Research Project Progress Report

Understanding citrus branch canker and dieback in the Southern California desert regions (not just Hendersonula)

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

Introduction

Growers in the desert regions of Southern California are concerned about a disease of grapefruit and lemon. This disease is characterized by branch cankers and dieback, bark cracking/splitting, gumming, and the presence of black fungal spores underneath the bark.

The causal agent was found to be the fungus *Neoscytalidium dimidiatum* (known as (*Hendersonula toruloidea*). Simply known as Hendersonula, this disease remains a problem for citrus growers in the desert regions.

During a previous statewide survey, samples collected from lemon and grapefruit trees in the desert regions of Riverside and San Diego counties yielded fungi not previously found in other counties sampled. These fungi were identified as species of *Eutypella*, which belong to the family Diatrypaceae.

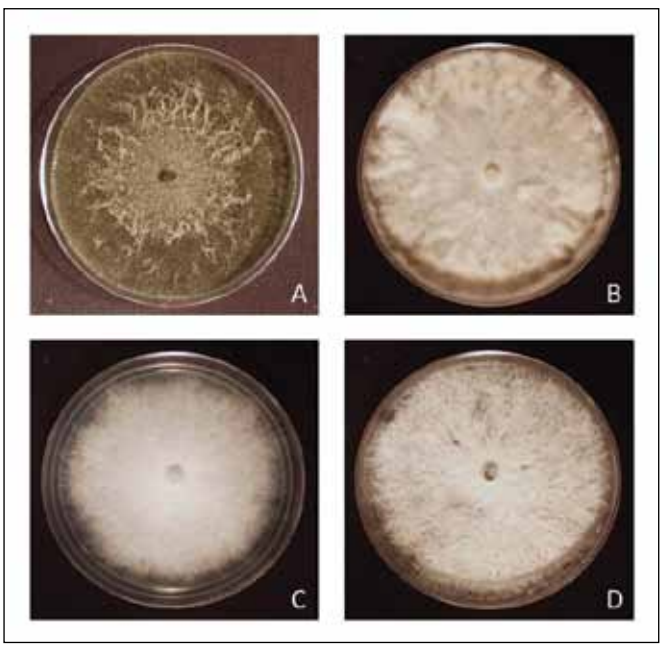

Figure 1. 10-day-old cultures on Potato Dextrose Agar of (A) *Neoscytalidium dimidiatum*, (B) *Eutypella microtheca*, (C) *Eutypella citricola*, (D) *Eutypella* sp.

Interestingly, several members of this fungal family are associated with grapevine decline in California, and in the Coachella Valley species of Eutypella are the predominant fungal species associated with grapevine cankers.

Although *N. dimidiatum* has been known as a pathogen of citrus in California since the 1950s, the association of *Eutypella* with citrus branch canker/dieback has been unknown. This leads to the question, do these *Eutypella* species play a role in causing citrus branch canker/dieback or do they exist in citrus as saprophytes?

The objectives of this study are to: (1) determine the fungi associated with citrus branch canker/dieback in the southern California desert regions, (2) assess the pathogenicity and aggressiveness of these fungi, and (3) develop management strategies for this disease.

How the study was conducted

Citrus groves throughout Riverside, Imperial and San Diego counties were surveyed for symptomatic trees showing signs of branch canker and dieback. Sampling was focused in the Coachella and Imperial valleys as well as the Borrego Springs region.

Symptomatic branch samples were collected from November 2011 to December 2012. Samples were taken from both lemon and grapefruit trees in commercial citrus groves but were also taken from other woody hosts that surrounded these citrus groves.

Isolations were made from symptomatic tissues, and the resulting fungi were identified morphologically. Those isolates belonging to the families Botryosphaeriaceae and Diatrypaceae based on colony and conidial (asexual, non-mobile spore) morphology were further characterized using molecular methods.

To identify fungi belonging to the fungal family, Botryosphaeriaceae, two gene loci, internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) and a portion of the translation elongation factor 1-α (EF1-α) gene, were

used for molecular phylogenetics to confirm the identities of these fungi.

Similarly for fungi belonging to the Diatrypaceae, the ITS region and a portion of the β-tubulin gene were used in the phylogenetic analysis.

To determine the pathogenicity of selected fungi, detached green shoots of 'Allen Eureka' lemon were stem-wound inoculated by removing a piece of cambium with a cork-borer and then placing agar plugs infested with one isolate of representative fungi on the wound. Control shoots were inoculated with uninfested agar plugs.

