Challenge plate testing to evaluate the inhibitory effects of *Bacillus amyloliquefaciens rm303* (Rhizo-max™) when combined with 2% w/v Sodium Bicarbonate against *Botryosphaeria* spp. root rot complex in Avocado.

Dr. Melinda McHenry, CQUniversity, 21st November 2014

Summary

RhizoMax ™ - a *Bacillus amyloliquefaciens (rm303)* preparation was combined with 2% w/v Sodium Bicarbonate ($NaHCO_3$) applied *in vitro* in a challenge plating experiment against *Botryosphaeria* sp. complex root rot pathogen isolated from Avocado bark sourced from a farm in the Bundaberg area. Following on from previous experimental work, which demonstrated that higher rates (e.g. 5%) of Rhizomax were required to hold the pathogen *in vitro,* four application rates were chosen – 2% Rhizomax, 5% Rhizomax, 2% Rhizomax/2% w/v $NaHCO_3$ and 5% Rhizomax/2% w/v $NaHCO_3$. The combination of Rhizomax (of either concentration) and the 2% w/v $NaHCO_3$ was highly successful in holding Botryosphaeria root rot complex after 72 hours incubation.

Aims

To determine if the *in vitro* addition of Rhizo-Max™ in solution with 2% w/v $NaHCO_3$ suppressed *Botryosphaeria* spp. complex, a root rot pathogen isolated from Avocado bark, foliage, and soil.

Materials & Methods

Sample Preparation

Suspected root rot pathogens (initially suspected to be *Phytopthora* spp.) were isolated from fresh Avocado soil, leaf and bark samples taken from an orchard in the Bundaberg region. Sabouraud Agar (SAB) plates, known to favour *Phytophtora* and to inhibit the growth of bacteria, were used to isolate fungi from sample material provided. Fungal colonies growing on plates were then re-isolated and grown separately on new SAB plates until pure cultures were obtained. Visual and laboratory preliminary inspections indicated pure samples similar in phenology to *Phytopthora* root rot pathogen, however, upon identification at Microgentix Laboratory in Melbourne (Appendix One), it was found that the isolated fungi was instead part of the *Botryosphaeria* spp. root rot complex. *Botryosphaeria* spp. are ubiquitous root rot pathogens and are commonly found in the stems of table grapes and pomes, although recently, the same complex was identified from infected tissues in other Avocado orchards in the Bundaberg region.

Challenge Plate Preparation

Once sporulating (~ 5 days after plating), pure colonies were excised from the SAB plates using a scalpel to cut a plate into a regular grid pattern. Five mm square plugs

were excised from the pure colonies, ready to be implanted into the centre of 13 SAB plates for challenge plating.

Efficacy of Rhizo-Max ™ and Sodium Bicarbonate

Rhizo-Max ™ was prepared at concentrations of 2% and 5% with sterile deionised water. Combination Rhizo-Max and Sodium Bicarbonate solutions were prepared by dissolving 2.00 g of $NaHCO_3$ in 100 ml of sterilised deionised water and swirling for 10 seconds, and then pipetting into the solution either 0.2 or 0.5 ml Rhizo-Max to make combined 2% Rhizo-Max/2% w/v $NaHCO_3$ or 5% Rhizomax/2% w/v $NaHCO_3$ solutions. In order to evaluate the possible inhibitory effects of the products against the pathogen, the product and the pathogen were applied to SAB plates using the *streak plate technique,* two parallel lines were drawn along the plate, approximately 20 mm from either 'side' of the plate. The products were applied along the lines using a sterile flame loop dipped in the corresponding concentration of the product. For each concentration of the product, there were three replicate SAB plates. In the centre of each plate, a 5 mm plug of the suspected pathogen was applied.

In order to determine efficacy of the products, streak plate sample diameters were measured at the start of the trial, and growth was compared to an untreated control. Successful inhibition of the pathogen was determined from challenge plates three days following inoculation if the suspected pathogen had not crossed the product barrier.

Measurements were recorded three days after incubations, due to the aggressive growth of the pathogen. The mean values of two measurements on each plate and standard deviations were presented. The percentage inhibition compared to the control was tabulated from the mean values.

Results & Discussion

Streak plate barriers were highly effective in reducing growth beyond the treated margins after three days, when the combined Rhizomax and Sodium Bicarbonate solutions were used (Table One). Despite previous *in vitro* assessments of the 5% Rhizomax solution working favourably against the pathogen, in this assessment, holding rates were low at ~ 16% efficacy (Figure 1).

Table One. Inhibition of *Botryosphaeria* spp. complex on plates streaked with Rhizo-max ™ at 2% and 5% application rates, with or without addition of 2% w/v Sodium Bicarbonate ($NaHCO_3$), as compared to a control (no treatment).

Treatment	**3 days incubation mean ± S.E. (mm)**	**Percent Inhibition**
Control	25.0 ± 0.0	Nil
2% Rhizo-max ™	23.0 ± 1.2	7%
5% Rhizo-max ™	21.0 ± 4.0	15%
2% Rhizo-max ™ + 2% w/v $NaHCO_3$	-1.7 ± 0.3	107%
5% Rhizo-max ™ + 2% w/v $NaHCO_3$	-2.3 ± 0.7	110%

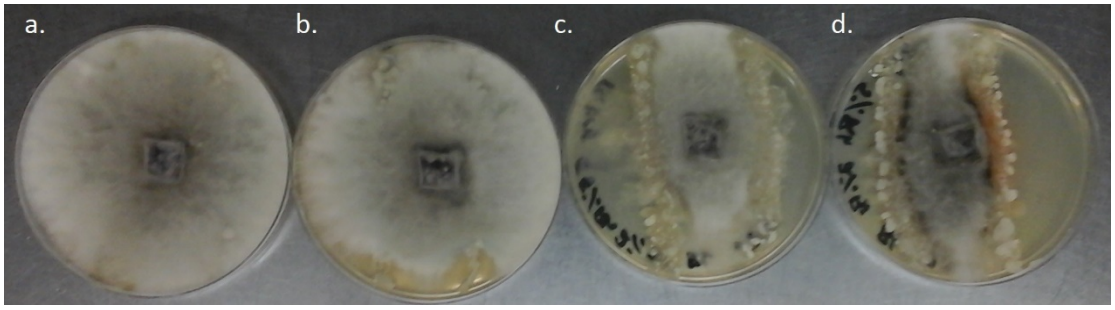

Figure One. *Botryosphaeria spp.* challenge plates. (a.) 2% Rhizo-max (b.) 5% Rhizo-max (c.) 2% Rhizo-max and 2% Sodium Bicarbonate (d.) 5% Rhizo-max and 2% Sodium Bicarbonate

Conclusion and recommendations

The aggressive growth of the isolated *Botryosphaeria* root rot pathogen *in vitro* suggests that frequent and/or highly concentrated treatments might be necessary to inhibit growth of the pathogen *in situ.*

The findings suggest that the combined Rhizomax and Sodium Bicarbonate treatments are most effective in suppressing and inhibiting the growth of *Botryosphaeria* spp. complex *in vitro,* and hence supplementary addition of $NaHCO_3$ might be considered beneficial to extend efficacy of the Rhizo-Max product without substantially reducing profit margins.

It is recommended that the impact of supplementary $NaHCO_3$ addition to the Rhizo-Max product on the survival and activity of the active ingredient, *Bacillus amyloliquefaciens,* be evaluated prior to using a combined product in a commercial setting.