

LS Alert E-blast sent locally from me on Aug. 13, 2010:

"Dieback of Simpsons stopper caused by *Neofusicoccum parvum* (*Botryosphaeria parva*) has been a relatively new development in nurseries over the last 3 years or so. We have had a few reports in the landscape, these haven't been confirmed with lab reports. But be on the lookout!



Please see the attached about some research on this disease on Simpsons stopper. Although this is a greenhouse study.....shock...more water (0.7 inch/day-again this was a nursery trial on 3 gallon plants) resulted in less *Botryosphaeria* dieback!! Hence, too little irrigation may contribute to disease development. This is a disease study!? Wow! Not what I would have predicted ! Fertilizer does not play a role.

Fungicides: petri dish studies: copper bombed, *Bacillus subtilis* (Serenade, Cease or similar products) treatments had NO fungal growth; propiconazole (Banner) did well also. That's what makes this type of research exciting! See symptoms on page 5,6 & 8 of the second third attachment." Doug Caldwell

Progress report on studies into the epidemiology and management of *Neofusicoccum* dieback on Eugenia: March 2010

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1) Introduction

Eugenia (scientific name=*Syzigium paniculatum*) is a popular ornamental that is often used in topiaries. A tree native to Australia, it is grown in the US in zones 10 and 11. Nurseries in Miami-Dade County produce an estimated 600,000 eugenia plants per year. After Hurricane Wilma in November 2005, a serious dieback disease of eugenia first appeared in South Florida. Affected nursery plants had wilted, defoliated branches, with dieback symptoms affecting anywhere from a few branches to the entire plant canopy. When diseased branches were cut longitudinally, the vascular tissue had a red discoloration.

The disease is caused by the fungus *Neofusicoccum parvum* (= *Fusicoccum parvum*, teleomorph *Botryosphaeria parva*). *Neofusicoccum parvum* is actually a complex of closely related species that have a wide host range, with 43 different hosts reported in the USDA-ARS Systematic Mycology and Microbiology Fungal Database. It also causes a dieback on *Syzigium cordatum* in South Africa, a native ornamental tree in that country. *Neofusicoccum parvum* has been associated with dieback on many other tropical and subtropical hosts, including avocado, guava, citrus, eucalyptus, and mango.

Disease Cycle and Epidemiology

Dieback occurs mainly in the late summer with the onset of high temperatures. Previous research conducted by Drs. Aaron Palmateer and Randy Ploetz on the effect of temperature and sunlight on disease development determined that the severity of external and internal symptoms increase as temperature increases. Because sunlight did not impact disease severity, the production of eugenia in full sun is not a contributing factor to the disease. Further research is needed to fully characterize the disease cycle and determine the environmental factors that contribute to disease development. Currently, the Palmateer lab is investigating the impact of irrigation and fertilization on disease levels.

Management

It is recommended to use good sanitation measures to manage this disease. This includes pruning symptomatic branches and removing the diseased cuttings from the nursery. Sanitize all tools used to prune or work with plants before each use, and sanitize pruning

shears between each plant. Some growers handle this by dipping shears in bleach followed by oil after drying (to minimize rust). Examples of disinfectants for tools include: 1) 25% chlorine bleach (3 parts water and 1 part bleach); 2) 25% pine oil cleaner (3 parts water and 1 part pine oil); 3) 50% rubbing alcohol (70% isopropyl; equal parts alcohol and water); 4) 50% denatured ethanol (95%; equal parts alcohol and water); 5) 5% quaternary ammonium salts. Soak tools for 10 minutes and rinse in clean water. **Do not mix quaternary ammonia with bleach.**

Chemical control options include applying a copper based fungicide according to the manufacturer's label. It is recommended to apply fungicides after pruning to minimize infection of the freshly cut tissue. Be certain to achieve good coverage, especially on new wounds made during pruning. Because it is likely that plant stress contributes to disease outbreaks, the maintenance of plant health is likely important for dieback management. A study to determine the efficacy of a range of fungicides in managing the disease is planned for Summer 2010.

