Identification and Pathogenicity of Fungal Pathogens Associated with Stem-End Rot of Avocado in California

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Abstract

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Stem-end rot of harvested avocado fruit commonly occurs wherever the crop is cultivated. Multiple fungal species have been described as causal agents. To determine the causal pathogens of stem-end rot in California, fungal isolations were conducted from symptomatic fruit, and fungi were identified by morphological and molecular techniques. In 2010 and 2011, a total of 177 isolates were recovered from 290 avocado fruit collected from seven orchards in one of the major avocado growing areas in Southern California. The majority of isolates was identified as *Neofusicoccum luteum* (65%), with the remainder either as *Colletotrichum gloeosporioides* (33%) or *Phomopsis* sp. (2%). In a pathogenicity test, *N. luteum* caused significantly ($P < 0.05$)

more severe stem-end rot than either *C. gloeosporioides* or *Phomopsis* sp. No significant ($P > 0.05$) differences in stem-end rot severity were observed between inoculations with *N. luteum* isolated from fruit stem-end rot and *N. luteum* or *N. parvum* isolated from branch cankers. This confirms that stem-end rot of avocado can be initiated by fungi causing branch cankers. Although low humidity and rainfall during much of the growing and harvest seasons in California are considered unfavorable conditions for the development of avocado stem-end rot, the identification of the causal pathogens is of value when decays have to be managed during outbreaks, and it stresses the importance of managing branch cankers.

Stem-end rot of avocado (*Persea americana* Mill.) has been reported from all major production areas worldwide (12,17). Crop losses may be severe when harvest occurs during rainy periods (9). The first visible symptom of stem-end rot is a shriveling around the stem base that is followed by decay, fruit discoloration, and softening. The decay ultimately consumes the entire fruit. Internally, the decayed flesh is brown and the vascular bundles in advance of the decay may darken. Despite the long period of exposure to potential pathogens, fruit rarely display symptoms before harvest. Thus, in commercial avocado production, stem-end rot mostly occurs during transit, in the packinghouse, and during or after marketing.

Various fungi cause stem-end rot of avocado. They include species in the Botryosphaeriaceae such as *Botryosphaeria lutea* A.J.L. Phillips (anamorph *Neofusicoccum luteum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips), *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. & De Not. (anamorph *Fusicoccum aesculi* Corda), *B. parva* Pennycook & Samuels (anamorph *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips), *B. ribis* Grossenb. & Duggar (anamorph *Neofusicoccum ribis* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips), and *B. rhodina* (Berk. & M.A. Curtis) Arx (anamorph *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl.). Other species reported include *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk (anamorph *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.), *Glomerella acutata* Guerber & J.C. Correll (anamorph *C. acutatum* J.H. Simmonds), *Nectria pseudotrachia* (Schwein.) Berk. & M.A. Curtis, and species of *Albonectria*, *Alternaria*, *Gibberella*, *Pestalotiopsis*, *Phomopsis*, and *Rhizopus* (12,13,17,20–22,26). *Colletotrichum* spp. can also cause fruit anthracnose (24).

Botryosphaeriaceae spp., which are generally recovered in greater numbers from stem-end rot of avocado fruit than other fungi and are often associated with severe rots (26), vary by

geographical region. For instance, the main cause of stem-end rot in Israel was reported to be *Lasiodiplodia theobromae*; in Australia and New Zealand, *Neofusicoccum luteum* and *N. ribis*; and in South Africa, *N. luteum* and *Nectria pseudotrachia* (17). In the United States, *B. dothidea* was reported as the causal pathogen (17), but extensive surveys were never done. Furthermore, in elucidating the etiology of stem-end rots, recent taxonomic revisions of the Botryosphaeriaceae based on molecular data (2) should be considered. Combined morphological characteristics and sequence data from multiple genes or gene regions have recently been used to distinguish and identify species within the Botryosphaeriaceae (2,23).

