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Abstract

We injected AGRA PHOS (Potassium Phosphite) 0-2.4-2, Propaconizole – 0.05%, Propaconizole plus Azoxystrobin – 0.117 and 0.135% respectively, Zn, Mn and Fe 0.105, 0.112, and 0.10% respectively, and Azoxystrobin – 0.137% using a low pressure injection system for the control of <u>Antrodia sinuosa</u> in lemon trees. The Propaconizole + 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.

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 30-35C (86-95F), 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 a greater threat to lemon trees than is *Coniophora*.

In 2011, Dr. Wright 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, headquartered in Córdoba, Spain <u>www.fertinyect.com</u>. The uniqueness of the system as presented, for agricultural purposes, 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 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 enTreeTM. Brandt has replaced the pouch technology with a bottle technology, and has benefitted from partnership between Fertinyect and other agrochemical firms, such as Bayer Crop Science Inc. Brandt has acquired the ability to fill the bottles at its Springfield Illinois main site, and

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will fill them with nutrient solutions and/or pesticides provided by the partners. While there is as yet no specific website for the enTree technology, an idea of the technologies use for the control of emerald ash borer can be found at http://treecarechicago.com.

Although much of the impact of this technology has been directed against insects (chiefly emerald ash borers, Asian citrus psyllids and red palm weevil), there is no reason why it might not also be directed against plant pathogens. This would particularly be the case for plant diseases that invade the plant vascular tissue and are difficult to control because they remain concealed there, unaffected by foliar applications of fungicides or bactericides. 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.

Materials and Methods

In early July, 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. The orchard had been used previously for another study that had similar methodology.

We introduced the fungus into the lemon trees by drilling a 1/8-inch hole in each of 3 branches of 2.0" or smaller diameter, and inserting an infested dowel.

Meanwhile, we received 50 pouches each of the following liquid treatments and code numbers on 23 July:

- AGRA PHOS (Potassium Phosphite) 0-2.4-2
- Propaconizole 0.05%
- Propaconizole plus Azoxystrobin 0.117 and 0.135% respectively
- Zn, Mn and Fe 0.105, 0.112, and 0.10% respectively
- Azoxystrobin 0.137%

To these treatments, we added a control (no fungicide or nutrients) however with the fungus, thus there were a total of 6 liquid treatments. Additionally, we decided to apply the liquid treatments to 50% of the trees three weeks before fungal introduction as a preventative treatment, and to introduce the fungus to the other 50% of the trees three weeks before the liquid treatments were applied as a curative treatment. Thus, there were in effect 12 treatments. Experimental design was randomized complete block, and there were 4 single-tree replications, and 3 branch replications for each of the 12 treatments, for a total of 48 trees in the experiment, and 144 branch measurements. (See Figure 1). Each tree had three sites where the fungus was introduced and two pouches (excepting the control treatment which had no pouches). Trees were 'Allen Eureka' lemon, 'Corona Foothills' lemon or 'Limoneira 8A Lisbon' lemon scions and one tree was 'Prior Lisbon'. Scions were budded to *Citrus macrophylla, Citrus volkameriana* or Rough Lemon rootstock. We attempted to distribute the treatments evenly among the scion/rootstock combinations.

On 27 July, we inserted the infested dowels in 50% of the trees (Figs. 2-5), and attached two pouches of the appropriate liquid treatment to 50% of the trees (Figs. 6 and 7). Then, on 17 August, we attached two pouches of liquid treatment to each of the trees that had previously been infested and inserted dowels in the branches. Trees were marked with colored flagging tape corresponding to the plot plan. All pouches were drained of their liquid within 24 hours.

We allowed the fungus to grow and the liquid treatments to counter that growth until January 5, 2013 before harvesting the branches. At that time, we cut off the infested branches and on January 7, we measured the length of the fungal growth within the wood. Examples of the fungal growth for each treatment are shown in Figures 8-19.

Results and Discussion

Results are presented in Figure 20. When the fungicides or micronutrients were applied first to the trees, the average lesion length was 107 mm, while when the fungus was applied first; the average lesion length was 180 mm. This represents a significant difference. The Propaconizole + 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. 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 Propaconizole, Propaconizole + 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 propose 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 Propaconizole, 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 two comparative treatments with the same micronutrient products, but at different concentrations.
- 4. Conduct the experiment on trees of a single rootstock and single scion so as to reduce variability.

