Research Project Progress Report

Multiple *Botryosphaeria* species causing 'Dothiorella' gummosis in citrus

Anthony Adesemoye, Akif Eskalen, Ben Faber and Neil O'Connell

The occurrence of branch or trunk cankers on citrus caused by members of the Botryosphaeriaceae is known as Dothiorella gummosis due to the gum or sap that may exude from the canker. Symptoms include grayish to brown discoloration on the bark, which could extend into the xylem (Fig. 1).

Though Dothiorella gummosis is viewed as a minor disease in citrus, in advanced stages it can lead to serious decline or death of branches, and in the case of young trees cause plant death. In a variety of woody hosts, many members of the Botryosphaeriaceae are known to cause branch and trunk cankers and occasionally contribute to stem-end rot.

Dothiorella gregaria (teleomorph: *Botryosphaeria ribis*) was long believed to be the pathogen causing Dothiorella gummosis. However, isolation of *Neofusicoccum vitifusiforme (Botryosphaeria* sp.*)* from citrus branch canker by Eskalen and McDonald stimulated our hypothesis that there is more than one species of *Botryosphaeria* causing Dothiorella gummosis in citrus.

Our objectives were to (a) determine the *Botryosphaeria* species causing branch canker and dieback on citrus and their geographical distribution in California, and (b) determine the pathogenicity and aggressiveness of the isolates.

How the study was conducted

In a survey of citrus orchards in California conducted in 2010, cankered branches were collected in six citrus growing counties in California, namely Fresno, Riverside, San Diego, San Luis Obispo (SLO), Tulare and Ventura. The branches were transported on ice to the laboratory at the University of California Riverside (UCR).

Small pieces of cankered woody tissue were plated onto growth media, and different isolates of *Botryosphaeria* were obtained from them. Isolates were identified using morphology and molecular methods. The molecular methods included the use of three different molecular markers (Internal Transcribed Spacer [ITS], Beta Tubulin, and Translation Elongation Factor). This was followed by sequencing, which was done at the UCR Genomics Core facility. The sequences obtained were then compared to the GenBank.

Teleomorph is a sexual reproductive stage, while anamorph is an asexual reproductive stage of the same fungus.

Pathogenicity tests were conducted for the strains by inoculating green shoots. Fresh wounds were made on 1-year-old citrus shoots using a 3-mm cork borer, and the freshly wounded surfaces were inoculated with mycelial plugs from 3- to 5-day-old cultures of each fungus growing on potato dextrose agar amended with 0.01% tetracycline (PDA-tet), a general medium used for culturing fungi. Control shoots were inoculated with sterile agar plugs. There were 10 replicates per isolate and the control.

Inoculated wounds and shoot ends were covered with petroleum jelly and wrapped with parafilm to prevent desic-

