# *Effect of SAR and micronutrients on lemon tree health – 2014*[1]

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## *Abstract*

*Brown wood rot (<u>Coniophora eremophila</u> and <u>Antrodia sinuosa</u>) are serious diseases affecting lemon trees in Southwest Arizona. We applied K-Phite (Mono- and di-potassium salts of Phosphorous Acid), Oxidate/Dioxysolv ($H_2O_2$), SAver (Salicylic acid), Sonata (Bacillus pumilus), and Serenade WP (Bacillus subtilis) to trees infested with <u>Antrodia sinuosa</u> in the hopes of eliciting an SAR response and reducing the length of the disease in infested branches. Application of the products improved yield in treated trees, though that improvement was not statistically significant. The treatments did not, however, reduce the length of infestation in inoculated branches.*

## 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 more aggressive and a greater threat to lemon trees than is *Coniophora*. Both are known collectively as "brown wood rot".

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 Propaconizole 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, using rubber bags, 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. While 2012 research showed a reduction in the length of the *Antrodia* decay columns with Azoxystrobin alone, Azoxystrobin + Propiconazole and a Zn + Mn + Fe treatment, the reduction was not significantly different than the control (Wright, 2015a). Further research in 2013 led to no significant differences among the treatments (Wright, 2015b). These

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results along with the high cost of the system has led to the realization that there needs to be an alternative method of control of brown wood rot

Systemic acquired resistance (SAR) is a natural plant defense mechanisms, and a natural biological defense response to fungal attack. One of the biochemical pathways involved in SAR is the salicylic acid pathway. This pathway involves the production of active oxygen species (hydrogen peroxide, peroxidase) that have been found to lead to fungal cell wall degradation, pathogen defense signaling, and thickening of the plant cell wall through increased lignifications and production of phenolic esters that strengthen cross linking in the cell wall. The pathway also leads to systemic and local accumulation of Pathogenesis Related Proteins (PR-Proteins), such as chitinases and ß-1, 3 Glucanase, and the systemic accumulation of anti-microbial compounds called phytoalexins.

Chitin is found in most fungal cell walls, and chitinases are enzymes that break down these walls. Activity of chitinases is greatly enhanced by Glucanase, and will lead to the degradation of cell walls and the release of other compounds that act as elicitors of defense reactions

Several products are currently sprayed on citrus for suppression of Huanglongbing (HLB) on citrus that are also involved in eliciting SAR. These include K-Phite (Mono- and di-potassium salts of Phosphorous Acid), Oxidate/Dioxysolv ($H_2O_2$), SAver (Salicylic acid), Sonata (*Bacillus pumilus*), and Serenade WP (*Bacillus subtilis*). None of these have ever been tested on trees infested with brown wood rot fungi in Arizona.

The objective of this study is to test several products currently used on citrus that are involved in inducing the SAR pathway to control the growth of brown rot fungi.

## Materials and Methods

Four treatments, each comprising 5 trees are were planned for this experiment:

1. Untreated Control
2. Application of Sonata - 1 gal per 500 gal $H_2O$, Serenade Max WP - 4 lbs. per 500 gal $H_2O$, Oxidate - 1 gal per 500 gal $H_2O$), Renew (3-18-20 w/ Boron, K-phite and Saver) - 10 gal per 500 gal $H_2O$, applied on 7/2 and 8/1/14. This treatment was applied prior to infestation with the fungi (See below).
3. Application of Sonata - 1 gal per 500 gal $H_2O$, Serenade Max WP - 4 lbs. per 500 gal $H_2O$, Oxidate - 1 gal per 500 gal $H_2O$), Renew (3-18-20 w/ Boron, K-phite and Saver) - 10 gal per 500 gal $H_2O$ applied on 9/4 and 10/3/14. This treatment was applied after infestation with the fungi (See below).
4. Application of Sonata - 1 gal per 500 gal $H_2O$, Serenade Max WP - 4 lbs. per 500 gal $H_2O$, Oxidate - 1 gal per 500 gal $H_2O$), Renew (3-18-20 w/ Boron, K-phite and Saver) - 10 gal per 500 gal $H_2O$ applied on 7/2. 8/1, 9/4 and 10/3/14. This treatment was applied prior and after infestation with the fungi (See below).

Applications of Sonata, Oxidate, and Renew were made to the foliage using an air-blast sprayer. Applications of Serenade were made to the orchard floor using a boom-sprayer. Treatment 2, initiated prior to infestation indicates whether these compounds can be used as preventatives. Treatment 3, initiated following infestation indicates whether these compounds can be used as curatives. Treatment 4 initiated before and after infestation indicates whether preventative application and curative application together is more effective than preventative and/or curative alone.

The foliar and soil treatments were each applied to blocks of 15 trees, and there were five blocks per treatment, thus each treatment was applied to 75 trees, and thus for the four treatments, there were 300 trees in the entire experiment.

Meanwhile, in July 2014, we collected some *Antrodia sinuosa* from a fallen tree limb in 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. Then, we chose and marked five trees from each of the blocks of 15 mentioned above. For each treatment, *Antrodia* was applied to at least four branches of each treated tree on 8/27/14 as follows: Holes were drilled in branches, and dowels were tightly inserted in holes, then holes were covered with pruning compound (Figs. 1-4). Thus, the tree branches were “inoculated” with the fungus. This is the methodology found in Matheron *et al.* (2006).

