current breeding programs is very difficult without specific guidelines to aid in the screening process.

The objectives of this study were to compare effectiveness of several methods for the evaluation of bacterial soft rot resistance in tubers. Simple and reproducible methods were sought that would best separate breeding lines and cultivars for resistance.

Materials and Methods

Sources of Test Material

Tubers of potato cultivars (cvs): Atlantic, Hilite Russet, Norchip, Norgold Russet, Norland, Russet Burbank, Russet NorKotah, and Superior were obtained from cultivar evaluation plots established by Drs. John Schoenemann and David Curwen in central (Hancock) and northern (Antigo) Wisconsin. At the Hancock Agricultural Research Station the soil is a loamy sand; at the Langlade County Agricultural Research Station in Antigo the test plot was on a silt loam sand. The cultivars selected were previously shown to differ in their resistance to bacterial soft rot caused by Ecc (36).

Tests were also completed with tubers of somatic hybrids produced by protoplast fusion between *Solanum brevidens* L., a diploid, non-tuber-bearing wild species, and a tetraploid potato, *S. tuberosum* L., tubers of the *S. tuberosum* fusion parent, two clonal lines of potato cvs (Katahdin and Russet Burbank) and tubers of the sexual progeny from crosses between the somatic hybrids and cv Katahdin. The production and field evaluation of somatic hybrids of R4 + 6A were described previously (1). Tubers of protoplast fusion plants and certain lines of their sexual progeny were shown to be resistant to bacterial soft rot previously (2). All tubers were stored at 7 C after harvest and tested after a six month storage period. Tests were initiated in February and completed by the end of March.

Bacterial Culture

Erwinia carotovora subsp. *carotovora* (Jones) Bergey *et al.* (Ecc-SR 394) originally isolated in Wisconsin from rotting carrot tissue in 1986 was used in this study. Suspensions for inoculations were prepared from cultures of Ecc-SR 394 following established procedures (12, 30, 36).

Screening for Soft Rot Resistance

1. Point titration assay.

Tubers (free of any obvious mechanical damage or disease) were washed and then immersed for two 20 min periods in 0.5% sodium hypochlorite (Clorox), rinsed with sterile deionized water, sprayed with 95% ethanol and allowed to dry in air. Polypropylene pipette tips containing 25 µl (one of three different concentrations of Ecc-SR 394, 1 x 10^5, 1 x 10^7 or 1 x 10^9 cfu/ml) were pressed in a randomized manner into the upper side of each tuber to a depth of 10 mm (30). Pipette tips containing sterile water were used as controls. Five tubers from each cultivar or breeding line were incubated at 25 C in a chamber at 95% relative humidity (R.H.) and five tubers in a chamber flushed with water-saturated nitrogen to provide low oxygen conditions at 20 C. After 72 h, tubers were sliced vertically through the point of inoculation and the width of decayed tissue was measured.

2. Mist chamber assay.

Potato tubers were bruised twice at one site per tuber with a pendulum bruiser (7). Immediately after bruising tubers were inoculated by placing a 10 µl of bacterial cell suspension at a concentration of 5 x 10^8 cfu/ml at the bruised site. Ten tubers from each cultivar or line were inoculated and then incubated in a mist chamber (7) at 20 C for 72 h. After incubation, tubers were sliced vertically at the inoculation site and width of decayed tissue was measured.

3. Slice assay.

Tuber surfaces were disinfested as described above. Ten tubers from each cultivar or line were cut into 5 mm thick slices following the procedure described by Dobias (14). Directly after cutting, slices were placed on a glass plate and a filter paper disc (5 mm diameter) was placed in the center of each slice. Slices were inoculated by pipetting 25 µl of bacterial suspension on each paper disc. Three different concentrations of bacterial suspension were used (5 x 10^5, 5 x 10^6 and 5 x 10^7 cfu/ml); ten slices from each cultivar were used for each inoculum level. Filter paper discs with sterile water were used as controls. Slices were incubated at 22 C in a chamber with R.H. of about 95%. After 72 h the diameters of decayed tissue areas were measured.