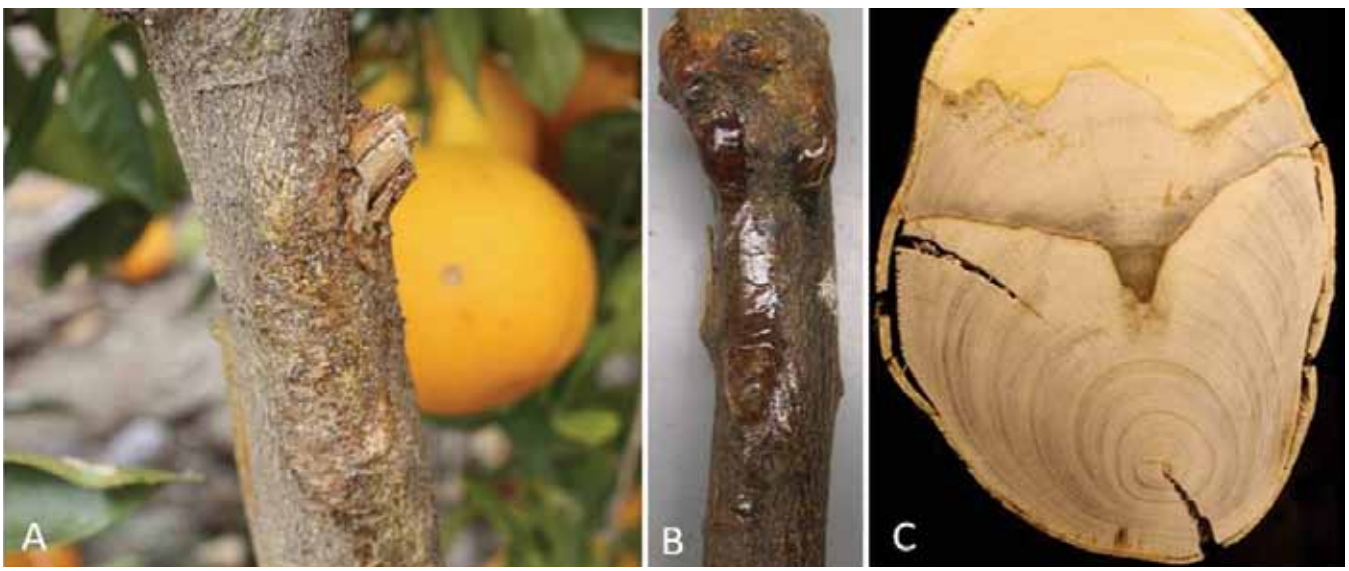
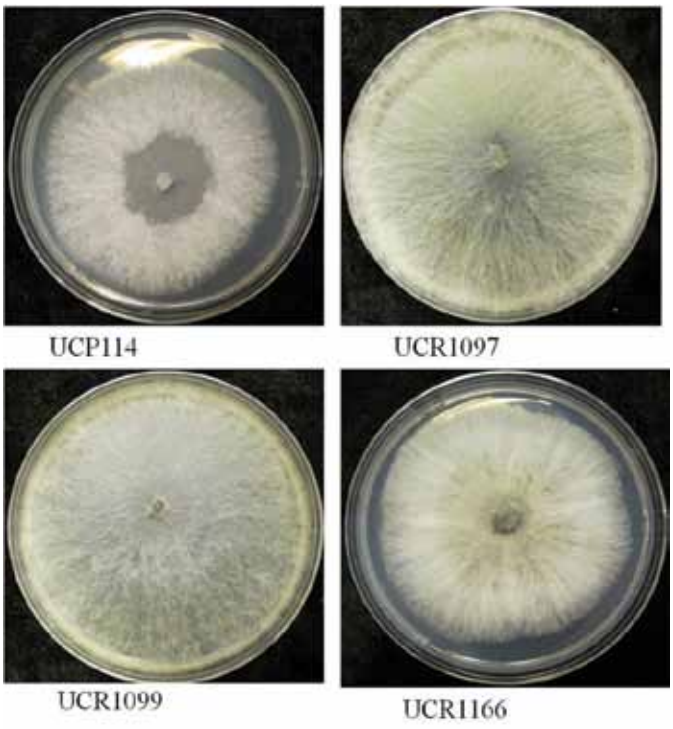


Fig. 1. Samples of gummosis and branch canker caused by *Botryosphaeria* collected from three different locations.



(Left) Fig. 2. Sample pictures of 3-day-old fungal isolates growing on plates in the laboratory at UCR (UCP114 - *Neofusicoccum mediterraneum*; UCR1097 – *N. luteum*; UCR1099 – *N. australe*; and UCR1166 – *N. parvum*).

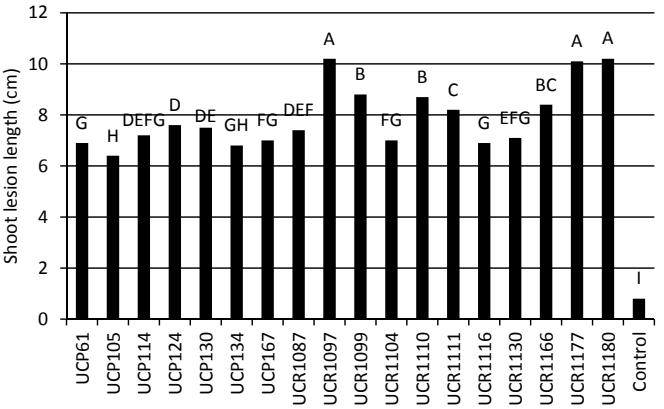


Fig. 3. Mean lesion length on shoots after test inoculation. Detail of names for listed strains can be seen in Table 1. Mean with different letters are significantly different at p=0.05.

cation. Shoots were incubated at 25°C in moist chambers for four weeks. Lesions were observed on all inoculated shoots except for the controls. The length of each lesion was recorded. Re-isolation from inoculated tissues was done to complete the pathogenicity of isolates.