There were two harvests for 2014, the first was on 9/15/14 and the second was on 10/27/14. For each block of 15 trees, five trees were selected for harvest. In each case, total yield per tree was calculated by tallying the number of 30 lb. picking sacks per tree. Then, approximately 70 lbs. of fruit from the entire five-tree subsample was passed through an automated portable fruit sizer, which provides fruit size and exterior quality data.

On January 9, 2015, infected branches were cut from trees, split, and the length of infestation in each branch was measured. An example of a split branch from a control tree is shown in Figure 5. Sometimes, if the infestation was severe, the branch broke while we were attempting to split it.

Data was analyzed using the SPSS statistical package. Analysis of variance and means separation tests were used with a p value of 0.05.

## Results and Discussion

Yield for the trees is shown in Figure 6. First harvest yield ranged from 178 to 288 lbs. per tree for the treated trees, and 208 lbs. per tree for the control. These values did not represent a significant difference. For the second harvest on 10/27, yields ranged from 530 to 640 lbs. per tree for the treated trees, with the July and August applications leading to the greatest yield. The yield for the control trees was just 460 lbs. per tree. These values, despite their apparent difference, were not significantly different due to variability among the blocks. Overall, the yields ranged from 665 lbs. per tree for the control treatments to 846 lbs. per tree for the trees treated four times from July through September.

There was no significant effect of treatment upon first harvest, second harvest or cumulative packout (data not shown). The majority of the lemons harvested were of size 165 (30 to 32%), followed by size 140 (25 to 28%) and size 115 (15 to 18%)

There was no effect of the treatments upon infection length. Control trees averaged 15.4 cm of infection. Trees treated in July and August averaged 15 cm of infestation, those treated in September and October averaged 12.cm, and those treated in all four months averaged 15 cm infestation. Meanwhile, the untreated control trees also averaged 15 cm of infestation.

It is disappointing that there was no effect of the treatments upon the length of inoculated fungal infestation. It is possible that more than one year of treatments will be needed to see an effect. The positive effect of the treatments upon yield suggest that there is some benefit to the tree with the use of these SAR elicitors, and that another year of research would be beneficial.

## Literature Cited

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Figure 1. Removing branches prior to insertion of dowel.

Figure 3. Drilling the hole for the dowel.

Figure 2. Petrie plate with dowels.

Figure 4. Dowel inserted in branch


stain. In this case, the fungus caused the branch to weaken and break.

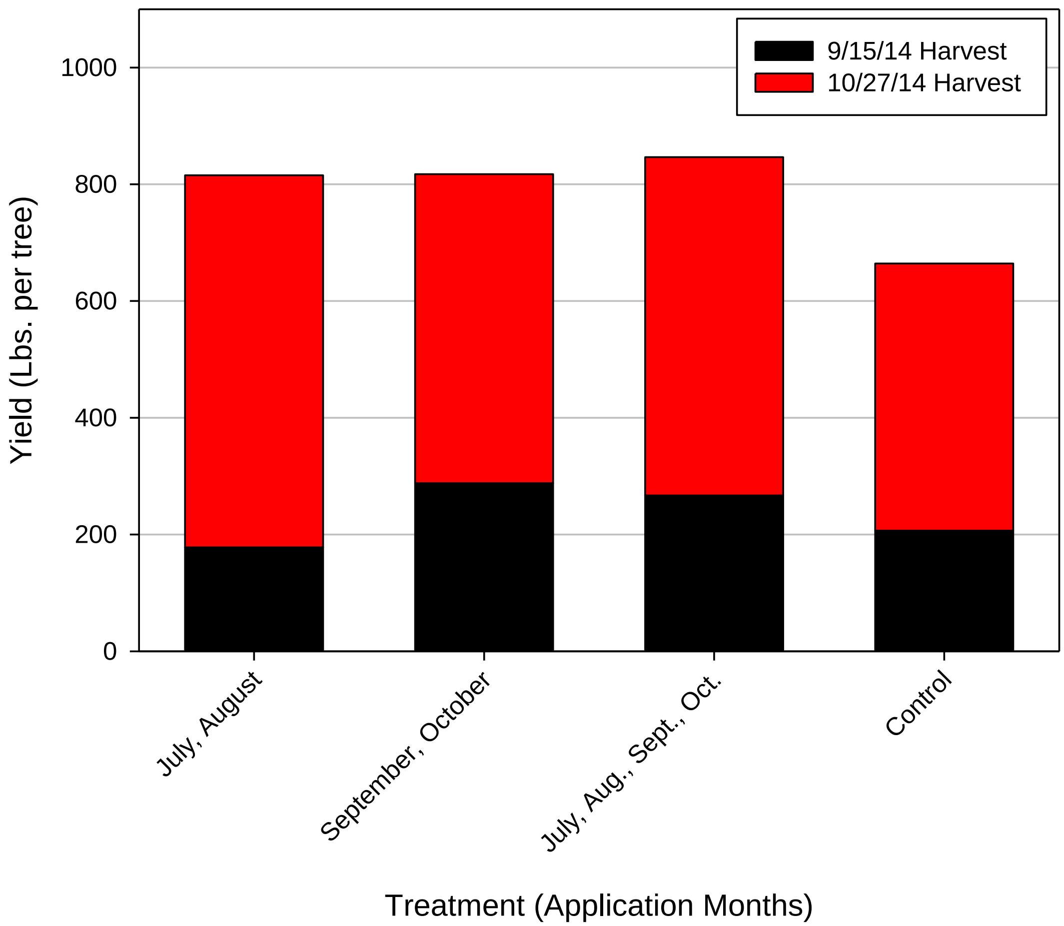


**Figure 6. Yield of lemon trees subject to applications of SAR elicitors.**