To determine whether position of the point of application of inoculum on a slice would influence disease severity, pairs of slices were prepared from 10 tubers of cv Russet Burbank. The filter paper discs were placed near the periderm on cortex tissue (first slice) or in the center (second slice); 25 µl of bacterial suspension (5×10^6 cfu/ml) were pipetted on each filter paper disc.

A second series of slice tests (10 tubers) was completed to determine whether position of the slice in the tuber would influence decay severity. Slices were taken in sequence from stolon to bud end from tubers of cv Russet Burbank and inoculated by placing 25 µl of bacterial suspension (5×10^6 cfu/ml) on a filter paper disc in the center of each slice. Incubation procedures and measurements were the same as those described above.

Dry Matter Determination

Potato tubers from each cultivar were cut into thin segments (3 samples per cv, each of 100 g fresh weight). Tuber tissue was initially dried in an oven at 65 C for 24 h; temperature was then increased to 100 C for the next 24 h. Dried samples were weighed and percent dry matter was calculated.

Statistical Analysis

Mean value of the width or diameter of decayed tissue was calculated for each cultivar and each type of inoculation technique. Data were analyzed using a Kruskal-Wallis Test (9). Evaluations with each inoculation technique were completed twice.

Results

A range in susceptibility was evident among the cultivars when tubers were screened with point titration assay (Fig. 1). Generally, tubers of cvs Hilite Russet, Russet NorKotah and Russet Burbank were more resistant to bacterial soft rot than those of the other cultivars in the point titration test under ambient oxygen conditions (dew chamber). The differences between these cultivars and the most susceptible cultivars, Norland, Superior and Atlantic, were significant for the intermediate level of inoculum 10^7 cfu/ml (Fig. 1). However, differences were not significant when results from three levels of inoculum were pooled together. Rankings of relative resistance of cultivars that were grown in Antigo and Hancock were essentially similar.

Cultivars that were relatively resistant to soft rot under ambient oxygen levels were more susceptible under limited oxygen conditions in the nitrogen chamber (Fig. 2). Severity of decay in cvs Norland, Superior and Atlantic, which were susceptible to soft rot under ambient oxygen conditions, was very similar to severity of decay under low oxygen concentrations

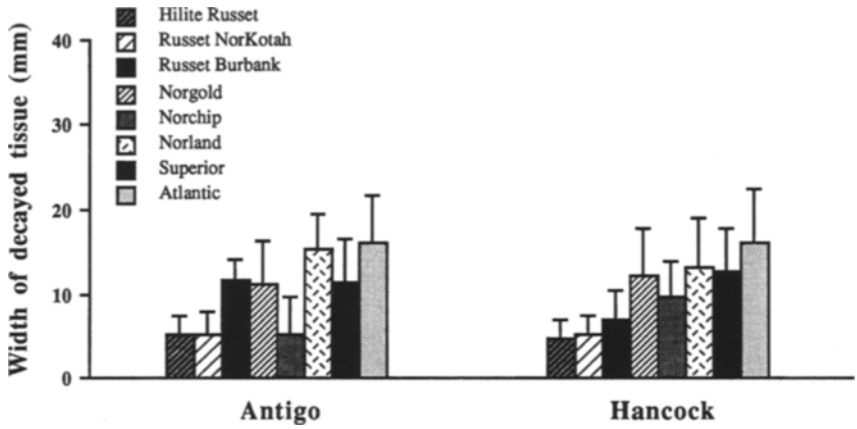

FIG. 1. Width of decayed tissue in tubers of different potato cultivars following insertion of pipette tips containing 25 µl of bacterial suspension (Ecc-SR 394, 1 x 10^7 cfu/ml). Pipette tips were pressed into tubers to the depth of 10 mm. Incubated for 72 h in a dew chamber at 25 C with relative humidity about 95%. Error bars indicate standard deviation calculated from two independent experiments.

