The Effects of Fungi and Commercial Antifungal Spray on *Brassica rapa* Growth

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Introduction

This study investigated the effects of yeast, black bread mold (*Rhizopus stolonifer*), and antifungal powder spray on fast plant (*Brassica rapa*) growth. Fungi can augment plant growth

by forming mutualistic associations called mycorrhizae with plant roots (Campbell, Reece, Taylor, and Simon 2006). Additionally, according to El-Tarabily and Sivasithamparam (2006), certain yeasts promote plant growth. However, fungi, particularly ascomycetes (to which yeasts belong), also cause roughly 80% of plant diseases (Campbell *et al.* 2006). *R. stolonifer* (a zygomycete), with its resistant zygosporangium and fast growth (Campbell *et al.* 2006), may be especially adept at infecting plants. By applying yeast and *R. stolonifer* to *B. rapa* plants, this study sought to discover whether these types of fungi aided, harmed, or uninfluenced plant growth. It was hypothesized that they would aid growth.

Chang, Chen, and Jao (2007) illustrated that natural antifungal agents can inhibit plant-pathogenic fungi and enhance growth in Chinese cabbage, a member of the mustard (Brassicaceae) family. This study sought to determine whether commercial antifungal agents affected growth in *B. rapa* plants, also mustards, that were infected with yeast or *R. stolonifer*. The researchers therefore applied over-the-counter antifungal powder spray containing 1% tolnaftate to the plants. With repeated use, this agent is designed to cure and prevent athlete's foot, a mycosis caused by the ringworm fungus (Campbell *et al.* 2006). In addition to expanding the knowledge on the effects of various fungi on plant growth, the results of this study were expected to indicate whether tolnaftate can inhibit a diversity of fungi. Furthermore, by applying the spray to some conditions before, and others after, germination, the study investigated whether the spray could not only inhibit, but also prevent fungal growth on plants. Based on the spray's ability to inhibit and prevent the ringworm fungus, it was hypothesized that, although unlikely, if it could inhibit yeast and *R. stolonifer*, it could also prevent them.

Methods and Materials

The experiment utilized the standard planting, watering, and lighting methods and materials of the Wisconsin Fast Plants™ (Anonymous). It omitted the anti-algal/anti-fungal

tablets to allow fungi to grow for the purpose of the investigation. Immediately after the *B. rapa* seeds were planted, the antifungal spray was topically applied to the soil of three quads (Figure 1). The process was repeated one week later (on day 8) after the seeds had germinated. The following week (on day 15), .5 grams of Fleischmann's® dry yeast and a small section of *R. stolonifer* were applied to the soil of two of these quads, one containing yeast and the other containing *R. stolonifer*. The third quad served as a control. The three quads received the topical spray treatment for the third time. Yeast and *R. stolonifer* were also applied to two other quads each (Figure 2). One week later (on day 22), after the fungi had time to grow, one of these quads received the spray treatment, while the other did not. Two additional controls were established, one of which received the spray treatment and the other of which did not. The three previously sprayed quads were sprayed for the fourth time (Figure 3). The next week (on day 29), each of the spray conditions received a final application of the antifungal treatment. In total, there were nine conditions: 1) control never sprayed, 2) yeast never sprayed, 3) *R. stolonifer* never sprayed, 4) control sprayed before and after germination, 5) yeast sprayed before and after germination, 6) *R. stolonifer* sprayed before and after germination, 7) control sprayed after germination only, 8) yeast sprayed after germination only, and 9) *R. stolonifer* sprayed after germination only.

Beginning on week 3 (on day 15), immediately before the fungi were applied, and continuing once a week for the next three weeks (on days 22, 29, and 36), the height of each plant was measured in millimeters. Following the experiment's completion, the average height of each condition was computed for weeks 3, 4, 5, and 6. Plots were created to represent each condition's growth rate, and a one-way analysis of variance was conducted to determine if there were statistically significant height differences between the nine conditions on weeks 3, 4, 5, or 6.

