Identification of Resistance in Sweetpotato to Rhizopus Soft Rot Using Two Inoculation Methods

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ABSTRACT

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Two inoculation methods were developed and compared to evaluate cured sweetpotato (*Ipomoea batatas*) storage roots for resistance to Rhizopus soft rot caused by *Rhizopus stolonifer* and *R. arrhizus*. Roots that had been cured and stored for at least 3 wk were first washed in a commercial washer. In the impact/dip method, roots were wounded by allowing them to drop approximately 1 m from the end of the washer into crates, after which they were dipped in a suspension of sporangiospores. In the puncture inoculation method, deep-threaded wood screws were dipped in a spore suspension and then hammered about 5–10 mm deep into the median of the root. Most genotypes were more susceptible to *R. stolonifer* than to *R. arrhizus*; thus, subsequent evaluations were made using only *R. stolonifer*. Ranking of genotypes was similar with both methods, and there was an overall correlation in soft rot incidence (R^2 = 0.22) between the methods. However, overall incidence of soft rot and differences among genotypes were greater by the puncture method. Genotypes with white-fleshed storage roots were uniformly susceptible, while those with orange flesh varied. Linear relationships were observed between inoculum concentrations and soft rot incidence on four different genotypes by the puncture method. By the impact/dip method, differences in soft rot incidence were not influenced as strongly by inoculum concentration. Of the commercial cultivars evaluated, Beauregard was the most resistant, Jewel and Hernandez varied from intermediate to susceptible, and the white-fleshed cultivars HiDry and Sumor were among the most susceptible.

Rhizopus soft rot, caused most frequently by *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill. (syn. *R. nigricans* Ehrenb.) and less frequently by *R. arrhizus* A. Fischer (syns. *R. oryzae* Went & Prinsen-Geerligs and *R. tritici* K. Saito), is the most widespread and destructive postharvest disease of sweetpotato (*Ipomoea batatas* (L.) Lam.) (4,5,11). Incidence of Rhizopus soft rot is greater on cured than on green sweetpotatoes and after sweetpotatoes have been washed and packed for shipment (4,5). Generally, the whole storage root is affected by a soft, watery rot and is completely destroyed within a few days. *R. stolonifer* causes most decay at about 20 C and *R. arrhizus*, at about 30 C (5,9).

For approximately 30 yr, packers/shippers in the United States have relied extensively on the fungicide dicloran (Botran) for control of Rhizopus soft rot in sweetpotatoes following removal from storage, washing, grading, and packing for shipment (14). In recent years, however, the production and availability of this fungicide have been inconsistent, and as a result, there has been interest in the United States in developing alternative control procedures (4). Re-curing roots after washing and packing was suggested (15) but has not been adopted because it is impractical to delay shipments and because Java black rot may increase during re-curing (13).

Use of resistance for disease control is attractive because there are few direct costs to the producer and because it does not require additional labor or effort to implement. It has been reported that sweetpotato genotypes vary in resistance to postharvest pathogens, such as *Fusarium solani* (Mart.) Sacc. (3,6), *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. (8,13), and *Erwinia chrysanthemi* Burkholder, McFadden, & Dimock (7). While variation in resistance to Rhizopus soft rot in sweetpotato has been suggested (11), there have been few reports on controlled studies to evaluate resistance to this disease. Harter and Weimer (9) cut wells in sweetpotato roots and filled them with a suspension of germinated sporangiospores of *R. stolonifer* in nutrient solution. They found that two cultivars, Nancy Hall and Southern Queen, had high incidence of soft rot but developed less decay in each root than the other cultivars tested. However, these two cultivars were susceptible to *R. arrhizus* (9). The present study was conducted to develop alternative methods for evaluating sweetpotato germ plasm for resistance to Rhizopus soft rot and to determine the relative resistance/susceptibility of available germ plasm.

MATERIALS AND METHODS

Inoculum. Isolates 92-RS-2 of *R. stolonifer* and 92-RA-6 of *R. arrhizus*, originally isolated from sweetpotato storage roots, were single-spored and maintained on silica gel at approximately −20 C. For inoculum production, mass transfers were made onto PDA plates and cultures were incubated in darkness at 28 C for 3 days. Sporangiospores were collected by flooding the culture with sterile distilled water, gently rubbing the surface with a flamed stainless steel spatula, and filtering the resulting suspension through four layers of cheesecloth. Sporangiospore concentration was adjusted from counts made with a hemacytometer. Except for experiments on effect of inoculum concentration, inoculum was routinely adjusted to 1.0×10^6 sporangiospores per milliliter.

