

**Effects of Biostimulant and Mycorrhizal Inoculant Applications
on Dry Direct-Seeded Rice**

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Abstract

For years, rice has been grown in flooded fields, but this production system uses up fresh water reserves, degrades soil, and releases methane. As an alternative, dry direct-seeded rice, is being increasingly explored and developed. Dryland production systems reduce negative environmental effects, and direct-seeded systems can reduce labour use and increase profitability. Yield can be reduced in dryland systems, but biostimulants and mycorrhizal inoculants may increase productivity and yield of rice, so they may be promising solutions to develop dry direct-seeded rice production systems. This study tested and compared the effectiveness of biostimulants and a mycorrhizal inoculant on the growth of dry direct-seeded rice in different levels of soil fertility, by measuring plant dry matter accumulation of roots and shoots. Dryland rice production was not significantly impacted by the biostimulants or mycorrhizal inoculant tested.

Introduction

Rice has been grown in flooded fields since 4,000 BC or earlier (Fuller et al. 2011). Flooding is used to suppress weeds and can increase grain yield (Alagbo et al. 2022; Vistan 2023), but flooded rice fields are conducive to methane production (Li et al. 2005). Moreover, flooding also uses up fresh water reserves and degrades soil quality (Liu et al. 2015), so researchers have worked to find solutions to manage water to increase resource efficiency and reduce these negative environmental impacts (Li et al. 2005; Liu et al. 2015). Alternatives have been explored to reduce environmental harm and increase economic efficiency: unflooded systems instead of flooded fields, and direct-seeded rice instead of transplanted rice (Liu et al. 2015; Fatima et al. 2020). Dry direct-seeded rice production systems can be more profitable and produce less methane (Fatima et al. 2020; Magar et al. 2022).

Biostimulants and mycorrhizal inoculants have also been tested as rice growth promoters. Krishnappa et al. (2024) found that a bacterial biostimulant increased immunity against some foliar pathogens and produced indole-3-acetic acid, which increased plant height and grain production. Al-Badiri and Al-Juthery (2022) found that biostimulants paired with potassium supplements increased plant height, grain yield, and agronomic efficiency. Zhang et al. (2016) found that mycorrhizal inoculation of wetland rice increased grain yield, while Das et al. (2021) reported that mycorrhizal inoculation of unflooded rice showed greater benefit in more water-stressed conditions.

Since dryland rice production is relatively new, there is not much research on the effects of biostimulants and mycorrhizal inoculants in unflooded conditions. By measuring plant dry matter accumulation of roots and shoots as indicators of growth, this experiment gathered more knowledge on the effects of biostimulants and mycorrhizal inoculation of rice in dry direct-seeded production systems with different fertility levels.

Objectives

To assess which amendments increase direct-seeded dryland rice growth, measured by plant dry matter accumulation in low and high fertility environments. The different fertility environments were to inform growers if they could use these amendments on their own or if they need to use these amendments with additional fertilizer or healthy soils.

The amendments and intended effects:

1. **Mykos Gold**: powder mycorrhizal inoculant, intended to increase water and nutrient uptake
 - a. Used with **K3NEO Seed Spray**: biostimulant intended to increase fertilizer uptake;

2. **Yeast Guard Foliar Spray**: biostimulant intended to increase the root system, tillering, and grain yield; and
3. **Tecamin MAX Foliar Spray**: liquid fertilizer, intended to increase water retention and absorption, and promote growth.

In general, all these amendments aim to promote growth.

Experiment Hypotheses

The null hypotheses of this study were:

- 1) Gaia Green has no effect on the growth of rice roots or shoots, measured by plant dry matter accumulation.
- 2) Mykos Gold has no effect on the growth of rice roots or shoots, measured by plant dry matter accumulation.
- 3) Yeast Guard has no effect on the growth of rice roots or shoots, measured by plant dry matter accumulation.
- 4) Tecamin MAX has no effect on the growth of rice roots or shoots, measured by plant dry matter accumulation.
- 5) There are no interactions between any of the products tested.

The alternative hypotheses were:

- 1) Gaia Green influences rice growth, measured by plant dry matter accumulation.
- 2) Mykos Gold influences rice growth, measured by plant dry matter accumulation.
- 3) Yeast Guard influences rice growth, measured by plant dry matter accumulation.
- 4) Tecamin MAX influences rice growth, measured by plant dry matter accumulation.
- 5) There is an interaction between the effects of the tested amendments.