Stem-end rot pathogens are present in living and dead branches, twigs, leaves, and in living pedicels (6,13) or sometimes in the soil (17). Inoculum is produced at high humidity and disseminated by rain splash, wind, or by shaking of branches when fruit are harvested (13). Although some infections originate from endophytic colonization that remains latent until after harvest (17), most stem-end infections probably occur at harvest when spores contaminating the stem surface are distributed by cutting tools (8,15). After infection, the fruit is gradually colonized. In contrast, pathogens that cause fruit body rots invade through the intact or injured fruit skin (26). Some pathogens, such as *Colletotrichum* and *Botryosphaeria* species, are also known to cause latent infections that become activated when physiological and biochemical changes that are associated with a reduction in the concentration of fungal inhibitors are initiated in the harvested fruit (15,21).

Stem-end rots are generally a relatively minor problem of avocados in California due to low relative humidity and rainfall during much of the growing season and harvest time (10). Early-season fruit harvested in spring, however, may develop a high incidence of stem-end rot due to a high level of fruit surface contamination by spores of the pathogens. Spores of fungi in the Botryosphaeriaceae and Diaporthaceae that may serve as inoculum for stem-end rot were detected in a spore trap study in eight avocado groves in the major avocado producing areas in Southern California during the rainy season, which extends from November to April (7).

Objectives of this study were to identify fungi associated with avocado stem-end rot in a major avocado growing area of California, evaluate their pathogenicity on avocado fruit, and compare

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their pathogenicity to that of several related pathogens previously isolated from branch cankers (16).

Materials and Methods

Fungal isolation. In 2010 and 2011, avocado fruit 'Hass' were harvested by clipping from seven orchards in the Fallbrook area, San Diego County, a major avocado growing region of California. Approximately 290 fruit were randomly harvested and transported to the laboratory and incubated for 2 to 3 weeks at 24°C to allow stem-end rot to develop. Small pieces of symptomatic flesh from the margins of decay were placed onto potato dextrose agar (PDA; Difco Laboratories, Detroit, MI) acidified with 10 ml of 25% (vol/vol) lactic acid per liter in petri dishes and incubated at 24°C for 3 to 5 days. Pure cultures were obtained by transferring mycelial tips from the colony margin onto fresh acidified PDA. Additional isolates recovered from avocado branch cankers between 2008 and 2009 were available from a previous study (16).

Fungal identification. Isolates were grown on PDA amended with 0.01% tetracycline hydrochloride (Sigma-Aldrich, St. Louis, MO) (PDA-tet) to morphologically distinguish species in the genera of *Botryosphaeria*, *Colletotrichum*, and *Phomopsis*. Cultures belonging to the Botryosphaeriaceae were transferred to 2% water agar amended with autoclaved pine needles or avocado wood chips to induce pycnidia production (16). Petri dishes were incubated at 25°C with a 12-h photoperiod for up to 4 weeks. Morphological characteristics were evaluated as previously described and compared to reference isolates (16).

For molecular characterization, DNA was extracted from representative isolates following the method described by Ceniz (1). Primers ITS4 and ITS5 were used to amplify the internal transcribed spacer ITS1 and ITS2, including the 5.8S region of rDNA (25). A partial sequence of the β -tubulin gene was amplified using primers Bt2a and Bt2b (11). A 25- μ l PCR reaction mixture con-

tained 0.25 μ M of each primer, 12.5 μ l of Promega master mix (Promega, Madison, WI), and 2 μ l of DNA template. PCR reactions were done in a thermal cycler (Bio-Rad Laboratories Inc., Hercules, CA) using the following programs: (i) initial denaturation at 95°C for 2 min; followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 60 s; and a final extension at 72°C for 5 min for the ITS region, and (ii) initial denaturation at 94°C for 2 min; followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 51°C for 45 s, and extension at 72°C for 90 s; and a final extension at 72°C for 5 min for the β -tubulin gene.

PCR products were purified using the QIAquick PCR purification kit (Qiagen Inc., Valencia, CA) and sequenced in both directions at the Institute for Integrative Genome Biology, University of California, Riverside. Sequences were edited using the program Sequencher 4.6 (Gene Codes Corp., Ann Arbor, MI) and aligned using Clustal X 2.1-Mac OSX (Conway Institute, UCD, Dublin, Ireland). Sequences of representative isolates of each morphological group were used to search for similar sequences in GenBank using the BLAST (version 2.0, National Center for Biotechnology Information, United States National Institutes of Health, Bethesda, MD).

