BIOLOGY, EPIDEMIOLOGY, AND MANAGEMENT OF SOUR ROT COMPLEX DECAY IN STONE FRUIT

PROJECT LEADER: Themis J. Michailides
COOPERATORS: Mohammad Yaghmour, David P. Morgan, and Kevin Day

BACKGROUND INFORMATION

Sour rot caused by *Geotrichum candidum* was reported on peaches from Georgia, Pennsylvania, New Jersey, and North Carolina (Burton and Wright, 1969). However, there was an earlier report in which *G. candidum* was isolated from peaches and other fruit in California and incited rot of 'Paloro' peach in 1960 (Butler, 1960). A short description of the disease also appeared later in the Agricultural Handbook No. 414 entitled "Market Diseases of Stone Fruit: Cherries, Peaches, Nectarines, Apricots, and Plums" that was published in 1972. More recently, in an article Adaskaveg and Crisosto provided also some information relevant to the epidemiology and control measures of the disease (2003).

In early July 2001, fruit samples of several nectarine and peach cultivars were brought from orchards in northern Tulare County or from packinghouses to our laboratory for diagnosis of an unusual decay. When the decay lesions originated close to the stylar end, leaking juice streamed from it. When the decay lesion was on the stem end of the fruit and touched the packing box, it developed a decay consisting of a ring of 0.5 to 2.0 cm inner and 1.0 to 3.0 cm outer diameter. The leaking juice dissolved the cuticle, the epidermis, and digested some of the fruit flesh, creating distinct furrows in the tissue. Samples with similar decay lesions were examined in 2001, 2002, and 2003. Isolations from the samples brought to the laboratory in 2001 revealed the presence of various yeasts with higher incidence of the organism *Geotrichum candidum*. Losses of up to 30% of the fruit due to sour rot have been reported. If one takes into account also the labor involved for sorting the decayed fruit, it is apparent that the losses could reach significant levels. Because we had identified the pathogen(s) and because this decay had the potential to become a major threat to the stone fruit industries in California, at the end of 2003 early 2004 we initiated a project on the ecology, epidemiology, and management of the sour rot decay of stone fruit. A short disease note on this disease has been published (Michailides et al., 2004)

The specific objectives of the project were:

OBJECTIVES

1) To identify all the organisms that can cause sour rot, confirm their pathogenicity on stone fruit, and determine possible relationships among them.

2) Identify sources of inoculum in orchard soils and fruit contamination and characteristics of orchards with the problem.

3) Determine the role nitidulid beetles, vinegar flies, and other orchard insects play in the epidemiology of sour rot in stone fruits.

4) Identify what sanitation practices in harvest equipment and the field can reduce sour rot decay.

PROCEDURES AND RESULTS

1. Identification of pathogens and pathogenicity studies (Koch postulates). Isolations from fruit with symptoms of sour rot consistently yielded in each year, two or three different yeasts, which were identified as *Geotrichum candidum, Issatchenkia scutulata*, and *Kloeckera apiculata.* All three yeasts were isolated from most of the samples, although sometimes different combinations of two of the yeasts were recovered. The most commonly isolated fungus-yeast was *G. candidum.* All the isolated yeasts were purified and stored in the fungal collection of the Kearney Agricultural Center. To complete Koch's postulates, each yeast was cultured on acidified potato dextrose agar at 77° F to prepare a dense (10^8) cell suspension. Eight mature Elegant Lady peach fruit were surface-disinfested in 0.1% sodium hypochlorite for 3 min, allowed to dry, and wounded once with a sterile nail (3 × 5 mm) on the fruit cheek. A 50-µl drop of the cell suspension was placed in each wound, and the peaches were incubated in containers with >95% RH at 81°F. Fruit inoculated similarly with a 50-µl drop of sterile water served as controls. In 2001, two containers containing eight fruit each were used for each yeast, and lesions started developing within 1 week after inoculation. The diameter of the decay lesion was measured after 10 days incubation of the fruit. Diameter of decay lesions ranged from 21 to 68 mm for *Geotrichum*, 30 to 55 mm for *I. scutulata*, and 9 to 39 mm for *K. apiculata* inoculations. The inoculation experiment was repeated by using two containers of eight cultivar Red Glo nectarine fruit per treatment yeast, under the same conditions as described above. Organisms recovered from the decay lesions were the same yeasts used for inoculating the peaches or nectarines. All three yeasts caused similar decay lesions in peaches, and the leaking effect was reproduced in both types of fruit. Symptoms were similar to those observed on fruit samples brought to our laboratory. Control fruit did not develop the characteristic decay lesions. We concluded that each of the yeasts on its own had the capacity to cause sour rot decay on stone fruit. We used *G. candidum* in most of our experiments since its incidence was the highest among the yeasts we isolated from fruit with sour rot.

