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Breakthrough in the Battle Against Fruit Flies

Figure 1. Symptoms of Colletotrichum Dieback on clementine mandarins: (A) shoot dieback symptoms; (B) gumming symptoms on an infected shoot; (C) branch dieback symptoms; and (D) wood discoloration and canker.

Colletotrichum Dieback of Mandarins and Navel Oranges in California

A new twig and shoot disease in the Central Valley

Akif Eskalen, Greg W. Douhan, Craig Kallsen and Joey S. Mayorquin

Project Summary

Recently, a twig and shoot dieback disease of clementine mandarins and navel oranges has occurred in the main citrus growing regions of California's Central Valley. The disease was first noticed in 2012 by several growers and nurserymen in various Central Valley orchards; however, the source of this dieback disease remained unknown. The purpose of the study was to determine the cause(s) of the twig and shoot dieback in Central Valley mandarins and navel oranges. Field surveys determined that Colletotrichum gloeosporioides *and* C. karstii *were among several fungi recovered from symptomatic tissues. Tests confirmed* C. karstii *as a pathogen of citrus, making this the first report of this citrus pathogen in California. Studies also determined that spores of* Colletotrichum *spp. generally are most abundant during rainy months. Although no management strategies exist for this new citrus disease, preliminary field trials determined that products containing pyraclostrobin were capable of reducing disease prevalence of Colletotrichum Dieback (CD).*

CD symptoms include leaf chlorosis, gumming on twigs and shoot dieback (**Figures1A** and **1B**) and, in severe cases, branch dieback of trees (**Figures 1C** and **1D**). The most characteristic symptoms of this disease are the gum pockets, which appear on young shoots either alone or in clusters, and the dieback of twigs and shoots (**Figures 1B** and **1C**). These symptoms primarily were reported by Farm Advisors and Pest Control Advisors from clementine, mandarin and navel oranges, but also have been seen on additional citrus varieties (data not shown). At this time, it is unclear which, if any, variety or cultivars are more susceptible or more resistant to this disease. Field observations indicate that symptoms initially appear during the early summer months and continue to be seen until the early fall. Trees showing dieback and gumming symptoms characteristic of this disease usually are spread out sporadically within an orchard, and generally only a few twigs or shoots are affected within a tree.

More than 100 species of *Colletotrichum* have been described, of which there are three well-known "species" of *Colletotrichum* based on traditional morphology – *C. gloeosporioides, C. acutatum and C. boninense*. With respect to citrus, two species of *Colletotrichum, C. gloeosporioides* and *C. acutatum*, have been associated with anthracnose diseases of citrus. These diseases, which include post-harvest anthracnose, post-bloom fruit drop (PFD) and key lime anthracnose (KLA), are of great economic importance (Timmer et al. 2000). However, recent evidence suggests that additional, previously unknown species of *Colletotrichum* are causing citrus diseases globally, particularly from the *C. boninense* species complex.

Colletotrichum karstii increasingly has been associated with anthracnose symptoms of citrus worldwide (Aiello et al. 2014; Huang et al. 2013; Ramos et al. 2016) and often occurs in association with other *Colletotrichum* spp., particularly *C. gloeosporioides*, which generally predominates within citrus hosts. *C. karstii* increasingly has been reported from anthracnose diseases of other crops like avocado (Silva-Rojas and Vila-Quezada 2011) and is considered the most common and widely distributed species of the *C. boninense* species complex (Damm et al. 2010). Although *C. karstii* has been reported from citrus in China, Italy and Portugal, it has not been reported from citrus species in the U.S.

To date, *C. gloeosporioides* has been the only species associated with anthracnose diseases of California citrus. Therefore, the objectives of this study were to:

1. identify *Colletotrichum* species associated with twig and shoot dieback, as well as branch canker of *Citrus* spp. in the Central Valley of California;
2. assess the pathogenicity of *Colletotrichum* spp. in twigs of *Citrus* spp.; and
3. determine when and under what environmental conditions spores of *Colletotrichum* spp. are dispersed within Central Valley citrus orchards based on spore trapping.

