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# Variable Response of Open-Pollinated Seedling Progeny of Avocado to Phytophthora Root Rot

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Phytophthora root rot, caused by *Phytophthora cinnamomi* Rands, is the most important disease of avocado (*Persea americana* Miller). In an attempt to identify root rot-resistant rootstocks that could ultimately be used under conditions in southern Florida, we screened open-pollinated progeny of avocado from the National Germplasm Repository in Miami. From 1996 to 1998, a total of 2,355 seedlings from 51 accessions were examined in potting mix artificially infested with *P. cinnamomi*. Most seedlings developed severe root rot, but tolerance was observed in some families (*i.e.*, progeny of certain accessions). Although the most susceptible families developed mean disease ratings of up to 97% root necrosis, mean ratings for the most tolerant families were less than 60%. There was also a strong relationship between the racial background of the female parent and the tolerance of seedlings. Seedlings of the West Indian race and hybrids between it and the Guatemalan race were significantly more tolerant than those from other parents ($P<0.05$). Individuals in several families developed <50% root necrosis, the arbitrary standard of tolerance in this study. Twelve families accounted for 82% (188 of 229) of the tolerant seedlings, and only two of these did not have a West Indian or Guatemalan × West Indian pedigree. Broad-sense heritability for PRR tolerance was 0.45. This is the first report on the inheritance of PRR tolerance in avocado and on the influence of genotype and racial pedigree under controlled conditions.

KEY WORDS: *Phytophthora cinnamomi*; *Persea americana* var. *americana*; *Persea americana* var. *guatemalensis*; avocado rootstocks; Phytophthora root rot.

## INTRODUCTION

Avocado, *Persea americana* Miller, is a significant fruit crop in the tropics and warm subtropics. In 2000, global production exceeded 2.4 million metric tons and the major producers were Mexico, Indonesia, South Africa and the USA (California and Florida) (1). Avocado originated in the Western Hemisphere. Three races were domesticated from primitive forms of the species that ranged from southern Mexico to Colombia (4). The Mexican race, *P. americana* var. *drymifolia*, arose in the highlands of southern Mexico, and the Guatemalan race, *P. americana* var. *guatemalensis*, arose in the highlands of Guatemala and Honduras. In contrast, the West Indian race, *P. americana* var. *americana*, arose in humid lowland environments in Colombia. Extant cultivars of avocado are either one of the above races or are racial hybrids.

Phytophthora root rot (PRR), caused by *Phytophthora cinnamomi* Rands, is the most serious disease of avocado worldwide (6,14). It has eliminated commercial production

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in many areas in Latin America and is the major factor limiting production in Australia, California, and South Africa. PRR causes its greatest damage in poorly drained and flooded soils (13). Unfortunately, the incidence of flooding in agricultural areas in southern Florida has increased in recent years. The cyclical occurrence of hurricanes and tropical storms has intensified, resulting in more frequent episodes of heavy rainfall. In addition, the Everglades National Park, which borders western agricultural areas, recently raised water tables in the park to assist wading birds. In total, these events have caused serious PRR-induced damage and mortality of avocado trees (12,13).

PRR-tolerant rootstocks provide an effective and environmentally benign means for managing this important disease. The selection and development of such rootstocks was pioneered by George Zentmyer at the University of California at Riverside (14). His research, now continued by John Menge, focused on screening large seedling populations from Central America and, more recently, from superior trees in California. In general, this program has relied on natural hybrids of the Guatemalan and Mexican races and, to a lesser extent, *P. americana* × *P. schiedeana* hybrids. Although many of these selections have good resistance to PRR, they perform poorly in the calcareous soils that predominate in southern Florida (11). Furthermore, they do not tolerate the lowland humid environment in the area as well as the West Indian race.

The objectives of the present research were to: (i) identify PRR-tolerant seedlings that had the potential to be used as rootstocks in southern Florida; (ii) examine previous reports that parents with a West Indian pedigree produced a relatively high proportion of tolerant seedlings; (iii) identify parents that produced high proportions of tolerant progeny; and (iv) determine the heritability of tolerance to this disease. Ultimately, results from this work will be used to focus our selection process on seedlings from superior parents that were identified in this study.

