Monilinia fructicola Resistance in the Peach Cultivar Bolinha

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ABSTRACT

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A search for *Monilinia fructicola* resistance among adapted peach cultivars and selections indicated that the best technique to obtain differences in degrees of resistance is to artificially inoculate maturing fruit. Twig and blossom inoculation showed no detectable differences. With the fruit inoculation technique, the peach cultivar Bolinha was more resistant than the selection Conserva 144, as indicated by the reduced rate of lesion development and sporulation per unit area. Field observations for a 3-yr period support these results, in that the incidence of brown rot infected fruit is less on Bolinha than on Conserva 144 or other commercial cultivars.

Brown rot, caused by *Monilinia fructicola* (Wint.) Honey, is considered to be the most important fruit-rotting disease of peaches in Southern Brazil (4). In this area, the temperature, rainfall, and relative humidity are favorable for disease development (6). Prolonged periods of wetness often occur during bloom (July to August) and fruit ripening (October to January). Losses exceeding 50% have been experienced because of unpredictable weather and difficulties associated with fungicide applications. Disease control with chemicals is costly because repeated applications of protective fungicides such as captafol and benomyl (2,3) are often necessary.

The use of resistant or tolerant cultivars is known to be one of the best means of disease control, yet peaches resistant to brown rot have not been reported. Many peach cultivars introduced from other countries such as the United States, Australia, and South Africa, where *M. fructicola* has been reported as the primary brown rot pathogen, showed high susceptibility under Southern Brazil conditions.

In view of the possible genetic resistance to brown rot of cultivars developed in Brazil and the need for a technique of screening cultivars and selections developed by breeders, we report results of different inoculation techniques and differences in Bolinha and Conserva 144 after fruit inoculation. A preliminary report of this study has been published (5).

MATERIALS AND METHODS

Plant materials. Trees used for inoculation of blossoms and twigs were located in an experimental test plot at the Centro Nacional de Pesquisa de Fruteiras de Clima Temperado (CNPFT), Pelotas, RS, Brazil. They were 4 yr old and in the second year of production. Routine cultural practices were employed, except that fungicide sprays were withheld while the experiments were in progress. For fruit inoculation, samples of Bolinha and Conserva 144 were obtained from commercial orchards (5 yr old), where the cultural practices had not been changed and the fruit had been paper bagged to prevent fungicide or insecticide residues.

Blossom inoculation. Ten blossoms per cultivar or selection were inoculated at the pink bud stage with 0.2 ml per blossom of conidial suspension of *M. fructicola* containing 1.0×10^5 spores per milliliter in August of 1981 and 1982. Ten blossoms inoculated with 0.2 ml per blossom of distilled water served as controls. Only one blossom per twig was inoculated and the rest removed. Observations for disease were made daily, and the appearance of blossom blight, incidence of twig infection, and sporulation on the blighted blossom parts were recorded. The blighted blossoms and the portions of the twig where the blighted blossoms were attached were plated on PDA to reisolate *M. fructicola*.

Twig inoculation. One-year-old twigs, 5–9 mm in diameter, were inoculated in the winter of 1981–1982. A 5-mm circular portion of the bark was removed with a cork borer in the middle portion of the twig and replaced with a 5-mm-diameter mycelial disk (with the mycelial side next to xylem). The inoculated portion was secured with masking tape. Control twigs were similarly inoculated, but with sterile potato-dextrose agar (PDA). Thirty days after inoculation, the twigs were harvested and the bark peeled to measure the necrotic tissue beyond the inoculated 5-mm portion under a stereomicroscope. To determine which portion of the bark or xylem was infected with *M. fructicola*, 1-mm-length portions of the discolored wood were plated onto PDA.

The diameter of the twigs was recorded to determine whether or not it influenced the length of the cankers.