Results

The results show a consistent pattern across the study's four measurement sessions in the relative differences between the conditions' average heights (Table 1 and Figures 4, 5, 6, and 7). All nine conditions experienced a steep increase in growth between weeks 3 and 4 and then gradually leveled off in weeks 5 and 6 (Figures 8, 9, and 10). In general, the conditions that never received the spray treatment started and ended the experiment with the highest average heights, followed by the conditions that were sprayed after germination only and the conditions that were sprayed before and after germination. From the first measurement session (on week 3, before the fungi were applied) to the last, the yeast condition that never received the spray treatment had the highest average height of the nine conditions. The *R. stolonifer* condition that was sprayed before and after germination consistently had the lowest average height. Despite these observable differences, the one-way analysis of variance revealed that there were no significant height differences between the nine conditions on any of the four weeks (Table 2).

Discussion

The results suggest that neither the yeast nor *R. stolonifer* had an effect on *B. rapa* growth. While the yeast condition that never received the spray treatment consistently had the highest average height of the nine conditions, this was the case before the yeast was applied. Likewise, while the *R. stolonifer* condition that never received the spray treatment consistently had the lowest average height of the three non-spray conditions, its growth rate followed the same pattern of the other two conditions (although it decreased at the end likely because one of its plants had broken). The *R. stolonifer* condition that was sprayed after germination only consistently had the highest average height of the three conditions sprayed after germination, but, again, had the same general growth rate of the other two conditions. These results, as well as the results of the one-way analysis of variance, suggest that any observable differences that existed between the nine conditions after the fungi were applied were due to random differences that

existed before the fungi were applied. The study's hypothesis that yeast and *R. stolonifer* would aid *B. rapa* growth was not supported.

These findings do not support El-Tarabily and Sivasithamparam's (2006) claim that certain yeasts promote plant growth; however, they do not negate it, as the experiment utilized only one form of yeast. The findings also have both advantageous and disadvantageous implications for *B. Rapa*: while the yeast and *R. stolonifer* do not appear to harm the plants' growth, they also do not appear to aid it by forming mycorrhizal associations with the plants' roots.

The results of the one-way analysis of variance also suggest that the antifungal powder spray did not affect *B. rapa* growth. However, Figures 8, 9, and 10 reveal that, generally, of the nine conditions, those that were sprayed before and after germination consistently had the lowest average heights, followed by those that were sprayed after germination only and those that were never sprayed. This pattern suggests that the antifungal spray hindered the early and late stages of *B. rapa* growth, and it is expected that the one-way analysis of variance would have considered the pattern significant had the sample sizes been larger.

These findings indicate that, unlike the natural antifungal agents that Chang, Chen, and Jao (2007) found to enhance growth in mustard plants, commercial antifungal agents designed to cure and prevent athlete's foot are disadvantageous to *B. rapa* growth. It is unclear whether this effect is caused by the spray's active ingredient, tolnaftate, or its inactive ingredients, such as alcohol.

There are several confounding variables that may have affected the experiment's results. For one, spores from the yeast or *R. stolonifer* conditions may have reached other plants, causing the nine conditions to be equally affected by the fungi and thus not differ in growth rate. Likewise, spores may have entered the plants' water reservoir and been carried to every

condition's soil. The antifungal spray may have also contaminated conditions that it was not meant to be applied to, lessening the differences between the nine conditions and rendering them statistically insignificant. Lastly, various factors may have caused the plants' height measurements to inaccurately reflect their actual growth. For example, as appears to have occurred in the *R. stolonifer* condition that never received the spray treatment, some plants may have broken prior to being measured.

Conclusions

Overall, the data from this experiment show that neither the yeast nor *R. stolonifer* affected *B. rapa* growth. While the antifungal powder spray did not have a statistically significant effect, it appears that it harmed *B. rapa* growth in its early and late stages.