Shoots were incubated at 25°C under humid conditions for two weeks. Resulting lesions were measured and isolations were made from these shoots to confirm pathogenicity.

Results and future outlook

Surveys conducted throughout the southern California desert regions revealed the predominate fungi associated with citrus branch canker and dieback are a *Neoscytalidium* species as well as three distinct *Eutypella* species based on morphology (Figure 1). These fungi have been detected in all three previously mentioned counties and can be found associated with both lemon and grapefruit trees.

Symptoms frequently observed included cankers -- which ranged in color from gray, chocolate brown to black -- splitting of the bark often accompanied by gum exudation, sloughing off of bark revealing a layer of black fungal spores underneath, and progressive dieback (Figure 2).

Samples collected from other woody hosts surrounding citrus groves, for example *Tamarix*, also show an association of *Eutypella* spp. with symptoms of branch canker and dieback; however, it is unknown if *Eutypella* is the cause of dieback on *Tamarix*. The *Neoscytalidium* sp. is the most common canker fungus isolated from symptomatic tissues.

Throughout this survey, numerous perithecia of *Eutypella* were observed on dead branches. These fungi were found associated with all varieties of citrus that have been sampled and all ages of trees are presumed to be susceptible to infection by these fungal species (Table 1).

Phylogenetic(s) – refers simply to the relationship among organisms inferred through an observable characteristic. In this study, DNA sequences of unknown fungi were compared to those of known fungi in order to determine fungal identity.

Morphological – refers to the physical characteristics (color, shape, size, etc.) of an organism.

Perithecia – flask shaped structure that contains sexual fungal spores (ascospores).

Phylogenetic analysis supports our morphological identification and confirms that the species *Neoscytalidium dimidiatum* causes the disease known as Hendersonula. These analyses also confirm two of the *Eutypella* spp. as *E. cit-*

