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 for three 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 used to harvest fruit, were assayed. In addition, the susceptibility of six different cultivars and the effect of eradication of cankered shoots from the fig trees as an effective method to combat the spread of the disease were also studied. Pathogenicity tests demonstrated that *N. dimidiatum* is able to induce cankers on fig, mainly on wounded shoots. Results from the remaining experiments reveal 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 non-stressed shoots. Brown Turkey, Conadria and Calimyrna cultivars (all non-persistent figs, meaning requiring pollination for fruit development) were less susceptible compared to more susceptible Kadota, Sierra, and Black Mission (all persistent figs, i.e., non-requiring pollination for fruit development). Canker removal from the orchard seems to be a good agronomic practice to avoid the spread of pre-existing disease.

Fig (*Ficus carica* L.) acreage in USA for 2017 reached 6,700 bearing acres with a production of 31,200 tons of fig (NASS, 2017). Although the world's top three producer countries are Turkey, Egypt and Morocco, USA is part of the top ten (FAOSTAT, 2018), with the State of California ranking first in the nation, accounting for

nearly 98% of all USA figs produced (AgMRC, 2018). The commercial cultivation of fig is an important crop providing economic income to many Mediterranean countries as well as 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 affecting 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 through 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 are able to 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, 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., 2019; 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 causing 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, Natrassia mangiferae, Scytalidium dimidiatum, S. hyalinum, Fusicoccum dimidiatum, Neoscytalidium hyalinum*). In 2006 Crous et al. 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, 1947), 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). The problem, however, seemed to gradually fade away, probably because acreage of the cv. 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 due to a severe dieback problem. On the basis of previous reports, and in order to better understand the limb dieback disease of fig in California, we conducted several experiments with the following aims: 1) to ascertain the incidence of the disease and the role of *N. dimidiatum* in the limb dieback etiology; 2) to study the influence of environmental and agronomic factors in the disease etiology; and 3) to evaluate the susceptibility of different fig cultivars to the disease in California.

Material 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 three years (2005 to 2007), collecting every year about 10-15 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 (Clorox Professional Product Company) at 10% (vol/vol) in sterile water for 3 min. Small pieces (3 to 5 by 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;; Microtech Scientific) 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 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-days-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 using a digital scale ruler. Mean data were converted to radial growth (mm). Data from two experiments were combined after checking for homogeneity of variances with *F* test. A nonlinear adjustment of the data were applied using 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.

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117 **Pathogenicity test on detached shoots**

118 Preliminary pathogenicity tests were conducted on detached shoots collected from an experimental orchard
119 at KARE under laboratory conditions. Current season shoots (cv. Calimyrna), 15-25 cm long, were surface
120 sterilized for 4 min in a dilute mixture of bleach and alcohol (160 ml bleach of 5.25% NaOHCl and 160 ml
121 ETOH/10 liters water) and allowed to air dry on plastic screens in plastic rectangular chambers of 30 × 23 ×
122 10 cm. A 7-mm in diameter plug from 14-days-old cultures of *N. dimidiatum* (isolates 3C02; 3C07) grown on
123 APDA was used to inoculate each shoot. Wounds were made with a 7-mm cork borer, and the mycelial plug
124 was placed on each wound upside down and covered with Parafilm® to prevent desiccation. Water was then
125 added to the bottom of the plastic container to create a humid environment and the containers were incubated
126 at 30°C. The experiment consisted of four treatments as follows: 1) wounded shoots non-inoculated; 2) non-
127 wounded and inoculated; 3) wounded and inoculated; and 4) non-wounded and non-inoculated. Ten shoots
128 per treatment served as replicates. The experiment was repeated once with a slight modification, using 4-
129 year old shoots instead of current shoots. Presence of cankers (disease incidence) and length of cankers
130 were recorded 40 days after the first experiment, and 30 days after the repetition.

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132 **Pathogenicity test in the field**

133 Based on the isolation results, pathogenicity tests in the field were conducted using *N. dimidiatum* (isolate
134 2D03), and *Phomopsis* sp., previously isolated from a symptomatic Calimyrna fig limb in Madera County.
135 These two species were inoculated onto 2-year-old shoots on 10 Calimyrna trees in the south row of an
136 experimental orchard located at KARE Center. A total of 15 shoots per treatment were used. Half of the
137 shoots were tied to a string attached to a plastic bag filled with dried soil as a weight to bend the shoots at a
138 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 due to shading 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 Parafilm® to prevent desiccation. Control consisted of a sterile plug of PDA. The length of canker at either side of the inoculation was recorded after one year and four months. Re-isolation from the margins of the cankers were done to fulfill Koch's postulates.

