Further Investigation on Limb Dieback of Fig (*Ficus carica*) Caused by *Neoscytalidium dimidiatum* in California

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Abstract

Fig limb dieback is a cosmopolitan disease caused by *Neoscytalidium dimidiatum* (Botryosphaeriaceae), characterized by branch and shoot cankers, discoloration of woody tissues, and dieback. The present study investigated the etiology of the disease in California that seems to have become prevalent among fig orchards in the last several years. During orchard surveys in Fresno, Kern, and Madera Counties over 3 years, we isolated consistently and evaluated the pathogenicity of *N. dimidiatum* under laboratory and field conditions. The effect of summer and winter pruning on the disease severity and the effects of different environmental and mechanical stresses, such as sunburn and wounding by mallets, were assayed. In addition, the susceptibility of six different cultivars and the effects of eradicating cankered shoots from the fig trees as a method to combat the spread of the disease were studied. Pathogenicity tests

demonstrated that *N. dimidiatum* induces cankers on fig, mainly on wounded shoots. Results from the remaining experiments revealed that summer infection leads to more severe canker lesions than those induced by winter infection and that stressed shoots are more susceptible to infection than nonstressed shoots. ‘Brown Turkey’, ‘Conadria’, and ‘Calimyrna’ cultivars (all nonpersistent figs, i.e., needing pollination for fruit development) were less susceptible than the more susceptible ‘Kadota’, ‘Sierra’, and ‘Black Mission’ (all persistent figs, i.e., not needing pollination for fruit development). Canker removal from the orchard seems to be a good agronomic practice to avoid the spread of disease.

Keywords: Botryosphaeriaceae, cankers, *Ficus carica*, fig, limb dieback, *Neoscytalidium dimidiatum*

Fig (*Ficus carica* L.) acreage in the United States in 2017 reached 6,700 bearing acres producing 31,200 tons of fig (www.nass.usda.gov). Although the world’s top three producer countries are Turkey, Egypt, and Morocco, the United States is in the top 10 (www.fao.org), with the state of California ranking first in the nation, accounting for nearly 98% of all U.S. figs produced (www.agmrc.org). The commercial cultivation of fig is an important crop, providing economic income to many Mediterranean countries and to the California fig industry, with the latter supporting research to improve the quality of the product, postharvest advanced technologies, and marketing (Crisosto et al. 2017).

Many diseases are reported to affect figs in California and worldwide (Michailides 2003). Figs grown in California can suffer extensive losses due to fruit decay caused by fungi such as *Fusarium moniliforme* (endosepsis) (Michailides and Morgan 1998), *Aspergillus niger* (smut), *Alternaria*, and *Ulocladium* (Doster et al. 1996; Michailides 2003). In addition, canker diseases could represent a serious threat for fig growers, causing progressive yield losses over the years. In California, diseases caused by Botryosphaeriaceae spp. and Diaporthaceae spp. have been extensively investigated over the years (Chen et al. 2014a, b; Moral et al. 2019), revealing the presence of numerous taxonomic groups of species affecting different crops (Inderbitzin et al. 2010). These pathogens can induce severe symptoms, such as branch, shoot, and trunk cankers, and also blight fruits and leaves. Many reports of canker and dieback diseases on *Ficus*