Susceptibility of stone fruit. Sour rot was recorded in many cultivars of nectarines and peaches. To determine susceptibility to sour rot, Fantasia nectarines were collected periodically from an orchard at Kearney Agric. Center. We wanted to determine at what stage this "resistant" cultivar will become susceptible to *G. candidum.* Replicated fruit were inoculated with a drop (20 µl) of 10^6 spores per ml of *G. candidum* and their firmness was measured. Fruit were incubated at 77° F for 7 days and incidence and severity (lesion size) were recorded. The results are shown in Fig. 1. Green fruit, with firmness >12 lbs-force, did not support any decay development; while fruit with lower than 11 lbs-force developed a high incidence of sour rot. Surprising there was not much difference between the incidence of sour rot in fruit of 11-12 lbs-force and those with

lower firmness (Fig. 1 left) What was different though was the size of the decay lesions, which increased as firmness decreased (Fig. 1 right). For instance, fruit with 11-12 lbs-force firmness had an average of 7 mm lesion diameter, while fruit with less than 2 lbs-force developed lesions of 43 mm in diameter. These results suggest that the pathogen can infect immature fruit if there is a wound and grows faster in softer rather than firmer fruit. Because fruit are infected when firmness is 11-12 lbs-force, some decay could develop even before the fruit is preconditioned if conditions in the orchard are favorable. Also the inoculation results suggest that sour rot can start and develop to some degree in fruit on trees if the conditions are right. In fact, in 2004 growing season, some growers of Summer Bright nectarines started losing fruit that were dropping to the ground about 10 days before becoming ripe and examination of these fruit showed that more than 75% had sour rot decay. These experiments will be repeated with low and high acidity nectarine cultivars.

Are wounds necessary for infection? To determine whether wounds are needed for *G. candidum* to infect, we collected Mayglo nectarines and we inoculated them with a drop of 20µl of a 10^6 spore suspension of *G. candidum.* For these experiments, we used one isolate of *G. candidum* recovered from soil of a stone fruit orchard and one from a fruit decayed with sour rot. Surface sterilization, inoculation procedure, and incubation temperature and time were the same for all inoculation experiments. In this particular case, half of the fruit in each replication were wounded with a sharp glass rod (2 mm in diameter) and the rest was not wounded, but the inoculation drop was placed in a pre-marked area on the cheek of each fruit. Fruit were incubated at 77° F for one week and incidence of fruit infection was recorded. Both the soil and the fruit isolates of *G. candidum* infected both the wounded and the non-wounded fruit (Figure 2). However, the incidence of infection was higher for the wounded than the non-wounded fruit. The results suggest that isolates from both soil and fruit can cause sour rot and that a wound is not necessary for infection to occur.

2. Sources of inoculum of *G. candidum* and relationship to sour rot decay. In cooperation with Kevin Day, Farm Advisor in Tulare County, we identified five orchards with a history of sour rot. In addition we used one orchard at the Kearney Agricultural Center in these studies. The goal was to determine if there was any relationship between the levels of propagules of *G. candidum* and sour rot incidence in fruit. Soil and debris were collected from these orchards in April and the levels of propagules of *G. candidum* were determined. Five grams of soil were placed in 10 ml of water and using the dilution plating technique on novobiocin potato dextrose agar plates we quantified the density of *G. candidum* propagules. (Propagules are either mycelial fragments or spores that develop colonies in agar media.)

At commercial harvest, 10 boxes of fruit were collected from four of these orchards, since the fifth orchard was harvested before we were able to collect the fruit samples. With the exception of one orchard, among the other three, there seemed to be a relationship with propagules of *G. candidum* in soil and incidence of fruit boxes with sour rot. Orchards with the higher numbers of propagules of *G. candidum* had higher incidence of boxes with sour rot and more fruit with sour rot per box than orchards with lower numbers of *G. candidum* propagules density (Figure 3). In one of the orchards, however, the propagules density in the soil was high (about 600,000 propagules per gram of dry soil) but the sour rot incidence was very low. Thus, this initial study suggests that there might be a relationship that should be investigated further by including more

orchards, as was initially proposed. We also need to investigate the reasons for the orchard with the high propagules density to have low incidence of sour rot?