Phylogenetic analysis and genetic variation. Sequences of representative isolates in the genus *Neofusicoccum* from stem-end rot and branch cankers, as well as several sequences from GenBank (Table 1), were used in a phylogenetic analysis. A *Guignardia philoprina* (CBS447.68) sequence from GenBank was chosen as an outgroup. The phylogenetic analysis was performed using MEGA 5.0. Maximum parsimony analysis was done using the heuristic search with a close-neighbor interchange (CNI) branch swapping option. Alignment gaps were treated as missing data. Support for each branch in the inferred trees was evaluated by 1,000 bootstrap replications. Prior to the heuristic search of the

Table 1. Isolates of species in the genus *Neofusicoccum* used in the phylogenetic analysis

Isolate ^x	Species	Host	Origin	GenBank accession ^w	
				ITS	β- tubulin
Isolated from avocado fruit with stem-end rot ^y					
UCR1359	<i>Neofusicoccum luteum</i>	<i>Persea americana</i>	California	JX869032	JX898979
UCR1211	<i>N. luteum</i>	<i>P. americana</i>	California	JX869033	JX898980
UCR1214	<i>N. luteum</i>	<i>P. americana</i>	California	JX869034	JX898981
UCR1273	<i>N. luteum</i>	<i>P. americana</i>	California	JX869035	JX898982
UCR1275	<i>N. luteum</i>	<i>P. americana</i>	California	JX869036	JX898983
UCR1336	<i>N. luteum</i>	<i>P. americana</i>	California	JX869037	JX898984
UCR1340	<i>N. luteum</i>	<i>P. americana</i>	California	JX869038	JX898985
UCR1347	<i>N. luteum</i>	<i>P. americana</i>	California	JX869039	JX898986
UCR1368	<i>N. luteum</i>	<i>P. americana</i>	California	JX869040	JX898987
Isolated from avocado branch cankers ^z					
UCR509	<i>Neofusicoccum australe</i>	<i>P. americana</i>	California	GQ857659	GQ857665
UCR739	<i>N. australe</i>	<i>P. americana</i>	California	GQ857662	GQ857668
UCR444	<i>N. luteum</i>	<i>P. americana</i>	California	HQ529764	HQ529773
UCR211	<i>N. luteum</i>	<i>P. americana</i>	California	GQ857658	GQ857664
UCR455	<i>N. luteum</i>	<i>P. americana</i>	California	HQ529756	HQ529726
UCR526	<i>N. luteum</i>	<i>P. americana</i>	California	HQ529763	HQ529733
UCR295	<i>Neofusicoccum parvum</i>	<i>P. americana</i>	California	HQ529765	HQ529734
UCR531	<i>N. parvum</i>	<i>P. americana</i>	California	GQ857661	GQ857667
UCR735	<i>N. parvum</i>	<i>P. americana</i>	California	HQ529766	HQ529735
UCR736	<i>N. parvum</i>	<i>P. americana</i>	California	HQ529767	HQ529736
UCR737	<i>N. parvum</i>	<i>P. americana</i>	California	GQ857660	GQ857666
Obtained from GenBank					
UCD1314 So	<i>N. australe</i>	<i>Vitis vinifera</i>	California	DQ008323	DQ008346
UCD1467 So	<i>N. australe</i>	<i>V. vinifera</i>	California	DQ233610	DQ233631
CMW6837	<i>N. australe</i>	<i>Acacia</i> sp.	Australia	AY339262	AY339254
UCD2089Te	<i>N. luteum</i>	<i>V. vinifera</i>	California	DQ233605	DQ233626
CMW9076	<i>N. luteum</i>	<i>Malus × domestica</i>	New Zealand	AY236946	AY236922
CMW10310	<i>N. luteum</i>	<i>V. vinifera</i>	Portugal	AY339259	AY339251

^w Two gene loci (internal transcribed spacer ITS1-5.8S-ITS2 regions and β -tubulin gene) were sequenced, and sequences were deposited in GenBank.

^x UCR = University of California, Riverside; UCD = University of California, Davis; CMW = Culture Collection Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa.

^y Representative isolates recovered from fruit stem-end rot.

^z Representative isolates recovered from branch cankers (16).

most-parsimonious tree, a partition homogeneity test with 1,000 random repartitions was performed in PAUP* (version 4.0b10; Sinauer Associates, Sunderland, MA) to determine whether ITS and β -tubulin sequence data could be combined. Tree length, consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated using MEGA 5.0.