spp. have been published, showing Botryosphaeriaceae (Al-Bedak et al. 2018; El-Atta and Aref 2013; Mayorquin et al. 2012; Mohali et al. 2017) and Diaporthaceae (Hampson 1981; Lima et al. 2005; Rehab et al. 2014) involved in these complex diseases. In addition to other *Ficus* spp., the cultivated fig is also attacked by botryosphaeriaceous and diaporthaceous fungi worldwide (Aiello et al. 2020; Banihashemi and Javadi 2009; Çeliker and Michailides 2012; Javadi and Banihashemi 2005). Among members of the Botryosphaeriaceae family, *Neoscytalidium dimidiatum* was reported in several countries to cause cankers and dieback on different *Ficus* spp., including the common fig (Al-Bedak et al. 2018; Elshafie and Ba-Omar 2002; Giha 1975; Mirzaee et al. 2002; Ray et al. 2010). This species produces two different asexual states known as synanamorphs: the coelomycetous morph, producing pycnidia with conidia (*Fusicoccum*-like), and the hyphomycetous morph, producing powdery arthric chains of conidia (*Scytalidium*-like conidia or arthrospores) (Farr et al. 2005; Nattrass 1933; Sutton and Dyko 1989), which is the reason why it has been characterized by a restless taxonomic process, going through different names and descriptions (*Torula dimidiata*, *Hendersonula toruloidea*, *Nattrassia mangiferae*, *Scytalidium dimidiatum*, *Scytalidium hyalinum*, *Fusicoccum dimidiatum*, *Neoscytalidium hyalinum*). In 2006 Crous et al. (2006) established the new genus *Neoscytalidium* (Crous and Slippers). In California, this species was reported with the old name *Hendersonula toruloidea* on fig (Paxton et al. 1964; Warner 1952), walnut (Wilson 1949), citrus (Calavan and Wallace 1954), and recently as an emerging pathogen on citrus, grape, and almond (Mayorquin et al. 2016; Nouri et al. 2018; Rolshausen et al. 2013). Furthermore, symptoms of a canker disease on ‘Kadota’ figs in California were also reported in the early 1950s, associated with *Phomopsis* species (English 1951, 1952; Hansen 1949). However, the problem seemed to gradually fade away, probably because acreage of ‘Kadota’ has decreased significantly over the years. In the last several years, fig growers in California have noticed a large number of trees in many orchards losing large limbs because of severe dieback. On the basis of previous reports, and to elucidate the limb dieback disease of fig in California, we conducted several experiments with the following aims: to ascertain the incidence of the disease and the role of *N. dimidiatum* in the limb dieback etiology, to study the influence of environmental and

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agronomic factors in the disease etiology, and to evaluate the susceptibility of different fig cultivars to the disease in California.

Materials and Methods

Field survey and fungal isolations. A total of 16 fig orchards located in Fresno, Kern, and Madera Counties (central and southern San Joaquin Valley in California) were surveyed for 3 years (2005 to 2007), collecting every year about 10 to 15 branches and shoots showing symptoms of cankers and dieback from five different cultivars, including 'Black Mission', 'Calimyrna', 'Conadria', and also a Stanford caprifig (male tree) and another unknown caprifig cultivar. Symptomatic tissues were surface disinfected with household bleach at 10% (vol/vol) in sterile water for 3 min. Small pieces (3 to 5 × 2 to 5 mm) from the margins of cankers were cut with a sterile scalpel and placed in Petri dishes containing 2% potato dextrose agar (PDA) acidified with lactic acid (2.5 ml of 25% [vol/vol] per liter of medium; APDA) to minimize bacterial growth. Petri dishes were incubated at 25 ± 3°C for 2 to 7 days, until fungal colonies were large enough to be examined. Occasionally tissue from stained wood segments distant from the canker margin, healthy-appearing wood away from the canker, infected lenticels or growth cracks, and tissue from insect borings were plated out. *N. dimidiatum* colonies were transferred to APDA dishes to obtain pure cultures, and single spore isolates were then stored in our collection. Recovered isolates used for further investigation are maintained in the culture collection of the Department of Plant Pathology at the University of California, Davis (Kearney Agricultural Research and Extension [KARE] Center in Parlier).