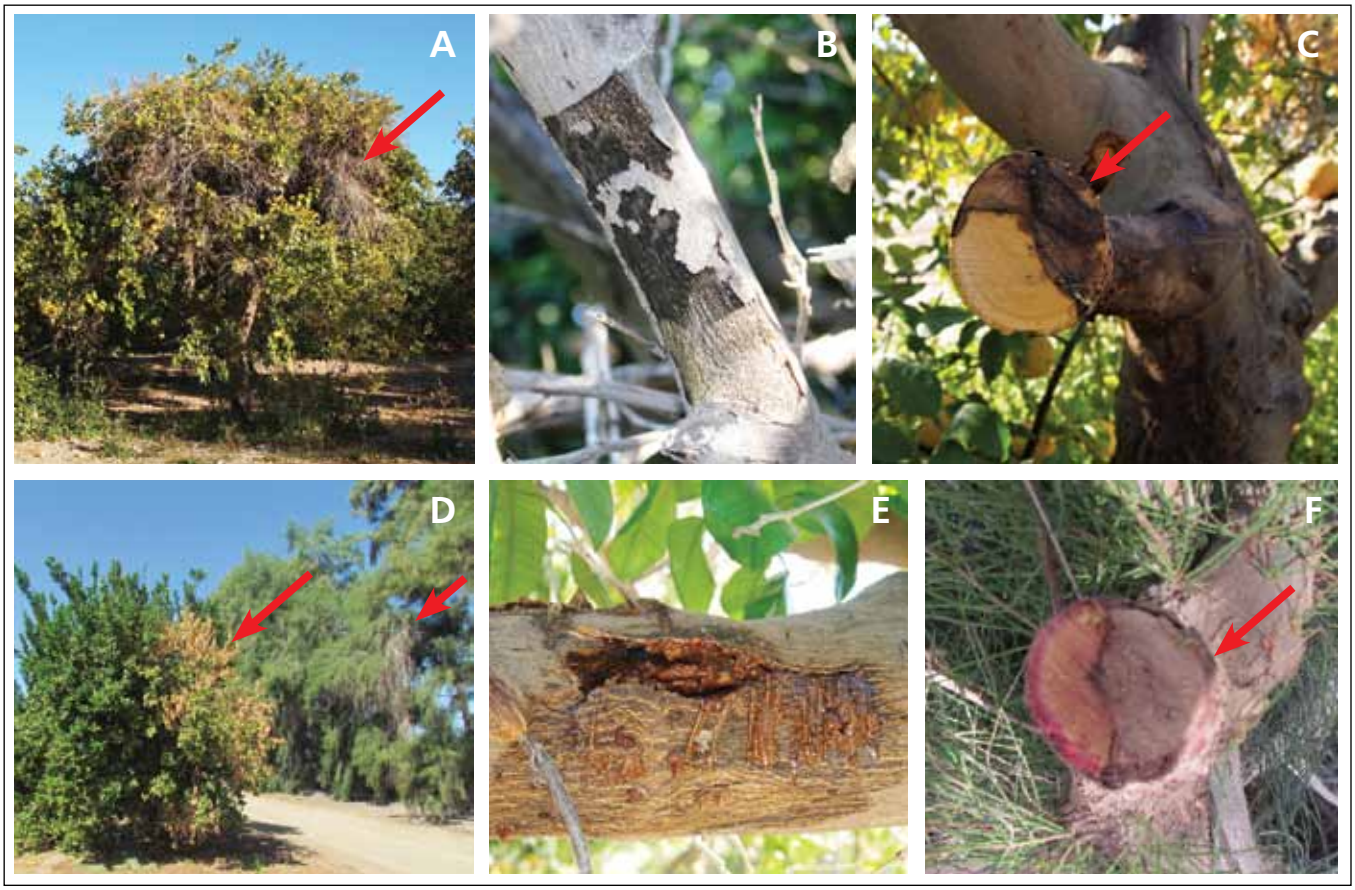

Figure 2. Photographs of various symptoms of citrus branch canker and dieback: (A) citrus tree in a commercial grove showing signs of dieback (red arrow); (B) branch of lemon showing bark peeling. Underneath the bark, fungal spores of *Neoscytalidium* can be seen as a black powdery mass; (C) cross section of a branch showing the brown necrotic canker (red arrow); (D) lemon tree and *Tamarix* (on the right) showing signs of dieback (red arrows); (E) branch showing bark splitting and exudation of gum; (F) cross section of cankered branch of *Tamarix* from photo D. Red arrow points to canker.

ricola and *E. microtheca*. The third *Eutypella* species remains unidentified at this time, but phylogenetic analysis shows it is closely related to *Peroneutypa scoparia*, formerly *Eutypella scoparia*. Future work will be aimed at determining the identity of this *Eutypella* species.

Based on the results of the detached shoot assay (Figure 3), *E. microtheca* and *Eutypella* sp. are pathogenic on citrus as both were able to produce lesions (~10mm) and could be isolated from inoculated shoots; however, *E. citricola* failed to produce lesions and could not be recovered from inoculated shoots. *N. dimidiatum* produced the largest lesions (~60mm) and was included in this experiment for comparative purposes even though it was reported as a pathogen of citrus in previous studies.

Statistical analysis was conducted to compare mean lesion lengths, and these results suggest that *Eutypella* spp. poses a low to moderate virulence in comparison to *N. dimidiatum*, as lesions caused by *Eutypella* spp. were not statistically different from the negative/healthy control.

Table 1: Representative *Neoscytalidium* and *Eutypella* isolates collected from this study

Species	Strain	County	Host	Variety
Neoscytalidium dimidiatum	DC08	Imperial	*Citrus paradisi*	Rio Red
N. dimidiatum	DC29	Imperial	*Citrus paradisi*	Rio Red
N. dimidiatum	DC132	Riverside	*Citrus limon*	Allen Eureka
N. dimidiatum	DC173	Imperial	*Citrus limon*	Lisbon
N. dimidiatum	DC176	Imperial	*Citrus paradisi*	Rio Red
Eutypella citricola	DC83	Riverside	*Citrus limon*	Allen Eureka
E. citricola	DC91	Imperial	*Citrus paradisi*	Rio Red
E. citricola	DC113	Imperial	*Citrus paradisi*	Rio Red
E. citricola	DC117	Imperial	*Citrus paradisi*	Rio Red
E. citricola	DC186	Imperial	*Citrus paradisi*	Ruby
E. citricola	DC272	San Diego	*Citrus limon*	Lisbon
E. citricola	DC291	San Diego	*Tamarix* sp.	–
E. citricola	DC293	San Diego	*Citrus limon*	Lisbon
Eutypella microtheca	DC09	Imperial	*Citrus paradisi*	Rio Red
E. microtheca	DC37	Imperial	*Citrus paradisi*	Rio Red
E. microtheca	DC148	Imperial	*Citrus limon*	Lisbon
Eutypella sp.	DC210	Imperial	*Citrus paradisi*	Ruby
Eutypella sp.	DC211	Imperial	*Citrus paradisi*	Ruby
Eutypella sp.	DC276	San Diego	*Citrus limon*	Lisbon
Eutypella sp.	DC287	San Diego	*Tamarix* sp.	–

It is necessary, however, to further explore the role of *Eutypella* spp. as pathogens of citrus and grapevine, particularly in regions where these two commodities are grown in close proximity.