We expect to further refine the treatments that will lead to reduction of the disease. It is anticipated that the work in 2013 will lead to field trials in commercial groves in 2014.

Literature Cited

Bigelow, D.M., Matheron, M.E., and Gilbertson, R.L. 1996. Biology and control of Coniophora eremophila on lemon trees in Arizona. Plant Dis. 80:934-939.

Bigelow, D.M., Gilbertson, R.L., and Matheron, M.E. 1998. Cultural studies of fungi causing brown rot in heartwood of living lemon trees in Arizona. Mycol. Res. 102:257-262.

Matheron, M.E., Gilbertson, R.L., and Matejka, J.C. 1992. Coniophora sp. implicated in rapid development of wood rot on living branches of lemon trees in Arizona (Abstr.). Phytopathology 82:1083.

Row → Tree No.	12	11	10	9	8	7	6	5	4	3	2	1						
V V																		
													ID	Color				
1		AB	RJN	RB		VN			NB			PN	A	Azul	Agraphos	12006		
													RJ R	Rojo Rosado	Propinaconazole Prop. + Azox.	12007 12008		
2	PB	NN		VB	RN						RJB		v	Verde	Zn, Mn, Fe	12009		
-													N		o Azoxystrobin	12010		
													Р	Purpurea	None	Fungi only		
3	VN		NB	PN					AN									
													N	Negro Blanco			/27, fungus on 8/17 27, product on 8/17	
4		RB	RJN					AB				PB	В	ыапсо		rungus on 7/	27, product on 8/1/	
4		TVD TVD	TO N															
5	AN	RJB				RN			VB		NN							
6	RJB					VN			NB	PN								
0	NJ D					VIN			IND	FIN								
7					RJN			AB	P B			NN						
8				PB			AN	RJB	RN		VB							
٥				гU			AN	NJ D	IVIN		VD							
9			AN			RJB	RN		VB	NN								
		DN			ND			DO	DIN			A D	ļ					
10		PN			NB	VN		RB	RJN			AB						

Figure 1. Plot Plan of the Brandt/enTree experiment 2012. Note that plot plan is partially in Spanish, as my workers speak that language.



gure 2. Removing branches prior to insertion of dowel.



Figure 4. Petrie plate with dowels.



Figure 3. Drilling the hole for the dowel.



Figure 5. Dowel inserted in branch



Figure 6. Trunk with pouches attached



Figure 7. Pouches after 24 hours

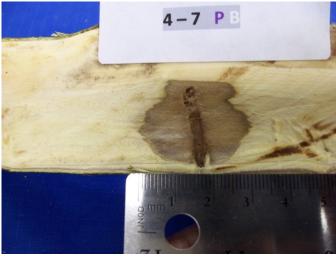


Figure 8. Branch with fungus only applied on 7/27.

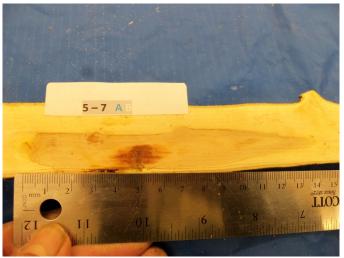


Figure 10. Fungus applied on 7/27 and Agrophos on 8/17.

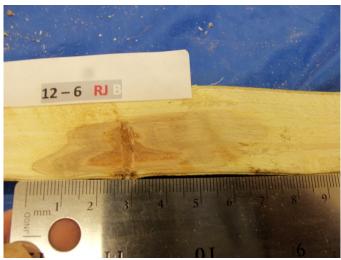


Figure 12. Fungus applied on 7/27 and Propaconizole on 8/17.

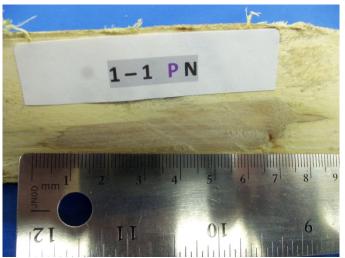


Figure 9. Branch with fungus only applied on 8/17.