Effect of summer and winter pruning on infection development

In order to study the effect of summer and winter pruning on infection development, trees of the Calimyrna experimental orchard at KARE Center were pruned and inoculated in August and February, using a spore suspension at the concentration of 1×10^5 spore/ml of the isolate 3B02 of *N. dimidiatum*. Inoculations were made on both summer and winter pruning cuts at different times after pruning, as follows: 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 in order to observe some difference in the length of the lesions, and then to evaluate the period of susceptibility of pruning cuts. Pruned but non-inoculated shoots served as controls. After spraying the inoculum on the pruning cut, the inoculated wounds were covered with Parafilm® to prevent desiccation. Length of the cankers were recorded twice: 1 and 2.5 years later the summer pruning, and 6 months and 2 years later for the winter pruning.

Effects of stress treatments on infection development

The effect of different environmental and/or agronomic stresses on the infection development was evaluated. Specifically, the effect of sunburn and mechanical injuries were studied. Eight treatments in all were conducted in this experiment, including: 1) shoots wounded with mallet and inoculated, 2) shoots wounded with mallets and sunburned, and then inoculated, 3) shoots wounded only with mallets and non- inoculated

4) shoots sunburned and then inoculated, 5) shoots inoculated but non-wounded (control 1), 6) shoots wounded but non-inoculated (control 2), 7) shoots wounded, inoculated, and painted with Whitewash, 8) shoots painted with Whitewash and then inoculated. Large limbs (3-4 years old) of 15-year old Calimyrna trees located at KARE Center were subjected to these stress treatments during August. Sun burning and/or heating of the shoots above ambient conditions were created by wrapping portions of the shoots with black plastic (Fig. 1E). In order to simulate wounding by mallets, shoots were wounded with the threaded end of a 7.94 mm × 304.8 mm (5/16 ×12-inch) carriage bolt. Cooling of shoots below ambient conditions was created by painting them with a White Tree Trunk Paint by Frazee Paint Company. 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 of mycelial plug (isolate 3C02). Symptoms were recorded 2 years and a half after the inoculations.

Susceptibility of fig cultivars to the infection

In order to evaluate susceptibility of various fig cultivars to the limb dieback, the following cultivars: Brown Turkey, Black Mission, Calimyrna, Conadria, Kadota, and Sierra have been 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 one-year-old shoots per tree were inoculated using a 7 mm mycelial plug from a 14-days-old colony of the isolate 3C02 on wounds made with a 7 mm cork borer. Inoculations were performed in September and results (length of cankers) were recorded twice, two months and one year and a half 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 from the “effect of summer and winter pruning on infection development” experiment,

and from half of the trees from "effects of stress treatments on infection development". Two years later the length of cankers from pruned and unpruned shoots were measured in order to see if the canker removal can contain the pathogen movement in the shoots, effectively. Re-isolations 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 using 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 ANOVA was performed. Mean differences were compared with Fisher's least significance difference test (LSD) 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 three years consistently showed the presence of *Neoscytalidium 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 under the space between the bark and the woody tissues from mycelia of the fungus that break to short pieces (Fig. 1D). Pycnidia were found embedded in the bark (Fig. 1C) and in general produced light color, unicellular pycnidiospores. The second most common fungus isolated from these surveys was *Phomopsis* spp., although its incidence seemed to decrease during the years of this study. *Phomopsis* spp. incidence was surprisingly high (100%) during 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, and presence of signs (pycnidia) of the pathogen in the bark, and arthrospores under the bark (Fig. 1A-D). The following isolates of *N. dimidiatum* were used in this study: 2D3/2D03, 3C2/3C02, 3C7/3C07, 3B02. Because of the uniformity and similar growth characteristics of all 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 three days of incubation results show that 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. The optimum growth temperature resulted 31.5 °C (Fig.2).