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Inoculation procedures. After preliminary experiments, two procedures were selected for comparison, a puncture inoculation method and an impact/dip method. In both methods, storage roots that had been cured (5–7 days at 30–35 C, 85–90% RH) and stored for at least 3 wk and not more than 6 mo at 16–19 C were run through a commercial washing machine. In the impact/dip method, the roots were allowed to drop approximately 1 m from the end of the washer into crates and were then dipped in a spore suspension. In the puncture method, roots were loaded carefully from the washer back into crates. Phillips flathead wood screws (7 × 3/4 in., steel-zinc) were dipped in a spore suspension and hammered about 5–10 mm deep into the root at the median and then pulled straight out. Roots were placed one to two layers deep in plastic, stackable baskets, which were arranged in a randomized complete block design in a storage room held at 19–24 C for roots inoculated with *R. stolonifer* or in a separate room at 22–29 C for those inoculated with *R. arrhizus*. In preliminary experiments, noninoculated controls for each method did not develop soft rot, while many inoculated roots developed extensive soft rot beyond the point of inoculation and "whiskers," typical signs of infection by *Rhizopus* spp. Thus, in all subsequent experiments, data were recorded as the number of roots developing soft rot 3 and 7 days, and in some experiments 10, 14, and/or 21 days, after inoculation. Percentage of roots with soft rot was converted by the $\sqrt{\text{arc}}$*sin transformation for statistical analyses.

Effect of inoculum concentration on reaction of genotypes. Reactions of four sweetpotato genotypes—Beauregard, Hernandez, Jewel, and T-30-13—were compared by the puncture and impact/dip methods. All roots used were harvested from the same plot at the Burden Research Plantation in Baton Rouge on 5 October 1993. The roots were cured and stored as above until 23 November 1993, when two replications of 10 roots each for each clone were inoculated with concentrations of 3.5×10^2, 10^3, 10^4, 10^5, or 10^6 sporangiospores per milliliter. The experiment was repeated using roots from the same source starting on 7 December 1993. Transformed data were analyzed by the ANOVA and REG programs in PC-SAS version 6.04 (SAS Institute, Cary, NC).

Screening. Reactions of Beauregard, Hernandez, and Jewel to inoculation with *R. stolonifer* by both methods were compared using four replications of 10 roots each. In a separate experiment, reactions to *R. stolonifer* by both methods and to *R. arrhizus* by the puncture method were compared among 37 sweetpotato clones during winter 1993; 10 roots of each clone were inoculated by each method. Because the overall incidence of soft rot and the differences in incidence among clones were consistently greater with *R. stolonifer* by the puncture method than by the impact/dip method, the puncture method was used in subsequent screening tests to compare reactions of sweetpotato clones. A total of 10 tests by the puncture method and three impact/dip tests were conducted with *R. stolonifer*. In each test, roots were harvested from the same field plot and stored under the same conditions prior to inoculation. Beauregard was included as a standard in all tests; Jewel and Hernandez also were included in each of the impact/dip tests and in five of the puncture tests.

RESULTS

Effect of inoculation procedure on reaction of genotypes. Regardless of the method of inoculation or sweetpotato genotype, individual roots either developed extensive soft rot (usually decay of the entire root) or showed no external decay. Thus, only data on incidence of soft rot were collected. When Beauregard, Hernandez, and Jewel were inoculated by the puncture method, the incidence of soft rot and the differences among the cultivars were greater than when they were inoculated by the impact/dip method (Fig. 1). In this experiment, soft rot was lowest on Beauregard and greatest on Jewel by both methods, but incidence on Beauregard was similar for both methods. When the reactions of 37 sweetpotato genotypes were compared by both methods, levels of soft rot and differences among genotypes were again greatest by the puncture method. There was a positive correlation ($R^2 = 0.22$, $P = 0.0016$) between percent soft rot by the puncture method and percent soft rot by the impact/dip method (Fig. 2). However, there were a number of genotypes for which there was not close agreement between the two methods of inoculation.