2) Research Update: Effect of irrigation and fertilization on Eugenia dieback development



Experiment duration: 10/21/09-12/15/09

Experimental Methods: Tissue cultured eugenia liners in two gallon pots were used for the experiment. Plants were treated with combinations of three irrigation and fertility rates (Table 1). A commercial slow release 18-6-8 fertilizer was applied once at the beginning of the trial. One week after treatments started, plants were inoculated with *Neofusicoccum parvum* or not inoculated, and disease ratings were taken bi-weekly. Three disease ratings were recorded: 1) a 0-5 rating for disease (0=no symptoms, 1=symptomatic branches slightly wilted, 2=symptomatic branches wilted with no necrosis, 3=symptomatic branches up to 50% necrosis, 4= symptomatic branches >50% necrotic, 5= symptomatic branches completely dead); 2) the total number of symptomatic branches; and 3) the total percent area of the plant exhibiting symptoms. Ratings continued for 7 weeks. At the end of the experiment, plant height, internal vascular discoloration, and wet and dry weights of roots and aerial portions of plants were measured.

Table 1. Rates of irrigation and fertilization for treatment combinations of irrigation-fertility experiment.

| | High | Medium | Low |
|-------------------------------------|-------------|---------------|-------------|
| Irrigation (mean inches/day) | 0.71 in/day | 0.25 in/day | 0.13 in/day |
| Fertilizer (18-6-8) | 50 g/pot | 35 g/pot | 25 g/pot |

Results and Discussion:

- Dieback symptoms developed on all inoculated plants, and began 10-12 days after inoculation. Symptoms increased in severity throughout the experiment period. Most inoculated plants developed severe dieback on the terminal of the plant, and many exhibited lateral branch dieback as well.
- In general, plants treated with the highest irrigation rate had slightly lower disease levels (fewer number of diseased branches and lower overall ratings), whereas there was no difference in disease between the medium and low irrigation rates (Fig 1-2).
- There was no apparent effect of fertilizer rates on disease.
- All inoculated plants exhibited extensive vascular discoloration, often extending the entire length of the main stem.
- Irrigation also had a significant effect on plant height and aerial mass of the plant. Higher irrigation levels resulted in larger plants overall.
- A single branch on one non-inoculated plant displayed dieback symptoms; all other non-inoculated plants were healthy with no symptoms. This indicates that the liners produced from tissue culture are disease-free. Nursery plants likely become infected in production, possibly through pruning with contaminated equipment.

Overall, this study suggests that fertility does not play a role in the development of eugenia dieback. Based on the disease data for the first experiment, too little irrigation may be a contributing factor for greater disease development. However, irrigation management alone will not eliminate dieback from a nursery. We are currently repeating the irrigation/fertilization experiment to determine if the same trends are observed.

Figure 1. Number of symptomatic branches per plant for each irrigation/fertility treatment. Only inoculated treatments are shown because disease did not develop on non-inoculated plants. Treatment codes: HW=high water; MW=medium water; LW=low water; HF=high fertility; MF=medium fertility; LF=low fertility; I=inoculated.