Literature Cited

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Campbell, N. A., J. B. Reece, M. R. Taylor, and E. J. Simon. 2006. Biology: Concepts and Connections. Pearson Education, Inc./Benjamin Cummings, San Francisco, CA.

Chang, W. T., Y. C. Chen, and C. L. Jao. 2007. Antifungal activity and enhancement of plant growth by Bacillus cereus grown on shellfish chitin wastes. Bioresource Technology 98: 1224-1230.

El-Tarabily, K. A., and K. Sivasithamparam. 2006. Potential of yeasts as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. Mycoscience 47: 25-35.

Figure 1

Seeds Planted	Seeds Planted	Seeds Planted	Seeds Planted	Seeds Planted, Quad Sprayed

Seeds Planted	Seeds Planted, Quad Sprayed	Seeds Planted	Seeds Planted, Quad Sprayed	

B. rapa Quad Layout on Week 1 (Day 1)

Figure 2

Fungi Not Added	Yeast Added	*R. stolonifer* Added	*R. stolonifer* Added	Fungi Not Added, Quad Sprayed for 3rd Time
Yeast Added	Yeast Added, Quad Sprayed for 3rd Time	Fungi Not Added	*R. stolonifer* Added, Quad Sprayed for 3rd Time	

B. rapa Quad Layout on Week 3 (Day 15)

Figure 3

Control 1: Quad Not Sprayed	Yeast 1: Quad Not Sprayed	*R. stolonifer* 1: Quad Not Sprayed	*R. stolonifer* 2: Quad Sprayed for 1st Time	Control 2: Quad Sprayed for 4th Time
Yeast 2: Quad Sprayed for 1st Time	Yeast 3: Quad Sprayed for 4th Time	Control 3: Quad Sprayed for 1st Time	*R. stolonifer* 3: Quad Sprayed for 4th Time	