Materials and Methods

Experimental Site

The experiment was conducted in the south-west corner of the KPU Farm at the Garden City Lands in Richmond, B.C. The study area was in an open space, near an irrigation source, lined with landscape fabric to separate the soil in the 32 experimental plots from external non-experimental factors. Each experimental plot was a 37 cm by 56 cm (0.2 m²) collapsible crate, about 25 cm deep. Each crate had two drip lines along the sides and was individually lined with landscape fabric to prevent erosion of the growing medium. The crates were spaced closely in an 8 by 4 grid.



Figure 1. Aerial view of KPU Farm, with approximate experimental site labelled.



Figure 2. The 32 experimental plots, arranged in an 8 by 4 grid. Photo taken August 27th, 2024.

Growing Medium

The crates were filled with a growing medium, composed of 50% sand, 23.5% perlite, 23.5% vermiculite, 3% biochar, with 3.78 g (18 g/m²) of Gaia Green 4-4-4 organic fertilizer in each experimental crate. This growing medium was modified from a study that similarly measured the roots of rice grown in containers in non-puddled conditions, using their ratio of sand, silt, and clay as a reference (Kato and Okami 2011). The mixture was primarily sandy to facilitate root-washing while ensuring minimal root loss; water- and nutrient-holding capacity were provided by the vermiculite and biochar. To ensure that the fertility levels were controlled, the sand was taken from a clean sand pile at the experimental site, the silt was substituted with perlite and vermiculite, the clay with biochar, and the nutrients were manually added by the organic fertilizer. The base fertilizer content was informed by local dryland rice growers and vegetable crop producers, to make sure that the rice could still grow in the medium.

Experimental Design

The plots were in a Completely Randomized Factorial Design, with four factors, two levels each, with two replicates:

1. Gaia Green: High or low fertility, explained in *Seeding & Treatments*
2. Mykos Gold: With or without
3. Yeast Guard Foliar Spray: With or without
4. Tecamin MAX Foliar Spray: With or without

Design Properties					
Factors	Levels	Treatments	Replicates	Plots	Seed
4	2 * 2 * 2 * 2	16	2	32	4050

Source	Degrees of freedom
Fertility	1
Seed treatment	1
Tecamin Max	1
Yeast Guard	1
Fertility * Seed treatment	1
Fertility * Tecamin Max	1
Fertility * Yeast Guard	1
Seed treatment * Tecamin	1
Seed treatment * Yeast Guard	1
Tecamin Max * Yeast Guard	1
Residual	21
Total	31

Figure 3. Table of the design properties, with the degrees of freedom.