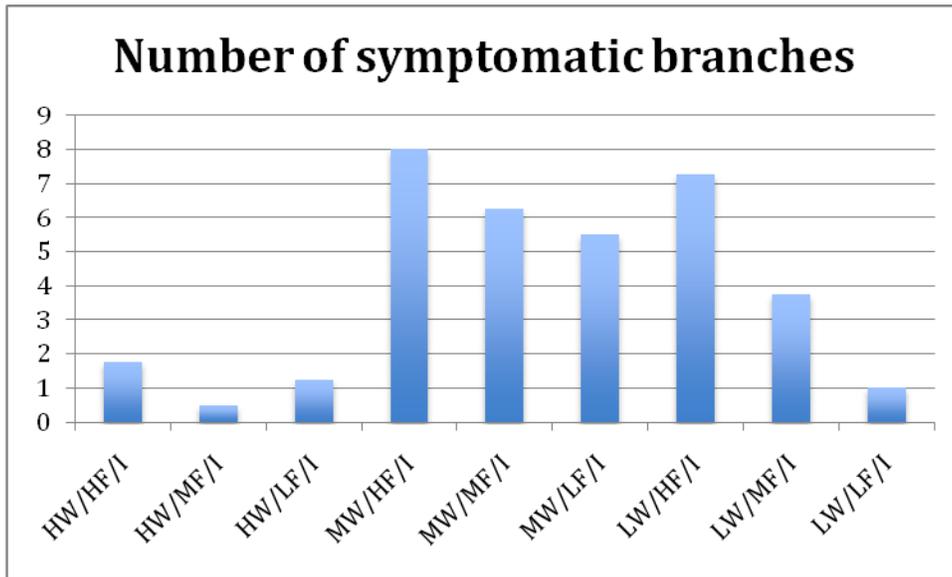
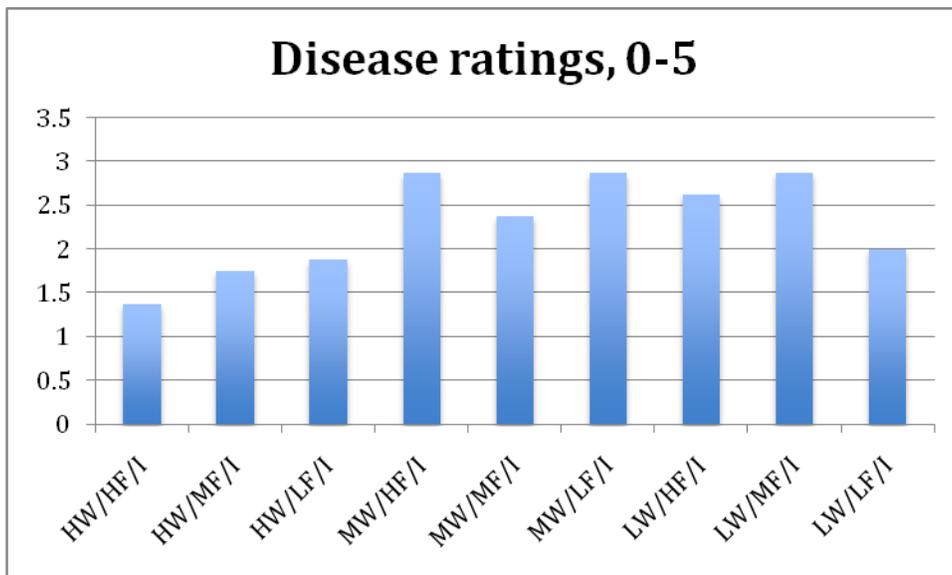


Figure 2. Final disease ratings for inoculated plants (11/30/09). Treatment codes are identical to those described in Figure 1.



3) Research Update: Efficacy of fungicides on *in vitro* growth of *Neofusicoccum parvum*.

Currently no data exists on the efficacy of fungicides for the management of eugenia dieback. Our study will first examine the inhibitory effect of several fungicides on the growth of the pathogen *Neofusicoccum parvum* on artificial growth medium, and then on efficacy against disease development in inoculated containerized plants.

Experimental Methods: Several fungicides were tested *in vitro* for their inhibitory effect on the growth of *N. parvum*. Potato dextrose agar (PDA) was amended with 0.001, 0.01, 0.1, 1, and 10 parts per million (ppm) of eight active ingredients (Table 2). Plates were then inoculated with agar plugs of *N. parvum*. Colony diameter was measured after three days and compared to the growth of *N. parvum* on non-amended agar.

Results and Discussion: The percentage that colony growth was inhibited for each of the concentrations of the eight active ingredients is shown in Table 2. Figure 3 compares inhibition for 10 ppm only. Figure 4 shows growth of *N. parvum* on PDA amended with 5 concentrations of propiconazole.

- Propiconazole, myclobutanil, and the biocontrol agent *Bacillus subtilis* inhibited colony growth completely at 10 ppm.
- The biocontrol agents *Bacillus subtilis* eliminated growth of the fungus at all concentrations tested.
- The strobilurins, azoxystrobin and pyraclostrobin were also effective, inhibiting growth by approximately 80% at 0.1ppm, but trifloxystrobin was less effective, with only 54-60% inhibition at any of the concentrations.
- The fungicide currently recommended for eugenia dieback, copper sulfate was the least inhibitory active ingredient.

In the summer of 2010, we are planning *in vivo* experiments to determine the efficacy of several of these active ingredients in inhibiting disease when applied to inoculated eugenia plants. Containerized eugenia plants will be inoculated with *N. parvum*, and several fungicides tested for their ability to decrease and/or prevent disease development.