Fruit inoculation. Fruit of Bolinha and Conserva 144 were enclosed with a double layer of glassine bag (Savage Universal Corporation, New York, NY) 2 mo before harvest to protect them from pesticide contamination and insect injuries. Except as otherwise stated, 30 fruit of approximately uniform size (6.4–6.9 cm diameter) and maturity were harvested and inoculated the same day. For each genotype, two stages of maturity designated as ripe (firm ripe) and mature green (ground color green to yellow blush) were used. Soluble solids (taken with a hand refractometer) and firmness (measured with a Magness Taylor Firmness Tester, AMETEK/Testing Equipment Systems, Lansdal, PA, with a 9/16-

TABLE 1. Soluble solids and firmness of fruits of peach cultivar Bolinha and selection Conserva 144 at two stages of fruit maturity

State of maturity and genotype	Soluble solids[x] (%)	Firmness[x] (lb)
Mature green fruits		
Bolinha	10.5 a[y]	13.5 a
Conserva 144	7.9 b	15.5 b
C.V.[z]	13.6%	14.9%
Ripe fruits		
Bolinha	11.4 a	8.2 a
Conserva 144	8.5 b	7.1 a
C.V.[z]	13.0%	24.7%

[x] Average of three replicates of 10 fruits.
[y] Values followed by the same letters are not significantly different according to the Duncan's multiple range test, $P = 0.05$.
[z] Coefficient of variation.

in. tip) were recorded for both maturity stages (Table 1).

M. fructicola (isolate AF32/81) inoculum consisted of a benomyl-sensitive culture obtained from a rotting peach fruit. Mycelial disks and spores were obtained from cultures grown on PDA for 5–7 days. When conidial suspensions were required, spores were removed by adding 20 ml of sterile distilled water solution containing 0.001% Triton X114 (octylphenoxy polyethoxyethanol) as a wetting agent and pouring the conidial suspension through four layers of sterile cheesecloth to minimize the presence of mycelial fragments. The inoculum concentration was adjusted to 2×10^5 conidia per milliliter with a hemacytometer.

Incubation of inoculated fruit or fruit parts was for 48 hr at 23–25 C in the dark at about 90% relative humidity. This was obtained by placing the fruit on stainless steel supports in plastic trays with pieces of cotton soaked in distilled water and enclosing them in sealed plastic bags.

Three methods of inoculation were compared:

1. Inoculation of uninjured fruit by depositing 10 μl of conidial suspension on the fruit surface. The fruit was previously determined to be free of visible mechanical injury by using a stereomicroscope. An approximately 1-cm area was first marked on four corners with a felt pen and the conidial suspension was deposited using a pipette tip that delivers a total of 10 μl (Fig. 1A). Data were taken on diameter of rot after 48, 72, and 96 hr and number of fruit with infection after 24, 48, and 72 hr.

2. Inoculation of artificially injured fruit (outer mesocarp tissue) consisted of injuring the fruit with a 4-mm-diameter nail (mounted in a wooden handle) to the depth of 3 mm and placing 10 μl of the conidial suspension in the wound with a Pipetman (Fig. 1B). Data were taken on diameter of rot 48, 72, and 96 hr after inoculation.

3. Inoculation of inner mesocarp tissue (flesh) was made on two sliced pieces from five fruits. A 5-mm mycelial disk was placed with the mycelial surface down on the center of the cut surface of the flesh (Fig. 1C). Diameter of decay was taken after 24, 48, and 72 hr and amount of sporulation after 7 days. To facilitate counting the conidia, only one slice of each fruit was used. In each slice, one 1-mm slice was taken below the sporulating surface from which two 8-mm disks were removed. The disks were macerated with a glass rod, in a solution containing 5 ml of distilled water, Triton X114 (0.05%), and formaldehyde (5%), then shaken and filtered through two layers of cheesecloth before counting the number of conidia with a hemacytometer.