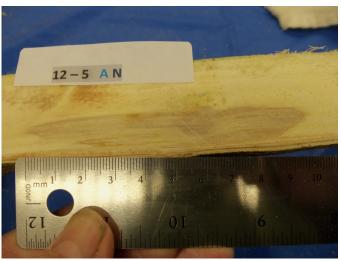


Figure 11. Agrophos applied on 7/27 and fungus on 8/17.

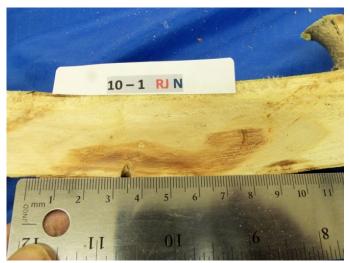


Figure 13. Propaconizole applied on 7/27 and fungus on 8/17.

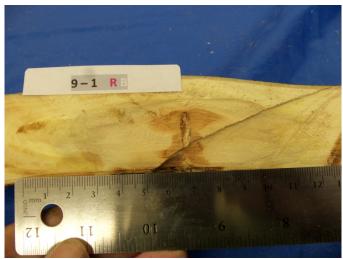


Figure 14. Fungus applied on 7/27 and Propaconizole + Azoxystrobin on 8/17.



Figure 16. Fungus applied on 7/27 and Zn + Mn + Fe on 8/17.



Figure 18. Fungus applied on 7/27 and Azoxystrobin on 8/17.

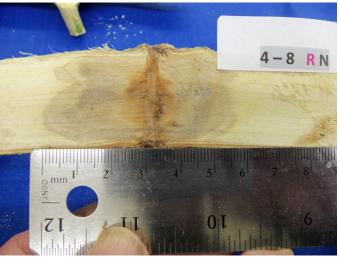


Figure 15. Propaconizole + Azoxystrobin applied on 7/27 and fungus on 8/17.

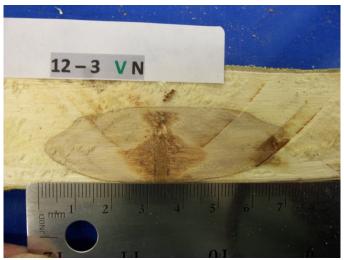


Figure 17. Zn + Mn + Fe applied on 7/27 and fungus on 8/17.

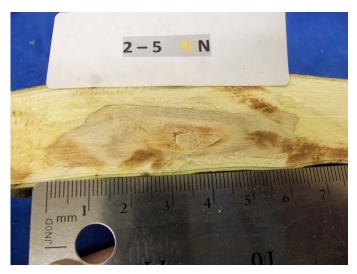
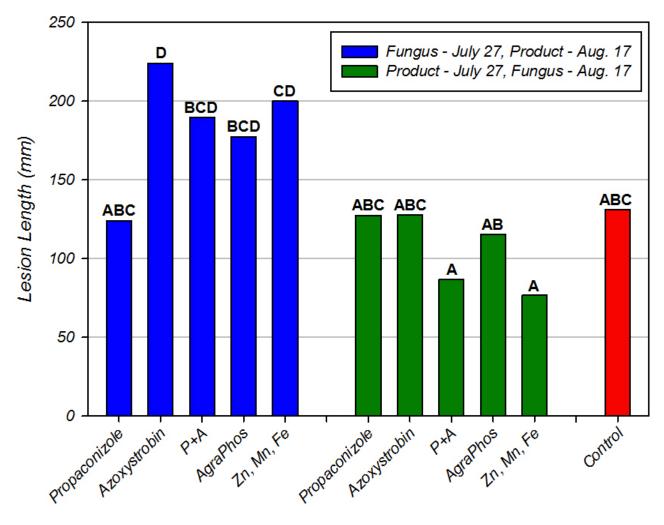


Figure 19. Azoxystrobin applied on 7/27 and fungus on 8/17.

Brandt Experiment to Control <u>Coniophora eremophila</u> and <u>Antrodia</u> spp. on Lemon - 2012



Fungicide or Product

Figure 20. 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).