## MATERIALS AND METHODS

A large collection of avocado germplasm resides in the National Avocado Germplasm Repository at the USDA-ARS Subtropical Horticultural Research Station in Miami. The collection was established in 1906 and currently comprises 208 genetically diverse accessions.

From 1996 to 1998, fruit was collected from 51 of the accessions in this collection (Table 1). Seeds were removed from fruit, dipped in benomyl fungicide (1.2 g $l^{-1}$), and planted within 2 days in a peat, bark and sand potting mix. Seedlings were grown for 3–4 months before they were replanted in potting mix that was infested with *P. cinnamomi* (10). Inoculum was of a virulent isolate of the pathogen that was grown for 2 weeks on autoclaved millet seed under ambient conditions in the laboratory ($\sim$ 24°C). It was mixed uniformly, at a rate of 2.2 g (fresh weight) $l^{-1}$ of potting mix, in a large Hobart blendor. No more than 25 seeds were planted per $0.7 \times 0.3$ m metal tray, and in a given year 40–50 seedlings of a given accession were screened.

After 6–8 weeks in an air-conditioned glasshouse (23–28°C), seedlings were removed from trays, washed gently, and examined for root necrosis (RN). A visual estimate was made of the percentage of roots on each seedling that were necrotic, and note was taken of the characteristics of seedlings that appeared healthy. RN data were segregated by family (*i.e.*, seedling progeny of a given accession/female parent) and racial pedigree, and subjected to analysis of variance using the Proc GLM procedure in SAS (SAS Institute, Cary, NC, USA). RN was transformed using an arcsine transformation to achieve normality. The statistical model can be written as follows:

$$Y_{ij} = \mu + R_i + F(R)_{ij+}\varepsilon_{ij},$$

where $Y_{ij}$ = the response of each individual seedling, $\mu$ = the experimental mean, $R_i$ = the effect of the *i*th racial background, $F(R)_{ij}$ = the effect of the *j*th family nested within the *i*th racial background, and $\varepsilon_{ij}$ = the residual term (2). For the estimation of variance components, all effects were considered random. Maximum likelihood estimates of variance components which correspond directly to terms in the model were produced using the Proc Mixed procedure in SAS. Broad sense heritability (H) was estimated from these variance components as follows:

$$H = \sigma^2_{F(R)}/\sigma^2_{F(R)} + \sigma^2\varepsilon_{ij}$$

## RESULTS AND DISCUSSION

A total of 2,355 open-pollinated seedlings from 51 maternal parents were screened in this study (Table 1). Although the availability of seed and glasshouse space did not allow us to screen seedlings of most of the parents during more than a single year, mean RN for progeny of six that were screened in both 1997 and 1998 were quite similar, verifying the merit and reproducibility of the screening approach [‘Courtright’ (85% and 93%), ‘Dade’ (84% and 93%), ‘Lula’ (90% and 94%), ‘Semil 34’ (75% and 85%), ‘Toltec’ (84% and 89%), and ‘Trapp’ (81% and 79%)].

In general, high levels of disease developed under the described conditions (Tables 1 and 2, Fig. 1). However, there was considerable variation among progeny of the different parents (seedling families). Highly significant differences were found among races and among families within a race ($P<0.0001$) (Table 3). Using an arbitrary standard of less than 50% RN to define tolerance, 31 of the 51 families had at least one tolerant seedling. Six of the families had mean disease ratings less than 60% (‘Arguello’, ‘Catalina’, ‘Family’, ‘Maxima’, ‘Simmonds’, ‘Tensen’, and ‘Winslowson Seedling’), and another six had mean ratings between 60% and 75% (‘Ishim’, ‘Aycock Red #3’, ‘Collins Seedling’, ‘Girrardin’, ‘Hiawassee’ and ‘Monroe’) (Table 1). In total, these 12 families accounted for 82% (188 of 229) of the seedlings that were PRR-tolerant.