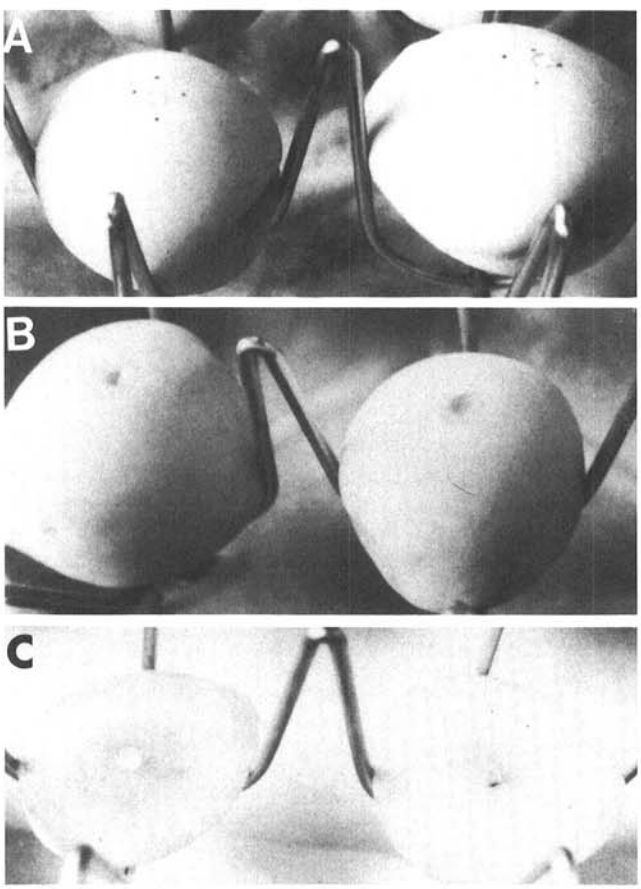

Fig. 1. Inoculated fruit on stainless steel support. **A,** Uninjured fruit; **B,** Injured fruit; **C,** Inner mesocarp tissue.

RESULTS

Blossom inoculation. No differences were noted among the genotypes tested (Diamante, Bolinha, Aldrighi, BR-2, Conserva 144, Topazio, Magno, and Capdeboscq) as to the time required for blossoms to blight, percent blighted flowers, and sporulation. Browning of petals, starting at the base, was observed 48 hr after inoculation. All inoculated blossoms wilted after 3–4 days and started to drop after the fifth day. Sporulation on the outer surface of the sepals was observed on 100% of the buds 5 days after inoculation. In the controls, all buds opened normally, with no incidence of brown rot infection. All blighted blossoms when plated on PDA yielded *M. fructicola*. However, attempts to recover the fungus from the peduncle scar or beneath the scar gave negative results.

Twig inoculation. No significant differences in canker length among genotypes were obtained in the 1981 (data not shown) and 1982 (Table 2) tests. Canker length was not correlated with the diameter of the twigs inoculated. *M. fructicola* could be isolated throughout the length of the cankered area up to the margins of the visible canker.

Fruit inoculation. *Uninjured fruit.* Data on percent infected fruit are given in Table 3. Percent infected fruit was significantly higher in ripe than in mature green fruit in both genotypes except after 72 hr, when no difference was obtained in Conserva 144. For both stages of fruit maturity, the percent infected fruit was the same or lower in Bolinha than in Conserva 144 at different periods after inoculation. There were no significant differences in lesion size

TABLE 2. Twig diameter of six peach genotypes and the corresponding length of cankers that developed 30 days after inoculation with *M. fructicola*

Genotype	Twig diameter (cm)[y]	Canker length (cm)[y]
Diamante	7.4 a[z]	4.2 a[z]
Bolinha	6.8 ab	3.0 a
Aldrighi	6.7 ab	3.5 a
BR-2	6.6 ab	3.6 a
Conserva 144	63. bc	3.6 a
Kakamas	5.5 c	3.4 a

[y] Data represent the average of 10 twig measurements.
[z] Values followed by the same letters are not significantly different according to the Duncan's multiple range test, $P = 0.05$.

TABLE 3. Percent infected fruit at time intervals after inoculation of uninjured mature green and ripe fruit of two peach genotypes[y]

Genotype and stage of fruit maturity	Hours after inoculation		
	24	48	72
Bolinha			
Mature green	26.7 c[z]	48.3 c[z]	63.3 b[z]
Ripe	55.0 b	85.4 a	92.0 a
Conserva 144			
Mature green	30.0 c	71.7 b	91.7 a
Ripe	72.2 a	90.0 a	96.4 a

[y] Average of three replications of 10 fruits.
[z] Values followed by the same letters are not significantly different according to the Duncan's multiple range test, $P = 0.05$.