Effect of inoculum concentration on reaction of genotypes. The two runs of the infectivity titrations did not differ significantly and were combined for analysis (Table 1). With the impact/dip method, incidence of disease was similar regardless of inoculum concentration or genotype at 3 and 7 days (Fig. 3). With the puncture method, there was a linear relationship between inoculum concentration and percent soft rot for clone T-30-13 and the cultivar Hernandez at 3 days after inoculation, but the incidence of soft rot was low at all concentrations for Beauregard and Jewel (Table 2). By 7 days, the incidence of soft rot had reached nearly 100% at the four highest concentrations for T-30-13 and the three highest concentrations for Hernandez (Fig. 3). For Beauregard at 7 days, incidence was low at all concentrations, and for Jewel, incidence was intermediate and the rise with increasing inoculum concentration from $\log_{10}$ 2.51 to 5.51 was not significant (Table 2).

In the tests in which various sweetpotato genotypes were compared by the puncture method, incidence of disease was consistently low in Beauregard (Fig. 4). Levels of soft rot in Jewel and Hernandez were more variable from test to test but were greater than those in Beauregard in all tests. In impact/dip tests, differences among the cultivars were not as great, although levels of soft rot were lower in Beauregard than in Jewel or Hernandez (Fig. 4). Incidence of soft rot was greater on Jewel and Hernandez by the puncture method than by the impact/dip method, but this was not true for Beauregard.

Germ plasm screening. Results of one test comparing 37 genotypes by the puncture method are presented in Figure 5. Soft rot development was less with *R. arrhizus* than *R. stolonifer*, and genotypes that were most susceptible to one species were usually among the most susceptible to the other. Most genotypes

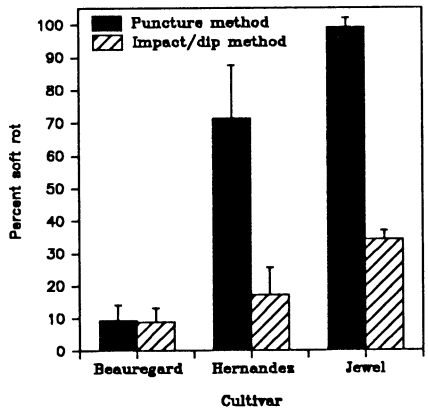

Fig. 1. Incidence of Rhizopus soft rot on sweetpotato cultivars Beauregard, Hernandez, and Jewel following inoculation with *Rhizopus stolonifer* by the impact/dip or puncture method.

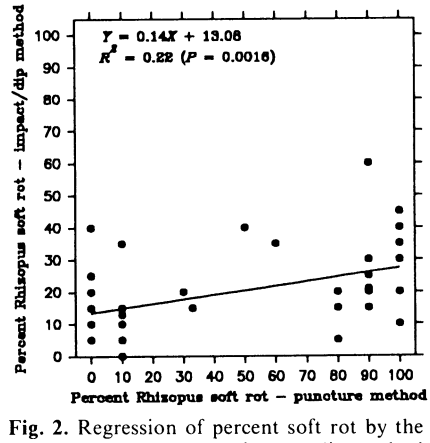

Fig. 2. Regression of percent soft rot by the puncture method on the impact/dip method of inoculating 37 sweetpotato genotypes with *Rhizopus stolonifer*. Each dot represents a data point for a different genotype.

Table 1. *F* values and probabilities to exceed F ($P > F$) from ANOVA of percent soft rot data collected at 3 or 7 days after inoculation of the sweetpotato genotypes Beauregard, Hernandez, Jewel, and T-30-13 with different concentrations of sporangiospores of *Rhizopus stolonifer* by the puncture and impact/dip methods

	3 days		7 days	
Source	*F* value	$P > F$	*F* value	$P > F$
Cultivar (Cv)	32.56	0.0001	45.86	0.0001
[Inoculum] (Inoc)	22.67	0.0001	6.89	0.0001
Inoc. Method (Meth)	75.20	0.0001	120.69	0.0001
Block	0.54	0.6524	4.54	0.0047
Cv × Inoc	3.55	0.0001	1.85	0.0466
Cv × Meth	26.66	0.0001	26.94	0.0001
Inoc × Meth	9.51	0.0001	3.65	0.0075

