Identification and Distribution of *Botryosphaeria* spp. Associated with Avocado Branch Cankers in California

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Members of the *Botryosphaeriaceae* family cause branch cankers and dieback on California avocado trees. With high-density planting becoming more common in the California avocado industry, more intensive pruning may increase the occurrence of branch canker. This study was undertaken to identify and characterize the *Botryosphaeriaceae* species involved in the branch canker disease complex in order to develop future management strategies. From 2008-2009, four to five trees from each of eight avocado groves in five California counties were sampled for symptomatic branch cankers. Six *Botryosphaeriaceae* species were identified based on morphology as well as phylogenetic analysis of the internal transcribed spacer region (ITS1-5.8S-ITS2) and a partial sequence of the β-tubulin gene. These six species included *Neofusicoccum australe, N. luteum, N. parvum,* an unknown *Neofusicoccum sp., Fusicoccum aesculi* and *Dothiorella iberica*. Members of the *Botryosphaeriaceae* were isolated from all avocado-growing regions sampled in California (Riverside, San Diego, Ventura, Santa Barbara and San Luis Obispo counties), however, incidence and distribution of species varied with location. *N. australe*, *N. lutea* and *N. parvum* were found in both the northern and southern counties with average air temperatures ranging from a low of 13.1° C in San Luis Obispo County to a high of 18.2° C in Riverside County. *F. aesculi*, *D. iberica* and the unknown *Neofusicoccum* sp. were all isolated only from the northern counties of San Luis Obispo and Ventura.

Keywords: Botryosphaeria, branch canker, dothiorella

Identificación y Distribución de *Botryosphaeria* spp. Asociadas con Cancro de Rama de Aguacate en California.

Miembros de la familia *Botryosphaeriaceae* causan cancros de rama y muerte regresiva en los árboles de aguacate en California. Es posible que la ocurrencia del cancro se aumente a causa del aumento en la densidad de arboles en las arboledas**,** que ahora es mas común en la industria de aguacate en California. Los objetivos de esta investigación fueron identificar y caracterizar las especies de *Botryosphaeriaceae* causantes de cancro de rama para desarrollar estrategias de control y manejo para el futuro. Se tomaron muestras de entre cuatro a cinco arboles de cada una de ocho arboledas situadas en cinco condados de California entre los años 2008-2009. Seis especies de *Botryosphaeriaceae* fueron identificadas a base de morfología, análisis filogenético del espacio interno transcrito (ITS1-5.8S-ITS2), y una secuencia parcial del gen β-tubulin. Estas seis especies incluyen *Neofusicoccum australe, N. luteum, N. parvum,* una *Neofusicoccum sp.*desconocida*, Fusicoccum aesculi* y *Dothiorella iberica*. Miembros de la familia *Botryosphaeriaceae* fueron aislados de todos los condados de los cuales se tomaron muestras en California (condados de Riverside, San Diego, Ventura, Santa Barbara y San Luis Obispo), pero la incidencia y distribución varía depende a la ubicación. *N. australe*, *N. lutea* y *N. parvum* fueron encontrados en los condados del norte y sur, donde la temperatura varía de 13.1° C en el condado de San Luis Obispo, hasta 18.2° C en el condado de Riverside. *F. aesculi*, *D. iberica,* y una *Neofusicoccum* sp. desconocida solamente fueron aislados en los condados de San Luis Obispo y Ventura, que están mas al norte de California.

Introduction

Canker on California avocado trees (*Persea americana* Miller) can occur on twigs, branches or trunks and appears to be caused by a complex of fungal species, many of which are in the family Botryosphaeriaceae (McDonald *et al*. 2009, Menge and Ploetz, 2003). Canker caused by Botryosphaeriaceae species was previously known as *Dothiorella* canker. This disease is distinct from another type of canker caused by *Phytophthora* spp. (Menge and Ploetz, 2003) which will not be discussed here.

Canker caused by Botryosphaeriaceae on avocado may exude reddish sap that dries to a whitish-beige powder. Bark may be cracked, darkly discolored, or slightly sunken (Fig. 1). Cankered inner bark and wood are red-brown to brown, instead of the normal pale color. In a cross section of a cankered branch, a characteristic wedge-shaped discoloration extending deep into the xylem may be

visible (Fig. 2). If the xylem is extensively colonized, the affected branch may rapidly wilt with leaves quickly turning brown but remaining attached (Fig. 3) (Menge and Ploetz, 2003).