Analysis of molecular variance (AMOVA) was performed using GenAlEx6 (19) to estimate the variance components between and within two groups of isolates of *N. luteum* recovered from stem-end rot or branch cankers.

Pathogenicity tests. Six isolates were used in the pathogenicity tests: three isolates from stem-end rot (UCR1301, *C. gloeosporioides*; UCR1359, *N. luteum*; and UCR1395, *Phomopsis* sp.) and three Botryosphaeriaceae isolates from branch cankers (16) (UCR295, *N. parvum*; UCR444, *N. luteum*; and UCR509, *N. australe*).

Mature Hass fruit freshly harvested by clipping at the Agricultural Operations field station of the University of California, Riverside, were surface disinfected by dipping into a 1.5% NaOCl solution for 1 min, rinsed with water, and allowed to air-dry. For each isolate, two methods were used for inoculation: (i) mycelial agar discs (5 mm diameter) from the edge of actively growing cultures

were placed into a hole on the pedicel (stem) that was made with a 5-mm-diameter cork borer and covered with Parafilm; and (ii) the pedicel was removed from fruits by snapping, and 50 μ l of a conidial suspension (1×10^5 conidia/ml) was placed onto the stem-end opening. For both methods, fruit were placed in plastic crispers and incubated at approximately 25°C and >80% relative humidity. Inoculated fruit and controls (mock inoculated with agar plugs or sterile distilled water) were arranged in a randomized complete block design with six replications. After 12 days, fruit were cut open lengthwise for evaluation. Stem-end rot was rated using a scale from 0 to 4, where 0 = no visible rot; 1 = 1 to 25%; 2 = 25 to 50%; 3 = 50 to 75%; and 4 = >75% of fruit surface decayed. Pathogenicity tests using both inoculation methods were conducted twice. Small pieces of the flesh removed from the margin of the rot were placed on PDA-tet. Fungal colonies were compared morphologically to those of isolates used in the inoculations.

Data analysis. Percent stem-end rot data from repeated experiments were tested for homogeneity of error variance by analysis of variance (ANOVA) in PROC GLM of SAS (version 9.2; SAS Institute Inc., Cary, NC). As no heterogeneity was detected, pooled data were tested by ANOVA in PROC GLM of SAS. Means were compared using Tukey's honest significant difference (HSD) mean separation test at $\alpha = 0.05$. Pearson's correlation (PROC CORR)

Fig. 1. Most parsimonious unrooted tree based on internal transcribed spacer (ITS)1, 5.8S rDNA, ITS2, and partial β -tubulin gene sequences of isolates belonging to the genus *Neofusicoccum* inferred from maximum parsimony analysis using MEGA 5.0. Numbers on branches are bootstrap values >50% in 1,000 replicates. Isolate CBS447.68 (*Guignardia philoprina*) deposited to the GeneBank by CBS (Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands), was added as an outgroup. FR = isolates recovered from fruit stem-end rot; BR = isolates recovered from branch cankers.

was used to establish the relationship between stem-end rot severity after inoculation with mycelial plugs and severity using conidial suspensions. A paired sample *t* test was conducted to examine whether there was a difference between means of the two inoculation methods.

Results

Fungal identification. A total of 177 fungal isolates were recovered from avocado fruit with stem-end rot. Morphological comparisons with reference isolates identified 114, 59, and 4 of these isolates as *N. luteum*, *C. gloeosporioides*, and *Phomopsis* sp., respectively. *N. luteum* isolates had buff-colored, aerial hyphae in patchy tufts that covered the surface of the medium in an 85-mm petri plate in 2 to 3 days. Conidia measuring $20.7 \pm 1.7 \mu\text{m}$ (length) and $7.3 \pm 0.5 \mu\text{m}$ (width) were hyaline, aseptate, and fusiform. Cultures were characterized by very pale yellow to bright yellow pigments in the center on the reverse of the plate. Colonies of *C. gloeosporioides* isolates were dense aerial, initially white to gray, becoming dark gray as the cultures aged. Conidia ($8.1 \pm 2.1 \times 5.3 \pm 2.1 \mu\text{m}$) were hyaline, aseptate, and cylindrical with obtuse apices. *Phomopsis* sp. isolates had whitish or pale to pale grayish colonies.

