DIAGNOSIS, ETIOLOGY, EPIDEMIOLOGY, AND MANAGEMENT OF CANKER DISEASES IN DRIED PLUMS

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OBJECTIVE

1. The overall objective of this research is to determine what are the causes causing canker diseases in dried plum trees and how trees get infected by canker fungi.
2. To develop management tools to prevent or reduce such infections and manage canker diseases.

INTRODUCTION

In the last several years, wood-cankers and branch killing of prune and other *Prunus* spp. have become of a major concern to growers in California. We continued helping farm advisors, pest control advisers, and also growers by diagnosing diseased samples. Over the years, we received samples from Tulare, Madera, Fresno, Sutter, Yuba, Butte, Colusa, Glenn, and Tehama Counties. We have isolated from these samples *Cytospora leucostoma, Lasiodiplodia citricola, Nattrassia mangiferae* (*Hendersonula toruloidea*), *Botryosphaeria dothidea,* other *Botryosphaeria* and *Phomopsis* species, *Diplodia seriata, Paecilomyces variotii,* and *Fusarium* species. Furthermore, from trees with heart rot we isolated *Schizophyllum commune* frequently, and two other basidiomycetes that may also play a role in heart rot attacking prunes. Among the fungi isolated from cankers some, such as *Cytospora, Lasiodiplodia, Botryosphaeria, Diplodia, Nattrassia, and Phomopsis*) are known canker pathogens, while others (such as *Paecilomyces, Fusarium,* and a couple of unknown fungi) are considered as putative pathogens of prunes that can cause cankers. All these fungi need to be investigated for their relative virulence on prunes and ability to cause cankers similar to those observed in the field.

Although *Cytospora* and *Schizophyllum* (together or on their own) were the most frequently isolated pathogens from samples, in some orchards, other fungi were also isolated, such as *Fusarium* species, *Paecilomyces variotii,* a basidiomycete and one unknown fungal species. Therefore, based on a large number of isolates from various samples, we will need eventually determine their virulence on potted prune trees. No previous, systematic work has been done to determine if pruning wounds themselves are susceptible to infection or if there are other means of infection in addition to bark damage

by the tree shaker, sunburn, freeze, and presence and predisposition by bacterial canker. In fact, some of the samples from the field show that infections may have been initiated from pruning wounds since the center of the canker is located in the area of pruning wound. Other times, branches which exhibit distinct signs of bacterial canker, they also have signs of *Cytospora* infection (characteristic pycnidia and mycelia among the decayed bark and staining in the wood). Furthermore, samples from some orchards in 2014 showed very extensive heart rot throughout the trees (trunks, scaffold, and branches) that one wonders whether these wood decay fungi started very early in the life cycle of the trees. It is not known how these fungi infect prunes, and our future studies will determine what the infection courts are, how infections occur, and how we can prevent infection.

No surveys have been conducted throughout various counties where prunes are grown to determine and characterize the extent of the wood canker problem in prunes. However, testimonials of growers have suggested that canker diseases and their damage on trees have become more severe in the last decade or so all the time. Furthermore to make things worse, and with the exception of information on *Cytospora canker*, there is not much recent research and knowledge about the etiology and epidemiology of canker diseases in prunes. Most importantly, the critical period of time and conditions in the field favoring infection are still for the most part unknown. We still do not know how these diseases initiate in a young orchard and develop within the plant tissues after infection, and what critical conditions affect the disease development. Although *Cytospora* is spread by water-splashed spores (conidia) there may be other canker pathogens that may produce both water-splashed and airborne conidia, thus complicating the spread of these fungi in the orchard. Understanding the biology of canker fungi and the epidemiology of the diseases they cause are essential for the development of effective disease management approaches.

The increase in nut crop acreage may have brought new pathogens proximal to prune orchards. For instance, species of fungi that cause cankers in almond, pistachio, and walnut are also found in cankers of prunes. A sample collected from a severely damaged prune orchard in Tulare County revealed all: *Cytospora* (a prune pathogen), *Nattrassia mangiferae* (a walnut pathogen), and *Botryosphaeria* species (pathogens of almond, pistachio, and walnut). Although this is only a hypothesis, the isolation of new canker pathogens from cankers of prune trees supports the contention that we are now dealing with new canker pathogens that had not been a problem to prunes in past years. We will continue our survey of canker diseases in both the San Joaquin (Tulare/Fresno/ Madera) and Sacramento (Yuba/Colusa, Glenn, Butte counties) Valleys where prunes are growing and record any differences in putative pathogens isolated from prune cankers.

