



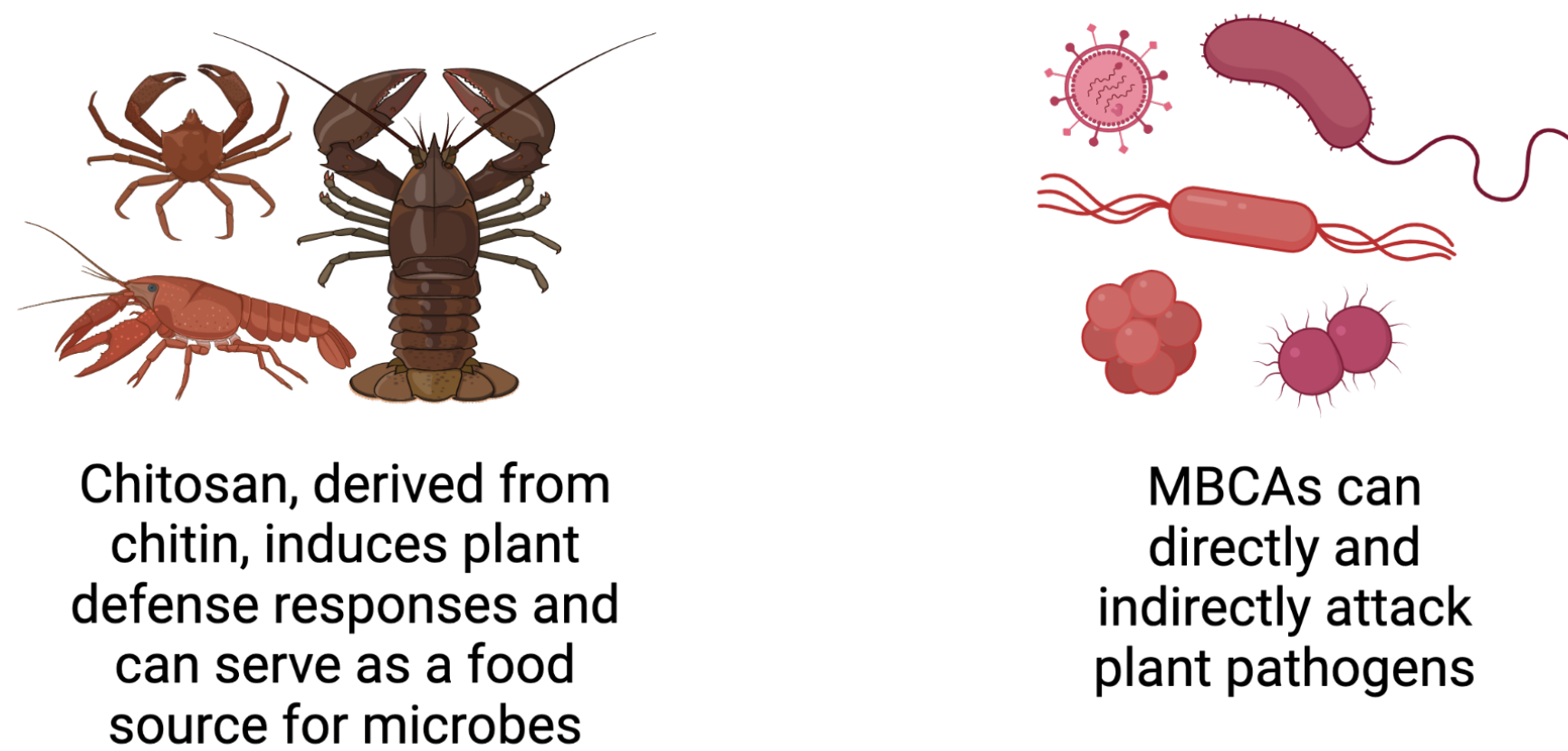
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Investigating the Effects of Chitosan Products on the Growth of Microbial Biological Control Agents

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Introduction

- Pathogens result in 17-30% of devastating crop loss worldwide (Ristaino et al., 2021)
- Natural products and biological methods offer sustainable solutions for crop protection
- Microbial biological control agents (MBCAs) indirectly inhibit pathogens by inducing plant resistance, priming plants to pathogen attack and competing for nutrients or space (Köhl et al., 2019)
- Chitosan products are derived from waste generated by the seafood industry, and can reduce pathogen infections in a greenhouse setting (DeGenring et al., 2023)
- While MBCAs and chitosan products are researched independently, little is known about how simultaneous use can affect their efficacy as plant disease mitigators (Figure 1).