Table 1: *Botryosphaeria* isolates fully identified morphologically and genetically.

Strain	Organisms	Teleomorph	County	Rootstock	Scion
UCP61	*Dothiorella viticola*	*Spencermartinsia viticola**	Tulare	Sour Orange	Valencia
UCP105	*Dothiorella viticola*	*Spencermartinsia viticola*	Tulare	Sour orange	Parent Washington
UCP114	*Neofusicoccum mediterraneum*	*Unknown*	Tulare	Carrizo	Fukumoto
UCP124	*Dothiorella iberica*	*Botryosphaeria iberica*	Tulare	Carrizo	Fukumoto
UCP130	*Diplodia mutila*	*Botryosphaeria stevensii*	Tulare	Carrizo	Fukumoto
UCP134	*Dothiorella viticola*	*Spencermartinsia viticola*	Tulare	Sour Orange	Parent Washington
UCP167	*Dothiorella viticola*	*Spencermartinsia viticola*	Tulare	Sour Orange	Valencia
UCR1087	*Neofusicoccum mangiferae*	*Botryosphaeria sp.*	San Diego	Sour orange	Red Blush grapefruit
UCR1097	*Neofusicoccum luteum*	*Botryosphaeria lutea*	San Diego	Unknown	Valencia
UCR1099	*Neofusicoccum australe*	*Botryosphaeria australis*	San Diego	Unknown	Valencia
UCR1104	*Dothiorella viticola*	*Spencermartinsia viticola*	San Diego	Unknown	Valencia
UCR1110	*Neofusicoccum australe*	*Botryosphaeria australis*	Riverside	Unknown	Satsuma
UCR1111	*Neofusicoccum australe*	*Botryosphaeria australis*	Riverside	Unknown	Satsuma
UCR1116	*Neofusicoccum mangiferae*	*Botryosphaeria sp.*	Fresno	Unknown	Mandarin Nova
UCR1130	*Dothiorellaviticola*	*Spencermartinsia viticola*	Riverside	Unknown	Eureka Lemon
UCR1166	*Neofusicoccum parvum*	*Botryosphaeria parva*	Ventura	Volkameriana	Meyer Lemon
UCR1177	*Neofusicoccum luteum*	*Botryosphaeria lutea*	SLO	Unknown	Lemon
UCR1180	*Neofusicoccum luteum*	*Botryosphaeria lutea*	SLO	Unknown	Lemon

*Spencermartinsia viticola *is also called* Botryosphaeria viticola (Basionym). *Basionym means the first or previous name that was given to the organism.*

Findings and the implications

As can be seen in Figure 2, some of the species of *Botryosphaeria* are very similar in morphology. Some are also very similar in genetic composition. The combination of morphology with three types of molecular markers helped to fully identify isolates and distinguish among closely related species. Hence, the 18 isolates listed in Table 1 have been fully identified. The common form of the organism seen in nature is the anamorph, but the teleomorph names are included on the table for better understanding.

Grower records were consulted to obtain information on the age of the trees, rootstock and scion, irrigation management, cultural and fertilization practices, and disease and pest histories in the orchard. The information shows that isolates were collected from both organic and non-organic farms. All ages of trees appear susceptible to the infection, as the hosts from which isolations were made varied from 5 to over 60 years old. Commonly used rootstocks – 'Carrizo', *Citrus volkameriana*, and sour orange – were infected.

Based on the pathogenicity tests conducted using green shoots in the laboratory (Figure 3), the isolates are pathogenic on citrus. All isolates were recovered 100% from lesions in each respective set of infected shoots. However, the study will continue with further tests in the greenhouse to better understand their pathogenicity and aggressiveness.

Results indicate that multiple species of *Botryospharia*,

including *B.australis*, *B. iberica*, *B. lutea*, *B.* species, *N. mediterraneum*, *B. parva*, *B. stevensii*, and *S. viticola*, were causing symptoms on citrus that were previously believed to be caused by *Dothiorella gregaria (B. ribis)*.

It was observed that these multiple species were widely distributed within counties. For example, in Tulare County where seven isolates were obtained, four of them were *S. viticola* while three were different species (*N. mediterraneum, B. iberica,* and *B. stevensii*). In San Diego, four different species were isolated (*B. lutea, B. australis, S. viticola,* and *B.* species). Two species were identified in Riverside (*B. australis,* and *S. viticola*) and one species each in Fresno (*Botryosphaeria sp.*), Ventura (*B. parva*), and San Luis Obispo (*B. lutea*).