Effect of different temperatures on mycelial growth of *N. dimidiatum*. To determine the cardinal temperatures of growth, a 4.76-mm plug of a 3-day-old colony of the isolate of *N. dimidiatum* 2D3 was removed and transferred to the center of 90-mm Petri dishes of APDA and incubated at eight different temperatures, from 5 to 40°C. Four Petri dishes were used for each temperature as replicates. The experiment was repeated once. After 3 days of incubation, the largest and smallest diameters of colonies were measured with a digital scale ruler. Mean data were converted to radial growth (in mm). Data from two experiments were combined after checks for homogeneity of variances via *F* test. A nonlinear adjustment of the data was applied via the generalized Analytis Beta model, as described by López-Moral et al. (2017), and the optimum growth temperature was calculated according to the formula provided by the same authors.

Pathogenicity tests on detached shoots. Preliminary pathogenicity tests were conducted on detached shoots collected from an experimental orchard at the KARE Center under laboratory conditions. Current season shoots ('Calimyrna'), 15 to 25 cm long, were surface sterilized for 4 min in a dilute mixture of bleach and alcohol (160 ml of 5.25% NaOCl bleach and 160 ml of ethanol/10 liters of water) and allowed to air dry on plastic screens in plastic rectangular chambers of 30 × 23 × 10 cm. A 7-mm in diameter plug from 14-day-old cultures of *N. dimidiatum* (isolates 3C02, 3C07) grown on APDA was used to inoculate each shoot. Wounds were made with a 7-mm cork borer, and the mycelial plug was placed on each wound upside down and covered with semitransparent film (Parafilm "M") to prevent desiccation. Water was then added to the bottom of the plastic container to create a humid environment, and the containers were incubated at 30°C. The experiment consisted of four treatments: wounded shoots noninoculated, nonwounded and inoculated, wounded and inoculated, and nonwounded and noninoculated. Ten shoots per treatment served as replicates. The experiment was repeated once with a slight modification, using 4-year-old shoots instead of current shoots. Presence of cankers (disease incidence) and length of cankers were recorded 40 days after the first experiment and 30 days after the repetition.

Pathogenicity test in the field. Based on the isolation results, pathogenicity tests in the field were conducted with *N. dimidiatum* (isolate 2D03) and *Phomopsis* sp. previously isolated from a symptomatic 'Calimyrna' fig limb in Madera County. These two species were inoculated onto 2-year-old shoots on 10 'Calimyrna' trees in

the south row of an experimental orchard located at the KARE Center. A total of 15 shoots per treatment were used. Half of the shoots were tied to a string attached to a plastic bag filled with dried soil as a weight to bend the shoots at a 45° angle and expose them more to direct sunlight to induce sunburn. The remaining shoots were left alone and were not exposed to direct sunlight because they were shaded by the foliage above them. A cork borer of 7-mm diameter was used to create a wound, and a mycelial plug of 7 mm of each fungus was used to inoculate each shoot. The wounds and the mycelial plugs were wrapped with semitransparent film to prevent desiccation. Control consisted of a sterile plug of PDA. The length of canker at either side of the inoculation was recorded after 1 year and 4 months. Reisolation from the margins of the cankers was done to fulfill Koch's postulates.

Effect of summer and winter pruning on infection development. To study the effect of summer and winter pruning on infection development, trees of the 'Calimyrna' experimental orchard at the KARE Center were pruned and inoculated in August and February in a spore suspension at a concentration of 1 × 10⁵ spore/ml of *N. dimidiatum* isolate 3B02. Inoculations were made on both summer and winter pruning cuts at different times after pruning, at 0, 1, 2, 3, 4, 5, 6, 7, 14, and 21 days after pruning, using 10 shoots per each treatment. These serial inoculations were performed to observe differences in the length of the lesions and then evaluate the period of susceptibility of pruning cuts. Pruned but noninoculated shoots served as controls. After the inoculum was sprayed on the pruning cut, the inoculated wounds were covered with semitransparent film to prevent desiccation. Length of the cankers was recorded twice: 1 and 2.5 years later for the summer pruning and 6 months and 2 years later for the winter pruning.