Chitosan, derived from chitin, induces plant defense responses and can serve as a food source for microbes

MBCAs can directly and indirectly attack plant pathogens

The use of these products together is still to be investigated and understood

Figure 1. The current knowledge regarding chitosan products and MBCAs, and how that information serves as a precursor for studying interactions between the two products.

Project Objectives:

1. To evaluate if mixing MBCAs and chitosan products affects survival of MBCAs.
2. To evaluate if long-term mixing MBCAs with chitosan affects the MBCA population and survival.

Long-term Goal:

Identify benefits and best-use practices of chitosan and MBCAs to provide growers with science-based recommendations for managing plant pathogen control.



Methods

Experiment 1: Evaluate if short-term mixing MBCAs and chitosan products affects survival of MBCAs.

This experiment was designed to simulate tank-mixing MBCAs with chitosan. Each MBCA (Table 1) was mixed with four concentrations of chitosan and plated to evaluate MBCA survival (Fig 2). Each treatment had four replicates. The experiment was conducted four times.

Table 1. Commercial MBCA and chitosan treatments used in experiment 1. Chitosan treatments were applied at 0, 0.1, 0.2, and 0.4% (v/v).

MBCA Products	Chitosan Products
Cease, <i>Bacillus subtilis</i> (QST 713)	Tidal Grow 2%
BotryStop WDG, <i>Ulocladium oudemansii</i> (U3)	ArmourZen 15%
Rootshield WP, <i>Trichoderma harzianum</i>	
BW165E WP, unknown species	