Disease responses also varied among seedlings with different racial pedigrees (Fig. 1). Of relevance to the objectives of this work, far more seedlings of the West Indian and Guatemalan × West Indian accessions were tolerant than any of the other pedigrees that were tested. Of the 12 families that accounted for most of the tolerant seedlings, only two did not have a West Indian or Guatemalan × West Indian background. When mean ratings were considered, Guatemalan × West Indian hybrids and West Indian progeny had significantly lower RN than any other pedigree (Table 2). Using the arbitrary standard of less than 50%, 14.6% (113 of 772) of the Guatemalan × West Indian seedlings and 15.7% (92 of 585) of the West Indian seedlings were tolerant, whereas only 3.1% (31 of 998) of the seedlings from all other pedigrees were so ranked. Of the latter seedlings, 23 came from just two parents, ‘Ishim’ and ‘Tensen’, both of which are Guatemalan × Mexican hybrids.

The selection of PRR-tolerant rootstocks in Israel and the Canary Islands was recently described (3,7,15). In both of these locations, tolerance was identified in progeny of the

TABLE 1. Parental cultivars, accession numbers (MIA#), race, number of seedlings screened, percent root necrosis (RN), standard error, and number of seedlings that developed <50% Phytophthora root rot in disease assays (P)

| Cultivar | MIA#[z] | Race[y] | Number of seedlings | Mean RN (%) | S.E. | P |
|---|---|---|---|---|---|---|
| Brooks Late | 22618 | G | 49 | 92.16 | 7.41 | 0 |
| Ishim | 657 | G | 33 | 64.66 | 18.41 | 10 |
| M00636 | 636 | G | 39 | 84.35 | 15.90 | 3 |
| Nabal | 656 | G | 52 | 92.96 | 7.95 | 0 |
| Nabal Seedling A | 20713 | G | 37 | 87.70 | 11.34 | 1 |
| Queen 8 | 20722 | G | 100 | 81.72 | 14.71 | 0 |
| EinVered | 23693 | GM | 50 | 96.68 | 2.21 | 0 |
| Netain | 24088 | GM | 96 | 90.91 | 11.02 | 2 |
| Sharwil | 23332 | GM | 44 | 93.54 | 6.84 | 0 |
| Tenerife | 17690 | GM | 17 | 81.76 | 8.82 | 0 |
| Tensen | 17114 | GM | 24 | 54.58 | 16.93 | 13 |
| Irwing 78 | 20716 | GMW | 79 | 94.62 | 4.82 | 0 |
| Irwing 134 | 20721 | GMW | 37 | 78.91 | 8.09 | 0 |
| Irwing 59 | 20715 | GMW | 40 | 84.00 | 8.41 | 0 |
| Marsheline | 20712 | GMW | 35 | 90.42 | 5.60 | 0 |
| Aycock Red #3 | 20022 | GW | 47 | 75.34 | 19.39 | 10 |
| Collinred Seedling B | 7826 | GW | 20 | 80.25 | 12.40 | 1 |
| Collins Seedling | 3897 | GW | 47 | 62.72 | 19.16 | 18 |
| Collinson | 400 | GW | 46 | 92.17 | 7.72 | 0 |
| Dunedin | 3896 | GW | 71 | 89.01 | 12.91 | 2 |
| Girrardin | 23343 | GW | 94 | 71.38 | 20.41 | 23 |
| Gripina 12 | 24223 | GW | 34 | 83.02 | 17.80 | 2 |
| Gripina 5 | 19769 | GW | 30 | 71.33 | 13.32 | 3 |
| Hiawassee | 26063 | GW | 50 | 72.50 | 20.93 | 12 |
| Lula | 7828 | GW | 69 | 91.95 | 4.34 | 0 |
| Monroe | 19852 | GW | 17 | 61.76 | 20.15 | 7 |
| Nesbitt | 22684 | GW | 45 | 83.02 | 16.48 | 4 |
| Semil 34 | 24224 | GW | 94 | 80.10 | 14.02 | 3 |
| Suardia | 17253 | GW | 33 | 85.90 | 8.33 | 0 |
| Tonnage | 19847 | GW | 38 | 90.44 | 7.24 | 0 |
| Winslowson Seedling | 3888 | GW | 37 | 45.54 | 18.72 | 28 |
| Toltec | 14768 | M | 210 | 86.44 | 10.13 | 2 |
| Courtright | 32154 | MW | 56 | 89.10 | 6.94 | 0 |
| Arguello | 21895 | W | 25 | 57.00 | 15.34 | 13 |
| Bernecker | 24244 | W | 21 | 86.52 | 9.29 | 0 |
| Biscayne | 26512 | W | 32 | 69.06 | 12.53 | 3 |
| Catalina | 17248 | W | 8 | 38.13 | 29.02 | 7 |
| Cellon's Hawaii Seedling | 6898 | W | 13 | 66.15 | 11.20 | 0 |
| Dade | 673 | W | 51 | 88.52 | 12.83 | 1 |
| Dr. Dupuis 2 | 25071 | W | 47 | 80.68 | 13.77 | 2 |
| Family | 3895 | W | 47 | 56.80 | 19.76 | 25 |
| General Bureau | 19331 | W | 50 | 80.40 | 16.37 | 5 |
| Jose Antonio | 17252 | W | 49 | 82.51 | 14.78 | 5 |
| Kilo 4 Ciba | 20026 | W | 8 | 90.63 | 4.96 | 0 |
| Maxima | 23271 | W | 8 | 53.75 | 5.18 | 5 |
| Pollock | 19846 | W | 49 | 72.24 | 18.62 | 12 |
| Simmonds | 670 | W | 14 | 43.21 | 7.49 | 12 |
| Trapp | 19849 | W | 60 | 79.75 | 12.60 | 1 |
| Waldin | 19850 | W | 50 | 80.50 | 9.96 | 1 |
| Wester | 675 | W | 11 | 74.09 | 14.97 | 0 |
| Wilson Popenoe | 3757 | W | 42 | 87.74 | 6.73 | 0 |