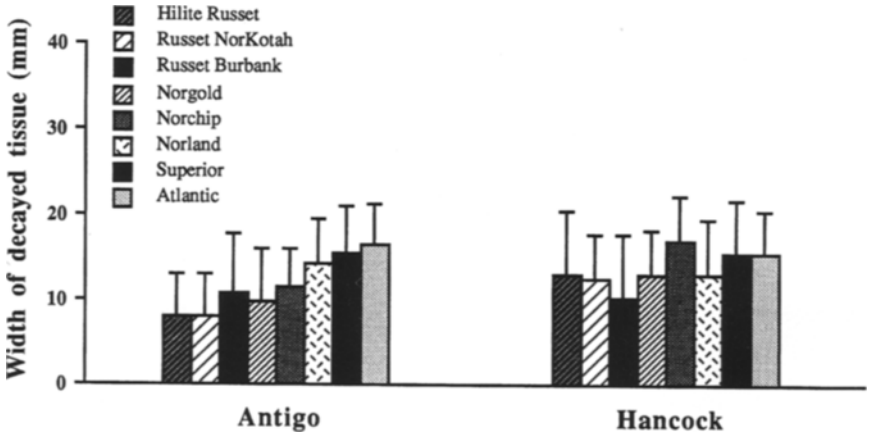

FIG. 2. Width of decayed tissue in tubers of different potato cultivars following insertion of pipette tips containing 25 µl of bacterial suspension (Ecc-SR 394, 1 x 10^7 cfu/ml). Pipette tips were pressed into tubers to the depth of 10 mm. Incubated for 72 h in nitrogen chamber at 20 C with relative humidity about 95%. Error bars indicate standard deviation calculated from two independent experiments.

(Figs. 1 and 2). Finally, under low oxygen levels, disease severity was high for all the cultivars (Fig. 2). Statistical analysis showed no significant differences between cultivars at any level of inoculum.

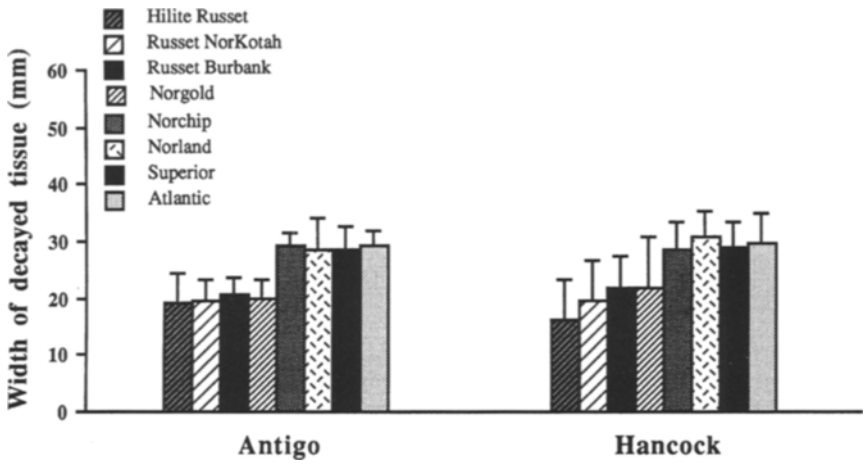

FIG. 3. Width of decayed tissue in tubers of different potato cultivars following bruising and inoculation with 10µl of bacterial suspension of Ecc-SR 394 containing 5 x 10^8 cfu/ml. Incubated for 72 h at 20 C in mist chamber. Error bars indicate standard deviation calculated from two independent experiments.