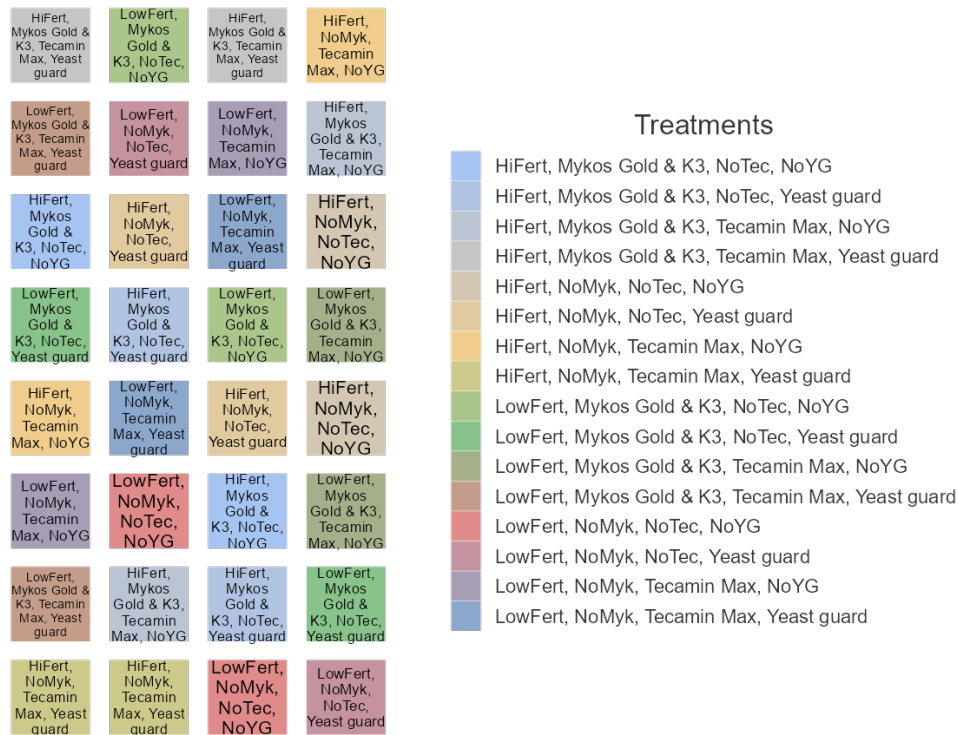


Figure 4. Plot map of the experimental design, with 32 plots, four factors, two levels per factor, and two replicates each. “HiFert” is refers to the High Fertility Treatment; “Mykos Gold & K3” is the Mykos Gold treatment, with the K3NEO included; “NoTec” is short for No Tecamin MAX; “NoYG” is short for No Yeast Guard; “NoMyk” is short for no Mykos Gold mycorrhizal inoculant or K3NEO treatment; and “LowFert” refers to the Low Fertility treatment.

Seeding & Treatments

Rice was directly seeded in the crates. The seeding rate was 10 kg of seed per 1000 m², or 2 g for each 0.2 m² plot; this translated to 12 seed clumps in each plot, 5 seeds per clump, about 2 cm deep. The first round of seeds was planted on May 29th, 2024. The following week, the crates seemed to have been tampered with by crows, many seeds dug up. All crates were covered with a row cover to let the first seeds germinate and see how much needed reseeding. By June 12th, the first seeds germinated, and the empty clumps were reseeded with three seeds each; the clumps were reseeded with three seeds instead of five to ensure that there would be enough seeds for all clumps, as the stock was running low. There was a difference in germination times because of the reseeding, and even after reseeding, there was a difference in the number of seeds per plot because of natural failed germination.

For the Mykos Gold factor, seeds were first coated with the K3NEO seed spray, which allowed the powder mycorrhizal inoculant to adhere to the seeds, as directed by the manufacturer, “at a rate of 10% of total dry seed weight on moistened seeds” (MYKOS GOLD WP n.d.). Yeast Guard and Tecamin MAX foliar sprays were sprayed for leaf coverage once every two weeks, as directed by the manufacturers. To prevent spraying other non-treated plots, treated plots were surrounded with cardboard barriers at each spray. For the fertility factor, Gaia Green was applied at a high and low fertility level. In the high fertility treatment, 200 g of Gaia Green was hand raked into the plots every two weeks. In the low fertility treatment, 200 g of Gaia Green was hand raked at seedling emergence and in “emergency,” when plants looked very nutrient-deficient, as directed by a local rice grower. The quantity and frequency of fertilizer treatments were informed by local dryland rice growers’ fertilizer application frequency and were adjusted to account for the sandy growing medium. The low fertility plots only had one instance of “emergency” application. By the end of the season, the low fertility plots received 400 g, and the high fertility plots 1,400 g.

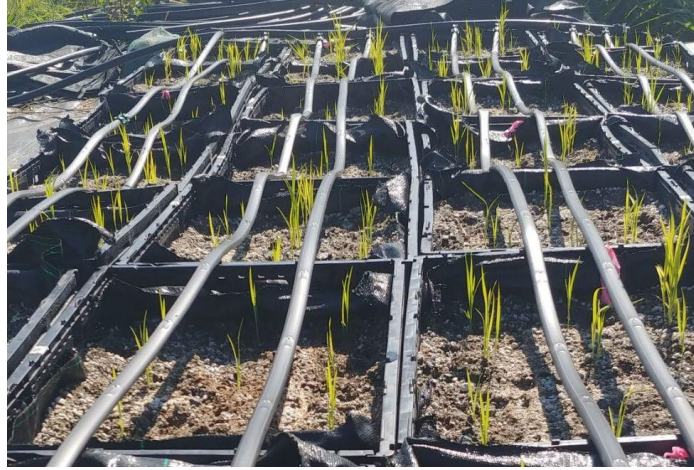


Figure 5. Rice were seeded into 12 seed clumps per plot, 5 seeds per clump.