Effects of stress treatments on infection development. The effects of different environmental and agronomic stresses on the infection development were evaluated. Specifically, the effects of sunburn and mechanical injuries were studied. Eight treatments were conducted in this experiment: shoots wounded with mallet and inoculated; shoots wounded with mallets, sunburned, and then inoculated; shoots wounded only with mallets and noninoculated; shoots sunburned and then inoculated; shoots inoculated but nonwounded (control 1); shoots wounded but noninoculated (control 2); shoots wounded, inoculated, and painted with whitewash (SurroundR, Nova-Source, Tessenlerlo Kerley, Inc., Phoenix, AZ, U.S.A.); and shoots painted with whitewash and then inoculated. Large limbs (3 to 4 years old) of 15-year-old 'Calimyrna' trees located at the KARE Center were subjected to these stress treatments in August. To sunburn or heat the shoots above ambient conditions, portions of the shoots were wrapped with black plastic (Fig. 1E). To simulate wounding by mallets, shoots were wounded with the threaded end of a 7.94- × 304.8-mm (5/16- × 12-inch) carriage bolt. To cool them below ambient conditions, shoots were painted with a white tree trunk paint. In this experiment, 10 replicates were used for each treatment, for a total of 10 trees. Three weeks after the beginning of each treatment, the bark was removed with a 7-mm cork borer, and the shoots were inoculated with a 7-mm mycelial plug (isolate 3C02). Symptoms were recorded 2 and a half years after the inoculations.

Susceptibility of fig cultivars to the infection. To evaluate the susceptibility of various fig cultivars to the limb dieback, 'Brown Turkey', 'Black Mission', 'Calimyrna', 'Conadria', 'Kadota', and 'Sierra' were planted in an experimental orchard at the KARE Center and used for the experiment. A total of eight trees per cultivar were used, and two 1-year-old shoots per tree were inoculated with a 7-mm mycelial plug from a 14-day-old colony of the isolate 3C02 placed on wounds made with a 7-mm cork borer. Inoculations were performed in September, and results (length of cankers) were recorded twice, 2 months and 1 and a half years after the inoculations.

Cankers eradication and pathogen recovery. Existing cankers of different inoculation experiments were pruned 5.08 cm (2 inches) below the canker margin from half of the trees in the "effect of summer and winter pruning on infection development" experiment and from half of the trees in the "effects of stress treatments on infection

development” experiment. Two years later the cankers from pruned and unpruned shoots were measured to see whether canker removal can contain pathogen movement in the shoots. Reisolations were made on acidified PDA from shoots subjected to this investigation to ascertain the possible recovery of *N. dimidiatum*.

Data analysis. Data of this study were analyzed in SAS (release 9.3; SAS Institute Inc.) and Statistix 10 (Analytical Software 2013). Data were tested for normality and homogeneity of variances, and then the analysis of variance was performed. Mean differences were compared with Fisher’s least significance difference test at $P = 0.05$.

Results

Field surveys, fungal isolations, symptoms, and signs of the disease. Results of the isolations from all 16 orchards surveyed in 3 years consistently showed the presence of *N. dimidiatum*, in both symptomatic and asymptomatic samples. Close examination of collected samples from trees with limb dieback revealed that the pathogen produced both arthrospores and pycnidia in woody tissues. Arthrospores are loose and develop in the space between the bark and the woody tissues from mycelia of the fungus that break into short pieces (Fig. 1D). Pycnidia were found embedded in the bark (Fig. 1C) and in general produced light-colored, unicellular pycnidiospores. The second most common fungus isolated from these surveys was *Phomopsis* spp., although its incidence seemed to decrease during this study. *Phomopsis* spp. incidence was surprisingly high (100%) in the second year of survey in one orchard of cultivar ‘Black Mission’ (Table 1). Symptoms observed in the field included internal wood discoloration, branch canker and dieback, signs (pycnidia) of the pathogen in the bark, and arthrospores under the bark (Fig. 1A to 1D). The following isolates of *N. dimidiatum* were used in this study: 2D3/2D03, 3C2/3C02, 3C7/3C07, and 3B02. Because of the uniformity and similar growth characteristics of these isolates, three random isolates, 2D3, 3C2, and 3C7, were molecularly identified on the basis of six loci in a study by Inderbitzin et al. (2010).