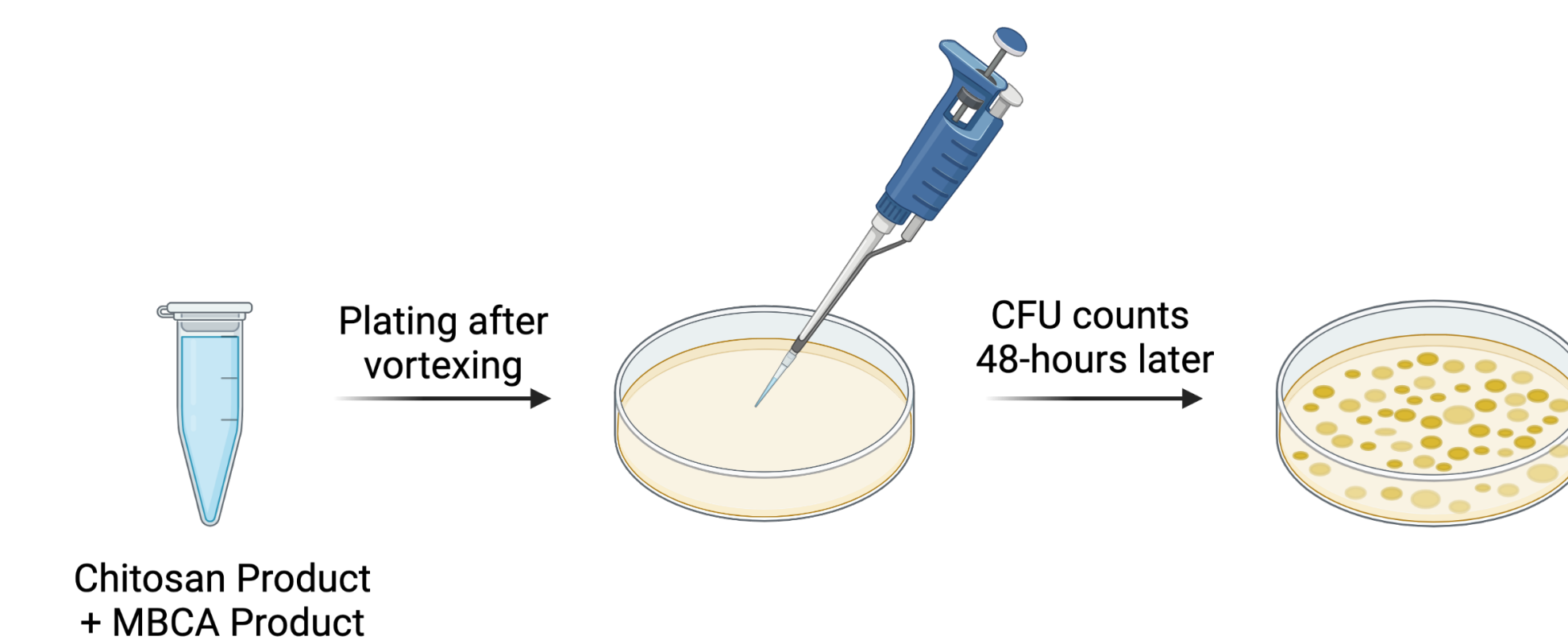


Figure 2. *In vitro* assays where chitosan and MBCA products were mixed then plated on 1/4 PDA for fungal MBCAs and on 1/4 TSA for bacterial MBCAs. Plates were then incubated at room temperature for 48-hours. Colony forming units (CFU) were counted 48-hours after plating.

Experiment 2: Evaluate if long-term mixing MBCAs with chitosan products affects the MBCA population and survival over time.

This experiment was designed to simulate prolonged tank-mixing of MBCAs with chitosan. Cease was mixed with five concentrations of Tidal Grow 2% in a broth mixture and incubated for five days (Fig 3). Each treatment had three replicates. The experiment was conducted twice.