To determine sources of inoculum, we collected fruit decaying on the ground or from trees in two orchards with a history of sour rot. The organisms decaying these fruit were examined with a compound microscope to identify the causal agent. For decaying fruit on the ground, *G. candidum* ranged from 17 to 73%, while the incidence of other yeasts ranged from 37 to 58%. In two of these orchards fruit also decayed by a *Fusarium* sp. (from 10 to 34%) (Table 1). We also observed in two other orchards that some fruit on the ground, after the flesh decayed, dried up, and became mummies with a white or gray appearance. *G. candidum* spores and mycelial fragments were observed from this white or gray film from 100% of these fruit. By scraping these spores from these dried up fruit, we recovered dehydrated spores that appeared to be rectangular in shape, suggesting that these spores may represent a resistant stage of the fungus, helping in its survival in the soil under dry conditions. After plating these dry spores in media with acidified potato dextrose agar, these rectangular spores absorbed moisture and became barrel shape (typical *G. candidum* spores), and more than 95% germinated and produced colonies of *G. candidum.* These findings were supported from samples collected in another orchard (Summer Flare nectarines) at a different location. The results in Table 1 and the discovery of the "resistant" spores of *G. candidum* suggest that fruit on the ground could decay by *G. candidum* easily, even if there were other decay organisms present [such as *Monilinia* (brown rot), *Rhizopus*, *Gilbertella, Aspergillus, Fusarium*, and others]. In addition, *G. candidum* seems to survive under dry orchard conditions on dried up fruit (mummies). Obviously, decaying fresh fruit and mummies on the orchard floor by *G. candidum* can serve as a source for increasing soil populations of *G. candidum*.

Table 1. Incidence of *Geotrichum candidum*, yeasts, and *Fusarium* sp. in fresh and mummified fruit collected from the ground and trees in three commercial peach orchards in Fresno and Tulare Counties.

Orchard	*G. candidum* (%)	Other yeasts (%)	*Fusarium* sp. (%)
Ground			
1	73	37	10
2	18	39	0
3	17	58	34
Tree			
1	28	39	0

* Nineteen to 48 fruit and mummies per orchard were examined.

To determine any sour rot development on fruit still on trees, samples of suspected fruit and fruit with indentation marks from touching the nearby twigs/leaves were also collected from a Summer Flare orchard. Microscopic examination of affected fruit and isolations on agar media revealed that up to 28% of these fruit were decaying with *G. candidum*, 33% with other yeasts, and 39% of the fruit had no decay. These results also support the contention that sour rot can start and develop to some degree in fruit on trees if the conditions are right in the orchard and if

there are enough propagules of the pathogen reaching the tree canopy. In a previous experiment, samples of immature stone fruit and leaves from peach and nectarine trees were washed with sterilized water using a shaker for 48 hours and the washings were plated on novobiocin potato dextrose agar (Butler, 1962) to determine possible propagules of *G. candidum.* A few propagules of *G. candidum* were recovered from both fruit and leaves of stone fruit, suggesting that they can easily reach the tree canopy most likely by means of wind dispersed soil particles. Additional research is needed in this area to determine when how long before harvest infection begins.

3. Insect transmission of sour rot.

Nitidulid beetles (*Carpophilus hemipterus*). Replicated wounded and non-wounded Elegant Lady fruit were enclosed in an insect cage and one fruit decayed by sour rot was also enclosed in the cage. In one experiment 5 nitidulid beetles and in another 10 beetles (*Carphophilus hemipterus*) were also enclosed in each cage. There were three cages with insects for each experiment and one cage without insects (control). The experiments using the nitidulid *C. hemipterus* indicated that they can successfully transmit the pathogen from infected to non infected fruit, but only to wounded fruit (Figure 4). For instance, when 5 and 10 beetles were caged in replicated plastic mesh containers with a fruit infected with *G. candidum* 20% and 53 % of the wounded fruit became infected, respectively; however none (0%) of the non wounded became infected. Only wounded fruit visited by the beetles were infected, while wounded fruit where beetles were excluded remained healthy (0% sour rot in the fruit kept in the control cages).

Vinegar flies (*Drosophila* spp.). Fruit flies were added to four cages, each containing one fruit infected with *G. candidum,* along with of three wounded and three non-wounded fruit. A fifth cage served as a control and had neither fruit flies nor an infected fruit. The wounded and non-wounded fruit were surface-disinfested for 4 minutes in a solution of 1.6% bleach (5.25% sodium hypochlorite), 1.6% ethanol, and 0.005% Tween 20 per liter. After dipping, fruit were wounded by making five small holes with a sharp end (2 mm diameter) of a capillary tube. In the first experiment six fruit flies were added per cage, while in the second experiment ten fruit flies were added per cage. Summer Flare nectarines were used in this experiment. We showed that vinegar flies (*Drosophila melanogaster* and other *Drosophila* species) transmitted *G. candidum* to 16% of either wounded or nonwounded nectarines (Figure 4). These experiments also suggest that vinegar flies play some role in the transmission of the sour rot disease in stone fruit orchards.

