# Assessment of biological control agents on the suppression of *Fusarium proliferatum* on *Cannabis sativa* production in a soilless system

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## Introduction

*Fusarium proliferatum* is a major disease in *cannabis* production. *Fusarium* species can affect the plants throughout their entire life cycle, infecting all tissues including roots, stem and flowers. *Fusarium* spp are difficult to control with current methods and limited number of registered pesticides for *cannabis* cultivation. This study aims to assess the efficacy of biological control agents (BCAs) suppressing the spread and severity of *F. proliferatum* on vegetative *cannabis.* Further, this study evaluates the rootzone colonization of the microbial species from the BCAs in a soilless cultivation system.

## Dual culture assays

To assess whether the BCAs are effective at controlling *F, proliferatum,* six biocontrol agents were tested in dual culture assays with *Fusarium*. Four of the six BCAs: Actinovate AG (*Streptomyces lydicus),* Jumpstart (*Penicillium bilaie*), Quickroots (*Trichoderma virens + Bacillus amyloliquefaciens*), Rootshield WP (*T. harzianum + T. virens*), showed greater than 50% reduction of pathogen growth, whereas Miicrobial Mass (*B. velezenesis, B. licheniformis, + B. megaterium*), and Dr Marijane (*B. subtilis, B. amyloliquefaciens, + Pseudomonas monteilii*) were not able to control the pathogen in vitro.

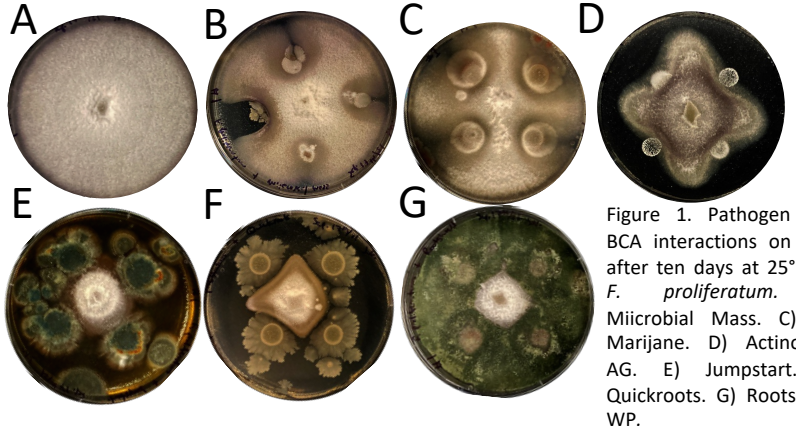


Figure 1. Pathogen and BCA interactions on PDA after ten days at 25°C. A) *F. proliferatum.* B) Miicrobial Mass. C) Dr. Marijane. D) Actinovate AG. E) Jumpstart. F) Quickroots. G) Rootshield WP.

## Plant assessment

Further testing of the four BCAs on their suppression of *Fusarium* occurred in a commercial-like setting on two strains of vegetative cannabis 'Duke Nukem` (DK) and 'Royal Goddess` (RG). This trial consisted of 6 treatments including a non-inoculated control, a *Fusarium* only inoculated treatment and four biocontrol's: Actinovate AG, Jumpstart, Quickroots, and Rootshield WP which were all inoculated with *F. proliferatum*($10^6$ CFU/mL) twice, 19-days apart. Treatments and *Fusarium* inoculum were all applied to the rootzone. Visual ratings of the spread of the pathogen on the tops of the rockwool blocks were taken weekly for 49-days.

Figure 2. Representative spread of *Fusarium* over the top of the rockwool block of each treatment after 49 days. Disease coverage was visually rated weekly for percent coverage of white mycelium of *F. proliferatum.* Left to right; Control – 16%, Fusarium – 60%, Actinovate – 28 %, Jumpstart – 48 %, Quickroots – 34%, Rootshield – 26 %.

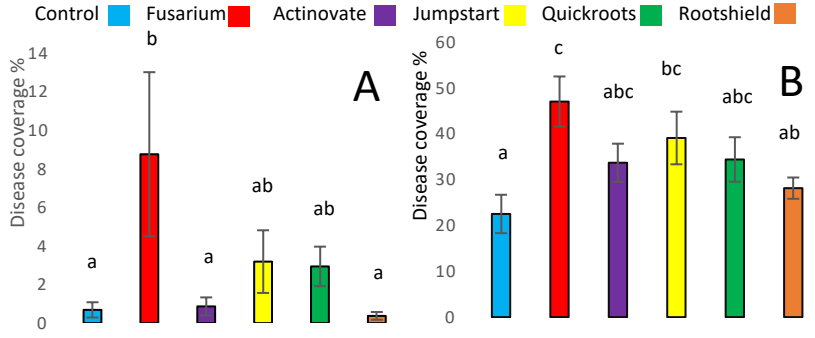


Figure 3. Disease spread on the tops of the rockwool blocks. No differences occurred between DK and RG, so the data was pooled. A) Disease coverage at 28-days post inoculation (dpi). B) Disease coverage at 49 dpi. One-way ANOVA was used at $p<0.05$, and Tukeys Post HOC test determined treatment differences as denoted by the letters. (n=95)

## Microbial recovery

Plant root tissues were sampled from central root system near the crown 49 dpi and surface sterilized with 70% ethanol followed by vortexing in 0.5% NaOCl for two minutes and rinsing thrice with sterile water. Root epidermis was removed, and the roots were plated on PDA amended petri dishes at 25°C.

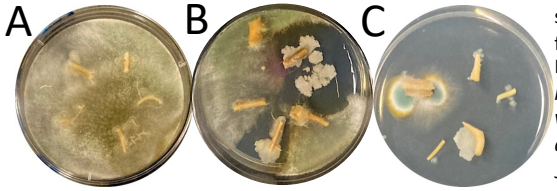


Figure 4. Recovery of microbial species of biocontrol agents from plant roots. A) Rootshield: *T. virens and T. harzianum*. B) Quickroots: *T. virens and B. amyloliquefaciens*. C) Jumpstart: *Penicillium bilaie*

## Conclusion

The objectives of this study were to determine the efficacy of BCAs *in vitro* and in a *cannabis* cultivation setting on the suppression of *F. proliferatum*. Dual culture assays demonstrate that BCAs: Actinovate AG, Jumpstart, Quickroots and Rootshield WP all exhibit >50% suppression of the pathogen *in vitro*. Next, observations in a cultivation setting demonstrated that the BCAs performed the same on two strains of *cannabis.* Actinovate AG can suppress the spread of *F. proliferatum* on the tops of the rockwool blocks for first 28-dpi, while Rootshield WP is significant up to 49-dpi . Further, the recovery of the microbial species of *Trichoderma, Bacillus* and *Penicillium* after 49-days from root tissue samples demonstrates these species can colonize and persist with *cannabis* plants in a soilless system. Additional research on flowering cannabis is being conducted currently in our lab to determine BCA treatment effects on yield and potency of secondary metabolites after inoculation *F. proliferatum*.

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