

Special Research Report # : Co-application of biopesticides and chitosan for optimized suppression of *Botrytis* infection in greenhouse floriculture

Disease Management

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BACKGROUND

Botrytis is a common plant pathogen in greenhouse production that can cause devastating losses during production, shipping, and in retail¹. To manage disease successfully and sustainably, an Integrated Pest Management (IPM) approach is necessary. Thus, it is ***critical for floriculture growers to have alternative control methods to use in rotation with fungicides***. Microbial biopesticides are an IPM tool for controlling disease while mitigating fungicide resistance risk². Adoption of biopesticides is growing but their variable performance under commercial conditions is still a challenge³. Advances in biopesticide research reveal that biopesticides work best when used in combination with other strategies. Several natural compounds have been found to enhance biopesticide efficacy with significant implications for IPM. In our previous AFE-funded research, we found that chitosan reduced symptoms caused by *Botrytis* on petunia leaves⁴. Chitosan is a natural biostimulant compound derived from chitin that has fungistatic and antimicrobial properties⁵. In this project, we investigated the use of commercially available chitosan products to enhance the efficacy of microbial biopesticides for control of *Botrytis* gray mold. Objectives of this research were to (1) identify chitosan application rates for use in floriculture; (2) determine compatibility between commercial chitosan products and biopesticides; (3) Evaluate effect of chitosan-biopesticide combinations on *Botrytis* suppression in vitro; and (4) Evaluate chitosan-biopesticide co-application to suppress gray mold on petunia.

MATERIALS AND METHODS

Objective 1. Identify chitosan application rates for disease suppression on flowers.

Since chitosan can cause phytotoxicity, commercial chitosan products were screened to identify application rates that are safe to use and suppress gray mold disease on flowers using petunia as a model crop. A detached flower assay was used for these experiments. Flowers were sprayed to glisten with chitosan treatments ranging from 0.1-0.3% chitosan. Treatments included Tidal Grow low molecular weight (MW), Tidal Grow high MW, ARMOUR-Zen 15, and a water control.

Objective 2: Evaluate the effect of chitosan on biocontrol agent growth.

To co-apply chitosan and biopesticides, research needs to be conducted to determine if biocontrol agents (BCAs) can grow or survive in the presence of chitosan. A simulated tank mix in vitro assay was used to evaluate the effect of commercial chitosan products on BCA viability. Four commercial biopesticides (Table 1) were mixed with seven chitosan treatments (Tidal Grow high MW and ARMOUR-Zen at 0.1, 0.2, and

0.4% chitosan compared to a water control). Stock solutions of each biopesticide was prepared at the label rate. The biopesticide stock solution was then mixed with each of the chitosan products at the three concentrations. The mixture was plated onto culture media and colony forming units (CFUs) were counted after 48 hours.

Table 1. Biopesticide products, active ingredients, and rates.

Product	Active Ingredient	Rate
Cease	<i>Bacillus subtilis</i> QST 713	6 qt / gallon
Rootshield WP	<i>Trichoderma harzianum</i> strain T-22	5.0 oz / gallon
BotryStop WDG	<i>Ulocladium oudemansii</i> strain U3	4 lb / 100 gallon
BotryStop WP	<i>Ulocladium oudemansii</i> strain U3	4 lb / 100 gallon

Objective 3: Evaluate chitosan-biopesticide combination on Botrytis suppression in vitro.

An in vitro dual-culture assay (Figure 1) was conducted to evaluate the effect of commercial chitosan products co-applied with biopesticides on *B. cinerea* growth in culture. Five treatments were evaluated for each chitosan product (Tidal Grow Low MW, Tidal Grow High MW, ARMOUR-Zen) and each biopesticide (Table 1). Treatments included a (1) chitosan + biopesticide, (2) chitosan only, (3) biopesticide only, (4) water only, and (5) chitosan activated biopesticide (the BCA was grown on chitosan-amended media prior to transfer to the plate with *B. cinerea*).