(data not shown) between green or ripe fruits of Bolinha and Conserva 144 after 48, 72, and 96 hr of incubation.

Artificially injured fruits. On artificially injured fruit, Bolinha developed significantly smaller lesion diameters than Conserva 144 on mature green as well as on ripe fruit during the three periods of incubation (Fig. 2A). The difference in lesion diameter of Bolinha in comparison with Conserva 144 was 0.8, 1.2, and 1.2 cm in mature green fruits and 0.7, 1.4, and 1.7 cm in ripe fruits after 48, 72, and 96 hr of incubation, respectively.

The rate of decay per 24-hr period, as determined by linear regression analysis, was faster in Conserva 144 than in Bolinha (2.50 vs. 1.80 cm) (Fig. 2B) and the same for the mature green and ripe fruit (2.0 vs. 1.95 cm) (Fig. 2C).

Flesh (inner mesocarp tissue). Lesion size on Conserva 144 did not differ from that on Bolinha after 24 hr incubation, but it was significantly larger after 48 and 72 hr at both stages of fruit maturity (Fig. 3A). The rate of decay per 24 hr, as determined by linear regression analysis, was higher in Conserva 144 than in Bolinha (1.78 vs. 1.58 cm) (Fig. 3B) and in ripe than in mature green fruits (1.80 vs. 1.55 cm) (Fig. 3C). Significant differences in spore production between the two genotypes was obtained. The number of spores in Bolinha was 27.8% less in mature green fruit and 39.0% less in ripe fruit than with Conserva 144 (Table 4).

DISCUSSION

The use of blossom and twig inoculations for evaluating differences in resistance or susceptibility to *M. fructicola* among peach genotypes has not successfully been demonstrated. Ogawa