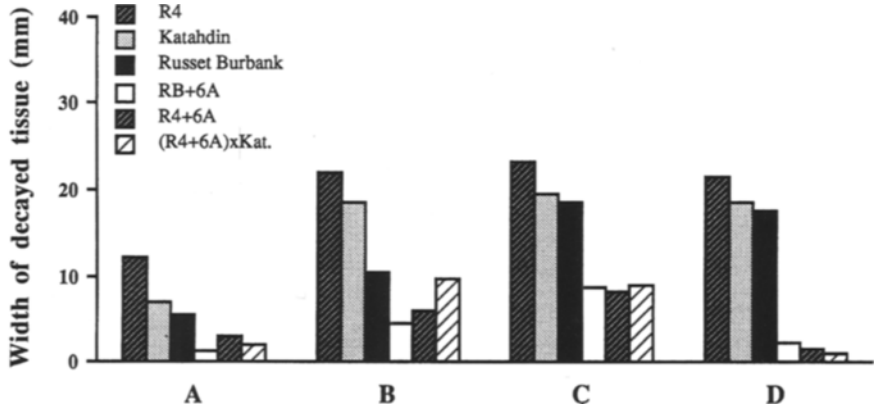

FIG. 4. Severity of soft rot decay on tubers of different potato cultivars and breeding lines following inoculation by four different procedures. A. Point titration and incubation in dew chamber; B. Point titration and incubation in nitrogen chamber; C. Tubers bruised twice with pendulum bruiser and incubated in mist chamber; D. Inoculation of slices, incubation in dew chamber.

When bruised tubers were inoculated and incubated in a mist chamber, disease severity was greater (Fig. 3) than when pipette tip inoculations were made (Figs. 1, 2). Tubers of cvs Hilite Russet, Russet NorKotah, Russet Burbank and Norgold Russet grown in Antigo were significantly

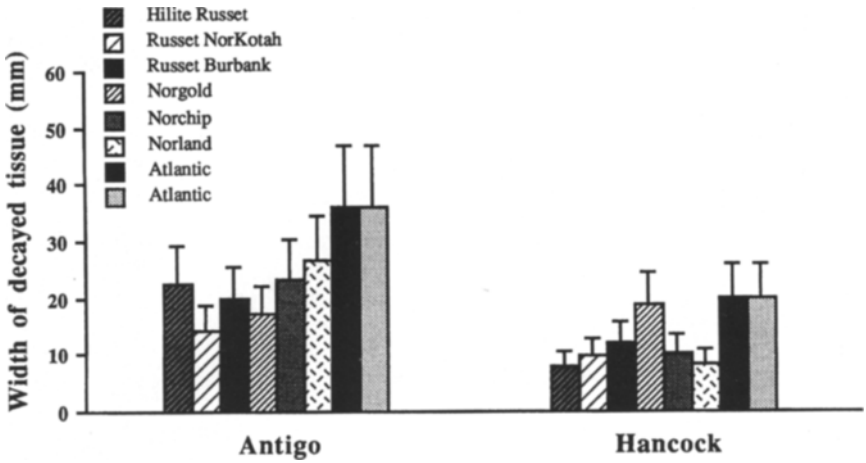

FIG. 5. Width of decayed tissue in slices of different potato cultivars following inoculation with 25 µl of bacterial suspension (Ecc-SR 394, 5 x 10^6 cfu/ml). Incubated for 72 h at 22 C with relative humidity about 95%. Error bars indicate standard deviation calculated from two independent experiments.

more resistant than tubers of cvs Norland, Norchip, Superior and Atlantic. Cultivars from both locations showed approximately the same ranking based on width of decayed tissue. In contrast to data obtained for cultivars, tubers of somatic hybrids of *S. brevidens* and *S. tuberosum* and their sexual progeny were significantly more resistant to bacterial soft rot than tubers of moderately resistant cultivars when evaluated by each of the assay procedures (Fig. 4).

In the test with tuber slices, differences among cultivars were not significant because of high variability in readings (Fig. 5). It was also true when analyses of data from each inoculum level were performed separately. Of the cultivars grown in Antigo, Russet NorKotah and Norgold were the most resistant. Of the cultivars grown in Hancock, Hilite Russet, Russet NorKotah, Norland and Norchip were the most resistant. Generally, slices of tubers grown in Antigo were more susceptible to soft rot than those from Hancock (Fig. 5). This site difference was not evident in tests with whole tubers.