[z] Accession numbers of the USDA-ARS National Avocado Germplasm Repository in Miami.

[y] Guatemalan (G), Guatemalan × Mexican hybrid (GM), Guatemalan × Mexican × West Indian hybrid (GMW), Guatemalan × West Indian hybrid (GW), Mexican (M), Mexican × West Indian hybrid (MW), and West Indian (W).

West Indian race. Our study corroborates that finding. Of the six families that produced the highest percentage of tolerant seedlings, five were of the West Indian race. Although no precautions were taken in the present work to ensure that cross-pollination of parental trees did not occur, it is probable that most of the seedlings that were tested resulted from self-pollination.

TABLE 2. Race, number of seedlings screened, mean root necrosis (RN) and Duncan grouping for transformed RN data[z]

| Race/pedigree[z] | Number of families[y] | N[x] | % RN[w] |
|---|---|---|---|
| MW | 1 | 56 | 89.11a |
| GMW | 4 | 191 | 88.59a |
| GM | 5 | 231 | 88.22a |
| M | 1 | 210 | 86.44b |
| G | 6 | 310 | 84.48b |
| GW | 16 | 772 | 78.46c |
| W | 18 | 585 | 75.67c |

[z] Mexican × West Indian hybrid (MW), Guatemalan × Mexican × West Indian hybrid (GMW), Guatemalan × Mexican hybrid (GM), West Indian (W), Mexican (M), and Guatemalan (G).

[y] Number of female parents screened.

[x] Number of individuals in a race/pedigree that were screened.

[w] Means followed by the same letter do not differ significantly at $P<0.05$ according to Duncan's grouping.

TABLE 3. Mean squares from the analysis of variance for the 51 families analyzed for percent root necrosis

| Source | df | Mean square | $F$ | $P>F$ |
|---|---|---|---|---|
| Race | 6 | 2.5626 | 47.15 | <.0001 |
| Family (Race) | 44 | 1.4925 | 27.46 | <.0001 |
| Error | 2304 | 125.2315 | | |
| Corrected total | 2354 | 209.3879 | | |
| Overall CV (%) = 22.99 | | | | |

*Persea americana* was previously assumed to outcross due to its complex reproductive system, floral dichogamy. The asynchronous production of pollen and the receptivity of the stigmatic surface that occurs in the species were thought to be evolutionary adaptations that ensured outcrossing. However, recent work in California and Florida indicates that avocado fruits in these states are usually selfs, rather than hybrids (5,9).