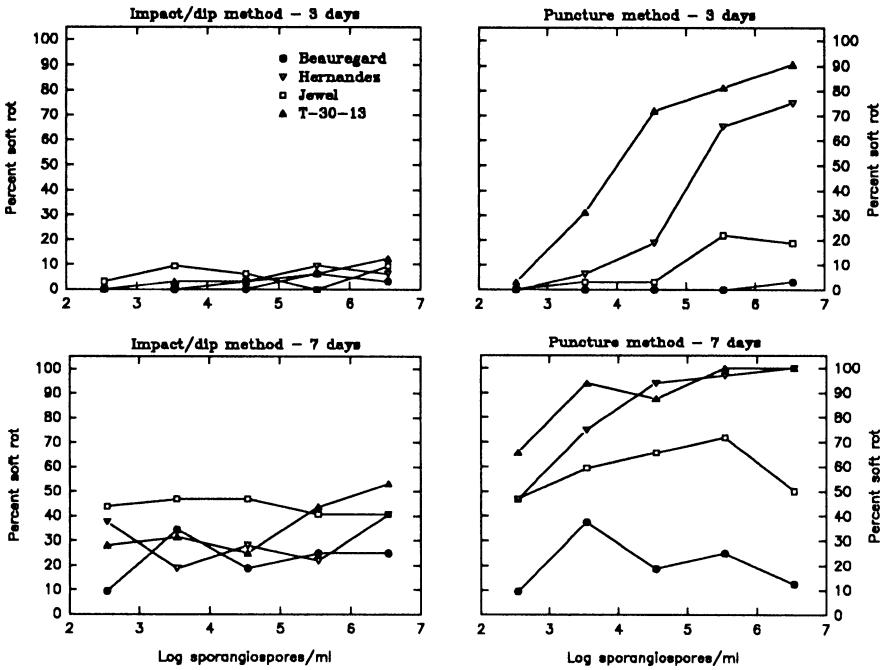

Fig. 3. Incidence of soft rot at 3 and 7 days after inoculation with varying concentrations of sporangiospores of *Rhizopus stolonifer* by either the puncture or the impact/dip method on sweetpotato cultivars Beauregard, Hernandez, and Jewel and the clone T-30-13.

Table 2. Regression analyses of $\sqrt{\text{arc}}$*sin transformation of percent soft rot by sweetpotato genotype and method of inoculation for Beauregard, Hernandez, Jewel, and T-30-13 inoculated with different concentrations of sporangiospores of *Rhizopus stolonifer*

Cultivar	Method	Days after inoculation	Adj. R^2	*P*	Regression equation
Beauregard	Impact/dip	3	0.11	0.0839	$Y = 2.07X - 6.29$
	Impact/dip	7	0.04	0.2092	$Y = 3.16X + 10.87$
	Puncture	3	0.06	0.1628	$Y = 1.04X - 3.67$
	Puncture	7	−0.04	0.6958	$Y = -0.93X + 28.11$
Hernandez	Impact/dip	3	0.18	0.0345	$Y = 3.34X - 9.51$
	Impact/dip	7	−0.05	0.7419	$Y = 0.80X + 27.21$
	Puncture	3	0.73	0.0001	$Y = 17.26X - 48.75$
	Puncture	7	0.50	0.0003	$Y = 10.99X + 23.23$
Jewel	Impact/dip	3	−0.05	0.9041	$Y = 0.23X + 7.12$
	Impact/dip	7	−0.03	0.5509	$Y = -0.76X + 44.71$
	Puncture	3	0.39	0.0019	$Y = 6.82X - 19.20$
	Puncture	7	−0.05	0.6810	$Y = 1.25X + 46.27$
T-30-13	Impact/dip	3	0.15	0.0491	$Y = 3.52X - 8.83$
	Impact/dip	7	0.22	0.0223	$Y = 3.87X + 18.84$
	Puncture	3	0.76	0.0001	$Y = 17.55X - 31.01$
	Puncture	7	0.53	0.0002	$Y = 8.01 + 40.54$

with white-fleshed storage roots developed 100% soft rot by 7 days after inoculation with *R. stolonifer*. White Beauregard, which is a mutation for storage roots with light-colored flesh selected from Beauregard, was an exception in that, like its progenitor, it did not develop soft rot. Orange-fleshed cultivars varied in reaction, with cultivars such as Hernandez and Georgia Red developing high levels of soft rot similar to those of their white-fleshed genotypes, while Beauregard did not develop soft rot (Fig. 5).