Pathogenicity test on detached shoots

Pathogenicity test conducted under laboratory conditions on detached current shoots, and 4-years-old detached shoots in the experiment 2, showed that *N. dimidiatum* causes canker on fig tissues. Specifically, in experiment 1, three statistical different groups emerged from the analysis. Results with isolate 3C7 on wounded shoots are significantly different by all other treatments, showing the highest length of 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 non-inoculated did not produce any lesion, 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 well as in the experiment 1, followed by the treatment with isolate 3C2 on wounded shoots, and the shortest lesion length with the same isolate on unwounded shoots. Interestingly, the isolate 3C7 inoculated on unwounded shoots did not produce any lesion this time and the incidence of infection was 0%. In the experiment 2, the isolates induced smaller lesions than those in experiment 1 (Fig. 3). Arthrospores were abundant under the bark on these shoots as well as pycnidia with mature pycnidiospores.

Pathogenicity test in the field

Pathogenicity tests in the field conducted on 2-years-old shoots with *N. dimidiatum* (2D03) and an isolate of *Phomopsis* sp. revealed significant differences between the two species. *N. dimidiatum* was able to induce lesions of 24.8 mm (no sunburn), and 19 mm (sunburn), but *Phomopsis* sp. did not induce any lesions (11.9 mm), since it was not significant greater than the discoloration resulted by the control (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, perhaps the heat stress was not sufficient to show any effect (Table 2). However, because we observed that cankers were frequently 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 two seasonal treatments. Length of cankers from summer pruning was significantly different compared to the lesion development after winter pruning. Lesions were measured twice, in two different times, and in both cases

significant differences were confirmed. In both evaluations the average of summer lesions length was 28.5 cm compared to the winter treatment, 9 cm. 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 in order to see if environmental and/or agronomic stresses could affect the infection establishment and development. Results demonstrated that limbs wounded with mallets and also sunburned had the largest cankers among all the treatments. Intermediate length of cankers was recorded for limbs wounded only 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 non-wounded but inoculated control (Fig. 4).

Susceptibility of fig cultivars to the infection

The first symptom evaluation conducted two months after the inoculation of the six fig cultivars revealed two statistically different groups, showing the cvs. Sierra, Kadota and Black Mission being more susceptible than the cvs. Brown Turkey, Conadria and Calimyrna. The second evaluation, performed one year and a half later, confirmed the results of the first evaluation, with the exception of the 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).

Cankers eradication and pathogen recovery

Results showed that pruning the shoots 5.08 cm below the obvious external margins of cankers effectively contained the pathogen movement within the shoots. Isolations from the cut surface of pruned shoots from trees in both experimental orchards at KARE 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 the epidemiology of the fig limb dieback in California. Results from our surveys conducted in the main fig production counties in California reveal the two most common species isolated from cankers of symptomatic branches and shoots were *N. dimidiatum* and *Phomopsis* spp. *Phomopsis* was reported in other Countries as an important fig canker pathogen. In Iran, its association with fig canker is known for over 25 years (Banihashemi, unpublished data). In California, *P. cinerascens* was reported in 1936 as an epidemic pathogen on the cultivar Kadota (Ferguson et al., 1990). Phomopsis canker can be found in all of the commercial figs cultivars but it is most devastating in the cv. Kadota (Obenauf, et. al., 1978). However, pathogenicity tests conducted in this study using both *N. dimidiatum* and *Phomopsis* spp revealed that *N. dimidiatum* was able to induce lesions as opposed to *Phomopsis*. Probably, the cv. Kadota was very susceptible to *Phomopsis* infection during those years, and its progressive displacement with other cultivars, perhaps less susceptible, led to a decrease of the incidence of this pathogen over the years. At the time 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 three years surveys of other fig cultivars showed high levels of both *N. dimidiatum* and *Phomopsis* spp. only in the first year, an indication that both these pathogens could co-occur in the same canker tissues. However,

in surveys of years 2 and 3, and with the exception of one Black Mission orchard where all the samples produced *Phomopsis* spp., *Phomopsis* spp. were not isolated or 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 very high in most of the samples during the 3 years survey. However, recent isolations showed the presence of *Phomopsis* spp associated with symptomatic samples, although at low percentage compared to *Neoscytalidium* frequency. Therefore, we can affirm that *Phomopsis* spp is in somehow associated to the disease, but further investigations are needed to confirm the interactions between these two species, and the disease development.