Kellam and Coffey (8) reported two components of resistance in PRR-tolerant rootstocks that were developed in California. No difference in root biomass was noted for 'Duke 7' grown in infested *vs.* noninfested soil, even though plants in the infested soil sustained significant levels of root rot (mean severities of 25% after 20 weeks), and populations of *P. cinnamomi* increased dramatically in soils in which it was planted (equal to those that developed with the susceptible 'Topa Topa'). The authors suggested that the tolerance of 'Duke 7' was based upon its ability to regenerate roots rapidly in infested soil, rather than high resistance of the roots to PRR. In contrast, although root biomass in 'G6' was reduced in infested *vs.* noninfested soil, it did not develop high levels of root necrosis (<10% after 20 weeks) and supported far lower populations of the pathogen than did either 'Duke 7' or 'Topa Topa'. The performance of 'G6' was thought to result from its resistance to PRR and the pathogen's reduced ability to reproduce on its roots.

Although our screening conditions differed from those used in the above study (PRR development is much more severe in organic potting mixes than in soil) and we have yet to test clonal progeny of these selections, the prolific and very robust root systems that these selections produce suggest that the root regeneration mechanism described by Kellam and Coffey (8) may be involved. Work to clarify how these and future rootstock selections respond to PRR is warranted.

Based on our results, it is likely that the inheritance of PRR tolerance is multigenic.

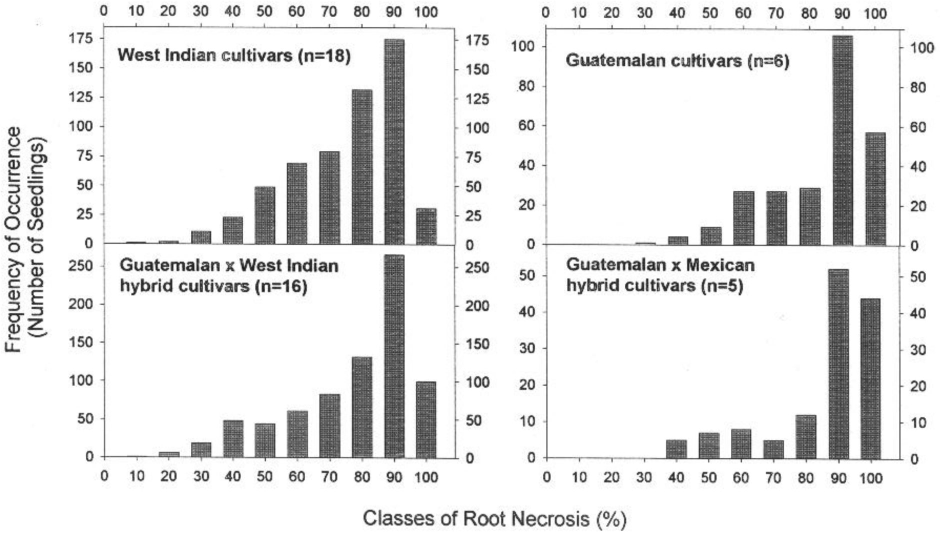

Fig. 1. Frequency distributions of root necrosis classes for different racial pedigrees of avocado. Data are for open-pollinated seedling progeny from the National Avocado Germplasm Repository in Miami that are listed in Table 1. Root necrosis classes were: 0–19% (10), 20–29% (20), 30–39% (30), 40–49% (40), 50–59% (50), 60–69% (60), 70–79% (70), 80–89% (80), 90–99% (90) and 100% (100), and n = the number of families that are represented for each racial background. Only accessions for which eight or more seedlings were rated are included in this figure and Table 1.