DISCUSSION

Consistent differences were found in the reaction of sweetpotato genotypes to *R. stolonifer*. While trends were similar for *R. arrhizus*, genotypes generally were less susceptible to this species than to *R. stolonifer*. These differences were most readily distinguished by the puncture method of inoculation but also were evident with the impact/dip method. The white-fleshed cultivars inoculated in this study, except for White Beauregard, appeared to be uniformly susceptible to Rhizopus soft rot. Although some orange-fleshed cultivars were as susceptible, overall they were more resistant. Beauregard was consistently the most resistant of the commercially grown cultivars. These results differ from those of Harter and Weimer (9) in several ways: 1) We did not observe differences in the severity or extent of decay, only differences in incidence; 2) Harter and Weimer described two cultivars with light-colored flesh, Nancy Hall and Southern Queen, as resistant to *R. stolonifer*, since the extent of decay was limited, although there was a high incidence of soft rot; and 3) Harter and Weimer found that although Nancy Hall and Southern Queen were resistant to *R. stolonifer*, they were susceptible to *R. arrhizus*, whereas we found that the genotypes evaluated ranked similarly in reaction to both species but were generally more resistant to *R. arrhizus*.

R. arrhizus occurs less frequently than *R. stolonifer* on sweetpotato (4,5,9) and causes soft rot at temperatures warmer than those at which sweetpotatoes are normally stored. Since sweetpotatoes appear to be generally more resistant to *R. arrhizus*, we agree with Harter and Weimer (9) that reaction to *R. stolonifer* is of greater importance than that to *R. arrhizus*.

The apparent difference in reaction of white-fleshed and orange-fleshed genotypes could be due to more carotene in the orange-fleshed types. Carotene content has been shown to be reduced following infection by *R. stolonifer* (19), but we are not aware of any reports of carotene content influencing disease resistance. Since some orange-fleshed genotypes were as susceptible as the white-fleshed genotypes, however, it is more likely that flesh color may be linked to

an unknown resistance factor as well as to carotene content. The resistance observed in Nancy Hall and Southern Queen (9) and White Beauregard may be explained by the fact that their flesh is not pure white and may be tinged with yellow to salmon color, whereas many of the other genotypes have truly white flesh. Thus, they may share with the orange-fleshed genotypes whatever trait is linked with resistance to *R. stolonifer*.

Development of soft rot requires suitable wounds for penetration and establishment of the pathogen (4,5). Srivastava and Walker (18) demonstrated that the type of wound is very important to successful establishment of the pathogen. Generally, it is thought that wounds resulting in crushing of host tissue are most likely to release nutrients from the plant cells that can be utilized by the fungus prior to infection. In this study, the puncture method provided more uniform wounding than the impact/dip method. Genotypes responded to increased inoculum concentration with the puncture method to a greater extent than with the impact/dip method. Possibly this is because the former results in crushing of adjacent tissue and inoculum is simultaneously deposited at the wound site. Response of potato cultivars to changing inoculum concentration of *Phoma exigua* Desmaz. var. *foveata* (Foister) Boerema also depends on type of wounding (1,2). However, differences among cultivars to increasing inoculum concentration were minimal when a uniform method of wounding was used and were greater when tubers were wounded by repeatedly running them on a grading table (2).

In potato, a distinction has been made between "tissue resistance" to the pathogen and resistance based on response of the cultivar to wounding (10). A similar phenomenon may occur with Rhizopus soft rot of sweetpotato. The large differences among genotypes in disease development and the response to changing inoculum concentration suggest that tissue resistance to *R. stolonifer* is expressed to a greater extent following puncture inoculation, where wounding is relatively uniform. On the other hand, wounding is more variable with the impact method, and differences among genotypes also may result from differences in sensitivity to bruising. Thus, we speculate that although Beauregard may have tissue resistance to *R. stolonifer*, as indicated by its consistently low incidence of soft rot in puncture wound tests, it may be more sensitive to bruising, since it had a higher incidence of soft rot in impact/dip tests even though most genotypes had a lower incidence in impact/dip than in puncture tests. Tissue resistance and wounding resistance would be expected to interact to determine the actual level of resistance expressed. The care taken in commercial postharvest handling would affect the extent of wounding and the degree to which resistance to Rhizopus soft rot could be exploited.