B. rapa Quad Layout on Week 4 (Day 22)

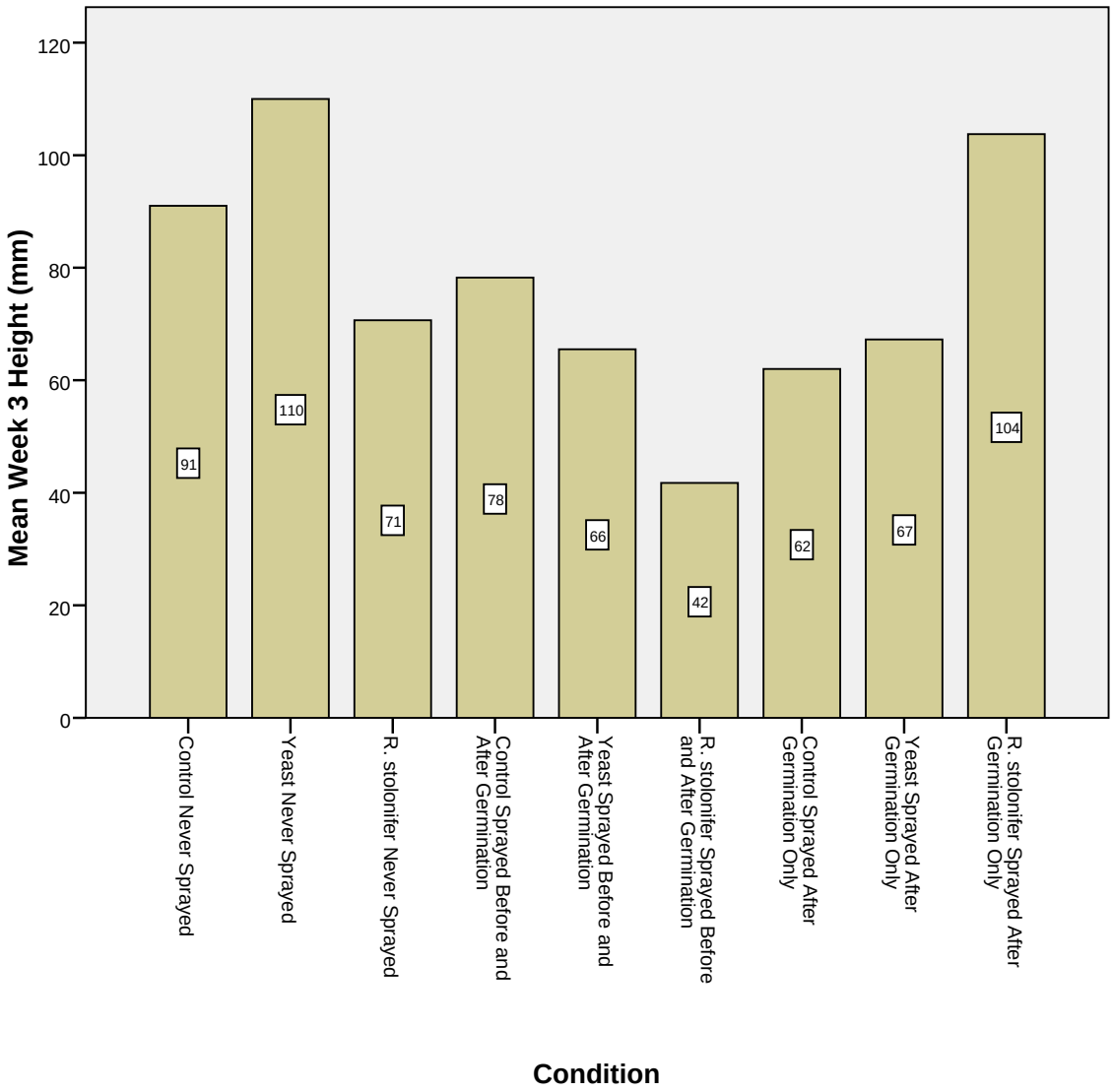
Table 1

Means and Standard Deviations for Each Condition's Week 3, 4, 5, and 6 Heights (mm)

Condition		Week 3 Height (mm)	Week 4 Height (mm)	Week 5 Height (mm)	Week 6 Height (mm)
Control Never Sprayed	Mean	91.00	166.50	181.50	188.75
	N	4	4	4	4
	Std. Deviation	21.463	63.211	74.411	75.429
Yeast Never Sprayed	Mean	110.00	191.00	196.67	206.67
	N	3	3	3	3
	Std. Deviation	58.924	77.091	63.509	54.848
R. stolonifer Never Sprayed	Mean	70.67	126.33	144.00	138.33
	N	3	3	3	3
	Std. Deviation	8.386	51.013	60.025	50.332
Control Sprayed Before and After Germination	Mean	78.25	136.25	153.50	156.25
	N	4	4	4	4
	Std. Deviation	39.886	35.444	48.260	43.851