The high variability of the data obtained from the slice test may be attributed to a number of factors that may modify the reaction of slices following inoculation. For example, the location in the tuber from which slices were cut may influence the severity of disease (Fig. 6). The statistical analysis completed on data for slices from 10 tubers of cv Russet Burbank indicated similar standard deviations between slices from individual tubers and from tubers of different cultivars (Table 1). Also, location of point of

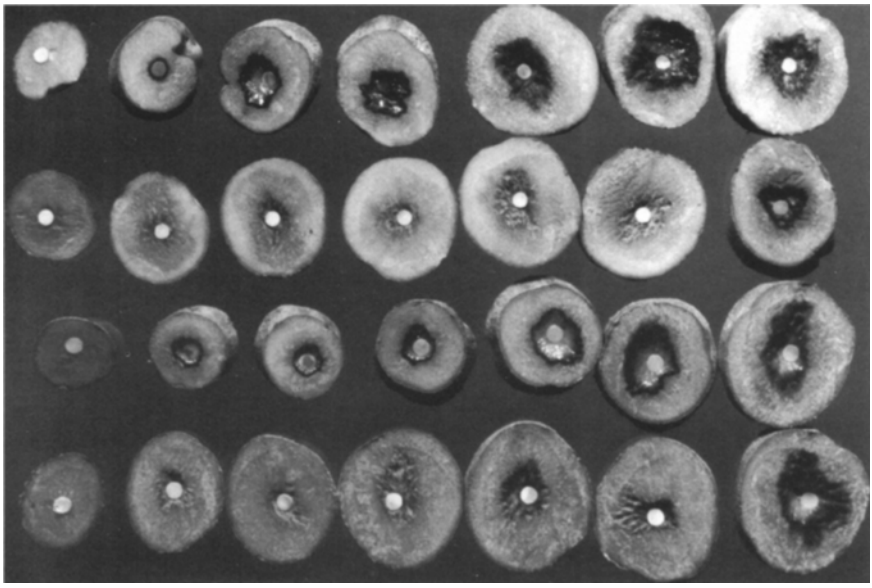

FIG. 6. Influence of position of slice in Russet Burbank tubers (from bud to stolon end) on susceptibility to bacterial soft rot caused by Ecc-SR 394. Slices in top row from left to right were taken in sequence from bud end to center of one tuber and in row 2 from right to left from center to stolon end. Sequence in bottom two rows was the same. All slices were inoculated by placing 25 µl of bacterial suspension (containing 5 x 10^6 cfu/ml) on a filter paper disc and incubating for 72 h at 22 C with relative humidity about 95%.

TABLE 1.—*Comparison of the results of slice test obtained for individual tubers of cv Russet Burbank and for different potato cultivars.*

Individual tubers of Russet Burbank	Width of rotting tissue mm	Cultivars	Width of rotting tissue mm
1	19.5±15.4	Atlantic	20.0±7.4
2	23.8±12.6	Hilite Russet	8.0±7.5
3	15.5±13.1	Norchip	10.3±8.3
4	22.5±18.9	Norgold	18.8±10.3
5	12.8±8.9	Norland	8.3±6.8
6	17.2±14.1	Russet Burbank	12.0±9.1
7	8.1±5.7	Russet NorKotah	9.7±7.3
8	17.2±14	Superior	23.4±11.1
9	21.7±18.3		
10	6.1±2.5		

Width of decayed tissue in potato slices following inoculation with 25 µl of Ecc-SR 394 (5 x 10^6 cfu/ml). Incubation 72 h at 22 C with relative humidity about 95%. Standard deviation was calculated from two independent experiments.

