In vitro sensitivity of a vascular wilt pathogen *Raffaelea lauricola* to paclobutrazol

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Introduction

Raffaelea lauricola is an invasive fungal vascular wilt pathogen that was first detected in Georgia, U.S.A., in 2002. Its infecting of genera in the Lauraceae, causing laurel wilt, has been devastating to native forested ecosystems across the Southeastern United States as far west as Louisiana. Recently, studies involving preventatively treating redbay (*Persea borbonia*), an abundant species integral to these environs, with systemic fungicides in the triazole group have shown to be promising[1]. Paclobutrazol (PBZ) belongs to the triazole group of fungicides and acts as a plant growth regulator (PGR). It is commonly used as a PGR in horticultural and arboricultural industries on ornamentals and shade trees.

PBZ has been shown to affect the growth of fungi and alter the disease progression caused by infections due to *Botrytis cinerea* on cut roses[2], *Verticillium dahliae* on cotton[3], and fungal infections on other economically important crops. Jacobs and Berg (2000) previously examined PBZ sensitivity on vascular wilt pathogen *Ophiostoma novo-ulmi* using fewer concentrations of PBZ and in their work fungi were grown on amended water cultures[4]. Jacobs and Berg also reported reduced growth rates *Armillaria gallica*, *Botryospheria dothidea*, *Ceratocystis fagacearum*, *Fusarium roseum*, *Ophiostoma novo-ulmi*, *Sirococcus clavigignenti-juglandacearum*, *Sphaeropsis sapinea*, and *Verticillium dahliae*[4].

The goal of this project is to determine whether PBZ affects the 2-dimensional growth rates of *R. lauricola* and whether the exposure to PBZ has any long-term effects on the fungus's growth once transferred to unamended MEA.

Figure 1. Experiment 1 - Two dimensional growth of *Raffaelea lauricola* on paclobutrazol-amended malt extract agar with concentrations between 0 and 1 ppm.

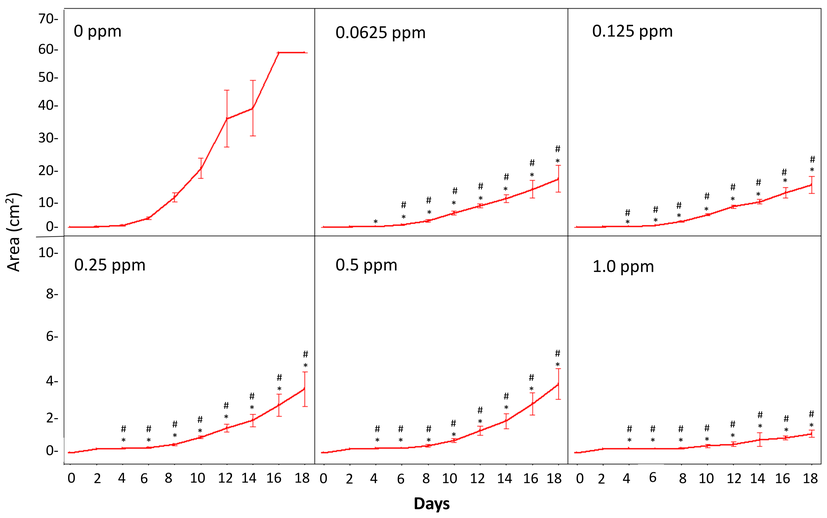


Figure 2. Experiment 2 - Two dimensional growth of *Raffaelea lauricola* on paclobutrazol-amended malt extract agar, replicate of experiment 1.

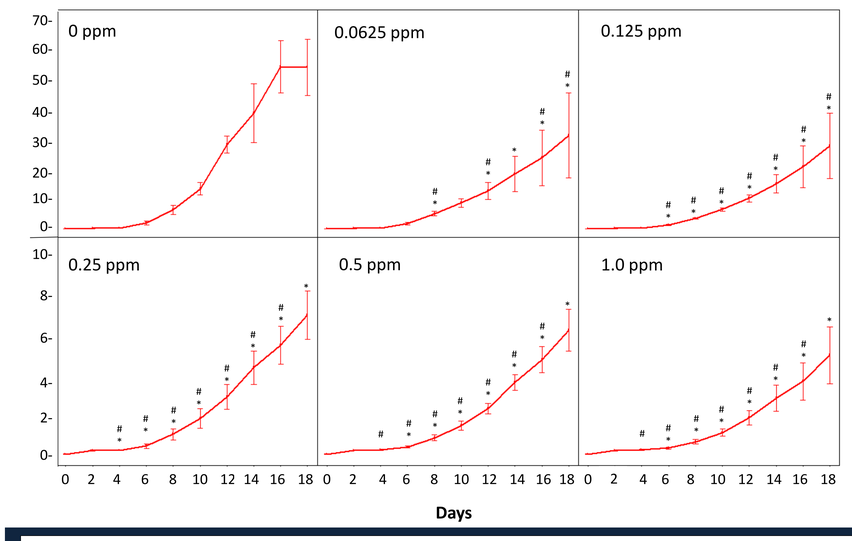
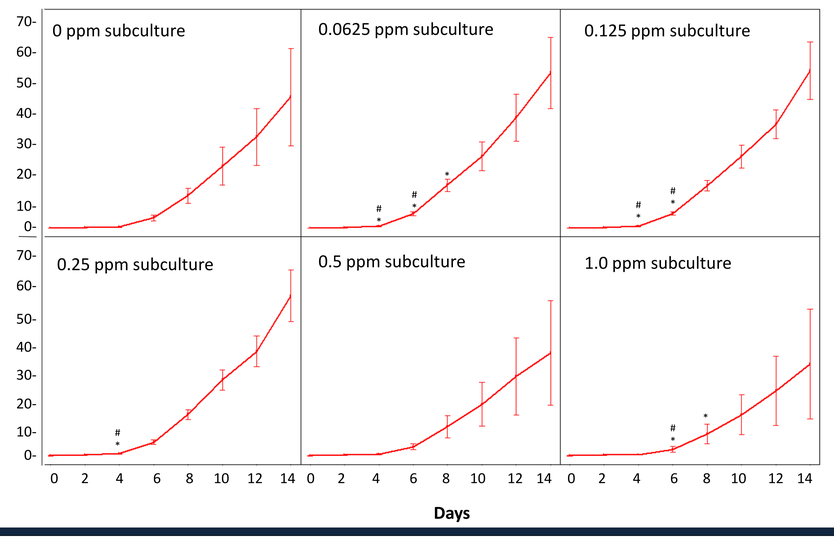