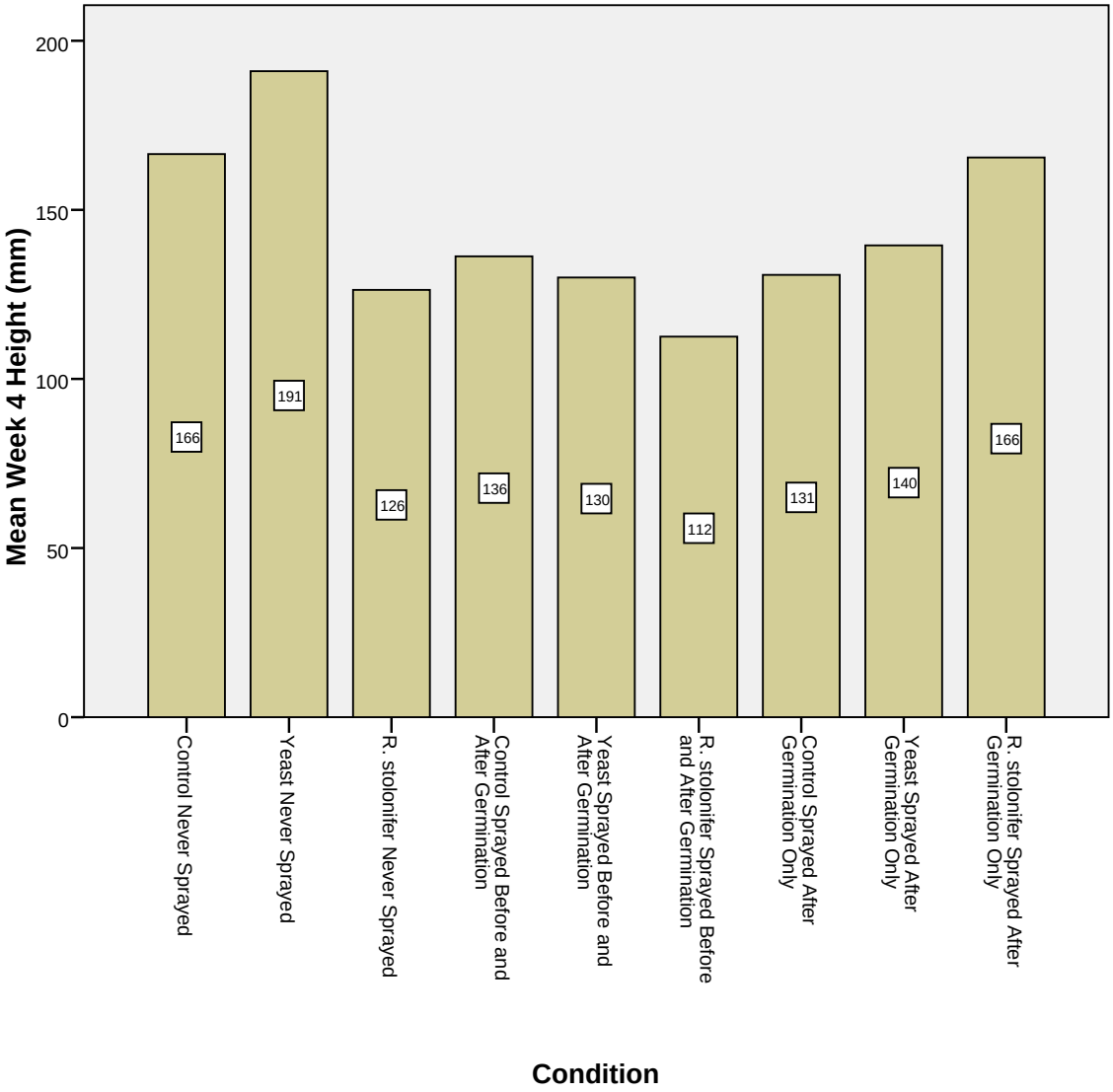
Yeast Sprayed Before and After Germination	Mean	65.50	130.00	152.50	158.75
	N	4	4	4	4
	Std. Deviation	1.732	21.863	32.275	32.243
R. stolonifer Sprayed Before and After Germination	Mean	41.75	112.50	128.75	131.25
	N	4	4	4	4
	Std. Deviation	27.585	48.398	43.277	49.728
Control Sprayed After Germination Only	Mean	62.00	130.75	140.50	158.75
	N	4	4	4	4
	Std. Deviation	23.664	22.794	20.567	30.653
Yeast Sprayed After Germination Only	Mean	67.25	139.50	157.25	160.00
	N	4	4	4	4
	Std. Deviation	17.231	37.117	39.559	40.825
R. stolonifer Sprayed After Germination Only	Mean	103.75	165.50	177.50	176.25
	N	4	4	4	4
	Std. Deviation	11.587	33.917	41.332	40.285
Total	Mean	75.88	143.41	158.47	163.38
	N	34	34	34	34
	Std. Deviation	31.277	45.278	46.823	46.851