The gumming, as seen in Figure 1B, was generally more common in the summer and early fall but was hardly seen in winter and spring. It has previously been shown in other hosts that both ascospores (sexual spores) and conidia (asexual spores) produced by *Botryosphaeria* in structures that can be found on dead bark, twigs and cankers are infective. The spores enter the host plant through fresh wounds such as pruning wounds, split branches from wind damage, frost damage, mechanical and grafting wounds. Also, wet weather, summer rain, high relative humidity, and furrow or sprinkler irrigation has been shown to be helpful in the infection process, especially in dispersing the spores.

Thus, disease management should be planned with consideration of the annual seasonal dynamics of the risk of infection. It is important to time pruning activity to dry periods of the year and totally destroy pruned materials. Overall, it is very important to maintain optimum tree health, free of wounds, and ensure high levels of sanitation in the orchard. Also, controlling damage by gophers and meadow mice seems to be important as the wounds can be points of entry.

Notably, *Dothiorella gregaria* was not isolated from any of the counties. The term "Dothiorella gummosis" now appears to be a misnomer, as anamorphs of many other species of *Botryosphaeria* have been determined to cause the same symptoms. It may be more appropriate to refer to the disease as "*Botryosphaeria* gummosis". To our knowledge, this is the first comprehensive study of *Botryosphaeria* gummosis on citrus in California. These findings are important and should be considered in the design of future studies on management techniques of *Botryosphaeria* gummosis in citrus.

Project leader Dr. Akif Eskalen is Cooperative Extension Specialist and Plant Pathologist, and Anthony Adesemoye is a postdoctoral scholar, Department of Plant Pathology and Microbiology, University of California Riverside. Dr. Ben Faber is a UC Cooperative Extension farm advisor in Ventura and Santa Barbara counties, and Neil O'Connell is a UC Cooperative Extension farm advisor in Tulare County.

Additional reading

Amponsah, N.T., Jones, E.E., Ridgway, H.J., and Jaspers, M.V. 2009. Rainwater dispersal of *Botryosphaeria* conidia from infected grapevines. New Zealand Plant Protection 62: 228-233.

Beckman, T. G., Pusey, P. L., and Bertrand, P. F. 2003. Impact of Fungal Gummosis on Peach Trees. HortScience 38 (6):1141-1143.

Crous, P. W., Slippers, B., Wingfield, M. J., Rheeder, J., Marasas, W. F. O., Philips, A. J. L., Alves, A., Burgess, T., Barber, P., and Groenewald, J. Z. 2006. Phylogenetic lineages in the *Botryosphaeriaceae*. Studies in Mycology 55:235-253.

Eskalen, A., McDonald, V., Citrus branch dieback and canker caused by Neofusicoccum vitifusiforme in California. 2008. 11th International Citrus Congress, Abstract book. Wuhan, China. P236.

Luque J., Martos S., Phillips, A. J. L. 2005. *Botryosphaeria viticola* sp. nov. on grapevines: a new species with a Dothiorella anamorph. Mycologia 97:1111-1121.

McDonald, V., Lynch, S., and Eskalen, A. 2009. First Report of *Neofusicoccum australe*, *N. luteum*, and *N. parvum* Associated With Avocado Branch Canker in California. Plant Disease 93 (9):967-967.

Pusey, P. L. 1989. Availability and dispersal of ascospores and conidia of *Botryosphaeria* in peach orchards. Phytopathology 79:635-639.

Slippers, B., Fourie, G., Crous, P. W., Coutinho, T. A., Wingfield, B. D., and Wingfield, M. J. 2004. Multiple gene sequences delimit *Botryosphaeria australis* sp. nov. from *B. lutea*. Mycologia 96: 1030-1041.

Timmer, L.W., Garnsey, S.M., Graham, J.H. 2000. Compendium of citrus diseases, 2ND Edition. APS press. 3340 Pilot Knob Road. St.Paul, Minnesota, USA. Pg 40.

Úrbez-Torres, J. R., Leavitt, G. M., Voegel, T. M., and Gubler, W. D. 2006. Identification and distribution of *Botryosphaeria* spp. associated with grapevine cankers in California. Plant Dis. 90:1490-1503.

Editor's Note: The research reported on in this article is from one of three studies under the research project titled "Investigating important diseases of citrus in California".