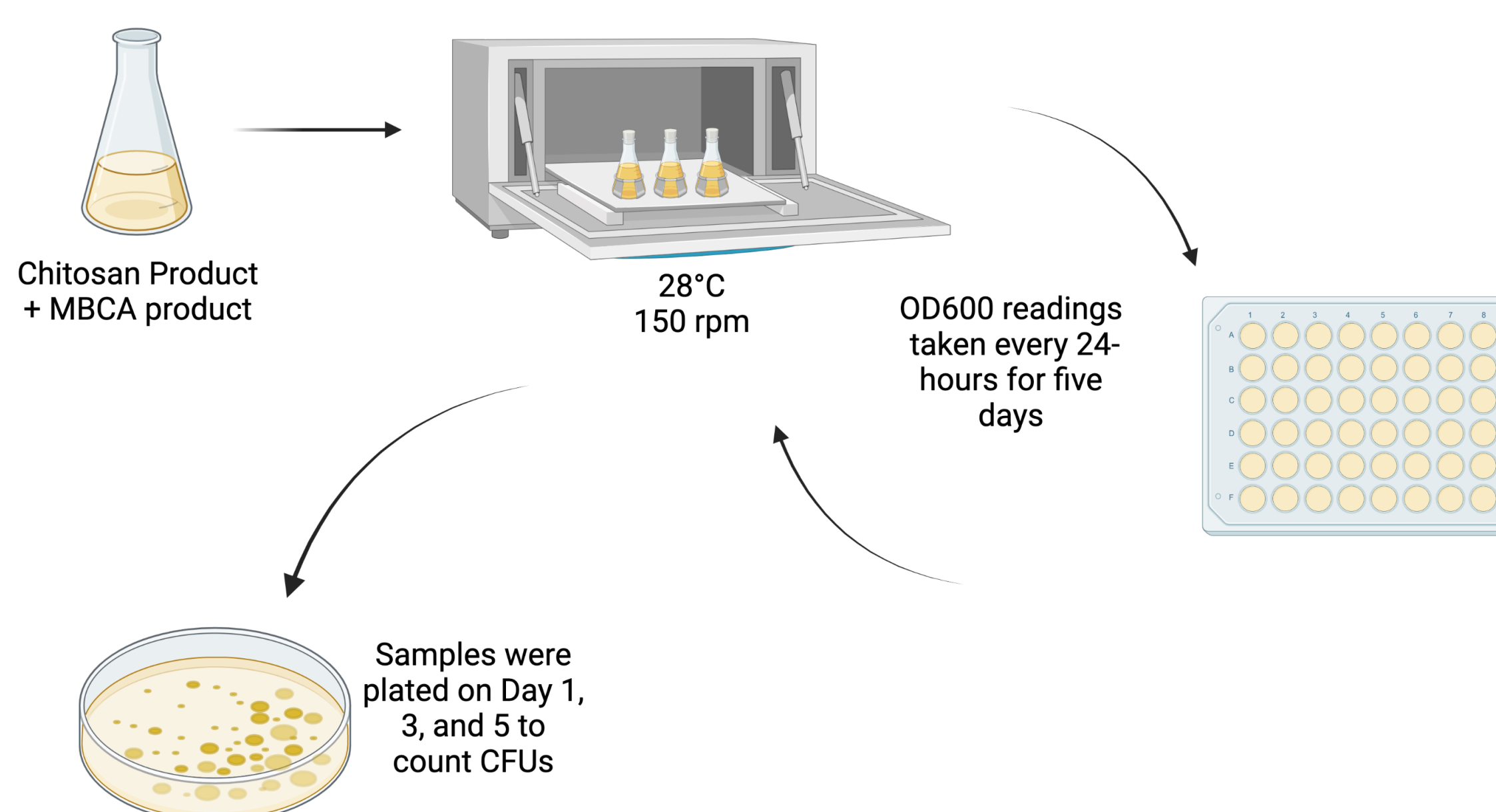


Figure 3. *In vitro* assays where chitosan treatments and MBCA products were used to inoculate broth cultures. OD600 was taken every 24-hours to measure growth of culture. Samples were taken from each flask and plated on days 1, 3, and 5.

Results

- There was no effect of chitosan treatments on CFUs of MBCAs in Rootshield WP (Figure 4.A) or BotryStop WDP (Figure 4.C) when mixed for a short period of time.
- Growth of MBCA in BW165E WP was increased when mixed with Tidal Grow 2% at 0.2% and 0.4% for a short period of time (Figure 4.B).
- Growth of MBCA in Cease was increased when mixed with Tidal Grow 2% at 0.1% and 0.2% and ArmourZen 15% at 0.1% for a short period of time (Figure 4.D).