How the study was conducted

Field surveys were conducted in ten commercial citrus orchards throughout Madera County (one orchard), Tulare County (four orchards) and Kern County (five orchards) beginning in the spring of 2014 to fall 2015. Citrus orchards were sampled once during the spring and re-sampled during the fall of that same year. In spring 2017, two Tulare County and two Kern County orchards that had been previously surveyed during 2014-2015 were resurveyed. The citrus varieties surveyed in citrus orchards from Kern and Tulare counties were clementine (cv. Clemenules) and navels (cv. Fukumoto and Washington). The citrus varieties surveyed in citrus orchards from Madera County were Valencia oranges and navels (cv. Fisher). The average age of all surveyed orchards was 11 years. Approximately 16 trees were sampled from each orchard during each sampling period, with twigs and shoots collected from trees showing signs of blighted twigs and shoots and cankered shoots.

Field Survey and Fungal Identification

By studying the physical characteristics of the fungi, as well as sequencing a specific genomic region, two distinct species of *Colletotrichum* (*Colletotrichum karstii* and *C. gloeosporioides*) were associated with twig and shoot dieback in the orchards. Interestingly, these *Colletotrichum* species also were isolated from cankers in larger branches. Other fungi isolated from symptomatic tissues were identified as *Alternaria* spp., *Penicillium* spp., *Fusarium* spp., *Quambalaria* spp., *Botryosphaeria* spp. and Diatrypaceae species. *Botryosphaeria* spp. were recovered from 27 of the 274 samples collected, and Diatrypaceae species were recovered from eight of the

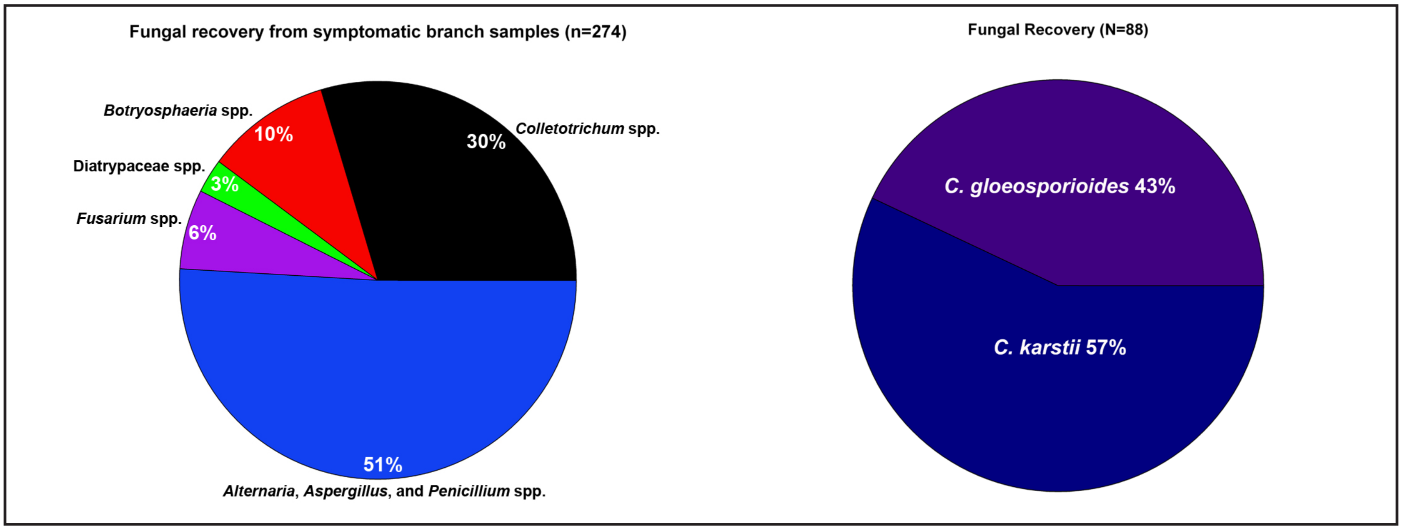

Figure 2. Fungal species recovered from twig and shoot samples.

274 samples. Both *Botryosphaeria* and Diatrypaceae species, known canker pathogens associated with citrus, were never co-isolated with species of *Colletotrichum* (**Figure 2**).