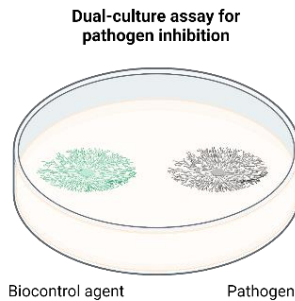


Figure 1: Dual-culture method in which the BCA treatments are grown in culture with *B. cinerea*. Growth of *B. cinerea* is measured to determine if the BCA treatments inhibit growth.

Objective 4: Evaluate chitosan-biopesticide co-application for suppression of gray mold.

Greenhouse experiments were conducted to identify any synergistic effects of commercial chitosan products combined with biopesticides for disease suppression. Four weeks after rooting, petunia plants were sprayed to glisten with the chitosan treatments (Tidal Grow Low MW or ARMOUR-Zen at 0.3%) or with water and allowed to dry. Next, plants were sprayed to glisten with a biopesticide (Cease, Howler, and Botrystop WP) or water.

Twenty-four hours after the treatment application, fifteen leaves per treatment (three from each plant) were collected for a detached leaf assay as described by DeGenring et al. (2023). Leaves were inoculated with 10 μ L drop of a 5.09×10^5 spores \cdot mL⁻¹ suspension of *B. cinerea*. Additionally, twelve flowers per treatment were collected and used for detached flower assay. Flowers were inoculated by spraying to glisten (~0.5 mL) with 1×10^4 spores \cdot mL⁻¹ suspension of *B. cinerea*. Leaves and flowers

were incubated for 48 hours, and disease severity measured using a 1-9 disease severity scale (Figure 2).

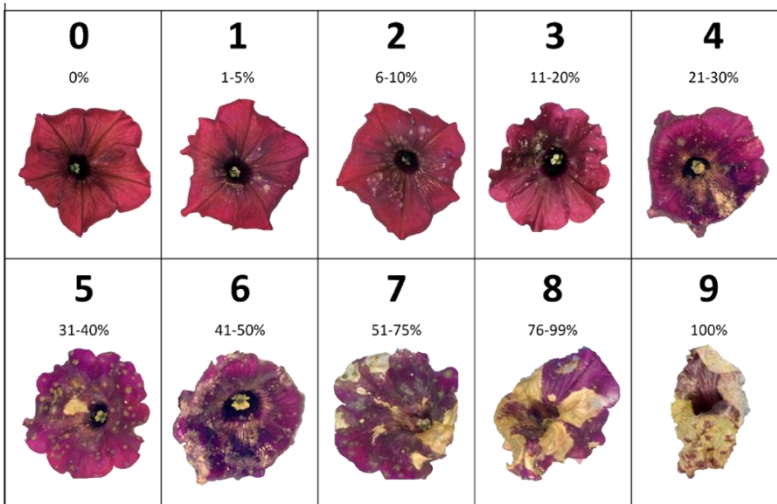


Figure 2. Rating scale used to assess *Botrytis cinerea* severity on Black Cherry petunia flowers. Percentages represent the leaf area showing symptoms. Each flower was assigned a rating (0-9) corresponding to the picture it most clearly resembled.

RESULTS

Objective 1. Identify chitosan application rates for disease suppression on flowers.

Products prepared at 0.2% and 0.3% (v/v) chitosan mixed with a nonionic surfactant CapSil (4oz/100gal) sprayed on flowers provided good spray coverage with low phytotoxicity on flowers (Figure 3).



Figure 3. Supertunia Black Cherry flowers 24 hours after being sprayed with three commercial chitosan products at various chitosan concentrations (0.1-0.3%) with the addition of the nonionic surfactant CapSil (4oz/100 gal).

Objective 2: Evaluate the effect of chitosan on biocontrol agent growth.

Chitosan did not have a negative effect on viability of the four BCAs tested. We noted that when treatments were applied to petri dishes containing culture media, the Biopesticide + Tidal Grow mixture resulted in higher concentrations of *B. subtilis* and *U. oudemansii* compared to the biopesticide + water treatment. However, there was no effect (positive or negative) of Tidal Grow on *T. harzianum* T-22 (Rootshield WP) growth compared to the water mixture. Additionally, ARMOUR-Zen did not have an effect

(positive or negative) on BCA growth when compared to the water mixture. These results indicate that chitosan is compatible with the biopesticides tested in this research.

Objective 3: Evaluate chitosan-biopesticide combination on Botrytis suppression in vitro.