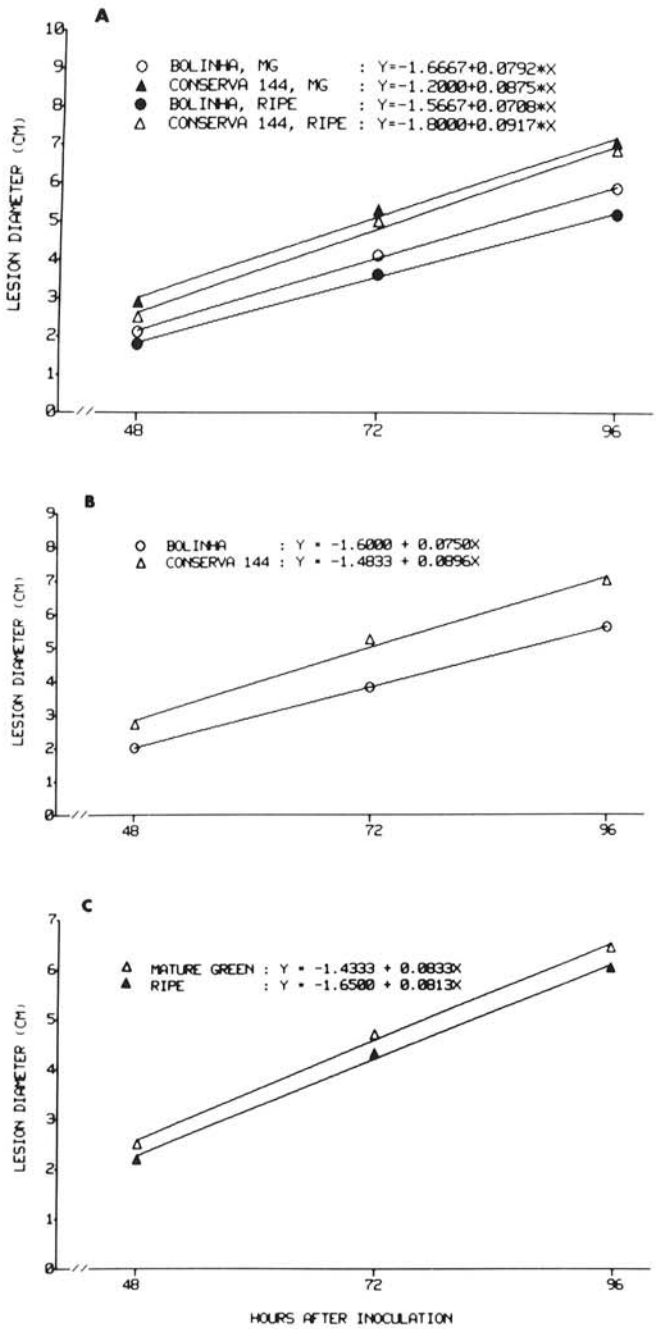

Fig. 2. Lesion diameter at time intervals after inoculation of artificially injured mature green and ripe fruit of peach cultivar Bolinha and selection Conserva 144 **(A)** and the rate of decay per 24-hr period as affected by genotype **(B)** and stage of fruit maturity **(C)**.