Pathogenicity and Fruit Decay Studies

Pathogenicity tests using clementine (cv. 4B) indicated that both *C. karstii* and *C. gloeosporioides* can produce lesions following inoculation of stems. *C. karstii* was the most aggressive fungal species producing the longest lesions after 15 months (**Figure 3**). Although *C. gloeosporioides* is known to cause anthracnose on citrus (a post-harvest disease causing fruit decay), it has not been reported to cause shoot dieback of citrus (Benyahia et al. 2003). *C. karstii*, however, has not been reported previously from citrus in California, and our laboratory confirmed the pathogenicity of this species in citrus. These findings confirm *C. karstii* as a new pathogen of California citrus.

It is known that *C. gloeosporioides* causes a post-harvest disease in citrus; however, it was unclear if *C. karstii* isolated from this study also could cause a post-harvest decay. Fruit decay studies using navel (cv. Washington), mandarin (cv. Murcott) and lemon (cv. Lisbon) revealed that when unwounded, neither *C. gloeosporioides* nor *C. karstii* caused any decay. When fruit were wounded, both *Colletotrichum* spp. caused decay, however a higher incidence and severity of decay was observed in fruit inoculated with *C. gloeosporioides*, particularly lemon and mandarin.

Spore Trapping

Relative humidity and precipitation in California citrus orchards play an important role in the epidemiology of *Colletotrichum* infection, whereby fungal spores dispersed by rain and humidity are conducive to pathogen spread. Based on spore trapping results, *Colletotrichum* species occurred most frequently during the months with the highest precipitation (January through May) (**Figure 4**); however, *Colletotrichum* spp. were not always correlated with rainfall. Wounding is also known to predispose plants to infection by

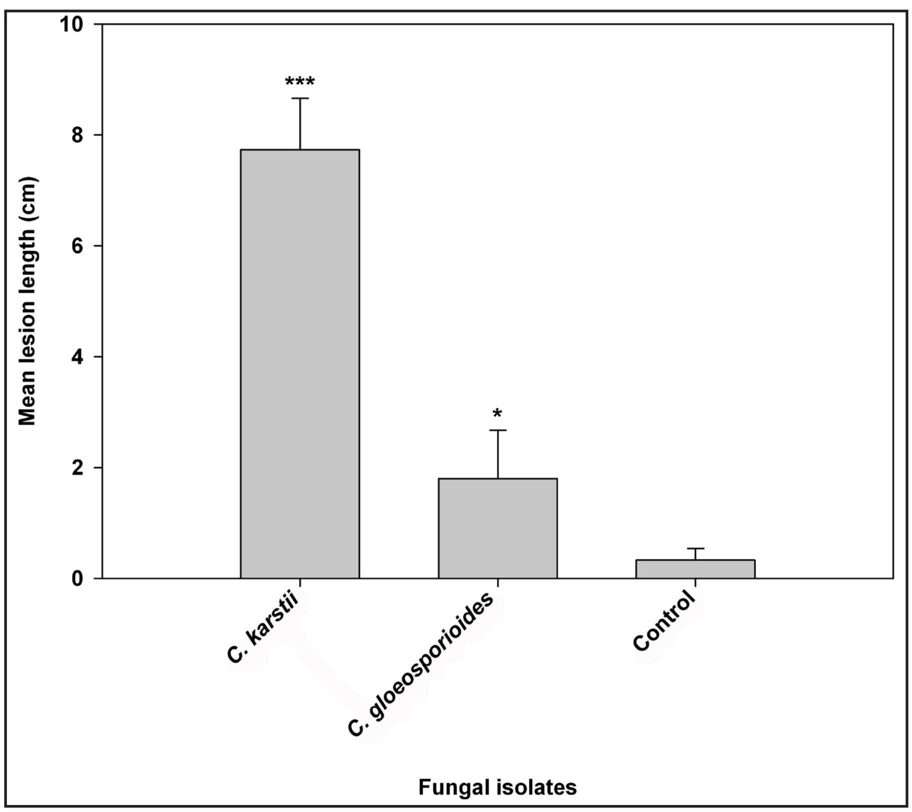

Figure 3. Pathogenicity of Colletotrichum *spp. on clementine mandarin (cv. 4B) after 15 months. Vertical lines represent standard error of the mean. Asterisks represent significance as follows: '*' P < 0.05 and '***' P < 0.001.*