Effect of different temperatures on *N. dimidiatum* mycelial growth. After 3 days of incubation, no mycelial growth was observed at 5 and 10°C. Mycelial growth was observed at all other temperatures, showing different mean values: 7.0 mm at 15°C, 11.1 mm at 20°C, 36.3 mm at 25°C, 39.6 mm at 30°C, 38.0 mm at 35°C, and 3.5 mm at 40°C. Optimum growth occurred at 31.5°C (Fig. 2).

Pathogenicity tests on detached shoots. Pathogenicity tests conducted under laboratory conditions on detached current shoots, and

4-year-old detached shoots in experiment 2, showed that *N. dimidiatum* causes canker on fig tissues. Specifically, in experiment 1, three statistically different groups emerged from the analysis. Results with isolate 3C7 on wounded shoots are significantly different from those of all other treatments, showing the largest cankers. Inoculations with isolate 3C2 on wounded and unwounded shoots, and 3C7 on unwounded shoots, did not reveal statistically significant differences, showing an intermediate level of canker length. However, treatments with wounded and unwounded shoots not inoculated did not produce lesions, showing a separate statistical group. In both experiments the highest incidence (100%) was recorded on wounded shoots inoculated with either isolate. In experiment 2, treatment with isolate 3C7 on wounded shoots produced the longest cankers, as it did in experiment 1, followed by treatment with isolate 3C2 on wounded shoots, and the shortest lesions with the same isolate on unwounded shoots. Interestingly, isolate 3C7 inoculated on unwounded shoots did not produce lesions this time, and the incidence of infection was 0%. In experiment 2, the isolates induced smaller lesions than those in experiment 1 (Fig. 3). Arthrospores were abundant under the bark on these shoots, as were pycnidia with mature pycnidiospores.

Pathogenicity test in the field. Pathogenicity tests in the field conducted on 2-year-old shoots with *N. dimidiatum* (2D03) and an isolate of *Phomopsis* sp. revealed significant differences between the two species. *N. dimidiatum* induced lesions of 24.8 mm (no sunburn) and 19 mm (sunburn), but *Phomopsis* sp. did not induce any lesions significantly larger than the discoloration that resulted from the control (11.9 mm vs 10.9 mm) (Table 2). This test in the field was performed also to determine effects of sunburn on the shoots inoculated with *N. dimidiatum*. In this experiment, there were no significant differences between the two treatments. Because maximum air temperature reached 32.2°C on only 4 days during the shoot inoculation experiment, the heat stress may not have been sufficient to show an effect (Table 2). However, because we observed that cankers were often associated with sunburned tissues, the hypothesis that sunburn affects *Neoscytalidium* canker was investigated separately in another experiment.

Effect of summer and winter pruning on infection development. Results from summer and winter pruning inoculations revealed significant differences between the two seasonal treatments. Cankers from summer pruning were significantly longer than those that occurred after winter pruning. Lesions were measured twice, at two different times, and in both cases significant differences were confirmed. In both evaluations the average length of summer lesions was 28.5 cm, compared with 9 cm for the winter treatment. Very small lesions in the last treatments (21 days after pruning) were observed from the serial inoculation time after pruning, suggesting that pruning cuts become less susceptible after 3 weeks (data not shown).

Effects of stress treatments on infection development. This experiment was performed to see whether environmental or agronomic stresses could affect infection establishment and development. Limbs wounded with mallets and also sunburned had the largest cankers among all the treatments. Intermediate length of cankers was recorded for limbs only wounded with mallets or only sunburned, followed by limbs only wounded with mallets or only sunburned without fungal inoculation. No significant differences were recorded between the cankers on whitewashed limbs before and after inoculation and the nonwounded but inoculated control (Fig. 4).