Fig. 1. Bleeding cankers on avocado tree

Fig. 2. Cross section of avocado branch canker extending into the xylem

Fig. 3. Branch dieback symptoms on avocado tree

For growers to remain competitive in the international market, they must find ways to manage their groves efficiently and significantly increase production. One way to do this is by decreasing spacing between trees from 20 x 20 ft to 10 x 10 ft, a practice already employed in Chile (Hofshi, 2001, 2004). More frequent pruning, such as would occur in a high density grove, could increase the dissemination of canker-causing members of the Botryosphaeriaceae from tree to tree, leading to an increase in canker incidence and a possible decrease in yield as cankered branches are pruned out.

The objective of this study was to identify the species of Botryosphaeriaceae associated with the avocado canker disease complex in California and evaluate their distribution. Identifying and characterizing the primary causal agents of this disease will assist in developing the appropriate control measures to reduce yield loss. Morphological and molecular methods were used to characterize these species. Anamorph names will be used for fungal species identified.

Current research

Between September 2008 and March 2009, four or five symptomatic avocado trees (cv. Hass) in each of eight avocado groves in five counties (Table 1, Fig. 4) were sampled for branch canker in order to identify the associated fungi. The groves selected were located in the major avocado production areas in California.

Table 1. Incidence of Botryosphaeriaceae spp. in the main avocado production areas of California, Sep 2008-Mar 2009.

County	No. groves[a]	No. branch cankers sampled	No. (%) Bot.[b]
San Luis Obispo	2	46	18 (39)
Santa Barbara	1	48	18 (38)
Ventura	2	71	34 (48)
Riverside	2	12	10 (83)
San Diego	1	10	2 (20)
Total:	8	187	82 (44)[c]

[a]Number of groves sampled.
[b]Number of branch cankers (and percentage of the total number of branch cankers sampled per county) yielding Botryosphaeriaceae.
[c]Total number of branch cankers (and percentage of the total number of branch cankers sampled) yielding Botryosphaeriaceae.

Fungi in Botryosphaeriaceae were present in all eight groves sampled and were isolated from 82 of 187 cankers (Table 1). Members of Botryosphaeriaceae were the most abundant fungi isolated from avocado branch cankers in California (Table 1). Other sporadically isolated fungi from avocado branch cankers were identified as belonging to the genera *Bionectria, Colletotrichum, Schizophyllum, Fusarium, Eutypella* and *Phaeoacremonium* based on morphological and DNA sequence evidence.

Morphological and molecular methods were used to identify the Botryosphaeriaceae fungi to the species level. Six *Botryosphaeriaceae* were found in California on avocado: *N. australe, N. luteum, N. parvum*, *Fusicoccum aesculi*, *Dothiorella iberica* and one unknown *Neofusicoccum* sp. (Table 2). Two other Botryosphaeriaceae species, *Lasiodiplodia theobromae* and *Diplodia mutila,* were also identified but were not included for further study as there was only a single isolate of each.

At least two Botryosphaeriaceae species were found in all counties sampled in this study (Table 2, Fig. 4). *N. luteum* was the most frequently isolated species from cankers followed by *N. australe, F. aesculi, N. parvum, D. iberica* and *Neofusicoccum* sp. Not all species were found in all counties. *N. australe* was found in all five counties. *N. luteum* was found in all counties, except for Riverside County. *N. parvum* was found in San Luis Obispo and Ventura counties, in addition to the southern county of San Diego. *F. aesculi*, *D. iberica* and the unknown *Neofusicoccum* sp. were found only in the two northern counties of San Luis Obispo and Ventura (Table 2, Fig. 4).

Table 2. Number of each Botryosphaeriaceae species and average air temperature, by county, in the main avocado production areas of California, Sep 2008-Mar 2009.

County	*N. australe*[a]	*N. luteum*[b]	*N. parvum*[c]	Unknown *Neo*. sp.[d]	*F. aesculi*[e]	*D. iberica*[f]	*P. theicola*[g]	Avg Air Temp (C°)[l]
San Luis Obispo	8	0	4	1	3	2	0	13.1
Santa Barbara	1	17	0	0	0	0	0	15.0
Ventura	13	9	1	2	8	1	2	16.3
Riverside	3	7	0	0	0	0	1	18.2
San Diego	1	0	1	0	0	0	1	15.7
Total:	26	33	6	3	11	3	4	

[a]*Neofusicoccum australe*. [b]*Neofusicoccum luteum*. [c]*Neofusicoccum parvum*. [d]Unknown *Neofusicoccum* sp. [e]*Fusicoccum aesculi*. [f]*Dothiorella iberica*. [l]Average air temperature March 2008 to March 2009.