N. dimidiatum was reported worldwide causing diseases on many other important crops (Dervis et al., 2019; Polizzi et al., 2009; Rolshausen et al., 2013; ; Türkölmez et al., 2019), and in the study of Marques et al. (2013) was considered the most aggressive among other 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 for an easy flow of inoculum (arthrospores) among the different tree species (Moral et al., 2019). Our results of optimum growth temperature are in accordance with those of Nouri et al. (2018) showing the optimum temperature of 31.5°C. In this study we showed a significant difference of lesion length in trees pruned in summer in comparisons with 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 the summer pruning where more severe infections and more abundant inoculum levels can occur.These results demonstrate that summer temperatures are an important factor for the infection development by *N. dimidiatum*, as stated by Hassan et al. (2011) and Sadowsky et al. (2007) . Both these reports in fact, showed the effect of heat stress treatments as a predisposing factor to infection by *N. dimidiatum*. In Israel, severe

symptoms on grapefruits appeared after extremely hot and dry weather events for several consecutive days (Oren et al., 2001). In Oman the infection on *Albizia lebbeck* occurred during the summer of 1998 when the temperature average was 40°C (Elshafie and Ba-Omar, 2002), as well as observed also in Iraq on different hosts (Hassan et al., 2009). Our results are in accordance 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, the recent study conducted in California on almond showed that *N. dimidiatum* isolates were able to infect almonds independently of the month of inoculations, 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 the tissues through the wounds and openings, especially when the host is stressed. Schoeneweiss (1975) suggested that stress plays a key role, exerting the most pronounced effect predisposing plants to facultative parasites, especially weak or non-aggressive pathogens. In this study, experiments conducted with different stress treatments demonstrated that the combination of mechanical injuries (mallet) and sunburn led to the highest canker lengths, followed by lesions derived from inoculations on wounded shoots (mallet), or only sunburned. In this experiment, as well as in the pathogenicity tests on 4-years-old detached shoots, wounds seemed to be necessary to initiate the infection process. However, in pathogenicity test 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 pre-existing wound. Similarly, Fullerton et al. (2018) on a study conducted on a dragon fruit canker disease caused by *N. dimidiatum*, concluded that the tissues most susceptible to infection were the tips of rapidly growing cladodes. Old mature cladodes were found highly resistant to infection. In 1972 Davison showed that *H. toruloidea* can infect an unwounded tree at the ideal temperature of 25°C, but wounding resulted in the higher disease incidence, reasons supporting our study that firmly suggests that injuries like sunburn, pruning wounds, or other mechanical injuries (i.e. mallet) are crucial in the infection

process, as it 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 at least 3 weeks. The same experiment revealed that shoots treated with Whitewash and inoculated did not produce any lesion, thus protected the shoots from infection by the pathogen. Treatments with Whitewash seem to be effective both in protecting trees from sunburn, and thus 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. requiring pollination to keep the fruit) are less susceptible than the cvs. Kadota and Sierra, which were more susceptible with the cv. Black Mission showing moderately susceptibility. Interestingly, these last three cvs. are persistent (i.e. they do not require pollination for fruit to develop). At the end of all experiments, it was ascertained that the prunings carried out 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 in 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 in understanding this destructive emerging pathogen in California fig orchards, and further research should aim at developing additional efficient control strategies.

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485 **Table 1.** Incidence (%) of fungal species emerged from three years surveys in Fresno, Madera and Kern Counties

486

Cultivar	Month	Incidence %			Year
		N. dimidiatum	*Phomopsis* sp.	*Botryosphaeriaceae* 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[a]	May	90	0		2
Black Mission[b]	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[c]	0		3
Black Mission	November	100[d]	0		3

487 **a** Isolations from infected lenticels or growth cracks; **b** isolations from tunnels of boring insects; **c** *N. dimidiatum* was isolated from 68% of the dark brown staining of the woody tissues in advance of
488 the cankers; **d** *N. dimidtiatum* was also isolated from 15% of symptomless shoots

489 **Table 2.** Pathogenicity test in the field.