Data Collection

All rice plants were harvested on October 9th, 2024; the first-seeded plants were 119 days old, and second-seeded were 133 days old. The plants were harvested early because it was supposed that they would not mature in time; grain yield was forgone, to be extrapolated instead from the plant dry matter accumulation, assuming that higher dry matter accumulation would translate into higher grain yield. Each plot of plants was washed in a tub of water to ensure minimal root loss, and double-rinsed to wash off as much growing medium as possible. The plants were patted dry until they no longer dripped, and roots were separated from shoots. Fresh weights were taken of the roots and shoots of all the plants from a plot, then put into bags, separated by roots and shoots. These bags were dried in a drying oven at 70°C, modified from the drying temperature found in Kato and Okami (2011), until the dry weights stabilized. An empty bag was also dried to subtract the bag weight from the plant dry weights accordingly.

Data were analyzed by ANOVA.

Results

The organic granular fertilizer increased plant growth ($p < 0.05$) (Table 1, Figure 6, Figure 7). No significant effects were found for the other amendments tested, Mykos Gold and K3NEO seed spray, Tecamin Max foliar spray, or Yeast Guard foliar spray (Table 1). No interactions were found between amendment effects (Table 1).

Table 1. ANOVA for average dry weight per plot. There was a significant effect found only in the Gaia Green fertility treatment, $p < 0.05$.

ANOVA - Dry weight (g)

	Sum of Squares	df	Mean Square	F	p
Gaia Green	59685.1	1	59685.1	9.98792	0.005
Mykos Gold	7564.5	1	7564.5	1.26587	0.273
Tecamin Max	1250.0	1	1250.0	0.20918	0.652
Yeast Guard	19503.1	1	19503.1	3.26372	0.085
Gaia Green * Mykos Gold	3960.5	1	3960.5	0.66276	0.425
Gaia Green * Tecamin Max	968.0	1	968.0	0.16199	0.691
Mykos Gold * Tecamin Max	55.1	1	55.1	0.00922	0.924
Gaia Green * Yeast Guard	8256.1	1	8256.1	1.38161	0.253
Mykos Gold * Yeast Guard	1740.5	1	1740.5	0.29126	0.595
Tecamin Max * Yeast Guard	4704.5	1	4704.5	0.78727	0.385
Residuals	125490.4	21	5975.7		

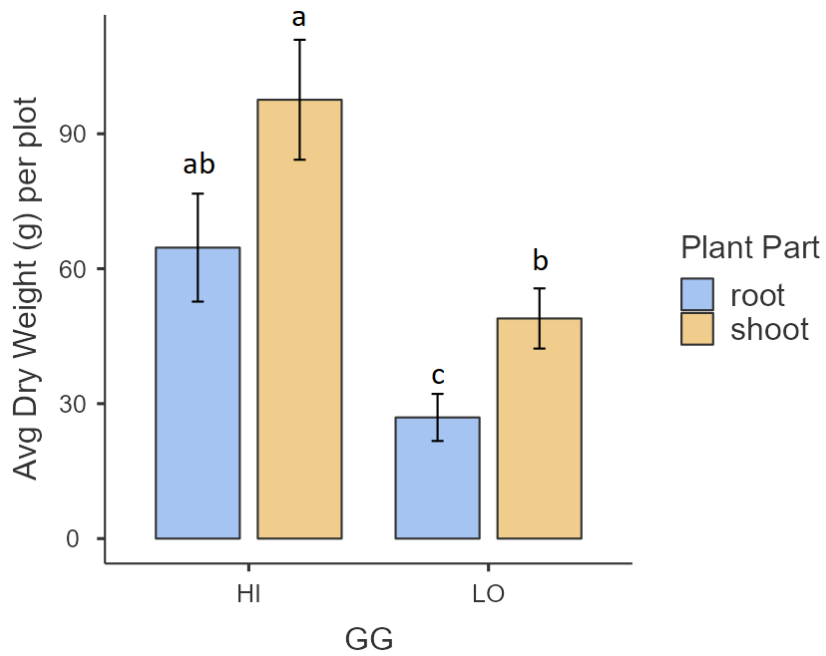


Figure 6. Average dry weight (g) of roots and shoots per plot in high and low fertility environments. Bars with the same letters do not differ significantly. Error bars denote standard deviation.