Table 2. Percent growth inhibition of *N. parvum* on PDA amended with eight different active ingredients at three different concentrations (0.1, 1, 10 parts per million).

| Fungicide active ingredient | Concentration (parts per million) | % growth inhibition |
|---------------------------------------|---|----------------------------|
| propiconazole | 0.1 | 64.2% |
| | 1 | 100% |
| | 10 | 100% |
| chlorothalonil | 0.1 | 10.5% |
| | 1 | 30% |
| | 10 | 86.8% |
| trifloxystrobin | 0.1 | 59.5% |
| | 1 | 60.7% |
| | 10 | 54.1% |
| azoxystrobin | 0.1 | 79% |
| | 1 | 73.5% |
| | 10 | 77.4% |
| pyraclostrobin | 0.1 | 79.8% |
| | 1 | 78.2% |
| | 10 | 78.2% |
| myclobutanil | 0.1 | 37% |
| | 1 | 64.6% |
| | 10 | 100% |
| <i>Bacillus subtilis</i> (biocontrol) | 0.1 | 100% |
| | 1 | 100% |
| | 10 | 100% |
| copper sulfate | 0.1 | 39.7% |
| | 1 | 40.9% |
| | 10 | 50.6% |

Figure 3. Percent growth inhibition of *N. parvum* on PDA amended with eight different active ingredients at 10 parts per million. 100% inhibition signifies no growth occurred for that fungicide.

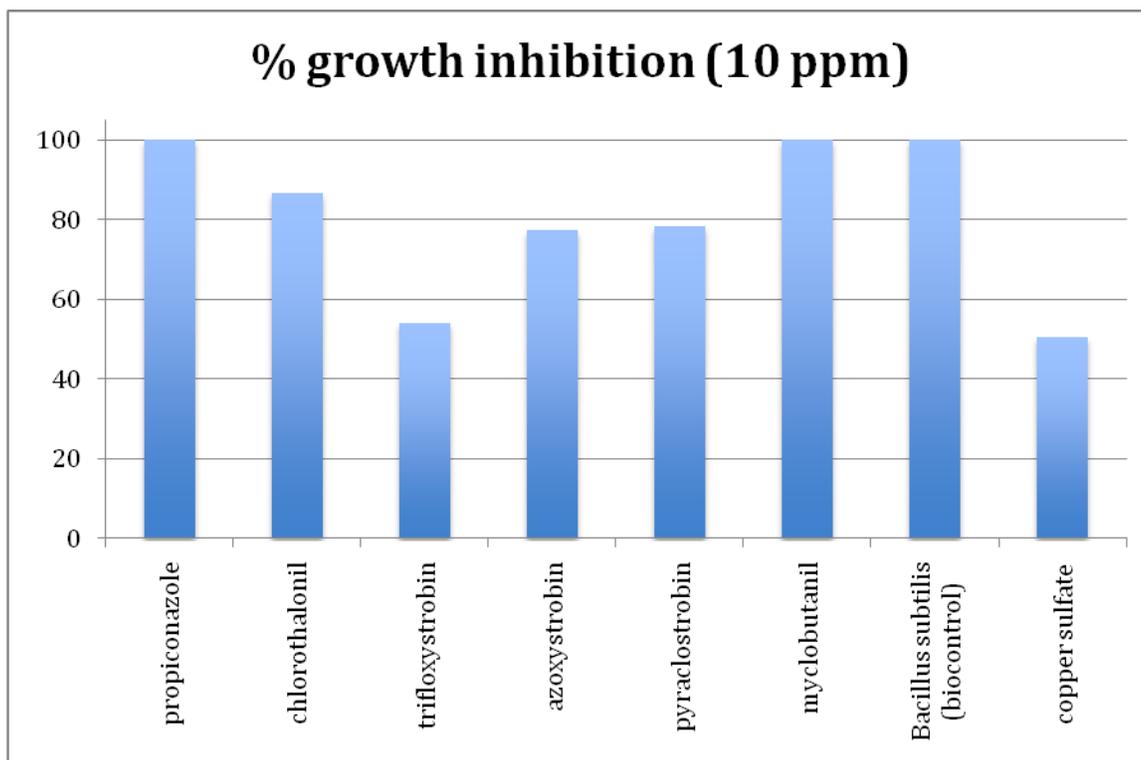


Figure 4. In vitro growth of *N. parvum* on PDA amended with propiconazole. Numbers above plates refer to parts per million concentration of fungicide.