Treatment	Species	Canker length (mm)
Sunburn	*N.dimidiatum*	19.0 a*
Non-sunburn	*N.dimidiatum*	24.8 a
	Phomopsis sp.	11.9 b
	Control	10.9 b

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

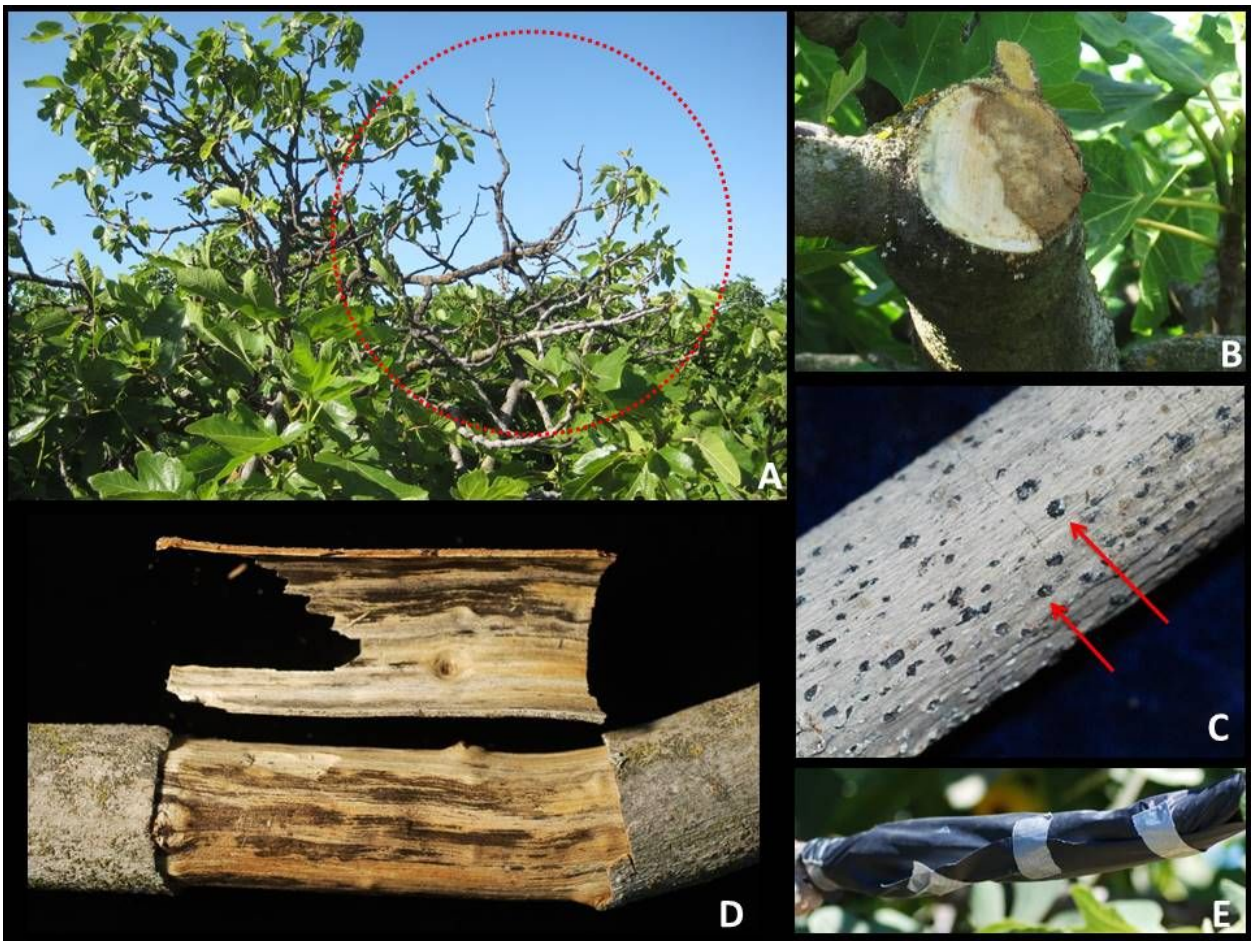

Figure 1. Symptoms and signs of *Neoscytalidium dimidiatum* on fig. **A**, Limb dieback of cv. Black Mission in the field. **B**, internal wood discoloration of cv. Black Mission. **C**, pycnidia observed on fig shoot. **D**, arthrospores developed under the bark. **E**, shoot wrapped with black plastic to create the sunburn treatment