Figure 3. Experiment 3 - Two dimensional growth of *Raffaelea lauricola* after being subcultured from PBZ-amended media (from experiment in Figure 1) onto MEA



* Indicates a significantly reduced area compared to the 0 ppm control at the $p = 0.05$ level
\# Indicates a significantly reduced daily growth rate (cm) compared to the 0 ppm control at the $p = 0.05$ level

Materials and Methods

Malt extract agar was amended with 18 different concentrations (ppm) of PBZ to assess whether the chemical affects the growth rate of *R. lauricola*.

PBZ concentrations tested were: 0, 0.0625, 0.25, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, 20, 40, 50, 100, 400 ppm. The PBZ was added to autoclaved MEA after the media cooled to 50°C. The amended media was stirred using a stir bar for 3 minutes each.

After 18 days all cultures from Experiment 8 were subcultured onto unamended MEA.

All cultures were grown at 25°C in the dark. Isolates were photographed every 48 hours for 14 or 18 days.

Daily growth rates were measured using ImageJ (National Institute of Health).

The data did not reliably meet the assumptions of normality and homogeneity of variance required for a standard ANOVA. Data was evaluated in R utilizing the ANOVA permutation test aovp of the lmperm library. Tukey's HSD test was applied to the resulting data to determine the concentrations which were statistically significant from the control at p=0.05.

1. Mayfield III, A. E., E. L. Barnard, J. A. Smith, S. C. Bernick, J. M. Eickwort, and T. J. Dreaden. 2008. Effect of propiconazole on laurel wilt disease development in redbay trees and on the pathogen *in vitro*. Arboriculture & Urban Forestry, 34: 317-324.
2. Shaul, O., Y. Elad, and N. Zieslin. 1996. Suppression of *Botrytis* blight in cut rose flowers with gibberellic acid. Effects of exogenous application of abscisic acid and paclobutrazol. Postharvest Biology and Technology, 7: 145–150.
3. Cimen, I., S. Basbag, M. Temiz, and A. Sagir. 2004. The effect of paclobutrazol, growth retardant, on cotton growth and *Verticillium* wilt (Verticillium *dahliae* Kleb.). Plant Pathology Journal, 3: 35–39.
4. Jacobs, K. A. and L. C.Berg. 2000. Inhibition of fungal pathogens of woody plants by the plant growth regulator paclobutrazol. Pest Management Science, 56: 407–412.

Results

- *R.* lauricola did not put on any lateral growth on media amended with 2 ppm and higher concentrations of PBZ. The fungus did develop thickened growth around the agar plug in each of the higher-concentration colonies (data not shown).
- The 2-dimensional area of the colonies (*) and the amount of daily growth (cm) (#) were significantly reduced when compared to the 0 ppm control at the p=0.5 rate. PBZ significantly reduced the growth rate of *R. lauricola* even at small concentrations, 1/16 ppm (Figures 1 and 2).
- The colonies subcultured from PBZ-amended media to MEA (Figure 3) did not have significantly reduced growth when compared to the 0 ppm control at the p=0.5 rate.

Discussion

- PBZ-exposed *R. lauricola* colonies did not show a reduction in growth after being subcultured back onto MEA. Mayfield, *et al.*[1] reported fungicidal effects on *R. lauricola* in concentrations of propiconazole and thiabendazole down to 1 ppm. This would indicate that PBZ is possibly not as toxic a fungicide as the other two triazole formulations.
- Colony morphology of *R. lauricola* can vary from strap-looking mycelial growth with undulating margins across the media to slimy dense colonies with smooth margins. These unpredictable and uncontrollable changes in morphology may have created unforeseen variability in the growth of the colonies.
- Examining the use of PBZ to manage laurel wilt by acting as a fungicide/fungistat is only one part of this research program. Future experiments will examine whether applying PBZ to redbay can change the morphology and physiology of the plant in a way that would mimic disease resistance. Many trees that are resistant to vascular wilt diseases have shortened and narrow vessel elements than susceptible clones of the same species. The following experiments will include:
  - Measuring the efficiency of net photosynthesis in response to PBZ treatments, *R. lauricola* inoculations, and both the treatment and inoculations.
  - Assessing tyloses production and vessel element sizes in PBZ treated and untreated trees in response to symptom development to laurel wilt.
- There are currently arboricultural and horticultural PBZ products, Cambistat (Rainbow Treecare Scientific Advancements) and Bonzi (Syngenta) (223,000 ppm and 4000 ppm, respectively), that are labeled for applications at concentration rates much higher than what is necessary to halt *R. lauricola* in this study. Bonzi was used in these current studies, both chemicals may be used in future studies depending on desired application concentrations.