Research Report
Control of Brown Wood Rot in Lemons with Low Pressure Injection 2013-14

Glenn C. Wright
Department of Plant Sciences, U. of A., Yuma Agriculture Center, Yuma, AZ

Abstract
We injected AGRA PHOS (Potassium Phosphite) 0-2.4-2, Propiconazole – 0.05%, Zn, Mn and Fe 0.105, 0.112, and 0.10% respectively, Zn, Mn and Fe 0.210, 0.220, and 0.200% respectively and Propiconazole – 0.05% + Zn, Mn and Fe 0.105, 0.112, and 0.10% respectively using a low pressure injection system for the control of Antrodia sinuosa in lemon trees. No treatment led to a significant reduction in fungal growth.

Introduction
Coniophora eremophila, a wood rotting fungus, was first reported in lemons in 1992 (Matheron, Gilbertson & Matejka, 1992). Another species, Antrodia sinuosa was found to be infesting lemons, and was isolated in 1997. Further research (Bigelow, Matheron & Gilbertson, 1996; Bigelow, Gilbertson & Matheron, 1998) found that Coniophora has been found sporulating on desert plants, but not been found sporulating on lemon wood, while Antrodia has been found sporulating on decaying fallen wood within lemon groves. Furthermore, the optimum temperature range for growth of Antrodia and Coniophora is 30-35°C (86-95°F), and the rate of decay for Coniophora and Antrodia in Lisbon lemon is higher than that for orange, tangelo and grapefruit trees. Finally, wood decay experiments suggest that Antrodia is more aggressive and a greater threat to lemon trees than is Coniophora.

Neither of the fungi are particularly difficult to control using commonly available fungicides. Artificial inoculation of excised branches with mixtures of fungi and fungicide led to significant control (Matheron, Porchas and Bigelow, 1996). Length of Coniophora wood decay columns was reduced by 94% and 80%, following treatment with Propiconazole and Azoxystrobin, respectively. Length of Antrodia decay columns was reduced by 79% and 71% following treatment with the same materials. The difficulty in controlling these diseases in the field is that there appeared to be no effective way to apply the fungicides to the diseased interior wood of a living tree in the orchard.

In 2011, the author became aware of a low-pressure injection technology system for trees that was being promoted as an alternative pesticide delivery system for control of pests and diseases in landscape and urban trees, for control of Asian Citrus Psyllid in citrus trees and for control of Red Palm Weevil in palms. The system was developed by a Spanish corporation, Fertinyect, www.fertinyect.com. The uniqueness of the system was that it consisted of a low-cost latex pouch, the contents of which could be passively injected into the xylem of a tree using only a power drill, a plastic connector tube, and the pouch of liquid pesticide or nutrient material. Materials could then be translocated throughout the plant. Unlike other methods, no high-pressure injection systems were needed that might damage the tree, and the pouches could be filled with pesticide active ingredients, nutrients, water or other chemistries. Subsequently, Fertinyect has licensed the product in the U.S. to Brandt Consolidated Inc., where it is marketed as Brandt enTree™ http://www.brandt.co/entree. Brandt has replaced the pouch technology with a bottle technology.

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and has benefitted from partnership with Bayer Crop Science Inc. Brandt acquired the ability to fill the bottles with nutrient solutions and/or pesticides provided by the partners.

Therefore, our lab initiated an experiment in 2012 to determine whether the Brandt enTree pouch system could be an effective delivery system that would allow for control of *Antrodia sinuosa* fungus in lemon trees. We applied two pouches of AGRA PHOS (Potassium Phosphite) 0-2.4-2, Propiconazole – 0.05%, Propiconazole plus Azoxystrobin – 0.117 and 0.135% respectively, Zn, Mn and Fe 0.105, 0.112, and 0.10% respectively and Azoxystrobin – 0.137%. Results of that work can be found at [http://extension.arizona.edu/pubs/control-brown-wood-rot-lemons-low-pressure-injection-2012](http://extension.arizona.edu/pubs/control-brown-wood-rot-lemons-low-pressure-injection-2012). The Propiconazole + Azoxystrobin treatment, the Azoxystrobin treatment, and the Zn + Mn + Fe treatment led to significantly less fungal lesion growth when applied prior to the introduction of the fungus, as compared to their application after fungal introduction (Fig. 1). These results suggested that subsequent research should address the following questions:

1. Research should focus on those treatments where the products were introduced prior to the introduction of the fungus.
2. If fungicidal concentrations will remain the same in subsequent work, emphasis should be placed on those treatments that appeared to be most effective, namely Propiconazole, Propiconazole + Azoxystrobin and Zn + Mn + Fe.
3. Different concentrations of the products should be tested.
4. Additional pouches per tree should be tested.
5. Subsequent work should be done in an orchard with the same scion/rootstock combination.