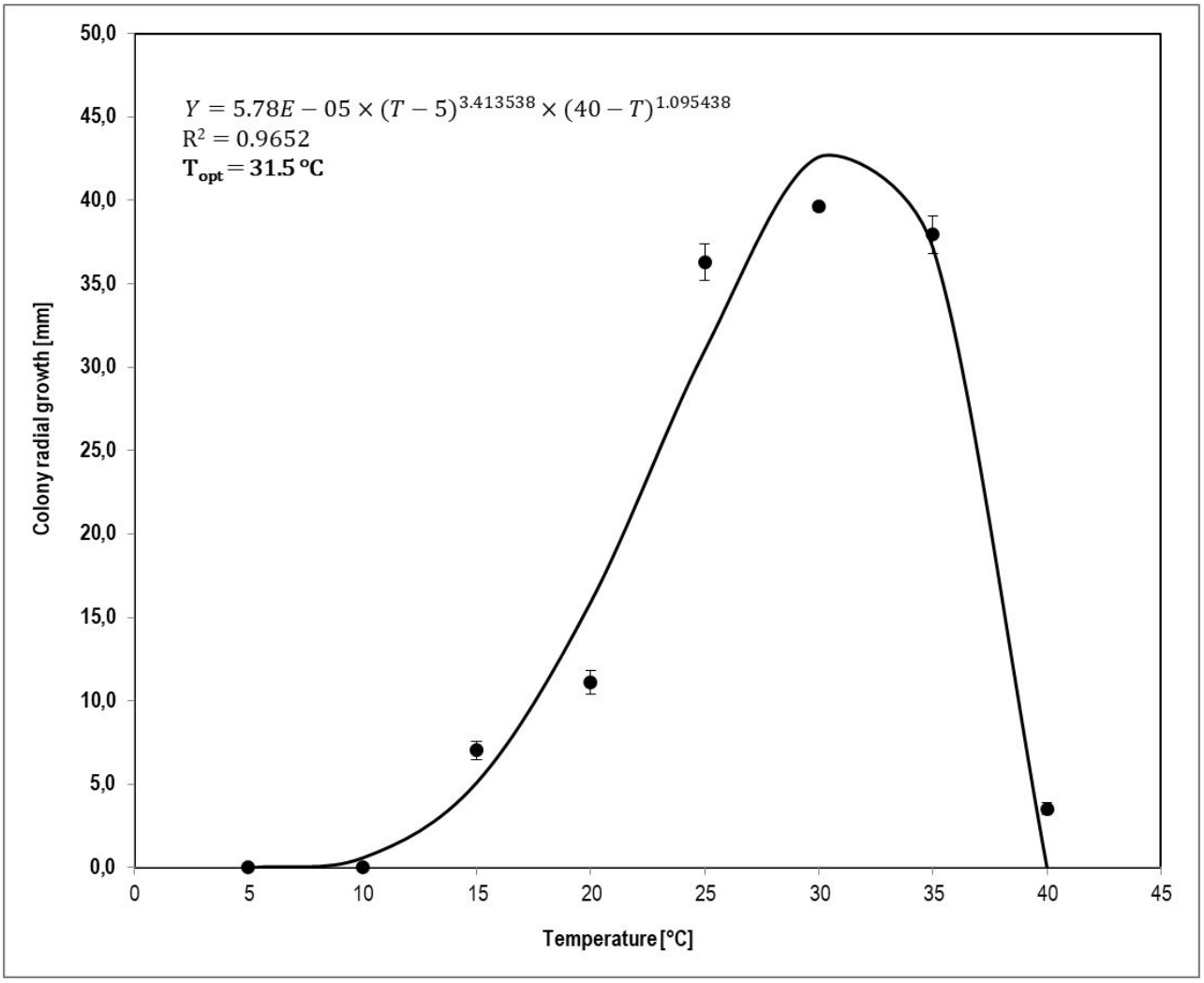

Figure 2. Effect of temperature on mycelial growth (mm) of the isolate D3 of *Neoscytalidium dimidiatum*. The average of radial growth over temperature were adjusted to a nonlinear regression curve using the Analytis Beta model. "Y" represents the standardized radial growth. Data points are the means and vertical bars are the standard error of the means.