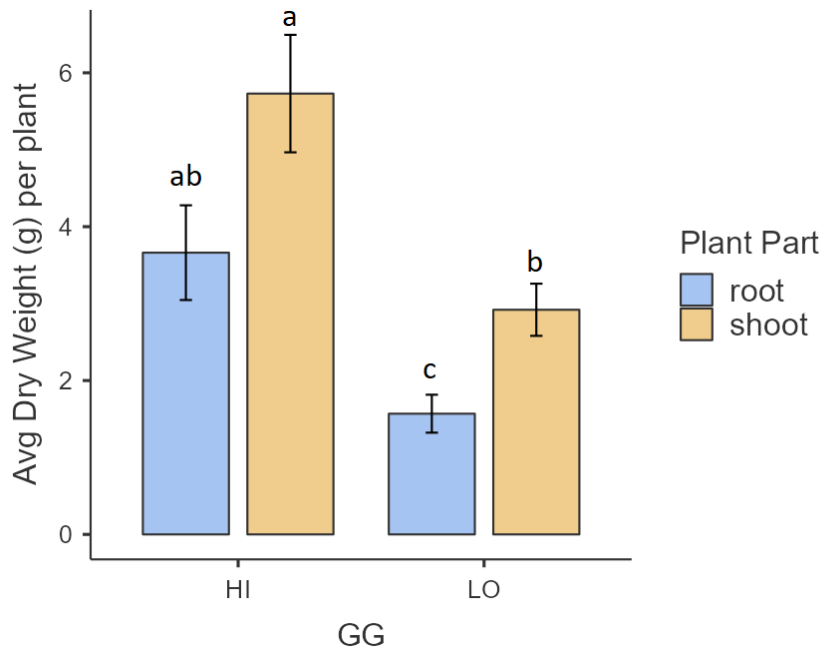


Figure 7. Average dry weight (g) of roots and shoots per plant in high and low fertility environments. Bars with the same letters do not differ significantly. Error bars denote standard deviation.

Discussion and Conclusion

The results provide evidence that the organic granular fertilizer improves rice growth. The results provide insufficient evidence to reject the null hypotheses that the other bio-stimulants and mycorrhizal inoculant have no effect on rice growth, measured by plant dry matter accumulation of roots and shoots.

There were a few possible variables that may have affected the results of this study. Firstly, there was some failed germination at initial seeding. While the statistical analyses accounted for this by taking the average weight per plant on top of average weight per plot, perhaps future studies could work with transplants instead, to ensure a standard number of plants across experimental plots. If a future study would still test these amendments on direct-seeded rice, using row cover at the start to ensure good germination across all plots could help plants be less stressed and more receptive to the amendments.



Figure 8. Picture of the plots after the first germination. Few plants survived. One plant is seen in the corner of this plot.

Secondly, the growing medium was not the type of soil typically used to grow rice. Amendments may work better if grown in a less sandy growing medium. Future experiments should 1) test these amendments on rice plants growing in less sandy soil; or 2) if using sandy soil — as it does make root-washing a lot easier and safer — use amendments at a higher quantity and / or frequency to account for the sandiness. Moreover, since these amendments are also relatively new to producers, testing them at different quantities and frequencies might also reveal optimal application rates.

Thirdly, there was uneven weed pressure among the plots; plots that received the high fertility treatment were weeded more frequently as the fertilizer was raked in. Future studies should keep all plots weed-free, or use a fertilizer treatment that would not have weeding as a secondary benefit. It could also be beneficial to add a factor of weeding and non-weeding treatments to see how amendments can affect plants in these different conditions.

Dryland rice production is a very promising development in Canada's agricultural sector, and finding amendments to facilitate this production system would be beneficial. While this study showed no evidence to support that Mykos Gold mycorrhizal inoculant with K3NEO seed spray, Tecamin MAX foliar spray, or Yeast Guard foliar spray provide any significant benefits, future trials with less variation in their methods could find better supporting evidence for the amendments' efficacies. However, until research can support the economic and agricultural value of these amendments, they are not recommended for purchase for dryland rice growers.

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