*PROJECT CONCLUDED – FINAL REPORT*

# Etiology, Epidemiology, and Management of Hyphoderma Gummosis

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Hyphoderma gummosis, a disease causing wood decay, serious dieback, tree decline, and eventual death of citrus trees was first observed in 2000 in several orchards in Tulare, Ventura, San Luis Obispo, and Riverside Co. causing serious losses to some lemon growers (Marais et al., 2001a,b). Mainly older lemon orchards (between 35 and 50 years old) are affected.

Early symptoms include wilting of the foliage. Cream- to salmon-colored fruiting bodies often first appear at the presumed infection sites, e.g., on branch stubs and along bark cracks and may later cover large areas of trunks and major limbs. Extensive gumming is often observed at this stage. Viable fruiting bodies, as assessed by spore production of the causal fungus, were found abundantly in orchards in Tulare Co. from February/March through June in most years. By early June, however, with the onset of hot, dry summer weather, most fruiting bodies were no longer viable.

These findings are in agreement with our studies where we demonstrated that viability of basidiospores quickly declines at temperatures of 36C and/or at low relative humidity values between 24 and 34%. The high-temperature susceptibility of the pathogen together with the long period of viable fruiting body occurrence indicates that for disease management, orchard pruning should be delayed as long as possible in the spring, but not delayed until summer if there is a possibility of sunburn injury to occur.

We also observed that in spring seasons with dry weather the incidence of new basidiomes was reduced, and consequently spread of the disease was likely reduced under these conditions. Still, once established in an orchard, Hyphoderma gummosis will continue to cause tree losses unless major changes in orchard management strategies are followed.

Thus, we observed that when judicious pruning and removal of diseased branches was done aggressively in severely affected orchards, the incidence and severity of Hyphoderma gummosis was much lower in the following season.

The organism implicated with the disease has been identified as the Basidiomycota fungus *Hyphoderma sambuci* that is commonly found on many other woody hosts. Re-inoculations of healthy branches in the field were successful, symptoms of the disease were reproduced, and the pathogen was re-isolated. Thus, Koch's postulates were fulfilled. Studies on the mode of infection indicated that *H. sambuci* can grow into the wood only when inoculations were done directly on the cut surfaces. This indicates that *H. sambuci* cannot infect its host through the intact bark but only through wood-exposing injuries such as pruning wounds.

Genetic studies on the outcrossing pathogen *H. sambuci* indicated that the fungus has a tetrapolar mating system with two genetic loci determining compatibility. We found multiple alleles for each of these loci, and the genetic make-up of fruiting bodies from trees within a small area in the orchard that were investigated was different. This indicated that (1) the pathogen population was highly heterogeneous, that (2) the disease is disseminated by basidiospores, that (3) multiple introductions of the pathogen were responsible for the disease epidemic, and that (4) new infective dikaryons capable of producing new fruiting bodies can be readily established. The highly heterogeneous population structure of *H. sambuci* was confirmed in our genetic analysis using random amplified polymorphic DNAs indicating that sexual recombination occurs and that basidiospore dissemination is the main mechanism of dispersal.

*Field symptoms of Hyphoderma gummosis on a lemon tree. Wilting foliage and dieback of scaffold branches infected with* hyphoderma sambuci.

In greenhouse studies on the host range of *H. sambuci*, potted plants of Navel and Valencia orange, Eureka lemon, Ruby Red Grapefruit, Mucott and Pixie Mandarin, Bears lime, and pineapple sweet orange were inoculated using two methods. Dieback was found on all cultivars except for Eureka lemon, and the highest incidence of dieback was observed on Navel and Valencia orange, as well as on pineapple sweet orange. The pathogen was reisolated with the highest incidence from Murcott, pineapple sweet orange, mandarin, and Valencia and Navel orange. Lower incidences of recovery were obtained for lemon, lime, and grapefruit. Thus, *H. sambuci* was found to be pathogenic and possibly more virulent to several citrus varieties other than lemon. Host-specificity of the pathogen in the field may depend on the type of wound or environmental factors that were not considered in our greenhouse studies. The occurrence of the disease only on lemon in the field may reflect the fact that lemons are more often affected by freeze damage and subsequent tissue wounding that creates entryways for the pathogen.

In our studies on disease management, we first evaluated the in vitro sensitivity of *H. sambuci* to selected fungicides. EC50 values for inhibiting germination of basidiospores, the primary inoculum for the disease, were determined to be between 0.092 and 0.165 ppm for azoxystrobin and between 0.006 and 0.028 ppm for pyraclostrobin. Spore germination was not inhibited by propiconazole or boscalid. Mycelial growth, however, was inhibited by the latter two fungicides. Azoxystrobin is registered on citrus for preharvest uses, and new studies are addressing improved usage. For the two biocontrol agents *Trichoderma viride* and *T. harzianum* there was no evidence for production of inhibitory antimicrobials, but a competitive growth interaction was observed on agar plates. This suggested that the *Trichoderma* species is antagonistic to *H. sambuci* by preventing its colonization of treated host substrates.

Pruning wound treatments with selected fungicides (propi- conazole, pyraclostrobin-boscalid, and azoxystrobin) and the two biocontrol agents were evaluated in several field experiments as a possible management strategy. For this, branches 1-2 cm in diameter were cut, treated, and then inoculated with basidiospores of *H. sambuci*. A significant reduction in pruning wound colonization was found for both protective biocontrol treatments. In contrast, treatments with any of the fungicides were ineffective.

Based on these data and working in cooperation with Bio- Works (the product manufacturer; Matt Needham is the cur- rent California representative) and Wilbur-Ellis (the product distributor), a registration of PlantShield HC (*T. harzianum*) on citrus was pursued. A Special Local-Need application was submitted to the Department of Pesticide Regulation to obtain a Section 24c registration in June 2007. BioWorks obtained exemption from food tolerance for the product in California (40CFR180.1201) and provided toxicity data against nontarget pests such as bees where no negative effects have been found. The Section 24c registration was approved in July 2008 (7/26/08).

The label that we assisted in preparing includes an application rate of 3-25 oz of product per acre in 100-500 gal of water (high-volume, non-air assisted (to prevent any microbial spore-related allergy problems). One or two applications should be made to tree pruning wounds and other wood- exposing injuries as soon as possible after pruning or within 4 to 5 days. Because a living organism is applied that has to be able to colonize the wood-exposing wounds as quickly as possible, applications should be done at temperatures above 48F. The treatment should not be applied within 30 days of harvest to prevent any issue with postharvest decay of fruit. Under normal packinghouse operations, sanitation programs will remove inoculum from fruit surfaces.

As indicated before, orchard pruning should be delayed as long as possible in the spring when no more rainfall is expected, but not delayed until summer if there is a possibility of sunburn injury to occur. Although the state approved this registration, the EPA restricted usage to exclude lemons because of reports of pathogenicity of *Trichoderma* spp. on lemon seedlings. We are currently disputing this claim for mature lemon trees, and new studies are being conducted to address this problem.

Other recommended management strategies include starting with clean nursery stock and preventing the establishment of the fungus by minimizing large wood-exposing wounds and using proper pruning (e.g., avoiding stub cuts) and irrigation (avoid getting the trunks and branches wet) practices. Sanitation measures include the removal of infected plant material from the orchard and burning. There are no curative treatments for this disease.