Figure 4



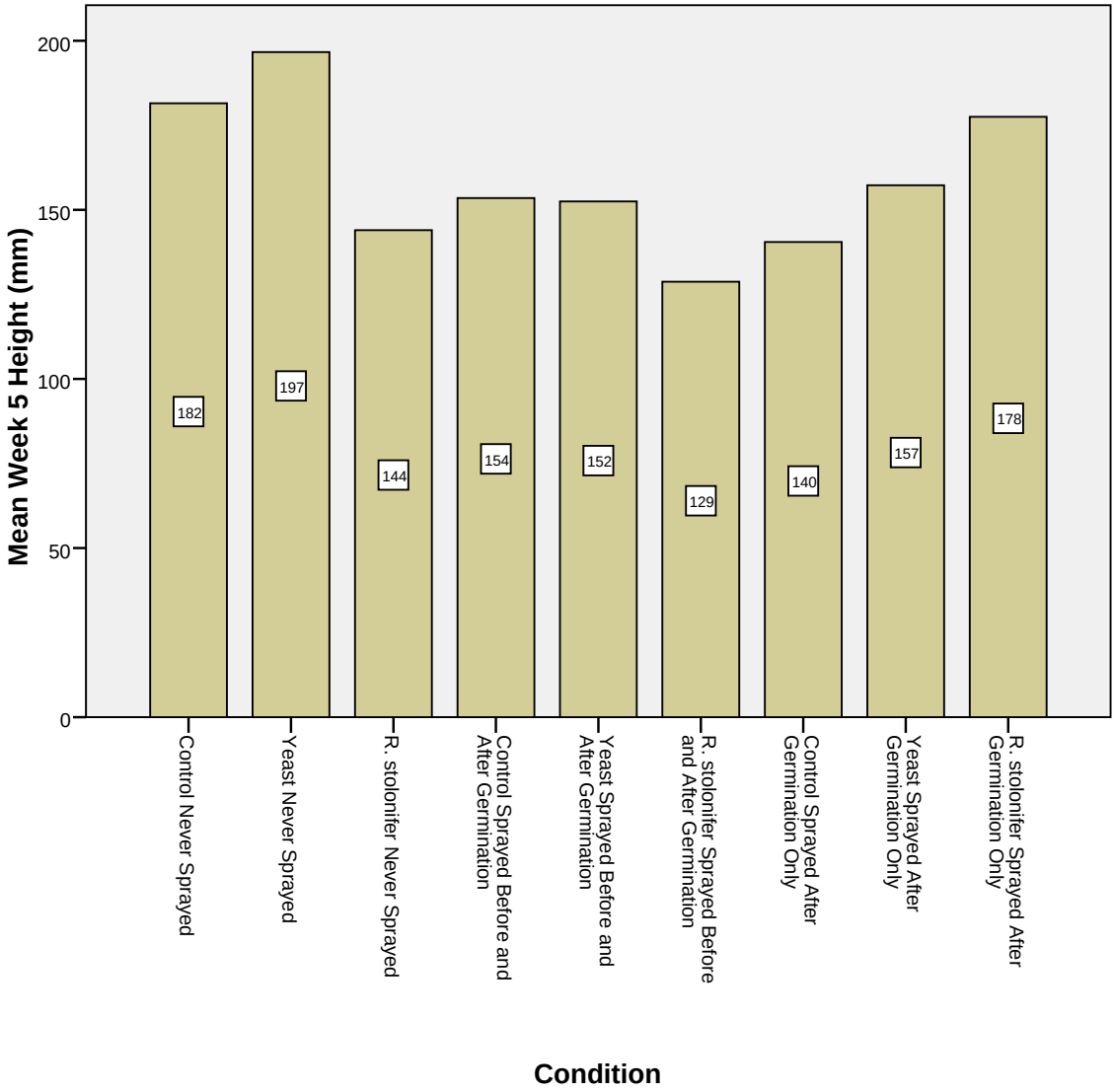
Average Week 3 Height (mm) for Each Condition