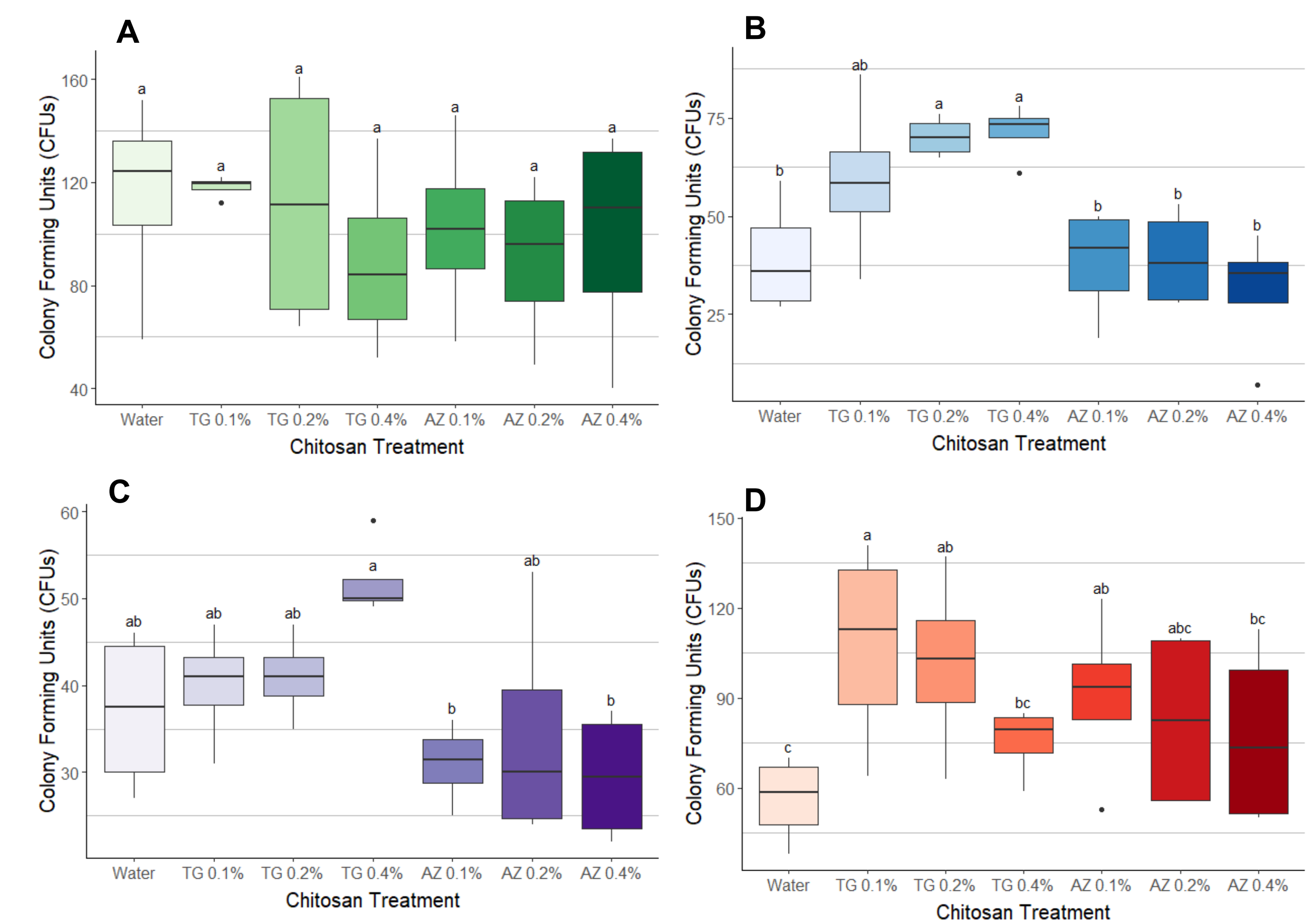


Figure 4. Results of Experiment 1. Effect of commercial chitosan products on growth of four different commercial MBCA products: (A) Rootshield WP, (B) BW165E WP, (C) BotryStop WDG, and (D) Cease. Created by L. DeGenring.

Ongoing and Future Work

- Experiment 2 is currently in progress
- Foundational work for Experiment 2 has already been conducted
 - Construction of a standard curve for the Cease isolate, *Bacillus subtilis* (QST 713) was completed prior to beginning Experiment 2
 - Standard curve will link OD600 and CFUs/mL

Takeaways

- Growth of MBCAs in products BW165E WP and Cease was enhanced when mixed with Tidal Grow 2%. While not significant, growth of MBCAs in BotryStop appears to increase when mixed with Tidal Grow 2% at 0.4%.
- There appears to be no effect of ArmourZen 15% treatments on MBCA growth.

Sources:

DeGenring, L. M., Dickson, R. W., & Poleatewich, A. M. (2023). *Plant Disease*, 107(3), 840-848. Ristaino, J. et al. (2021). *Proceedings of the National Academy of Sciences*, 118(23). Köhl, J., Kolnaar, R., & Ravensberg, W. J. (2019). *Frontiers in Plant Science*, 10.

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