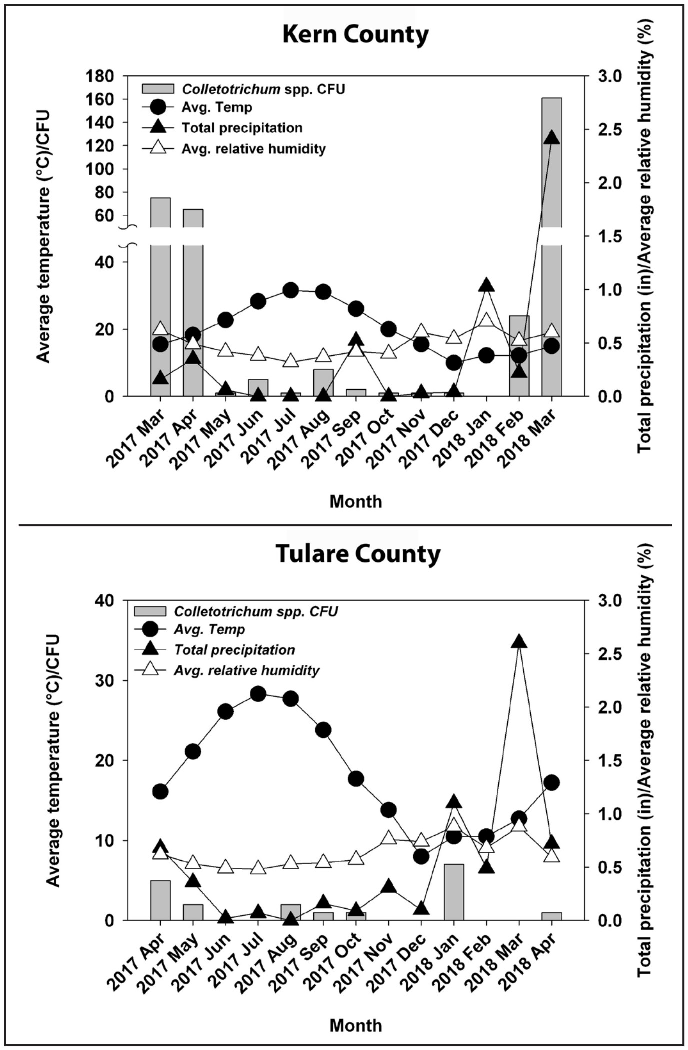

Figure 4. Relationships of monthly spore trap counts to temperature (°C), precipitation (mm), and relative humidity (%) for (a) Kern and (b) Tulare counties. Vertical bars represent total colony forming units (CFU) counted from each citrus orchard by month. Lines represent average monthly temperature (°C), relative humidity (%) and total monthly precipitation (mm).

Colletotrichum spp., and typical agricultural practices and the environment in California citrus groves (pruning, shearing, wind/sand damage) give *C. gloeosporiodes* and *C. karstii* the opportunity to colonize citrus trees. During this study, symptoms were observed during the late spring and summer months, with no new symptoms being observed in fall, winter or early spring. This suggests that young, tender tissues developing in the late spring likely are necessary for initial pathogen colonization.

Fungicide Sensitivities and Field Trial

Germination of *Colletotrichum* spp. spores was inhibited *in vitro* at concentrations below labeled rates for fungicides tested: azoxystrobin, pyraclostrobin, trifloxystrobin (all strobilurins) and fenbuconazole (a triazole) (**Table 1**). The half-maximal effective concentration (EC_{50}), as used in this report, refers to the dose of a fungicide that causes 50 percent inhibition of spore germination. Strobilurins had EC_{50} values (between 10 to 50 parts per billion) approximately 100-fold lower than EC_{50} values (between 500 to 600 parts per billion) of fenbuconazole. At the request of citrus growers, copper also was studied to determine its inhibitory effects on spore germination of *Colletotrichum* spp. *In vitro* experiments determined that copper concentrations of 150 parts per million or greater were completely inhibitory to spore germination of all isolates tested.