4. Sanitation practices in harvest equipment and the field to reduce sour rot decay. In order to proceed with this objective we need to understand the ecology of *G. candidum* and the other yeasts, find the major sources of contamination of fruit and the harvest containers, and then design experiments towards sanitation practices. Some progress towards understanding the ecology and epidemiology of the sour rot in stone fruit has been made in 2004, and we will proceed with the proposed sanitation experiments as we have proposed.

CONCLUSIONS

1. Some stone fruit orchards have very high populations of *G. candidum* and other yeasts.
2. Mummies of stone fruit can be sources for survival of *G. candidum.*
3. Dried up rectangular spores of *G. candidum* may serve as "resistant" (over-wintering and/or over-summering) structures of the pathogen.
4. Fruit decaying with *G. candidum* on the orchard floor can increase the soil population of this fungus and reach the tree canopy.
5. There is an indication that there may be a correlation between propagule numbers in the soil and sour rot incidence in fruit.
6. Fruit with more than 12 lbs-force firmness does not support the development of *G. candidum,* while more mature fruit (less than 12 lbs.-force) facilitates rapid symptom development and increased incidence of this disease.
7. Propagules of *G. candidum* were recovered from fruit and leaves still hanging on the trees.
8. Decay by *G. candidum* usually initiates close to the stem end and shoulder of fruit, and can even begin when they are still on the trees.
9. Under certain conditions some of these infected fruit drops about a week to 10 days before harvest.
10. The nitidulid *Carpophilus hemipterus* transmitted *G. candidum* only on wounded fruit while *Drosophila melanogaster* transmitted *G. candidum* to both wounded and nonwounded fruit. Numbers of beetles were positively correlated with disease incidence.

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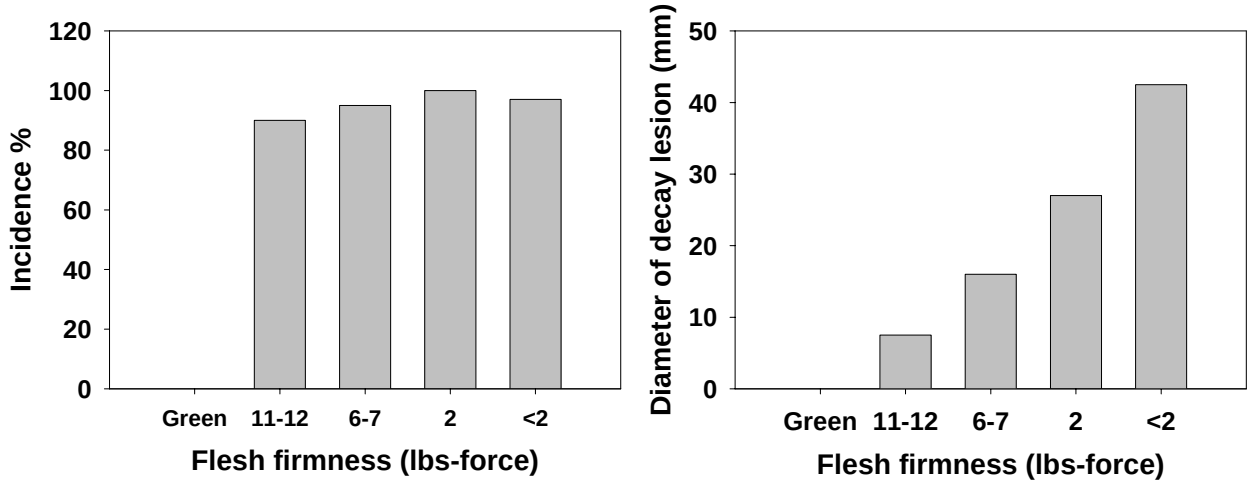

Figure 1. Incidence (left graph) and diameter of lesions (right graph) of sour rot decay of different maturity Fantasia nectarines caused by *Geotrichum candidum* after 5 days incubation at 77° F.