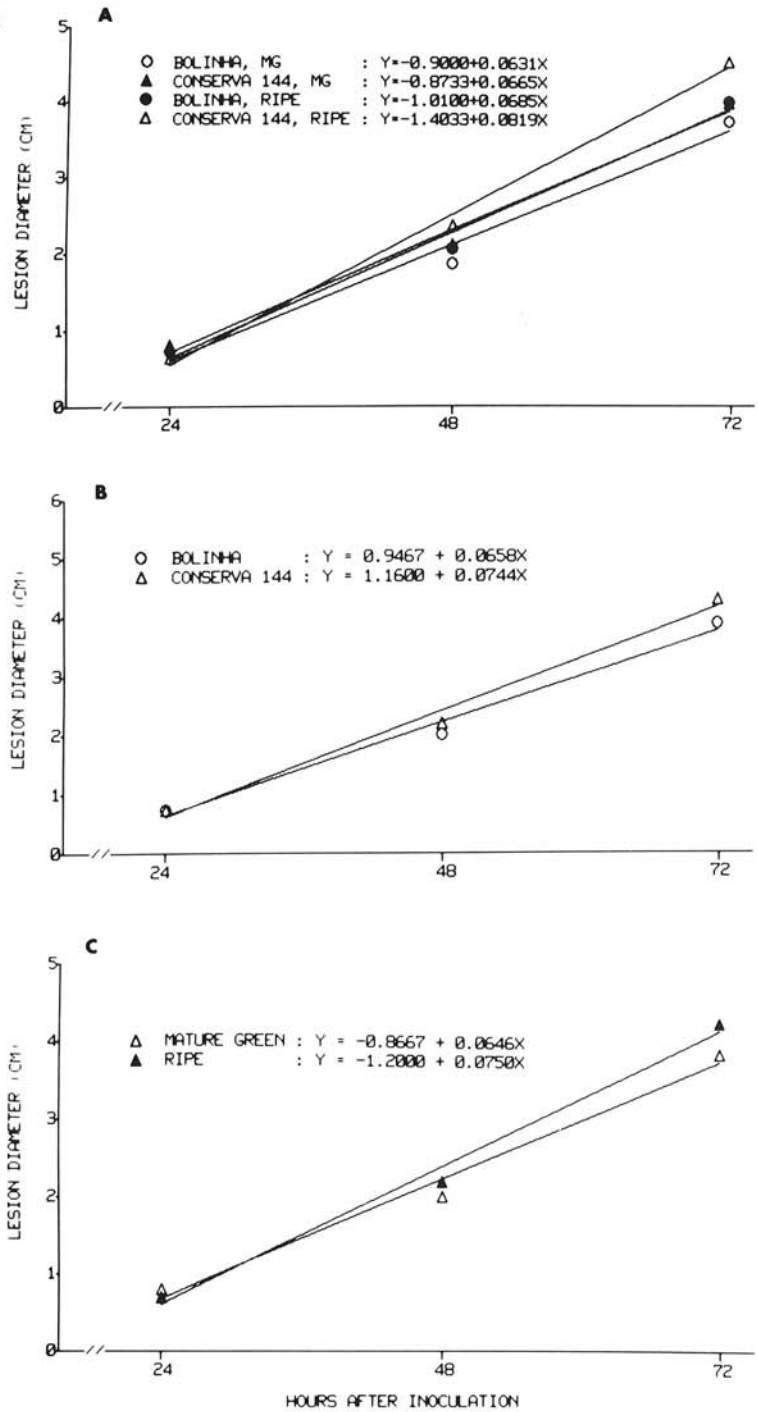

Fig. 3. Lesion diameter at time intervals after flesh inoculation of mature green and ripe fruit of peach cultivar Bolinha and selection Conserva 144 **(A)** and the rate of decay per 24-hr period as affected by genotype **(B)** and stage of fruit maturity **(C)**.

TABLE 4. Spore production on Bolinha and Conserva 144 seven days after inoculation of fruit flesh

Genotype	Number of spores per ml
Mature green fruit	
Bolinha	54.1×10^4 a[y]
Conserva 144	74.9×10^4 b
C.V.[z]	20.3%
Ripe fruits	
Bolinha	44.1×10^4 a
Conserva 144	72.3×10^4 b
C.V.[z]	27.8%

[y] Mean separation for each stage of fruit maturity by Duncan's multiple range test, $P = 0.05$.
[z] Coefficient of variation.

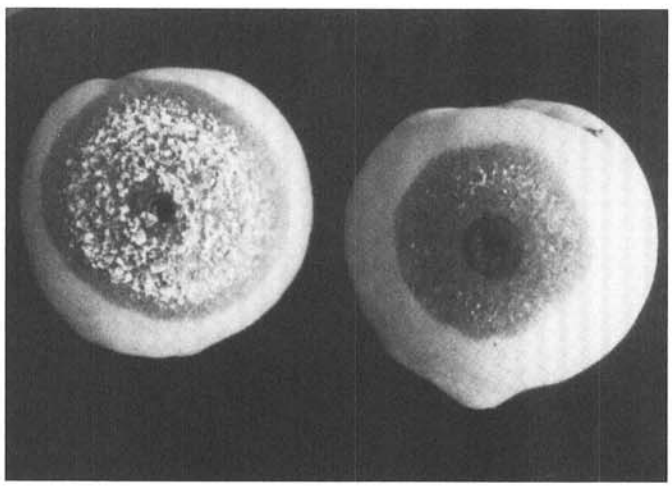

Fig. 4. Typical lesion size and sporulation of Conserva 144 (left) and Bolinha (right) 72 hr after inoculation of artifically injured fruit.

and English (7) were able to obtain significant differences in reaction to twig inoculation between *Prunus* species, whereas Crossa-Raynaud (1) demonstrated that mean growth rate of cankers on young branches can be used in appraising resistance to *M. laxa* (Aderh. & Ruhl.) in almond and apricot cultivars. In our study, however, no significant differences in canker length or percent blighted flowers between the resistant peach cultivar Bolinha and the susceptible Conserva 144 were obtained. Significant differences between Bolinha and the other genotypes were noted in the fruit inoculations. In the preliminary and final experiments, using fruit inoculation techniques, Bolinha proved to be the most resistant and Conserva 144 the most susceptible among the genotypes tested, as indicated by lesion size and sporulation (Fig. 4). Results of the laboratory tests confirm the observed resistance of Bolinha in the field. In a survey (unpublished data) of blossom blight and fruit rot infections in a commercial orchard in Pelotas, RS, from 1981 to 1983, a range of 0.6–1.0% blossom blight for cultivars Bolinha, Capdeboscq, Magno, and Conserva 144 was obtained. Fruit rot at harvest was 5.9% for Bolinha, 66.5% for Capdeboscq, 43.3% for Magno, and 77.0% for Conserva 144. The differences in percent fruit rot at harvest did not appear to be related to blossom blight infection or to the maturation period. Of the four genotypes, Conserva 144 is the earliest maturing and Magno, the latest. Capdeboscq and Bolinha ripen at approximately the same time. Lower incidence of decay of Bolinha in the field may be attributed to slower growth of brown-rot lesions and less sporulation of infected fruit.