The high broad-sense heritability of this trait, 0.45, indicates that significant progress could be made by screening additional progeny from the USDA–ARS collection. The large percentage of seedlings of ‘Winslowson Seedling’, ‘Arguello’, ‘Catalina’, ‘Family’, ‘Maxima’, ‘Simmonds’ and ‘Tensen’ that were tolerant in this study indicates that they should be used as parents in future selection trials, and a second group that produced a lower proportion of tolerant seedlings, ‘Pollock’, ‘Monroe’, ‘Hiawassee’, ‘Girardin’, ‘Collins Seedling’, ‘Aycock Red #3’ and ‘Ishim’, also merit further evaluation. By concentrating on the above parents, a higher proportion of PRR-tolerant seedlings could be produced than if those from random genotypes were used. Furthermore, in addition to using open-pollinated progeny from these parents (half-sib families), it will now be possible to select intelligently which parents should be used in controlled pollinations to produce full-sib families. Ultimately, clones of the best selections will be grafted with commercial scions and tested in flooded and nonflooded calcareous soil infested with *P. cinnamomi*.

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## REFERENCES

1. Anon. (2001) FAOSTAT online database at: http://www.fao.org/default.htm
2. Becker, W.A. (1975) Manual of Quantitative Genetics. 3rd ed. Program in Genetics. Washington State Univ. Press, Pullman, WA, USA.

3. Ben-Yaacov, A., Zilberstaine, M. and Sela, I. (1992) A study of avocado germplasm resources, 1988-1990. V. The evaluation of the collected avocado germplasm material for horticultural purposes. *Proc. World Avocado Congr. II* (Orange, CA, USA), pp. 559-562.
4. Bergh, B.O. (1975) Avocado. *in:* Simmonds, N.W. [Ed.] Evolution of Crop Plants. Longman, London, UK. pp. 148-151.
5. Davenport, T.L., Parnitzki, P., Fricke, S. and Hughes, M.S. (1994) Evidence and significance of self-pollination of avocados in Florida. *J. Am. Soc. Hortic. Sci.* 119:1200-1207.
6. Erwin, D.C. and Ribiero, O.K. (1996) Phytophthora Diseases Worldwide. APS Press, St. Paul, MN, USA.
7. Gallo-Llobet, L. and Siverio, F. (1995) Evaluation of West Indian avocado seedlings' tolerance/resistance to *Phytophthora cinnamomi* Rands compared with clonal resistant root-stocks Duke 7, Thomas and Toro Canyon, under field conditions. *Abstr. Bookl. Proc. World Avocado Congr. III* (Tel Aviv, Israel), p. 56.
8. Kellam, M.K. and Coffey, M.D. (1985) Quantitative comparison of the resistance to Phytophthora root rot in three avocado rootstocks. *Phytopathology* 75:230-234.
9. Kobayashi, M., Lin, J.-Z., Davis, J. and Clegg, M.T. (2000) Quantitative analysis of avocado outcrossing and yield in California using RAPD markers. *Sci. Hortic.* 86:135-149.
10. Ploetz, R.C., Haynes, J. and Schnell, R.J. (2002) Phytophthora root rot-resistant avocado rootstocks for South Florida: Selection of open-pollinated seedling progeny. *Proc. Fla. State Hortic. Soc*. (in press).
11. Ploetz, R.C., Ramos, J.L., Parrado, J.L., Schaffer, B. and Lara, S.P. (1989) Performance of clonal avocado rootstocks in Dade County, Florida. *Proc. Fla. State Hortic. Soc*. 102:234-236.
12. Ploetz, R.C. and Schaffer, B. (1987) Effects of flooding and Phytophthora root rot on photosynthetic characteristics of avocado. *Proc. Fla. State Hortic. Soc*. 100:290-294.
13. Ploetz, R.C. and Schaffer, B. (1989) Effects of flooding and Phytophthora root rot on net gas exchange and growth of avocado. *Phytopathology* 79:204-208.
14. Zentmyer, G.A. (1980) *Phytophthora cinnamomi* and the Diseases it Causes. Monogr. No. 10, Am. Phytopathol. Soc., St. Paul, MN, USA.
15. Zilberstaine, M., Ben-Yaacov, A. and Sela, I. (1995) Selection of avocado rootstocks resistant to root-rot in Israel – update report. *Abstr. Bookl. Proc. World Avocado Congr. III* (Tel Aviv, Israel), p. 51.