Climatic factors do not provide an explanation for differences in the frequencies or location of isolation of the six Botryosphaeriaceae species found during this study. *N. australe*, *N. lutea* and *N. parvum* were found in both the northern and southern counties with average air temperatures ranging from a low of 55.6°F in San Luis Obispo County to a high of 64.8°F in Riverside County. *F. aesculi*, *D. iberica* and the unknown *Neofusicoccum* sp. were all isolated only from the northern counties of San Luis Obispo and Ventura. In a study of grapevine canker in California, which encompassed a large number of northern California counties, as well as some southern California counties, *N. australe* (*B. australis*) and *N. parvum* (*B. parva*) were detected as far north as Sonoma County, *F. aesculi* (*B. dothidea*) as far north as Mendocino County, and *N. luteum* (*B. lutea*) only in Riverside County (Urbez-Torres *et al*. 2006). Based on the field information from the grapevine canker study and the current study, as well as *in vitro* studies which showed that Botryosphaeriaceae can tolerate a range of temperatures (5), it seems unlikely that climatic conditions are an important factor in the distribution of species in California.

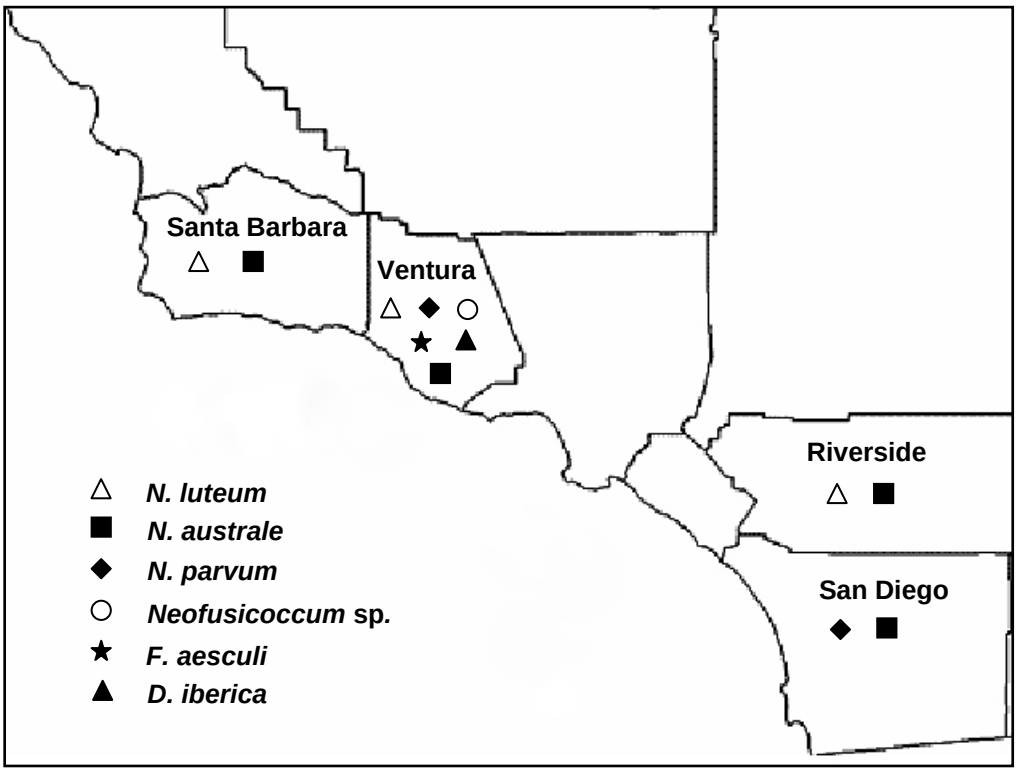

Fig. 4. California counties in which *Botryosphaeriaceae* spp. were detected in this study.

As high-density planting becomes more frequent in California avocado groves, management strategies must be developed to protect pruning wounds from invasion by canker-causing pathogens. Currently, there are no effective fungicides used by growers to prevent infection of pruning wounds on avocado trees. Preliminary *in vitro* studies (V. McDonald, *unpublished*) have shown there are chemicals on the market that are effective against Botryosphaeriaceae spp. Studies designed to evaluate the most effective fungicides to protect pruning wounds in the field are now underway.

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