Among the fruit inoculation techniques, inoculation of artificially injured fruits and evaluation of lesion diameter after 72 and 96 hr appeared to be the most consistent method, as shown by the low coefficient of variation (Table 5). In the estimation of the spores produced per unit area, however, the use of inner mesocarp tissue (flesh) is preferable because of the ease of macerating the flesh as compared with the epidermis. In preliminary screening of cultivars and selections at CNPFT, therefore, we are employing the technique of inoculation of artificially injured fruits and measuring lesion diameter after 72 hr. These data are compared with those of Bolinha and Conserva 144. When a promising genotype is detected, inoculations of inner mesocarp tissue to determine sporulation are conducted.

It is interesting to note that in artificially injured fruits of both Bolinha and Conserva 144, the lesion diameter of mature green is greater than that of ripe fruit. The same result was obtained in a preliminary test in California using the susceptible California peach cultivar Halloween. Although firmness in plum (8) and low sugar content in apple (9) have been associated with host resistance to brown rot, these two factors cannot explain the resistance of Bolinha nor the difference in susceptibility between the green mature and ripe fruit.

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TABLE 5. Comparison of three different techniques of fruit inoculation to detect differences in diameter (cm) of brown rot lesions at different times after inoculation

Genotype and stage of fruit maturity	Uninjured fruit* (epidermis)			Artificially injured fruit* (outer mesocarp tissue)			Flesh (inner* mesocarp tissue)		
	48 hr	72 hr	96 hr	48 hr	72 hr	96 hr	24 hr	48 hr	72 hr
Bolinha: Mature green	X[a] = 0.19 s[b] = 0.32 cv[c] = 166.8%	X = 0.83 s = 1.12 cv = 135.4%	X = 1.56 s = 1.81 cv = 115.7%	X = 2.07 s = 0.43 cv = 20.9%	X = 4.08 s = 0.50 cv = 12.3%	X = 5.87 s = 0.69 cv = 11.8%	X = 0.74 s = 0.26 cv = 34.6%	X = 1.88 s = 0.44 cv = 23.2%	X = 3.77 s = 0.79 cv = 21.0%
Bolinha: Ripe	X = 0.62 s = 0.57 cv = 92.9%	X = 1.69 s = 1.22 cv = 72.3%	X = 2.90 s = 1.91 cv = 65.9%	X = 1.76 s = 0.49 cv = 27.8%	X = 3.61 s = 0.57 cv = 15.9%	X = 5.22 s = 0.86 cv = 16.4%	X = 0.74 s = 0.27 cv = 36.9%	X = 2.07 s = 0.44 cv = 21.4%	X = 4.03 s = 0.63 cv = 15.7%
Conserva 144: Mature green	X = 0.11 s = 0.35 cv = 321.7%	X = 0.61 s = 1.14 cv = 187.3%	X = 1.24 s = 1.92 cv = 154.9%	X = 2.87 s = 0.21 cv = 7.2%	X = 5.35 s = 0.48 cv = 8.9%	X = 7.12 s = 0.31 cv = 4.3%	X = 0.82 s = 0.31 cv = 38.3%	X = 2.12 s = 0.29 cv = 13.5%	X = 4.01 s = 0.49 cv = 12.2%
Conserva 144: Ripe	X = 0.56 s = 0.68 cv = 120.5%	X = 1.90 s = 1.73 cv = 90.9%	X = 3.43 s = 2.51 cv = 73.3%	X = 2.55 s = 0.23 cv = 9.1%	X = 4.95 s = 0.30 cv = 6.1%	X = 6.92 s = 0.36 cv = 5.2%	X = 0.64 s = 0.33 cv = 51.3%	X = 2.37 s = 0.41 cv = 17.3%	X = 4.57 s = 0.45 cv = 9.9%

[a] Average of three replications of 10 fruits.
[b] Standard deviation.
[c] Coefficient of variation.

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