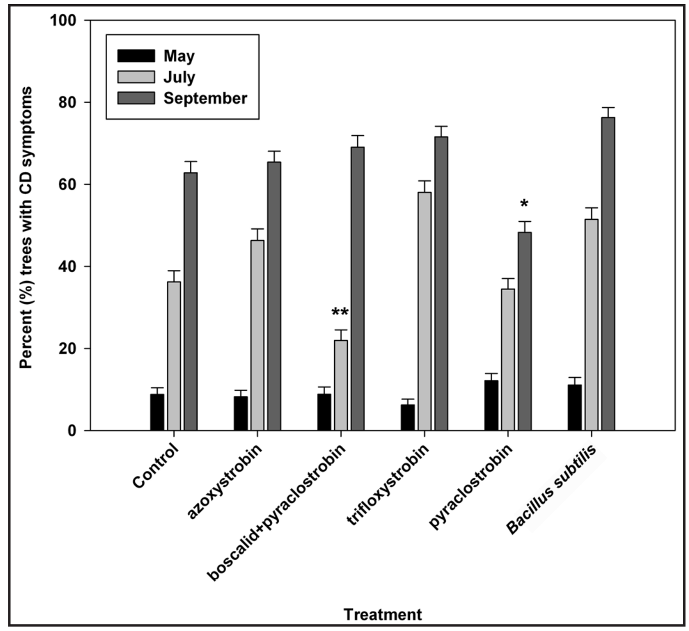

Figure 5. Prevalence of Colletotrichum Dieback (CD) symptoms in citrus trees by month after fungicide application in June. Vertical bars represent the mean percent of trees displaying symptoms of CD. Asterisks denote the following significance: '*' **$P < 0.05$** ***and '**"*** **$P < 0.01$.**

During a one-season field trial, single applications of commercial fungicides containing pyraclostrobin reduced CD symptoms for at least one month when tested in the field (**Figure 5**). Treatment with a premix of boscalid and pyraclostrobin showed a significant reduction ($P < 0.01$) in CD symptoms during the month of July, while treatment with pyraclostrobin alone showed a significant reduction ($P < 0.05$) in CD symptoms for September when compared to untreated control trees. All other treatments showed higher disease prevalence when compared to untreated controls for the months of July and September. It is important to note that the results of this field trial are preliminary and further field trials will be conducted to confirm the efficacy of these fungicides in managing CD.

Conclusions

Adherence to cultural practices recommended for the management of canker and dieback pathogens should be followed. These practices include maintaining trees in good condition through appropriate irrigation regimens and

Table 1. EC_{50} values of several fungicides tested for spore germination inhibition of *Colletotrichum karstii* and *C. gloeosporioides in vitro*.

Fungicide A.I.	Concentration applied (µg/ml)	EC_{50} values (µg/ml)			
		C. karstii		*C. gloeosporioides*	
		Mean±SD	Range	Mean±SD	Range
Fenbuconazole	500	0.518±0.201 a	0.238-1.056	0.622±0.252 a	0.302-1.190
Azoxystrobin	10	0.039±0.019 b	0.020-0.099	0.055±0.021 b	0.020-0.111
Trifloxystrobin	10	0.008±0.004 c	0.004-0.017	0.008±0.004 c	0.004-0.018
Pyraclostrobin	10	0.002±0.001 d	0.001-0.004	0.002±0.001 d	0.001-0.005

Values were determined from four isolates per fungal species. No inhibition is indicated by NI. Levels connected by the same letter are not significantly different using Tukey's honest significant difference (HSD) at $\alpha = 0.05$.

proper fertilization, removal of infested branches and pruning debris during dry periods followed by immediate disposal of infested material and sanitizing pruning equipment. Chemical management using fungicides is being investigated, and these methods may become part of an integrated pest management strategy for controlling this disease. Additional work also is being conducted to investigate the diversity of both *C. gloeosporiodes* and *C. karstii* to better understand the epidemiology of these pathogens and thus gain further insights into controlling these pathogens.

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***Akif Eskalen, Ph.D. is a cooperative extension specialist and lecturer in the Department of Plant Pathology at the University of California, Davis. Greg W. Douhan Ph.D. (Tulare County) and Craig Kallsen (Kern County) are farm advisors at the University of California Cooperative Extension. Joey S. Mayorquin, Ph.D. is a research associate at the Citrus Research Board, where he also serves as associate science editor of* Citrograph. *For more information, contact* aeskalen@ucdavis.edu.**