Figure 5



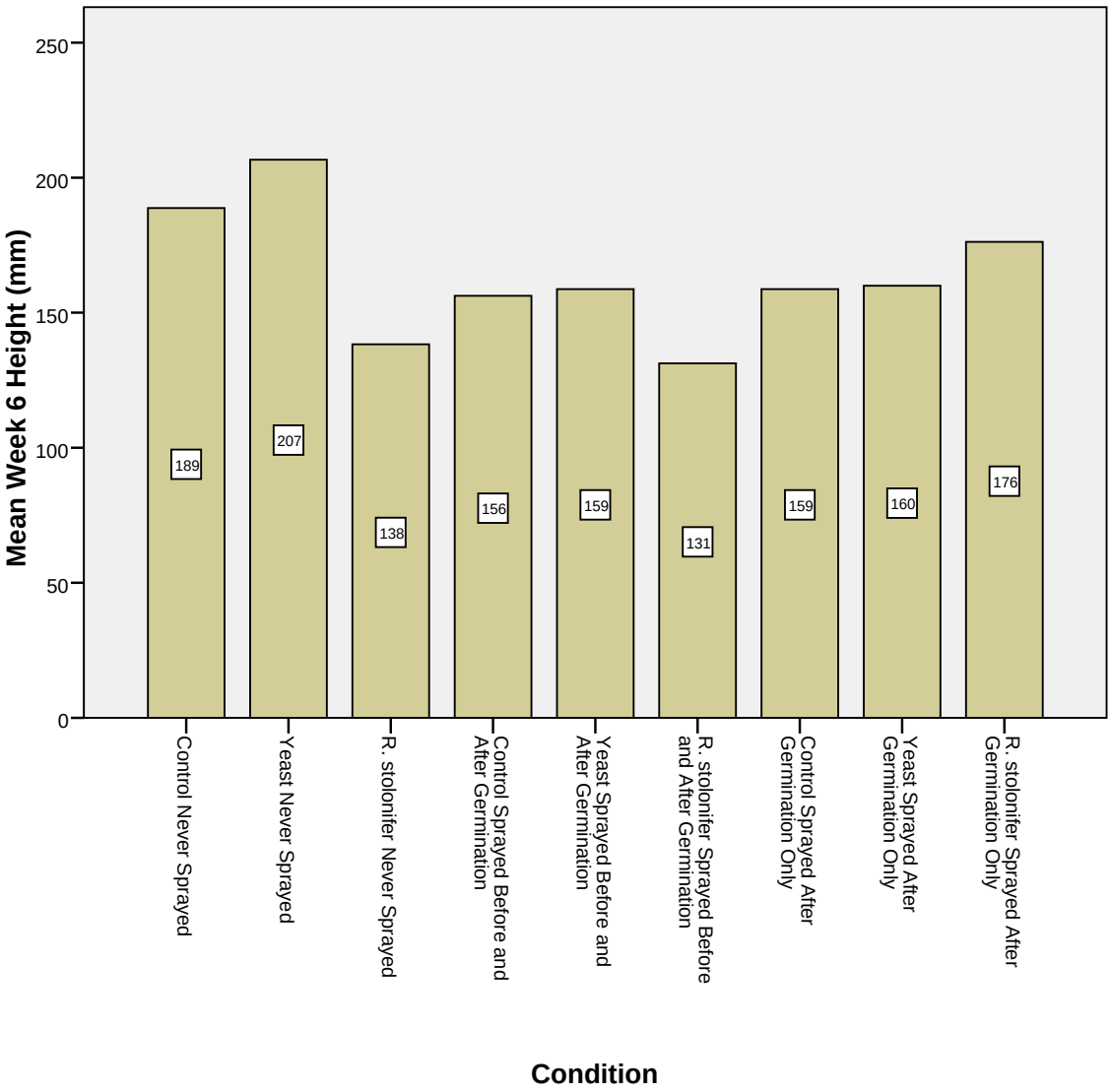
Average Week 4 Height (mm) for Each Condition