In contrast to leaf and fruit diseases, which in high levels can cause an epidemic only during the growing season, canker diseases demonstrate more complexity and their

management is very difficult. The critical period and environmental conditions during the season that favor infection are still unclear. Development of infection by canker-causing fungal pathogens in the woody tissues of prune trees could last as long as one year or longer before symptom appearance with various disease development features. Such complicated features in the incubation and reproduction phases of disease require systematic and periodic observations throughout several years to acquire important knowledge that will help us understand the development of latent infections (phase of the disease before the expression of any symptoms) and subsequent canker expansion and production of sporulation structures. Similarly, disease management strategies also require systematic investigation on efficacy of fungicides and application methods over the seasons. Evaluation of disease management for this type of disease is complex and requires a longer time to obtain useful information than management methods of foliar and/or fruit diseases. Some of these more complicated "efficacy trials" have been initiated in 2014 in both in a commercial orchard and in our experimental orchard of French prune at Kearney.

PROCEDURES

Diagnosis of prune samples and systematic survey of prune orchards in two major geographic areas.

We handled 3 types of prune samples for diagnosis of canker diseases in 2014: a) Samples sent by farm advisors, pest control advisers, and growers. Results of isolated fungi and their frequency are presented in Table 1. b) Samples collected by our crew after visiting problematic orchards. Usually, samples are collected in individual plastic bags and when the weather is hot samples are placed in an ice chest and transported to the laboratory. And c) Samples brought to Kearney Agric. Center directly by a FA, PCA, or grower. All samples are recorded so that when it is necessary to examine historical data on prune diseases will be easy to do. Observations and isolations are made either on the same day or 1-2 days after getting a sample. For isolations, we mainly use acidified potato-dextrose agar for putative fungal pathogens and regular PDA or Kings medium B for bacterial isolations (*Pseudomonas syringae,* etc.). At least four unique situations and the putative canker fungi are presented from commercial orchards along with the incidence of isolation (Table 2-5).

Because of the drought no problematic orchards were pointed to us in counties of the San Joaquin Valley in 2014, thus the majority of diseased samples for diagnosis were collected from orchards with canker problems from counties in the Sacramento Valley.

Epidemiology and development of management tools to prevent or reduce infections by canker fungi.

Effect of temperature on growth of* Cytospora leucostoma *isolates. Because symptoms of cankers caused by *Cytospora* become worst during the summer, we wanted to determine what is the optimum temperature of this pathogen. For this purpose, three isolates were selected and plated on acidified PDA plates and the plates were incubated in incubators set at 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, and 40°C (corresponding to 41, 50, 59, 68, 77, 85, 95, and 104 F). Radial growth of mycelia of each colony for each isolate were determined after 7 days. Results are presented in Figure 1.

Effect of pruning wound age on infection by Cytospora leucostoma, Lasiodiplodia citricola, and Paecilomyces variotii. To determine how long pruning wounds remain susceptible to infection by canker-producing fungal pathogens, three experiments were set, one each for *Cytospora leucostoma, Lasiodiplodia citricola,* and *Paecilomyces variotii* at the experimental prune orchard at Kearney Agric. Center. Sixty shoots were pruned in February 2014, and 10 each were inoculated 0, 3, 7, 15, and 30 days after pruning by spraying a 50,000 spores/ml suspension of each of the above fungi onto the pruning wound and covering the inoculated surface with a sandwich plastic bag until the next morning when the bag was removed. Ten shoots pruned in the same way but not inoculated served as controls. Infection and length of canker will be determined 1 year after inoculation in February 2015.

Effect of fungicides in protecting pruning wounds from infection. A literature review of recent reports on controlling canker diseases indicated that a) fungicides of the dicarboxamide class performed quite well in preventing infection; b) the benzimidazoles did well, but probably they will be difficult to get them registered; c) the myclobutanil (the active ingredient of Rally fungicide) had little to no effect; and d) strobilurin and dicarboxamide mixtures showed the most efficacy against canker diseases. Based on this information, we chose the following treatments for the fungicide efficacy trial in the field. Because of the drought, we had to wait to time these experiments just before a rain event so that conditions for spore spread were maximized. The experiment was set in a commercial orchard that had enough replant trees for these experiments. To determine the risk of Cytospora canker in this orchard, a random sample was collected of shoots with infected pruning cuts which it was shown that 75% of them were infected by *Cytospora.* One trial was set up on 6 November and a second on 12 November 2014, just before a rain storm in the afternoon of 12 and during the 13th November 2014. Ten pruned shoots were used per fungicide treatment and 10 shoots were not treated and served as controls. Evaluation of disease will be done 3, 6, and 12 months after treatment.