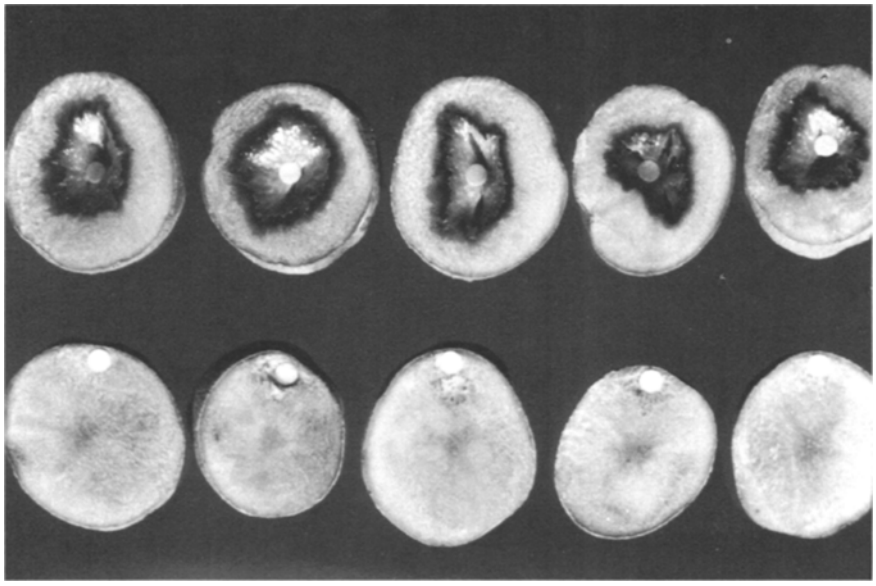

FIG. 7. Relation of inoculation position on tuber slices to severity of bacterial soft rot caused by Ecc-SR 394. Slices were inoculated by placing 25 µl of bacterial suspension (containing 5 x 10^6 cfu/ml) on a filter paper disc on each slice and incubating for 72 h at 22 C with relative humidity about 95%. Top row, one disc placed in center of each slice; bottom row, one disc placed close to periderm in cortex tissue of each slice.

inoculation on the slices could influence disease severity (Fig. 7). In this case medullary tissue was significantly more susceptible than cortex tissue. Diameters of decay in medullary and cortex tissue were respectively, 23.2±13.5 and 3.1±2.5 mm. This variability can affect ranking of lines for resistance when slices are used instead of whole tubers.

Evaluation of dry matter content in the tissue of examined cultivars ranged from 16.5% for cv Norland to 22.4% for cv Atlantic. Cultivars from Antigo and Hancock showed the same ranking of dry matter content.

Discussion

Many different methods of screening tubers for soft rot resistance have been used in different laboratories (3, 7, 10, 13, 14, 22, 36). The factors that may influence screening procedures are: 1) selection of the type of material to be inoculated, *i.e.* intact, wounded or bruised tubers vs. slices, cylinders or discs (3, 23); 2) point of inoculation on tuber or slice: bud or stolon end and cortex or medullary tissue (32); 3) timing of inoculation directly after wounding or bruising or after short wound-healing period (15, 19, 28); and 4) incubation conditions after inoculation as influenced by the following factors: temperature, humidity, oxygen level and

light (8, 13, 19). Other important sources of variability are: virulence of the strain or strains selected for inoculations, level of inoculum, and incubation conditions specific for the particular species of bacterium to be tested.

Relatively few reports compare results obtained on same material with different screening methods (3, 23). Lack of a generally accepted method for screening potato tubers for soft rot resistance indicates the need to compare the effectiveness of several different evaluation procedures.

Data obtained for each of the four methods were influenced by different factors that may affect soft rot resistance of the tested tubers. As a result cultivars differed in specific rank order from test to test. Data obtained in tests completed with whole tubers (three assays) were very similar, however. Hilite Russet, Russet NorKotah, Russet Burbank and Norgold Russet were significantly more resistant than Atlantic and Superior which were more susceptible than the other cultivars (Figs. 1, 2, and 3). Tubers of Russet Burbank and Norgold Russet were also more resistant to bacterial soft rot than other commercial cultivars in previous studies in which the mist chamber assay was used with freshly harvested inoculated tubers (36). Tests completed on tuber slices indicated that cultivars differed in ranking, but data showed high variability (Table 1, Fig. 5).

