Rhizospheric Microbiome's Effect on Rooted Cuttings in Cannabis Using a Proprietary Microbial Solution from GMB: A Validation Study

Dr. Robert Flannery
Dr. Robb Farms, Parcel 12, Coachillin Cannabusiness Park, Desert Hot Springs, CA 92282

Introduction

A growing body of evidence contradicts the once accepted notion that the soil-root interface is merely a source of nutrients for plants (Bonkowski, et al., 2009). The rhizosphere hosts a diverse community of micro- and macrobiota that form a wide range of ecological relationships with plants (competitive, amensal, neutral, commensal, mutualistic). Plant science research historically has focused on the commensal and mutualistic relationships through both biotic and abiotic interactions with roots and the rhizospheric microbiome for targets for microbial supplementation in economically important crops (Zhang, et al., 2013; Meena, et al., 2017). For over 30 years, the plant science community has focused on adding specific bacterial strains (such as those found in the genera *Pseudomonas* and *Azospirillum*) known for promoting vigor and crop productivity (Burr, et al., 1978; Lin, et al., 1983). However, focusing research on the effects of singular strains of microbes has shifted to researching diverse microbiomes with strains representing multiple taxa in rhizospheric supplementation. A wide variety of microbial activity in the rhizosphere promotes plant health through several mechanisms: increased access to nutrients, nitrogen fixation, and microbial production of phytohormones to promote growth (Compant, et al., 2019; Köberl, et al., 2013). Diverse rhizospheric microbiomes have shown to increase both crop yields and the accumulation of secondary metabolites such as terpenoids (Pagnani, et al., 2018). Additionally, a diverse rhizospheric microbiome promotes increased resistance to pathogenic infection in the roots of plants (Kusari, et al., 2013; Scott, et al., 2018).

Beyond the mutual benefit of promoting growth in both the microbial and plant populations, a healthy and diverse rhizospheric microbiome protects the crop from pathogenic infection. Some rhizospheric microbes produce exude antibiotics that reduces the virulence of specific pathogens (Kim, et al., 2006). Specific strains of *Trichoderma* can outcompete fungal competitors by utilizing antimicrobial compounds of plant pathogens (Elad, et al., 2008). The crops and microbiomes that inhabit the environment directly around the crops form a superorganism that benefits both the microbe and plant (Mendes, et al., 2013). The microbes evolved methods to target plant pathogens to reduce their virulence for the benefit of the greater "superorganism".

Cannabis sativa subsp. *sativa* and *Cannabis sativa* subsp. *indica* are examples of economically important crops that are grown for their secondary metabolites (cannabinoids and aromatic terpenes) and are susceptible to root-borne infections by pathogens such as *Fusarium oxysporum*, *Fusarium solani*, *Fusarium brachygibbosum*, *Pythium dissotocum*, *Pythium myriotylum*, and *Pythium aphanidermatum*. This combination of potential benefits to a diverse

rhizospheric microbiome in commercial cannabis production prompts the question of whether the addition or supplementation of microbes to the rootzone would provide a benefit to the crop.

Anecdotal evidence suggests that the introduction of microbiotics provides a benefit throughout cannabis production, from cutting and rooting from mother stock to vegetative production to the flower cycle. Successful rooting programs are essential in cannabis production. Minimizing time from cutting to emergence of first root and increasing the number of rooted cuttings over the number of total cuttings are the primary metrics used to determine the success of a rooting program. The application of microbiotics to cuttings may improve the success of rooting.

GMB has a proprietary microbial supplement (Green MicroBiotics) that will be tested for its potential plant growth effects starting at cloning. This experiment was designed to validate the effects of different concentrations of GMB's supplement (from here on referred to as "GMB") as it pertains to the amount of time it takes to root from cutting and the percentage of cuttings that survive.