RESULTS AND DISCUSSION

Diagnosis of prune samples and systematic survey of prune orchards in two major geographic areas.

Overall, the following fungi were isolated from various samples collected and/or sent to the laboratory in 2014. *Cytospora leucostoma, Lasiodiplodia theobromae, Botryosphaeria dothidea, Diplodia seriata, Nattrassia mangifera, Paecilomyces variotii, Phomopsis* sp., *Fusarium* spp., *Schizophyllum commune,* and two unknown fungi (one being a basidiomycete fungus) (Table 1). In comparing the fungi isolated from samples of previous years with those isolated in 2014, it is obvious that *C. leucostoma* was the dominant fungus isolated followed by *Schizophyllum*. Among these, *Cytospora, Lasiodiplodia, Diplodia,* and *Botryopshaeria* species.. The fungi *Cytospora, Botryosphaeria, Eutypa, Lasiodiplodia, Diplodia, Nattrassia,* and *Phomopsis* are known to cause canker diseases on trees and vines. However, *Paecilomyces* and *Fusarium* are not known as canker-pathogens of trees. It has been lately reported that among isolates of *P. variotii* some were able to cause cankers on pistachio trees in Iran (pathogenic isolates) while other isolates were not pathogenic (did not cause any canker symptoms). Furthermore, *Schizophyllum commune* and *Chondrostereum purpureum* (isolated in 2013) are basidiomycetes and decay wood causing heart rot as it was a severe case of an orchard in Tehama County (Table 5). In this orchard, the majority of the trees had both "systemic" heart rot and cankers caused mainly by *Cytospora.* Other examples of isolations from orchards in Tehama and Yuba Counties suggest that *Cytospora* is the main culprit (Tables 2 and 4). *Paecilomyces variotii* and *Fusarium* sp. were also frequently isolated from these cankered shoots. Interestingly, samples of trunks of prune trees with cankers from an orchard in Glenn and Tehama Counties had an unknown yellow-brown fungus isolated at high incidence (60 to 100%) among the plated tissues (Table 3). Other fungi isolated were *Schizophyllum commune* (10-13%), and *Phomopsis, Botryosphaeria, Nattrassia* (each at 3% level), and *Colletotrichum* sp. at 3 to 17% levels. Interestingly, no *Cytospora* was isolated from any of the pruning wounds of these samples in this orchard (Table 3). All the isolates, both those known to cause cankers, the putative canker producers and the unknown fungi were stored in our long storage culture collection for further studies. The first experiment scheduled to be completed in 2015 is to check pathogenicity and relative virulence of each fungus using potted French prune trees. This experiment is essential to help us determine which fungi we should focused on in addition to *Cytospora* and *Schizophyllum*.

Epidemiology and development of management tools to prevent or reduce infections by canker fungi.

Effect of temperature on growth of* Cytospora leucostoma *isolates. Three isolates of *Cytospora* were tested. All three isolates grew at a range of 50 to 95 F temperatures. Two isolates had an optimum temperature for growth at 77 to 85F while the third isolate did not grow well at 85 but its optimum temperature for growth was 77F. None of the isolates grew at 104 F. More isolates will be tested to compare *Cytospora* isolates from the San Joaquin Valley to those from the Sacramento Valley and determine whether *Cytospora* populations from the Central San Joaquin

Valley can do better at higher temperatures than those from the Sacramento Valley. Also it will be epidemiologically important for the development and management of the disease to determine whether isolates from the Central Valley have a shorter reproduction time, an advantageous feature to complete their life cycle under conditions of less rainfall than their counterparts in the Sacramento Valley. Such differences may affect the time span one has to leave the prunings in the orchard or remove them as soon as possible where the *Cytospora* is capable producing spores in short time.

Effect of pruning wound age on infection by Cytospora leucostoma, Lasiodiplodia citricola, and Paecilomyces variotii. For these inoculations, we selected three fungi based on the frequency of isolation throughout the last few years and their growth speed on media in the laboratory. Depending on the results of these inoculations (results will be recorded in February 2015), future inoculation experiments will be adjusted accordingly. One fungus which was not included initially was *Schizophyllum commune*, which was found in 2014 to create major heart rot in prune trees and in combination with Cytospora canker to affect, weaken, and reduce productivity of prune trees in mature orchards.

Effect of fungicides in protecting pruning wounds from infection. The fungicides used in these two trials are listed in Table 7. Because we depended on natural spread of the disease these experiments were delayed until November of 2014. Data on the efficacy of the fungicides and fungicide combinations used will be recorded in the spring and summer 2015.