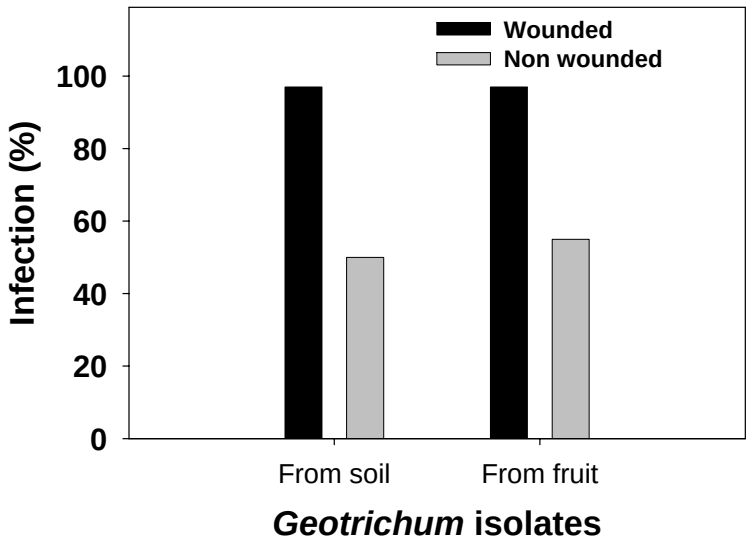

Figure 2. Infection of wounded and non wounded Mayglo nectarines by *Geotrichum candidum* isolates from soil and fruit (fruit were inoculated and incubated for 7 days at 77° F).

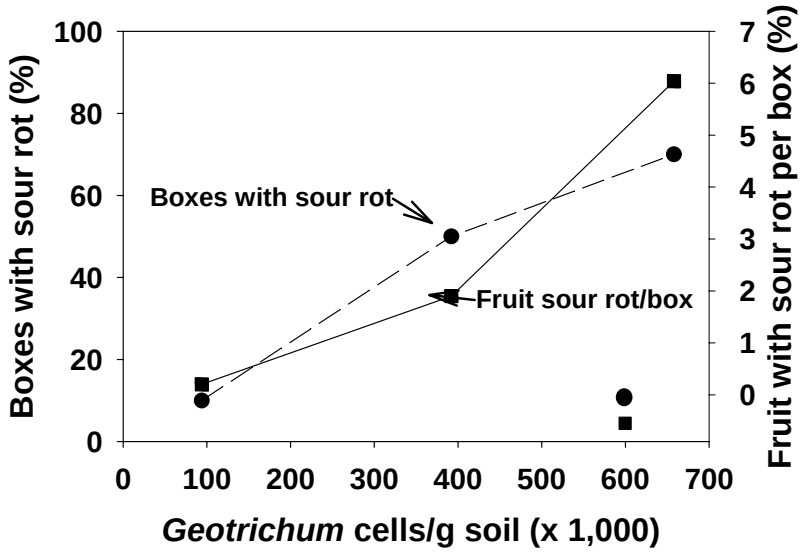

Figure 3. Relationship between propagules of *Geotrichum candidum* per gram of soil and incidence of boxes with sour rot and sour rotted fruit per box for four commercial orchards..

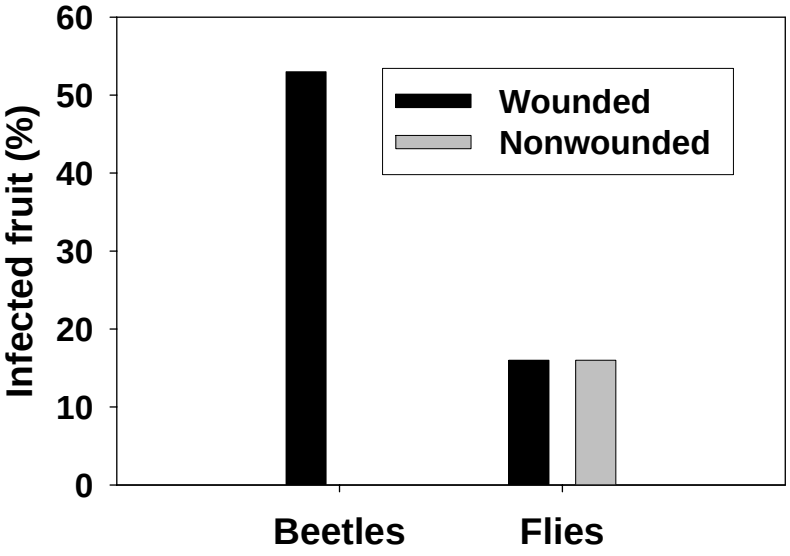

Figure 4. Transmission of *Geotrichum candidum* causing sour rot on stone fruit by nitidulid beetles and vinegar flies. (experiment with nitidulid beetles used Elegant Lady peaches and experiment with vinegar flies used Summer Flare nectarines). Source of sour rot was a fruit decayed by *G. candidum* per insect cage.