ITS and β -tubulin sequences were obtained from 57 isolates, and representative sequences were deposited in GenBank with accession numbers indicated in Table 1. Sequence comparisons in GenBank assigned 37 isolates to *N. luteum*, 18 isolates to *C. gloeosporioides*, and 2 isolates to *Phomopsis* sp., and these assignments matched the morphological identifications.

Phylogenetic analysis and genetic variation. The nonsignificant partition-homogeneity test ($P = 1.00$) indicated congruence between ITS and β -tubulin sequence sets, and thus, both sets were combined for further phylogenetic analysis. Of the 1,154 nucleotides analyzed, 54 were informative. Maximum parsimony analysis yielded a total of 740 trees (length = 232, CI = 0.98, RI = 0.98, RC = 0.96, and HI = 0.04) with three well-defined clusters with little sequence variability among isolates within each cluster (Fig. 1). The first cluster consisted of nine isolates of *N. luteum* from fruit stem-end rot and seven isolates of *N. luteum* from branch cankers. The second and third clusters consisted of five isolates each of *N. australe* and *N. parvum*, respectively, obtained from branch cankers. Results from AMOVA revealed a significant ($P < 0.01$) genetic differentiation within and between two groups of isolates of *N. luteum* recovered from stem-end rot or branch cankers. The variance between groups was 20%, and that within groups was 80% (Table 2).

Pathogenicity tests. Although severity of stem-end rot using both inoculation methods was significantly different ($P = 0.018$ for two-tailed *t* test), Pearson's correlation indicated a strong positive relationship between both methods ($r = 0.91$, $n = 96$, $P < 0.0001$). Isolates of three fungal species recovered from fruit stem-end rot differed significantly ($P < 0.05$) in causing stem-end rot of Hass avocado fruit (Table 3). Fruit inoculated with *N. luteum* caused the highest stem-end rot severity with 84.4 and 78.3% of the cut-open fruit surface decayed using mycelial plugs and conidial suspensions, respectively. Isolates of *Phomopsis* sp. and *C. gloeosporioides* were less virulent and resulted in a similar ($P > 0.05$) low stem-end rot severity using both inoculation methods. A high severity of stem-end rot also developed from inoculations with fungal species from branch cankers. An isolate of *N. luteum* from branch cankers was similarly virulent (severity 74.5 to 83.0%) as the isolate of the same species obtained from stem-end rot (Table 3). Furthermore, inoculations with isolates of *N. parvum* and *N. australe* originating from branch cankers also resulted in a similar ($P > 0.05$) high severity of stem-end rot, although decay development after inoculation with mycelial plugs of *N. australe* was lower. In reisolations, fungi used in the inoculations were successfully recovered (80 to 100%) when small pieces of decayed tissue were placed onto agar medium.

Discussion

Avocado stem-end rot in a major avocado growing region of California was found to be caused by *N. luteum*, *C. gloeosporioides*, and a *Phomopsis* species. The most frequently isolated species in our 2-year survey was *N. luteum*. This species was previously reported to be a major avocado stem-end rot pathogen in Australia, New Zealand, and South Africa (4,12,18). Similar to studies conducted in New Zealand (9), species of *Phomopsis* were found to be of minor importance in causing stem-end rot in our study. *B. dothidea*, previously reported as the primary species causing stem-end rot in the United States (17), was not recovered from avocado fruit with stem-end rot in our study; however, it may occur in avocado growing areas that were not sampled. Alternatively, with the more recent use of molecular methods in fungal identification and changes in the taxonomy of the Botryosphaeriaceae (2), the causal agent of stem-end rot may have been misidentified in earlier studies. Based on DNA sequence data, most of the isolates first identified as *B. dothidea* were reassigned to different species, primarily within the genus *Neofusicoccum* (23).

In previous research conducted in Southern California, 82% of the fungi associated with avocado branch cankers belonged to the

Table 2. Analysis of molecular variance between and within two groups of isolates of *Neofusicoccum luteum* recovered from either stem-end rot or branch cankers

Source of variation ^z	df	Sum of squares	Mean squares	Variance component	% Variation	P value
Between groups	1	2.863	2.863	0.239	20	<0.01
Within groups	14	13.762	0.983	0.983	80	<0.01
Total	15	16.625	...	1.222	100	

^z Hierarchical analysis based on groups of nine and seven isolates from avocado stem-end rot and branch cankers, respectively.