Table 1. Fungi recovered from prune, peach, and cherry samples with canker diseases collected or submitted to our laboratory.

Year	Prune	Peach	Cherry
2006	*Cytospora leucostoma* *Phytophthora* (roots)	*Cytospora leucostoma* *Armillaria* (roots)	*Cytospora leucostoma*
2008	*Cytospora leucostoma*		*Cytospora leucostoma*
2009	*Cytospora leucostoma* (*Leucostoma sincta*) *Diplodia seriata*	*Lasiodiplodia theobromae*	*Cytospora leucostoma*
2010	*Cytospora leucostoma* *Lasiodiplodia theobromae* *Nattrassia mangiferae* *Paecilomyces variotii, Phoma* species		*Cytospora leucostoma*
2011	*Cytospora leucostoma*		*Cytospora leucostoma*
2012	*Cytospora leucostoma* *Fusarium* species	Bacterial canker	Bacterial canker *Botryosphaeria* sp. *Cytospora, Fusarium, P. variotii*
2013	*Cytospora leucostoma, Paecilomyces variotii, Chondrostereum purpureum, Botryosphaeria* spp., Foamy canker	*Cytospora leucostoma* *Lasiodiplodia citricola*	Blast (*Pseudomonas syringae*) *Cytospora leucostoma*
2014	*Cytospora leucostoma* 28% *Schizophyllum* 28% *Botryosphaeria* + *Phomopsis* 22%	*Cytospora leucostoma* *Lasiodiplodia*	*Paecilomyces variotii* and Flathead borer.

	Fusarium 11%; *Paecilomyces variotii* 11%; *Eutypa lata* 5%; *Nattrassia 5%*	*citricola* *Botryosphaeria* sp. *Phomopsis* sp.	**Apricot:** *Cytospora leucostoma*

(Table 1 continued)

Table 2. Isolations from cankers of prune trees collected from a commercial orchard in Tehama County in 2014.

Tree sample	**Tree part**	**Putative fungal pathogen isolated**
Tree #1	Trunk	*Cytospora leucostoma* + *Paecilomyces variotii*
	Shoot 1	*Cytospora leucostoma* + *P. variotii*
	Shoot 2	*Cytospora leucostoma* + *P. variotii*
	Shoot 3	*P. variotii*
	Shoot 4	*P. variotii*
	Shoot 5	*P. variotii*
Tree #2	Bark shavings	*Cytospora leucostoma*
	Shoot 1	*Cytospora leucostoma* + *P. variotii* + *Schizophyllum* sp.
	Shoot 2	*P. variotii*
	Shoot 3	*Cytospora leucostoma* + *P. variotii*
	Shoot 4	*P. variotii* + *Schizophyllum* sp.
	Shoot 5	*Cytospora leucostoma* + *P. variotii*

Table 3. Isolations from cankers of prune trees collected from a commercial orchard in Glenn and Tehama Counties in 2014.

Tree part	**Putative canker fungi isolated**	**Incidence (%)**	
		Tehama Co.	**Glenn Co.**
Trunk #1	*Schizophyllum commune*	70	10
	Botryosphaeria dothidea	3	3
	Unknown brown fungus	23	60
	Phomopsis	---	3
	Nattrassia mangiferae	---	3
Trunk #2	*Schizophyllum commune*	---	10
	Unknown brown fungus	100	33
	Paecilomyces variotii	---	3
	Colletotrichum sp.	---	3
Trunk #3	Unknown brown fungus	93	100
	Schizophyllum commune	---	3
Trunk #4	Unknown brown fungus	60	70
	Schizophyllum commune	---	13
	Colletotrichum sp.	17	---
	Nattrassia mangiferae	---	3

Table 4. Isolations from cankers in scaffolds, branches, and pruning wounds of French prunes

from a commercial orchards in Tehama and Yuba Counties in 2014.

Plant part	Putative fungal pathogen isolated	Incidence of isolation (%)
Scaffold (6" diam.) (Tehama Co.)	*P. variotii* *Fusarium* species	85
		35
Scaffold (5"' diam.) (Yuba Co.)	*Cytospora leucostoma* *Phomopsis* sp. *Fusarium* sp.	75 25 5
Branch (2" diam.) (Tehama Co.)	*Cytospora leucostoma* *Fusarium* sp.	85 75
Cankers from pruning wounds (Tehama Co.)	*Eutypa lata* *Botryosphaeria* sp.	38 20

Table 5. Isolations from a declining orchard with cankers and heart rot in Tehama County in 2014.