Susceptibility of fig cultivars to the infection. The first symptom evaluation, conducted 2 months after the inoculation of the six fig cultivars, revealed two statistically different groups, with cultivars ‘Sierra’, ‘Kadota’, and ‘Black Mission’ being more susceptible than cultivars ‘Brown Turkey’, ‘Conadria’, and ‘Calimyrna’. The second evaluation, performed a year and a half later, confirmed the results of the first evaluation, with the exception of cultivar ‘Black Mission’, which showed moderate susceptibility between the less susceptible ‘Brown Turkey’, ‘Conadria’, and ‘Calimyrna’ and the more susceptible ‘Kadota’ and ‘Sierra’ (Fig. 5).

Canker eradication and pathogen recovery. Pruning the shoots 5.08 cm below the obvious external margins of cankers effectively contained pathogen movement within the shoots. Isolations from

Fig. 1. Effect of temperature on mycelial growth of isolate D3 of *Neoscytalidium dimidiatum*. The averages of radial growth and temperature were adjusted to a nonlinear regression curve via the Analytis Beta model. Y represents standardized radial growth. Data points are the means and vertical bars are the standard error of the means.

the cut surface of pruned shoots from trees in both experimental orchards at the KARE Center produced no *N. dimidiatum*. Otherwise, the fig canker pathogen was consistently recovered from the margins of cankers in unpruned shoots that remained on the trees in this orchard (frequency average = 43%).

Discussion

The present study investigated the etiology and epidemiology of fig limb dieback in California. Results from our surveys conducted in the main fig production counties of California reveal that the two most common species isolated from cankers of symptomatic branches and shoots were *N. dimidiatum* and *Phomopsis* spp. *Phomopsis* has been reported in other countries as an important fig canker pathogen. In Iran, its association with fig canker has been known for >25 years (Banihashemi, unpublished data). In California, *Phomopsis cinerascens* was reported in 1936 as an epidemic pathogen on the cultivar 'Kadota' (Ferguson et al. 1990). *Phomopsis* canker can be found in all commercial fig cultivars, but it is most devastating in 'Kadota' (Obenauf et al. 1978). However, pathogenicity tests conducted in this study using both *N. dimidiatum* and *Phomopsis* spp. revealed that *N. dimidiatum* induced lesions, unlike *Phomopsis*. 'Kadota' probably was susceptible to *Phomopsis* infection during those years, and its progressive displacement with other cultivars, perhaps less susceptible, led to a decrease in the incidence of this pathogen over the years. When the surveys of orchards with putative canker diseases were performed, no commercial orchards of 'Kadota' were located in counties of the central San Joaquin Valley in California. The 3-year surveys of other fig cultivars showed high levels of both *N. dimidiatum* and *Phomopsis* spp. only in the first year, an indication that these pathogens could co-occur in the same canker tissues. However, in survey years 2 and 3, with the exception of one 'Black Mission' orchard where all samples produced *Phomopsis* spp., *Phomopsis* spp. were not isolated or were isolated at low levels in a few orchards (Table 1). Obviously, the incidence of *Phomopsis* spp. was reduced in years 2 and 3 from surveyed symptomatic tissues. In contrast, the incidence of *N. dimidiatum* was high in most samples during the 3-year survey. However, recent isolations have shown the presence of *Phomopsis* spp. associated with

symptomatic samples, although at low percentage compared with *Neoscytalidium* frequency. Therefore, we can affirm that *Phomopsis* spp. is in somehow associated with the disease, but additional investigations are needed to confirm the interactions between these two species and disease development.