Materials & Methods

A simple validation experiment took place at the Parcel 12 Hybrid Indoor cannabis cultivation facility managed by Dr. Robb Farms. Four treatments and one control group consisting of 10 cuttings in each group were taken on April 15, 2021. All of the cannabis cuttings were taken from a group of 5 mother stock plants (*Cannabis sativa* cv. 'FX') of the same age. The control cuttings were treated with a demineralized water dip (0x), whereas the treatment plants were given varying concentrations of GMB (1x, 2x, 3x, non-diluted stock solution). After the cuttings were taken from the mother stock, the stem was scraped and dipped in 0.10% indole-3-butyric acid rooting powder. The cuttings were then dipped and submerged from the top of the shoot down to just above the rooting hormone application on the stem with either demineralized water or the various concentrations of GMB. The treated cuttings are placed in pre-treated (with nutrient solution at 0.8 dS/m) 2-inch rockwool cubes. The cuttings are then placed in clone trays with transparent hoods, where they remained for 15 days. The cuttings were monitored each day for the emergence of roots or root primordia. Each day the number of cuttings with roots were counted to determine total number of rooted cuttings and the first day of root emergence.

Results

The only experimental groups that had a 100% success rate in rooting were the 3x and non-diluted (ND) treatments at the end of the 15-day trial. The control group showed an 80% success rate, while the 0x, 1x, and 2x treatments had 80%, 90%, and 80%, respectively. The control group (0x) and the 1x treatment did not achieve their final rooting rates until 13 days after cutting. The ND treatment achieved their final rooting rate of 100% after 10 days in the trial (3 days prior to the control group). The ND treatment's first day of root emergence was also 3 days prior to the control group's first root emergence (Fig. 1; Table 1). The non-diluted treatment reached a 50% success rate within 8 days of being cut (faster than any of the treatments). The 0x treatment did not achieve above a 50% success rate until day 10, which is the same day that the

non-diluted treatment reached a 100% success rate. The non-diluted treatment spent 40% of its time in the trial with 100% of the cuttings having rooted (the 3x treatment spent 33% of its time with all of the cuttings showing roots). The 0x treatment achieved an 80% final success rate, of which the group spent 20% of its time during the trial. The plants in the non-diluted treatment only spent 20% of the trial's time without a single plant showing roots compared to the 0x, 1x, & 2x treatments which saw 40% of their time in the trial without roots (3x at 33% of its time without roots).