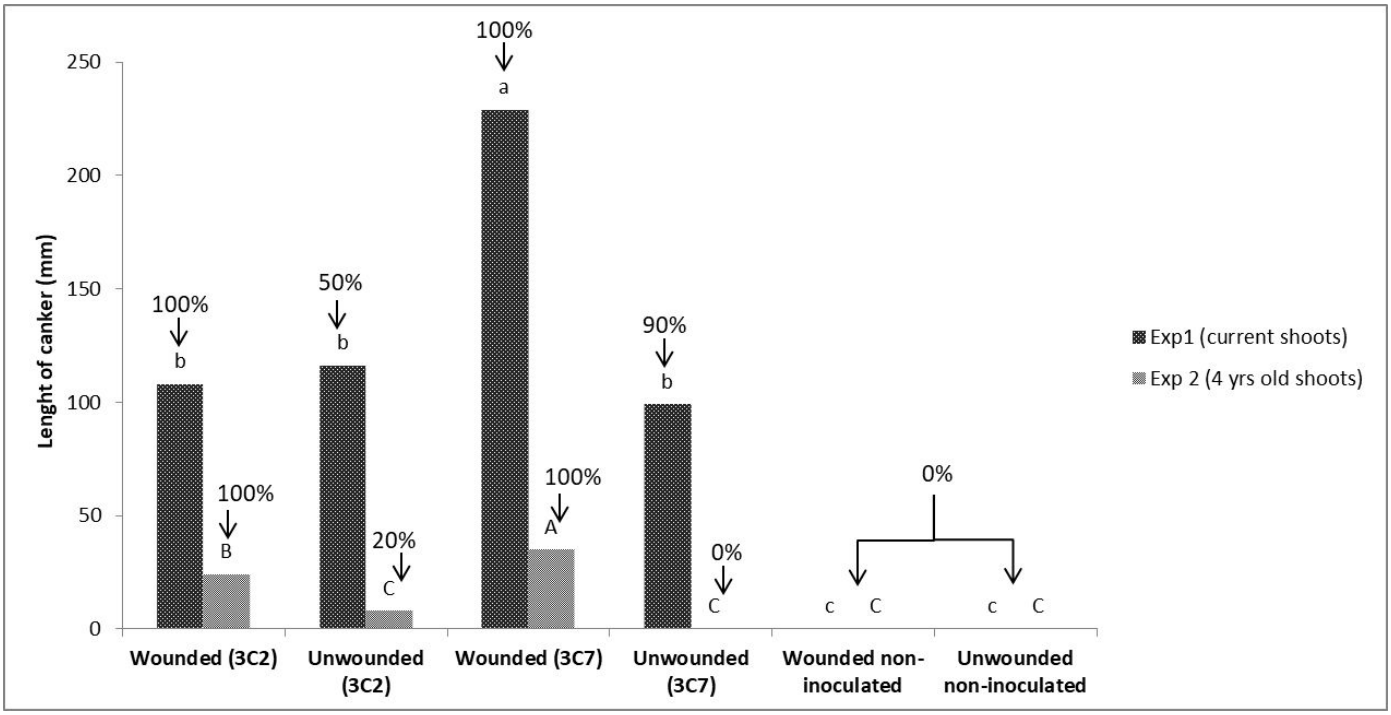

Figure 3. Pathogenicity of *Neoscytalidium dimidiatum* on detached fig shoots. Average lesion length (mm) resulting from inoculation with a mycelium plug of *N. dimidiatum* onto current and 4-years-old shoots. Percentages above the columns indicate the incidence of infection. Letters above the columns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