The “activated” biopesticides (BCAs grown on chitosan amended media) were more effective at reducing *B. cinerea* growth in vitro compared to the biopesticide + chitosan treatments (Figure 4). Additionally, this activation enhanced biopesticide efficacy as it was often more effective at reducing *B. cinerea* growth compared to the biopesticide alone treatment.

While results varied between biopesticide product, chitosan product, and concentration, all treatments significantly reduced *B. cinerea* growth in vitro compared to the water control (Figure 4). The biopesticide treatment alone was more effective at reducing *B. cinerea* growth than the chitosan treatment alone. While the combination of the biopesticide + chitosan had greater reduction of *B. cinerea* growth in vitro compared to the chitosan alone, it was no different compared to the biopesticide treatment alone. However, there were synergistic effects observed when the biopesticide was “activated” on chitosan amended media and then plated against *B. cinerea* in the dual culture assay.

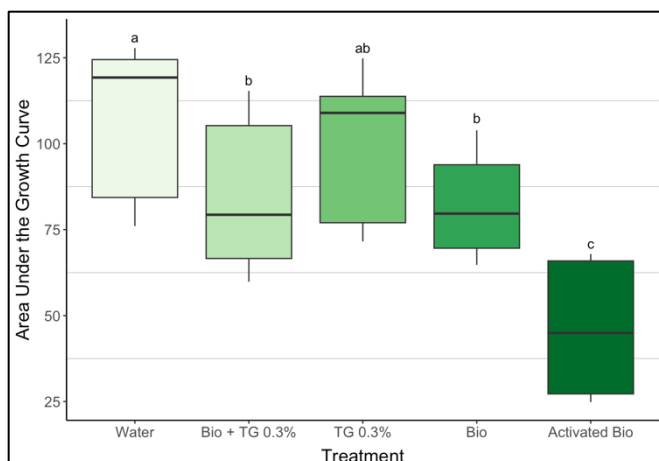


Figure 4. Effect of BotrytStop WP and Tidal Grow high MW at 0.3% on growth of *B. cinerea* alone and in combination compared to water. This is an example of data captured for Objective 3 and the overall trend observed.

Objective 4: Evaluate chitosan-biopesticide co-application for suppression of gray mold.

As observed in previous experiments, leaves from plants treated with Tidal Grow or ARMOUR-Zen alone had smaller lesions than leaves from plants treated with water. While there was an enhanced effect of the biopesticide + chitosan treatments on reducing lesion size, this was largely due to the reduction in disease caused by the chitosan product. Only leaves from plants treated with the Tidal Grow + Cease combination had less disease than leaves from plants treated with Tidal Grow alone or Cease alone, suggesting a true synergism is occurring between *B. subtilis* and chitosan.

Disease suppressive effects and chitosan-biopesticide synergisms were more clearly observed on leaves compared to flowers. This may be because flowers are more susceptible to *B. cinerea* and inoculated flowers often have high disease severity, regardless of treatment. However, there was a 28% reduction in disease severity on

flowers treated with ARMOUR-Zen and Cease compared to flowers just treated with Cease.

CONCLUSIONS

- Chitosan products can be applied to petunia plants during flowering at 0.2% and 0.3% (v/v) mixed with a surfactant.
- There was no negative effect of chitosan on in-vitro growth of the biopesticide products. In some cases, the mixture of Tidal Grow with the biopesticide resulted in higher concentrations of the BCA.
- “Activating” the biopesticide agent on chitosan amended media prior to dual culture assay enhanced the biopesticide suppression of *B. cinerea* growth in culture.
- In-planta, chitosan was more effective at reducing disease caused by *B. cinerea* compared to the biopesticides, but a synergistic effect was observed on leaves of plants treated with Tidal Grow + Cease.

INDUSTRY IMPACT

These results confirm that chitosan is an effective tool for managing *Botrytis* disease. The combination of chitosan + biopesticides did not substantially increase efficacy as expected, however, “activated” biopesticides grown on chitosan amended media were the most effective. This suggests that formulating biopesticides in a chitosan amended solution, may enhance their efficacy and eliminate the need for two separate spray applications.

REFERENCES

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