For 2013, we proposed to repeat some of the previous year’s work with significant modifications. Specifically, we will:

1. Include only treatments where products will be introduced in advance of the fungus.
2. Include only Propiconazole, micronutrient and phosphite treatments, as those are already registered on citrus or will not require EPA registration, and can be used more readily by the citrus grower.
3. Include mixtures of the treatments that appeared to be successful in 2012, and in the case of the nutrients, we added a double concentration.
4. Conduct the experiment on trees of a single rootstock and single scion so as to reduce variability.

**Materials and Methods**

In July 2013, we collected some *Antrodia sinuosa* from a local lemon orchard, and made several cultures of the fungus on agar in petri plates. Then, we purchased some 1/8-inch wood dowels, cut them into ½-inch lengths, and placed them in the petri plates, for the purpose of infesting the wood with the fungus. Within 2 weeks, all the dowel pieces were well-infested. The experimental site was a lemon orchard at the University of Arizona Yuma Mesa Agriculture Center, Block 20. The orchard consisted of the ‘Limoneira 8A Lisbon’ cultivar on *Citrus volkameriana* rootstock.

Meanwhile, we received 50 pouches each of the following liquid treatments in mid-July:

1. AGRA PHOS (Potassium Phosphite) 0-2.4-2
2. Propiconazole – 0.05%
3. Zn, Mn and Fe 0.105, 0.112, and 0.10% respectively
4. Zn, Mn and Fe 0.21, 0.22, and 0.20% respectively
5. Propiconazole – 0.05% + Zn, Mn and Fe 0.105, 0.112, and 0.10% respectively
To these treatments, we added a control (no fungicide or nutrient pouch) for a total of 6 treatments.

On July 1, we applied the fungicide and nutrient treatments. Trees were marked with colored flagging tape corresponding to the treatment. Each tree had two identical pouches (excepting the control treatment which had no pouches). We drilled holes in the trunks of the treated trees below the infestation site, and then inserted a plastic tube which was then connected to the pouch. Within 24 hours, the liquid in all the pouches had entered the tree as a result of transpiration. The pouches were flaccid, and we removed them and the plastic tubes.

On 25 to 29 July, we inserted the infested dowels. We drilled 1/8” holes in limbs above the site of the pouches, inserted the infested dowels and sealed the area with wax. Examples of pouch and dowel insertion are shown in Figures 2 – 7.

Experimental design was randomized complete block, with four blocks. Within each block there were five trees treated with each treatment, and so there were 15 single-tree replications, and there were 3 branch replications for each tree. Thus, for each of the 6 treatments, there were 15 trees, and 45 infested branches, and for the complete experiment, there were a total of 90 trees and 270 infested branches.

We allowed the fungus to grow and the liquid treatments to counter that growth until November 25-27, 2013 when we harvested the branches. On December 3rd, we split the branches and measured the length of the fungal growth within the wood. This is generally the same procedure that we followed in the 2012 report. Additional photos of the procedures and fungal growth within the wood can be found in the 2012 report referenced above.

Results and Discussion

Results are presented in Figure 8. There were no significant differences due to treatment. This may be because there were insufficient pouches of material, thus the concentration of the materials in the xylem, or because the xylem vessels at the point where the material entered the tree did not lead to the point of infection. Further research will be needed to determine how to proceed with this work.

Literature Cited


Figure 1. 2012-13 Growth of fungal lesions in lemon wood in the presence of fungicides or micronutrients applied via low pressure injection. Treatments whose associated letters are different are significantly different. Mean separation using Duncan's Multiple Range Test (α=0.05).
Figure 2. Removing branches prior to insertion of dowel.

Figure 3. Drilling the hole for the dowel.

Figure 4. Petrie plate with dowels.

Figure 5. Dowel inserted in branch

Figure 6. Trunk with pouches attached

Figure 7. Pouches after 24 hours
Brandt Experiment to Control Coniophora eremophila and Antrodia spp. on Lemon - 2013

Figure 8. 2013 Growth of fungal lesions in lemon wood in the presence of fungicides or micronutrients applied via low pressure injection.