There have been several studies of abrasion or skinning of sweetpotato, and methods have been developed to quantify this type of injury (12,21). However, there is a lack of information for sweetpotato on bruise wounds that are more likely to favor infection by *R. stolonifer*. Several methods have been developed for evaluating bruising on potato (16,17). Future research on resistance to postharvest pathogens in sweetpotato should endeavor to more accurately characterize the types of wounds that favor infection by different pathogens and to integrate information on tissue resistance with measures of resistance to relevant types of wounding and wound healing characteristics of the different genotypes (20). In addition, research is needed to determine the feasibility of using resistance for control of Rhizopus soft rot and other postharvest diseases under varying storage conditions. In particular, it should be determined whether factors known to predispose sweetpotato to Rhizopus soft rot (e.g., chilling injury, different types of wounding) negate the effectiveness of resistance.

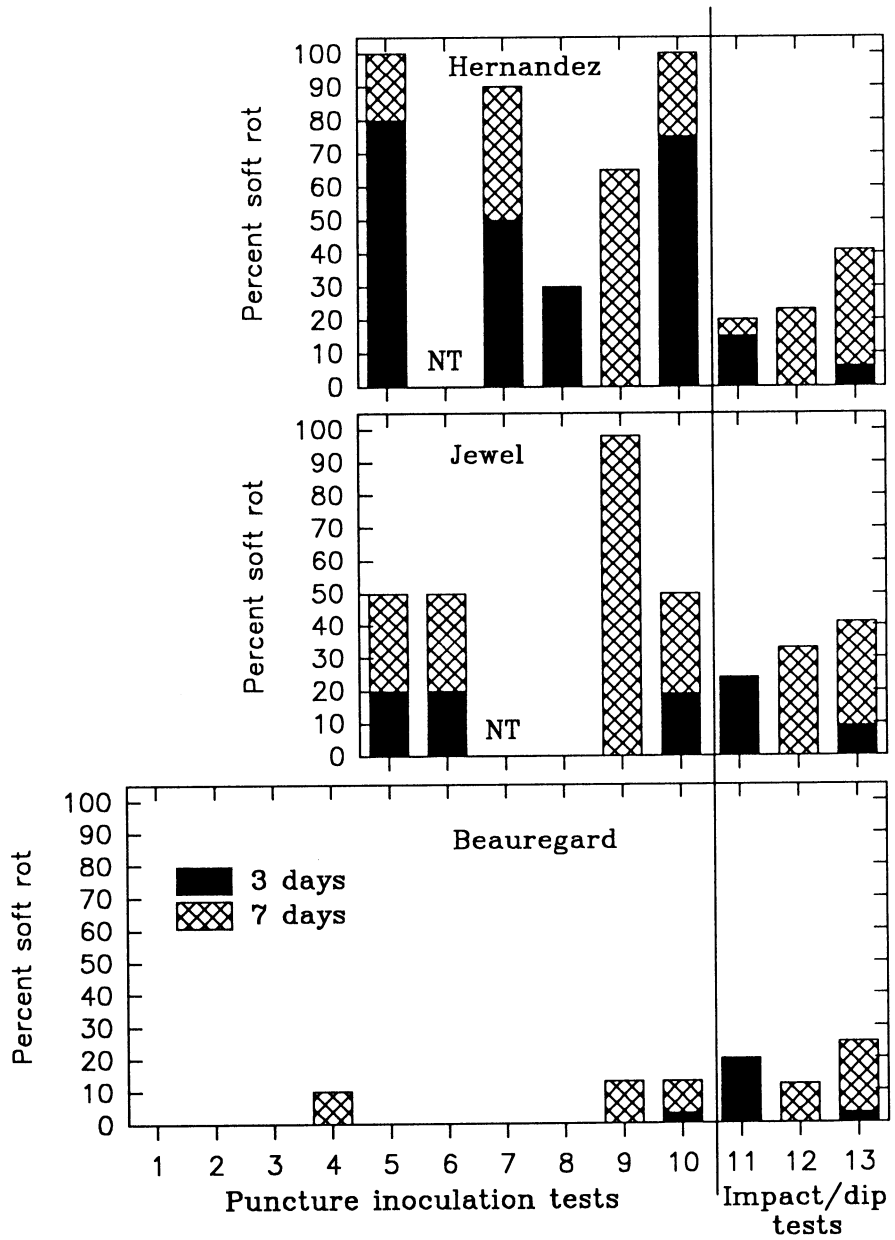

Fig. 4. Summary of results from 10 tests with the puncture method and three tests with the impact/dip method of inoculation with *Rhizopus stolonifer*. Percent soft rot at 3 and 7 days after inoculation is shown for sweetpotato cultivars Hernandez, Jewel, and Beauregard. NT = not tested.