Tree-sampled	Tree part sampled	Putative pathogens isolated and incidence of isolation (%)
Tree #1	Branch #1; canker Branch #2; canker Branch #3; canker Branch #4; canker	*Cytospora leucostoma* + *Botryosphaeria* sp. *Cytospora leucostoma* *Cytospora leucostoma* *P. variotii*
Tree #2	Branch #1; heart rot Branch #1; canker Branch #2; heart rot Branch #2; canker	*Schizophyllum* (80%) *Cytospora* (10%) + *P. variotii* (10%) *Schizophyllum* (70%) *Cytospora* (10%) + *P. variotii* (10%)
Tree #3 Tree #4	Branch #1; heart rot Branch #1; canker Branch #2; heart rot Branch #2; canker Trunk; heart rot	*Schizophyllum* (85%) + Unknown Bsidiomycete Basidiomycete (60%) *Schizophyllum* (55%) + *P. variotii* (50%) *Cytospora leucostoma* (80%) *Schizophyllum* (100%)

Table 6. Effect of of different age pruning cuts on infection by *Cytospora leucostoma.*[1]

Treatment	Date of pruning	Date of inoculation [2]	Flag/ Treatment color
0 days	2/21/2014	2/21/2014	OrBLK
3 days	2/18/2014	2/21/2014	PBL
7 days	2/20/2014	2/27/2014	RBL

15 days	2/19/2014	3/6/2014	GBL
30 days	2/18/2014	3/20/2014	RW
Not inoculated (control)	2/21/2014	---	Lime

(Table 6 coninued)

[1] Similar experiments were done with inoculations with spore supsensions of *Lasiodiplodia citricola* or *Paecilomyces variotii;* inoculations will be recorded in spring and summer 2015.
[2] Pruning wounds were sprayed with a 50,000 spores per ml suspension.

Table 7. Fungicde trials in a commercial orchard to determine efficacy against *Cytospora* canker in Yuba County (two experiments).

Treatment	**Rate per liter**
Topsin (thiophanate –methyl)	5 g a.i.
Quilt Ecel (azoxystrobin + propiconazole)	5 g a.i.
VitiSeal	1:10 dilution
Pristine + Pentre Bark	5 g a.i. + 1 oz
Tebuconazole	5 g a.i.
Pristine + VitiSeal	5 g a.i.+ 1:10 dilution
Untreated control	---

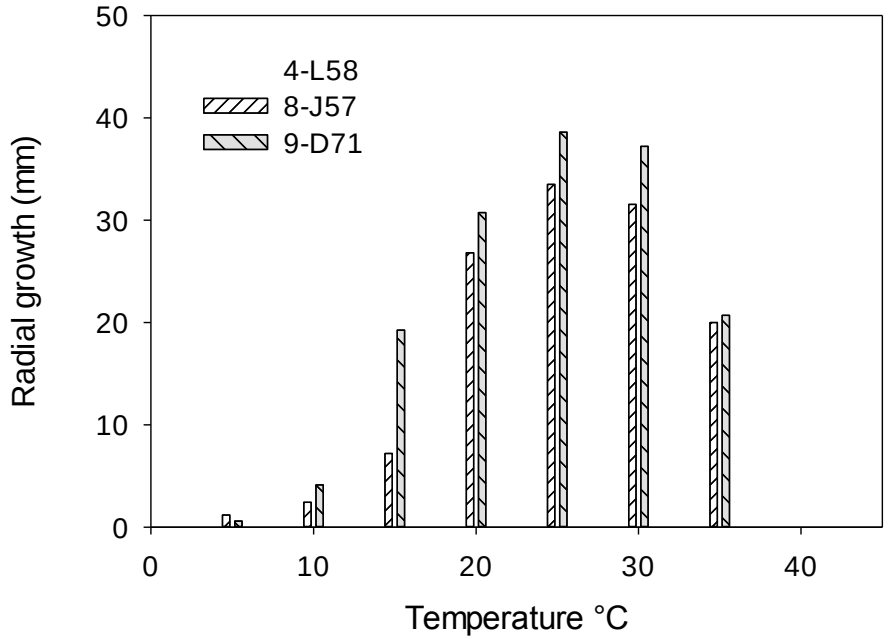

Figure 1. Radial growth of three isolates of *Cytospora leucostoma* under different temperature regimes. (5°C = 41°F; 10°C = 50°F; 15°C = 59°F; 20°C = 68°F; 25°C = 77°F; 30°C = 86°F; 35°C = 95°F; and 40°C = 104°F).