N. dimidiatum has been reported worldwide, causing diseases on many other important crops (Derviş et al. 2019; Polizzi et al. 2009; Rolshausen et al. 2013; Türkölmez et al. 2019), and in the study of Marques et al. (2013) it was considered the most aggressive of all Botryosphaeriaceae. A recent study conducted in California on almond branch and trunk cankers showed a high incidence of *N. dimidiatum* and confirms that this disease appeared to be widespread, suggesting a recent increase of this pathogen and the diseases it causes (Nouri et al. 2018). In California, the presence of different susceptible crops in contiguous areas allows an easy flow of inoculum (arthrospores) between tree species (Moral et al. 2019). Our optimum growth temperature results accord with those of Nouri et al. (2018), showing an optimum temperature of 31.5°C. In this study we found a significant difference in lesion length between trees pruned in summer and those pruned in winter. Lesions from summer pruning were always significantly longer than those on winter-pruned trees. Also, winter pruning is less problematic than summer pruning, when more severe infections and higher inoculum levels can occur. These results demonstrate that summer temperatures are an important factor for the development of infection by *N. dimidiatum*, as stated by Hassan et al. (2011) and Sadowsky et al. (2007). In fact, both reports showed the effect of heat stress treatments as a predisposing factor to *N. dimidiatum* infection. In Israel, severe symptoms on grapefruits appeared after extremely hot and dry weather events for several consecutive days (Oren et al. 2001). In Oman, infection on *Albizia lebbek* occurred in the summer of 1998 when the average temperature was 40°C (Elshafie and Ba-Omar 2002), as was observed in Iraq on different hosts (Hassan et al. 2009). Our results accord with previous research of English et al. (1975), who described the canker pathogen *H. toruloidea* in California as extremely temperature sensitive, able to cause appreciable infection only in summer. However, a recent study conducted in California on almond showed that *N. dimidiatum* isolates infected almonds regardless of the month of inoculation,

Table 1. Incidence of fungal species emerging from 3 years of surveys in Fresno, Madera, and Kern counties

Cultivar	Month	Incidence (%)			Year
		<i>Neoscytalidium dimidiatum</i>	<i>Phomopsis</i> sp.	<i>Botryosphaeriaceae</i> sp.	
Black Mission	May	26.4	66.4	1.0	1
Calimyrna	May	78.3	13.3		1
Conadria	June	82.2	7.8	7.8	1
Calimyrna	June	65.5	32.2	3.3	1
Black Mission	June	11.3	55.5	1.7	1
Black Mission	June	6.7	93.3		1
Calimyrna	May	75	17		2
Black Mission	May	59	0		2
Black Mission	May	8	100		2
Conadria	May	86	0		2
Caprifig	May	85	0		2
Conadria	May	99	0		2
Black Mission	May	97	17		2
Black Mission ^w	May	90	0		2
Black Mission ^x	May	100	0		2
Stanford caprifig	May	90	3		2
Calimyrna	May	100	0		2
Calimyrna	May	85	10		2
Calimyrna	May	100	0		2
Calimyrna	May	32	39		3
Black Mission	June	84 ^y	0		3
Black Mission	November	100 ^z	0		3

^w Isolations from infected lenticels or growth cracks.

^x Isolations from tunnels of boring insects.

^y *N. dimidiatum* was isolated from 68% of the dark brown staining of the woody tissues in advance of the cankers.

^z *N. dimidiatum* was also isolated from 15% of symptomless shoots.

Fig. 2. Symptoms and signs of *Neoscytalidium dimidiatum* on fig. **A**, Limb dieback of cultivar 'Black Mission' in the field. **B**, Internal wood discoloration of cultivar 'Black Mission'. **C**, Pycnidia observed on fig shoot. **D**, Arthrospores developed under the bark. **E**, Shoot wrapped with black plastic to create the sunburn treatment.