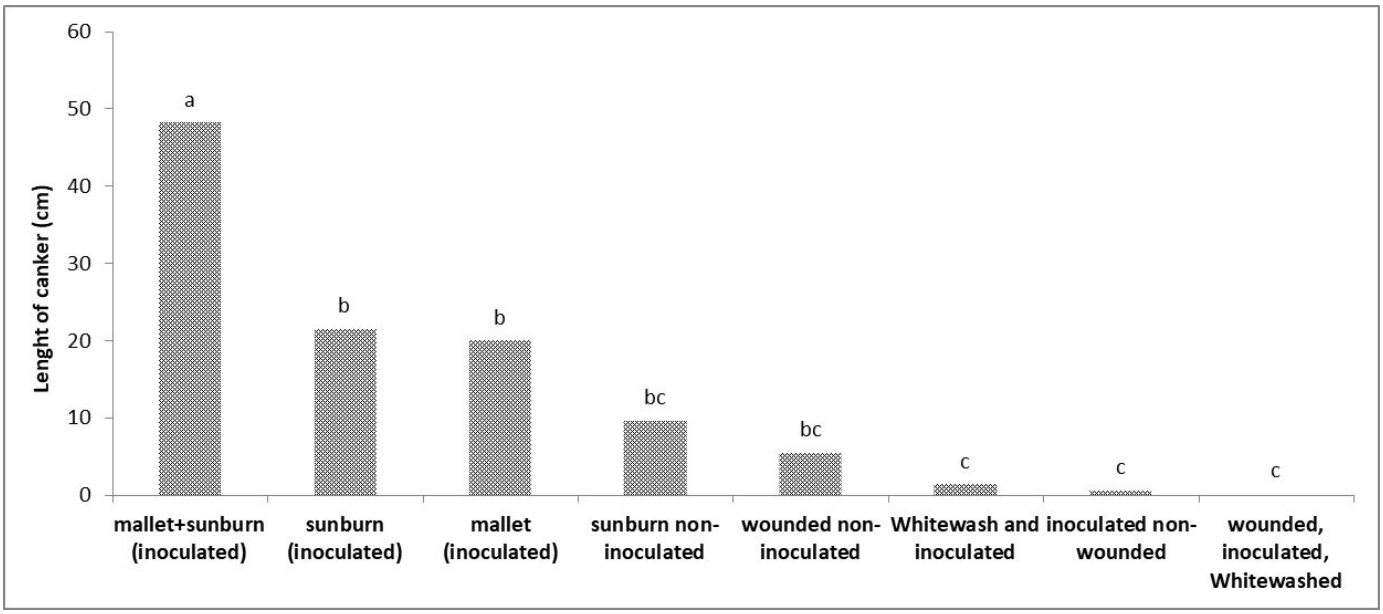

Figure 4. Effects of different stress factors on lesion length (in cm) after inoculation of fig shoots with *Neoscytalidium dimidiatum*. Different letters above columns indicate significantly different treatments ($P < 0.05$).

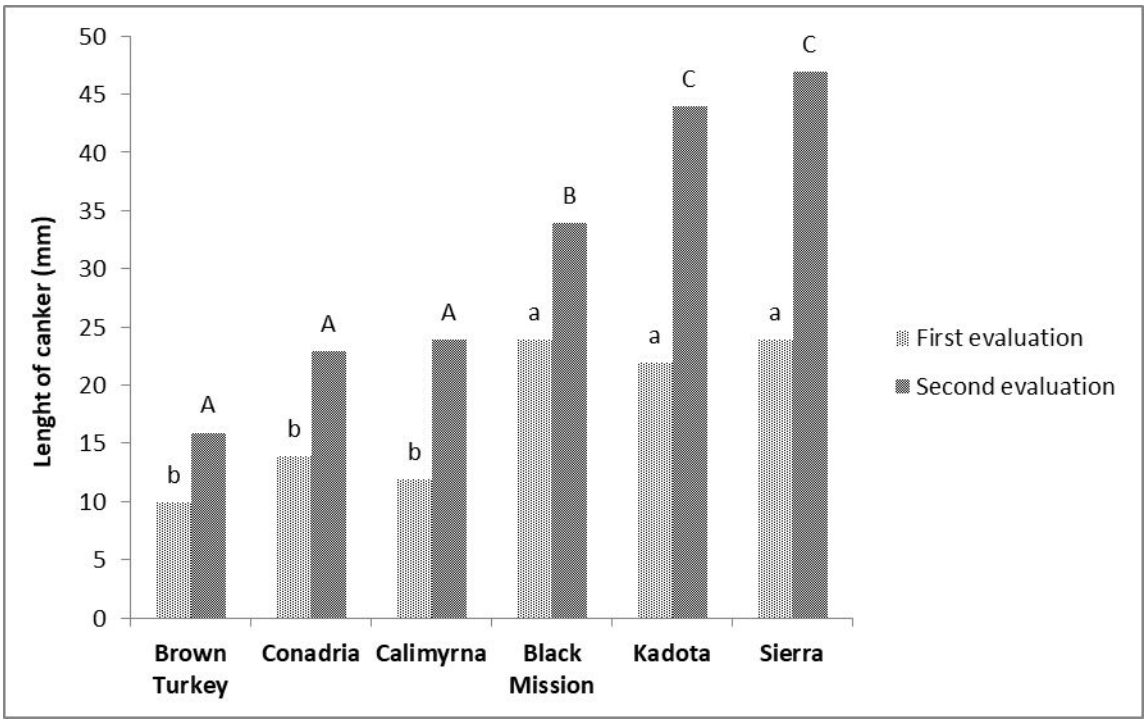

Figure 5. Cultivar susceptibility (measured in mm lesion length) to limb dieback disease caused by *Neoscytalidium dimidiatum*. Different letters above columns indicate significantly different treatments ($P < 0.05$). Letters refer to specific (first and second) disease evaluation.