For example, the predominance of *Eutypella* in the Coachella Valley region likely suggests an adaptation to the climate of these regions which experience wide fluctuations in temperature.

Results from a growth rate assay of fungi in different temperatures reveal optimal growth temperatures for both *N. dimidiatum* and *Eutypella* spp. to be between 30°C and 35°C (Figure 4). Studies are underway to explore the effect of temperature on disease expression and development.

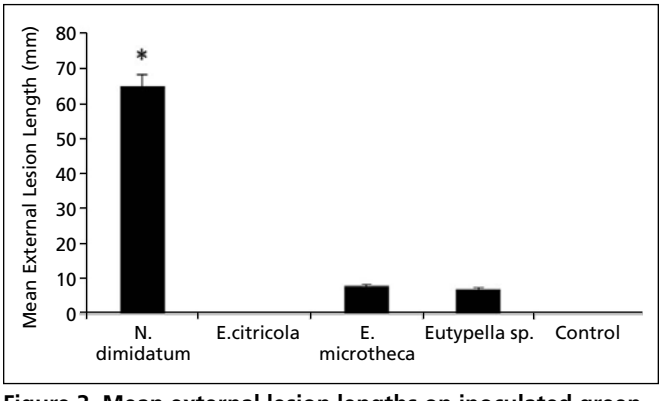

Figure 3. Mean external lesion lengths on inoculated green shoots of 'Allen Eureka' lemon. Asterisk denotes means that are significantly different at p=0.05.

Additionally, studies are being planned to investigate the interaction between *N. dimidiatum* and *Eutypella* spp. In a few cases, both *N. dimidiatum* and *Eutypella* spp. could be isolated from the same diseased branch sample, raising questions regarding the interaction of these two fungi.

The nature of this interaction is unknown, but of particular interest is whether or not this interaction has any effect on disease development. If a degree of antagonism exists between *Neoscytalidium* and *Eutypella*, then it is possible that colonization of the plant by one fungus could preclude or at least limit later colonization of the plant by the other fungus. Conversely, these two fungi could act together to produce a greater degree of disease than either one individually.

These greenhouse and field studies will aid in better understanding the role of these fungi in the overall dieback of citrus in this region.

Management of this disease is currently under investigation, but growers are advised to maintain good cultural practices to ensure proper grove hygiene. These practices include: avoiding excessive pruning/mechanical damage, as open wounds serve as infection sites for these pathogens; removing diseased branches and properly disposing of them; and, disinfecting contaminated tools so as to reduce the risk of spreading the pathogen.

In cases where branch canker and dieback are severe, growers may benefit from the use of chemical applications. Efforts are being made to screen commercial fungicides currently on the market that may be efficacious in the control of these pathogens. Initial results from an *in vitro* fungicide

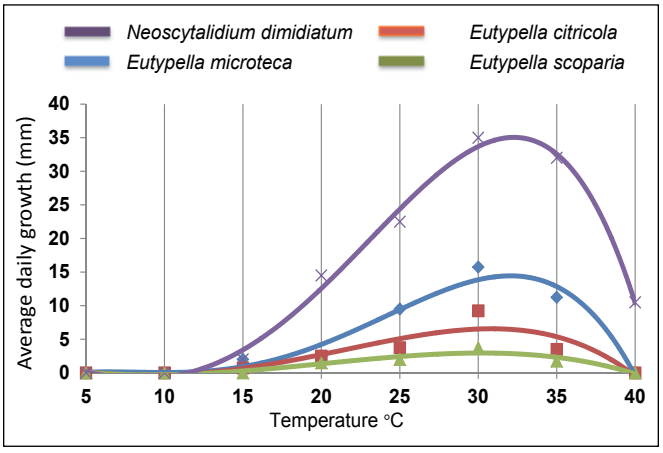

Figure 4. The growth rate of fungi in different temperatures.

screen show that a number of commercial products currently registered on citrus are capable of reducing fungal growth, some of which are highly effective at very low concentrations.

Field studies will be necessary to evaluate fungicide efficacy and appropriate application methods. These results will be made available to growers through the UC IPM online website (http://www.ipm.ucdavis.edu).

Taken together, these results underscore the need for further investigation into this unique disease complex faced by desert growers. Conclusion of these studies will provide the industry with the appropriate management strategies to maintain lucrative production in this area.

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Additional reading

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