Fig. 3. Pathogenicity of *Neoscytalidium dimidiatum* on detached fig shoots. Average lesion length resulting from inoculation with a mycelium plug of *N. dimidiatum* onto current and 4-year-old shoots. Percentages above the columns indicate the incidence of infection. Letters above the columns indicate treatments significantly different ($P < 0.05$). Letters refer to the specific experiment.

although the authors affirmed that the winter of 2015 was particularly dry and warm (Nouri et al. 2018). Many authors refer to *N. dimidiatum* as a weak or opportunistic pathogen, invading tissues through wounds and openings, especially when the host is stressed. Schoene-weiss (1975) suggested that stress plays a key role, exerting its most pronounced effect by predisposing plants to facultative parasites, especially weak or nonaggressive pathogens. In this study, experiments conducted with different stress treatments demonstrated that the combination of mechanical injuries (mallet) and sunburn led to the longest cankers, followed by lesions derived from inoculations on wounded shoots (mallet) or only sunburned shoots. In this experiment, and in the pathogenicity tests on 4-year-old detached shoots, wounds seemed to be necessary to initiate the infection process. However, in pathogenicity tests on current shoots, the infection occurred also on unwounded ones, probably because the tissues were not lignified enough and the pathogen was able to penetrate without a preexisting wound. Similarly, in a study conducted on a dragon fruit canker disease caused by *N. dimidiatum*, Fullerton et al. (2018) concluded that the tissues most susceptible to infection were the tips of rapidly growing cladodes. Mature cladodes were highly resistant to infection. Davison (1972) showed that *H. toruloidea* can infect an unwounded tree at the ideal temperature of 25°C, but wounding resulted in the higher disease incidence, supporting our results by suggesting that injuries such as sunburn, pruning wounds, and other mechanical injuries (i.e., mallet) are crucial in the infection process, as was also shown by other authors (Calavan and Wallace 1954; Nouri et al. 2018; Oren et al. 2001). Our results also showed that pruning wounds are susceptible to infection for ≥ 3 weeks. The same experiment revealed that shoots treated with whitewash and inoculated did not produce lesions. Treatments with whitewash seem to be effective at both protecting trees from sunburn, thus

Table 2. Pathogenicity test in the field

Treatment	Species	Canker length (mm)
Sunburn	<i>Neoscytalidium dimidiatum</i>	19.0 a ^z
Non-sunburn	<i>N. dimidiatum</i>	24.8 a
	<i>Phomopsis</i> sp.	11.9 b
	Control	10.9 b

^z Average lesion length (mm). Letters indicate treatments that were significantly different ($P < 0.05$).

Fig. 4. Effects of different stress factors on lesion length after inoculation of fig shoots with *Neoscytalidium dimidiatum*. Different letters above columns indicate significantly different treatments ($P < 0.05$).

preventing development of cracks and other wounds on shoots, and suppressing infection by the pathogen and canker formation.

In this investigation, six commercial fig cultivars were evaluated for their susceptibility to canker formation by *N. dimidiatum*. Among these cultivars, 'Brown Turkey', 'Conadria', and 'Calimyrna' (non-persistent figs, i.e., needing pollination to keep the fruit) are less susceptible than cultivars 'Kadota' and 'Sierra', which were more susceptible, with the cultivar 'Black Mission' showing moderately susceptibility. Interestingly, these last three cultivars are persistent (i.e., they do not need pollination for fruit to develop). At the end of all experiments, we ascertained that pruning 5 cm below the canker could successfully remove the pathogen from the infected shoots. The pathogen was never recovered from shoots that were pruned 5 cm below the canker, an indication that the pathogen has difficulty advancing more than 5 cm internally beyond the external canker margin. This information should be used by growers as a best practice to safely remove cankers from their fig orchards. This investigation represents a contribution to our understanding of this destructive emerging pathogen in California fig orchards, and future research should aim at developing efficient control strategies.

Fig. 5. Cultivar susceptibility (as indicated by lesion length) to limb dieback disease caused by *Neoscytalidium dimidiatum*. Different letters above columns indicate significantly different treatments ($P < 0.05$). Letters refer to specific (first and second) disease evaluation.

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