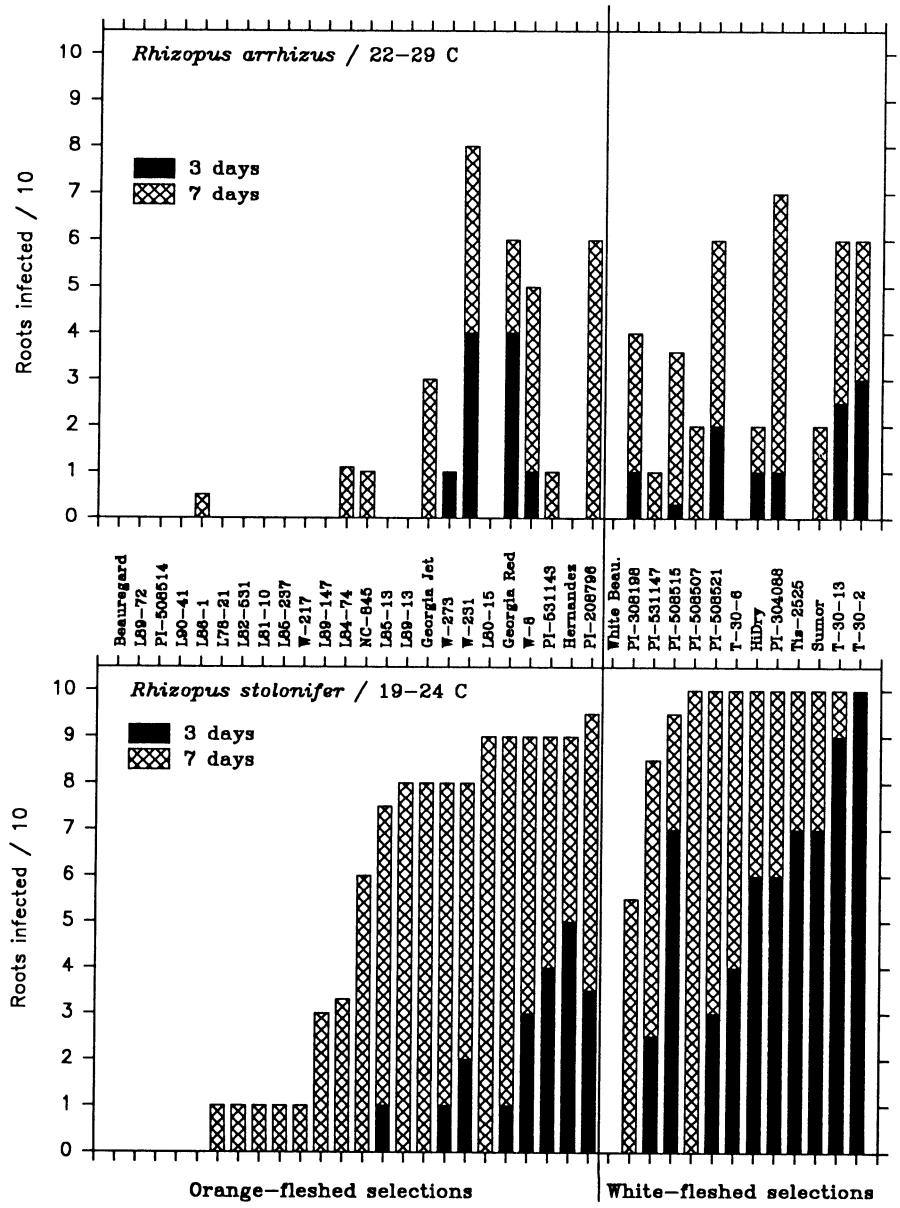

Fig. 5. Incidence of soft rot in different orange- and white-fleshed sweetpotato genotypes 3 and 7 days after inoculation by the puncture method with either *Rhizopus arrhizus* (incubated at 22–29 C) or *R. stolonifer* (incubated at 19–24 C).

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