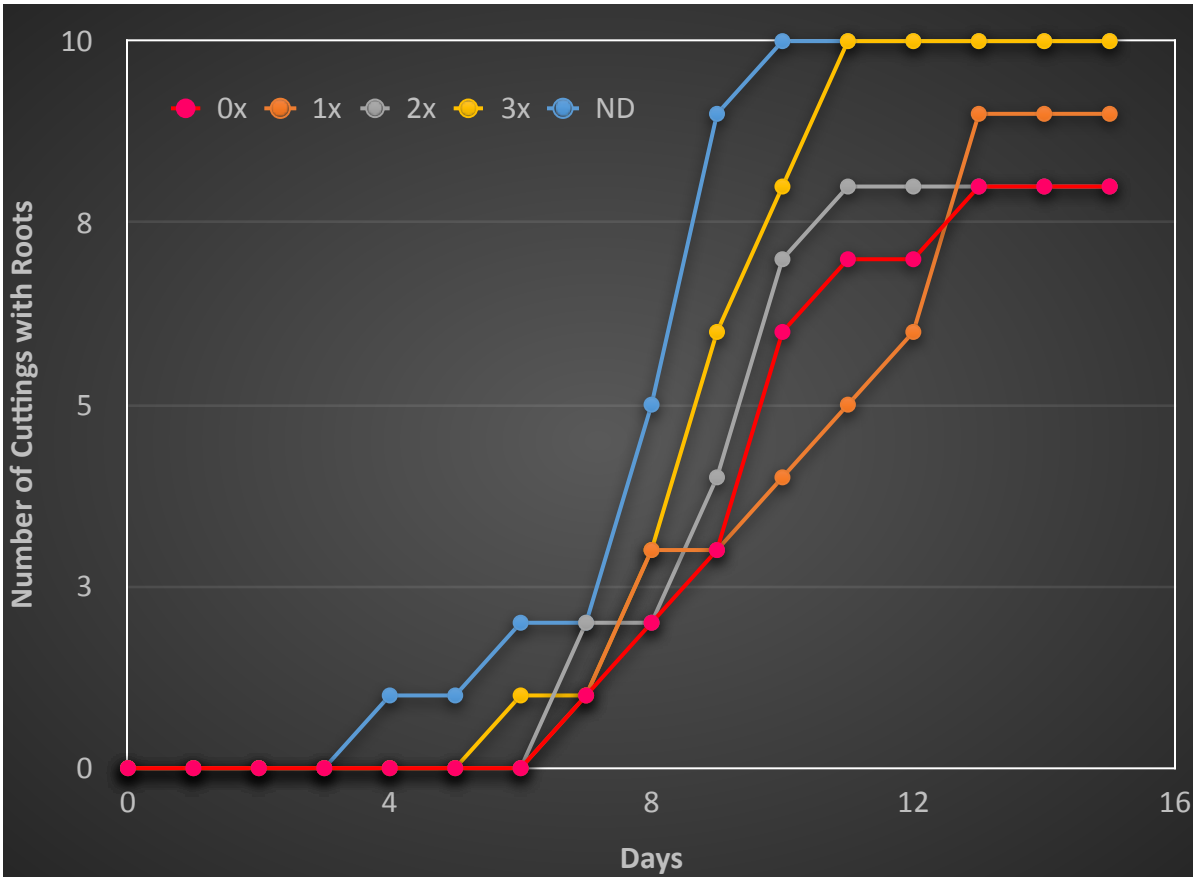

Figure 1. The number of cuttings with roots over the number of days after being cut from the mother stock with cannabis cuttings treated with no GMB, 1x, 2x, 3x, and non-diluted solutions.

	0x		1x		2x		3x		ND	
Day	Rooted Cuttings	% of Rooted Cuttings	Rooted Cuttings	% of Rooted Cuttings	Rooted Cuttings	% of Rooted Cuttings	Rooted Cuttings	% of Rooted Cuttings	Rooted Cuttings	% of Rooted Cuttings
1	0	0%	0	0%	0	0%	0	0%	0	0%
2	0	0%	0	0%	0	0%	0	0%	0	0%
3	0	0%	0	0%	0	0%	0	0%	0	0%
4	0	0%	0	0%	0	0%	0	0%	1	10%
5	0	0%	0	0%	0	0%	0	0%	1	10%
6	0	0%	0	0%	0	0%	1	10%	2	20%
7	1	10%	1	10%	2	20%	1	10%	2	20%
8	2	20%	3	30%	2	20%	3	30%	5	50%
9	3	20%	3	30%	4	40%	6	60%	9	90%
10	6	60%	4	40%	7	70%	8	80%	10	100%
11	7	70%	5	50%	8	80%	10	100%	10	100%
12	7	70%	6	60%	8	80%	10	100%	10	100%
13	8	80%	9	90%	8	80%	10	100%	10	100%
14	8	80%	9	90%	8	80%	10	100%	10	100%
15	8	80%	9	90%	8	80%	10	100%	10	100%

Table 1. Total number of rooted cuttings per day after initial cutting from mother stock and percentage success rate with cannabis cuttings treated with no GMB, 1x, 2x, 3x, and non-diluted solutions.

Figure 2. Day 4 of non-diluted treatment showing root emergence.

Figure 3. Non-diluted treatment on day 14.

Figure 4. 0x treatment on day 14.

Figure 5. 0x treatment with no root emergence on day 14.

Figure 6. 1x treatment on day 14.

Figure 7. 2x treatment on day 14.

Figure 8. 3x treatment on day 14.