The results obtained for the two tests that employed the point titration methods and later incubation of inoculated tubers in the dew chamber (Fig. 1) or the nitrogen chamber (Fig. 2) confirmed previous observations that limiting oxygen supply increased susceptibility to soft rot (13, 30). This was especially evident in cultivars relatively resistant to this disease under ambient oxygen conditions. Our results confirm other reports indicating that low oxygen conditions enhance tissue maceration by soft rot erwinias (8, 13, 29, 30). Oxygen deficiency also inhibits the formation of wound periderm and suberin (20), decreases activity of enzymes such as polyphenoloxidase peroxidase and phenylalanine-ammonialyase or synthesis of compounds such as phytoalexins (27, 29, 34).

With the point titration method it is possible to determine not only the extent of tissue maceration, but also the bacterial population necessary for initiation of lesions. Higher populations of bacteria were required for the initiation of decay in tubers of Hilite Russet, Russet NorKotah and Russet Burbank than for Atlantic and Superior (data not shown). This was evident when tubers were incubated either at ambient or low oxygen levels.

In the method that used pipette tips, bacteria were introduced directly into both cortical and medullary tissue. In contrast, bruising of the tubers with the pendulum bruiser, followed by application of inoculum at the bruise site, introduced bacteria mainly to the cortex tissue. Cortex tissue has been reported to be more resistant to soft rot than medullary tissue (3, 32). Our results indicated higher disease severity after inoculation at bruised sites than by other inoculation methods. First, the type of injury

obtained in bruises vs other inoculation procedures was quite different with respect to type and size of wounded tissue, and number of cell layers damaged by each procedure (35). Second, bruised tubers were incubated in a mist chamber in which conditions were very favorable for soft rot (anaerobic conditions inside the tubers as a result of a water layer covering the tubers) (7, 8). In contrast surfaces of tubers incubated in the dew chamber were free of a water film.

Although data obtained after inoculation of medullary or cortex tissue of tuber slices showed higher resistance of the cortex layer (Fig. 7), the bruising effect obviously alters the responses in the cortex favorable for wound healing in whole tubers.

Great variability in ranking of cultivars for resistance was evident in tests with tuber slices (Table 1; Fig. 5). This method was described by Dobias (14) and has been used by other investigators (21, 23); high variability of results with this assay has been noted previously (23).

Cultivars and clonal lines showed differences in dry matter content, but in contrast to results obtained by Hidalgo and Echandi (16), correlations based solely on dry matter content could not be made. For example, tubers of cv Atlantic had the highest dry matter content of the material tested, but it was one of the cultivars highly susceptible to bacterial soft rot.

Direct comparison of the results obtained by the various screening procedures used in this study is very difficult. Each of the methods involved different inoculation procedures and may also involve effects of different factors on mechanisms for resistance in tuber tissue. Results of the point titration test involving incubation of the tubers in a nitrogen chamber should be evaluated carefully in screening of potato cultivars since anaerobic conditions inhibit the expression of resistance mechanisms in potato tubers (20, 29, 34). With this method only extremely resistant material such as somatic hybrids of *S. brevidens* and *S. tuberosum* showed a low level of tissue maceration (Fig. 4).

However, the point titration test followed by incubation in the dew chamber and the mist chamber-bruise tests showed a similar pattern of resistance for cultivars that were used in these experiments. These two methods are appropriate for screening potato tubers of different cultivars. Each of the methods evaluated for testing has advantages and disadvantages. Point titration methods are very useful if only limited numbers of tubers are available. This method could be recommended also when susceptibility of the tubers to different bacterial strains needs to be evaluated. In this case instead of different inoculum concentrations each tuber could be inoculated with several bacterial strains. However, this method is time consuming and could not be recommended for screening large numbers of breeding lines or cultivars.

The mist chamber-bruise test is simpler than the others; however, to

obtain reproducible results large numbers of tubers are required. Also a special chamber with temperature controls and an effective misting device is necessary.

Because of the great variability of the results obtained in inoculation of slices the reliability of this approach can be questioned as a standardized method for evaluation of resistance.

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