Table 3. Stem-end rot severity of 'Hass' avocado fruit inoculated with fungi isolated from stem-end rot or branch cankers on avocado

Isolate	Species	Source	Stem-end rot (%) ^y	
			Mycelial plugs	Conidial suspension
UCR1359	<i>Neofusicoccum luteum</i>	Fruit	84.4 a	78.3 a
UCR1301	<i>Colletotrichum gloeosporioides</i>	Fruit	33.3 c	27.8 b
UCR1395	<i>Phomopsis</i> sp.	Fruit	18.8 c	25.3 b
UCR444	<i>N. luteum</i>	Branch canker	83.0 a	74.5 a
UCR295	<i>Neofusicoccum parvum</i>	Branch canker	77.3 ab	70.8 a
UCR509	<i>Neofusicoccum australe</i>	Branch canker	65.6 b	67.5 a
Control ^z			1.1 d	1.3 c

^y Fruit were inoculated with mycelial plugs or a conidial suspension of each isolate. Data are mean values of stem-end rot severity that was based on percentage of the longitudinally cut open decayed fruit. Means followed by the same letter within columns are not significantly different ($P < 0.05$) according to Tukey's honest significant difference (HSD) mean separation test.

^z Fruit were inoculated with agar plugs without fungal mycelium or with sterile distilled water.

Botryosphaeriaceae, with *N. luteum* and *N. australe* being the predominant species (16). Similarly to the current stem-end rot survey, *Phomopsis* sp. was associated with a lower incidence in the avocado branch canker study (16). *N. australe*, however, was not recovered from stem-end rot, although we demonstrated it to be pathogenic and highly virulent in stem-end inoculations of avocado fruit. Additionally, in our comparative pathogenicity studies, stem-end rot and branch canker isolates of *N. luteum* caused a similar severity of stem-end rot. An isolate of *N. parvum* from a branch canker was also as virulent as *N. luteum* on avocado fruit. These results indicate that branch cankers can be a source of inoculum for fruit infections and that species not detected in our stem-end rot survey in California can potentially cause stem-end decay. Conversely, it is possible that species from stem-end rot lesions could cause branch cankers. *N. parvum* is known as an avocado stem-end rot pathogen in Australia, New Zealand, and South Africa (13,17), but *N. australe* has never been reported to cause avocado stem-end rot.

Hierarchical analysis of genetic variation indicated that isolates of *N. luteum* from branch cankers could not be genetically separated from those obtained from stem-end rot. There was low genetic diversity within this species, and 20% of the diversity was distributed between the two groups of isolates (isolates from stem-end rot or branch cankers), while 80% was distributed within groups. This indicates that both groups are closely related.

C. gloeosporioides was a weaker stem-end rot pathogen in our inoculation studies compared to the three members of the Botryosphaeriaceae. This species, however, also causes anthracnose of avocado fruit that can result in serious postharvest crop losses. Previous studies by others also identified *C. gloeosporioides* as a weak avocado stem-end rot pathogen (26). It was reported as an important pathogen, however, when present in combination with other stem-end rot fungi, such as in the *Dothiorella/Colletotrichum* rot complex (3).

Results of this research emphasize the potential impact of branch canker and dieback diseases on the occurrence of fruit stem-end rot of avocado and the importance of integrated management of orchard diseases. Preharvest and postharvest fungicide applications for the control of stem-end rot have been used with some success in other parts of the world (5,14), but not in the United States. Thus, a critical aspect for minimizing stem-end rot is orchard sanitation and optimal cultural practices such as pruning and harvesting only during dry conditions, disposing of dead wood and old fruit away from avocado trees, providing sufficient irrigation, and correcting nutritional stresses (26). Fruit should be picked by clipping instead of snapping the pedicels, and clippers should be frequently sterilized (13). Harvested fruit should be stored at optimal temperatures, and proper sanitation practices should be followed in the packinghouse.

To further elucidate the etiology of avocado stem-end rot in California, follow-up studies should be done in additional locations and during years of high rainfall. Clearly, species other than the ones described in this study may be involved in causing the disease in California. Furthermore, based on the occurrence of the disease under different climatic conditions, additional preharvest and postharvest management practices may need to be developed.

Acknowledgments

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