Figure 6



Average Week 5 Height (mm) for Each Condition

Figure 7



Average Week 6 Height (mm) for Each Condition

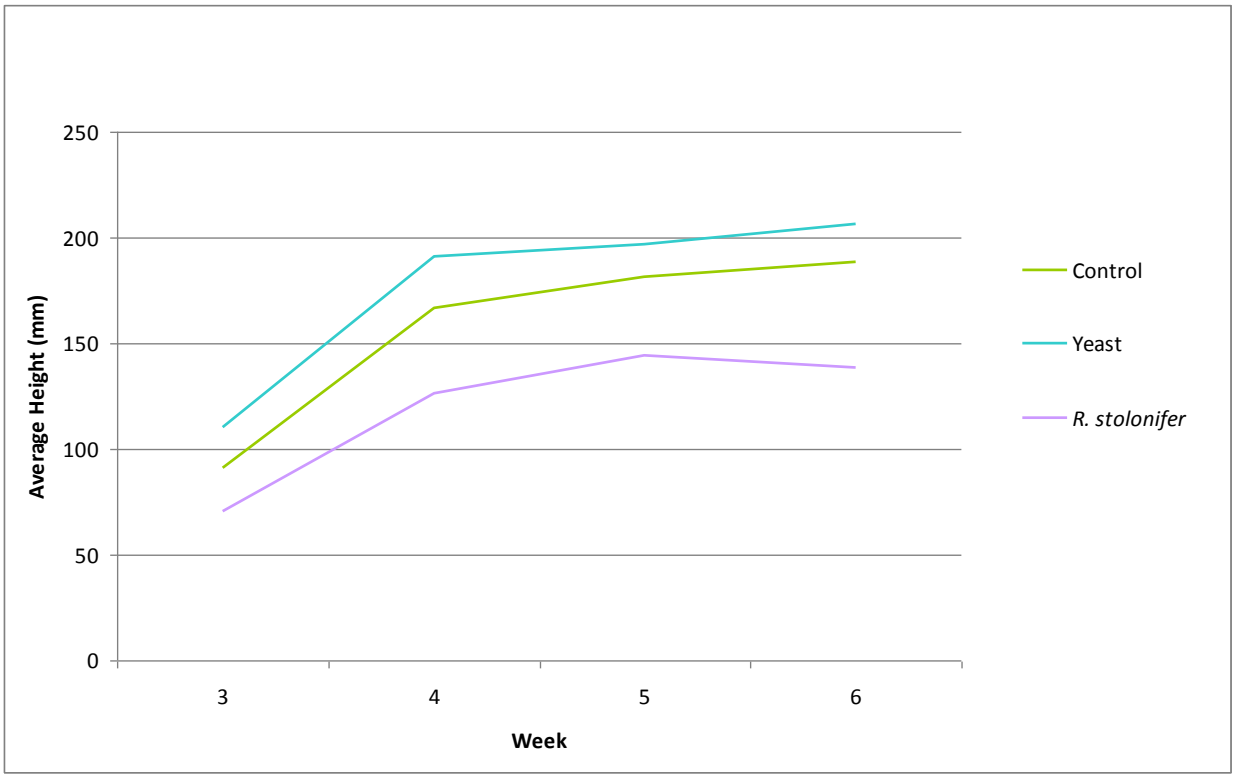
Table 2

One-Way Analysis of Variance Testing the Height (mm) Differences Between the Conditions on Weeks 3, 4, 5, and 6

		Sum of Squares	df	Mean Square	F	Sig.
Week 3 Height (mm)	Between Groups	13776.863	8	1722.108	2.327	.051
	Within Groups	18504.667	25	740.187		
	Total	32281.529	33			
Week 4 Height (mm)	Between Groups	17202.069	8	2150.259	1.066	.417
	Within Groups	50450.167	25	2018.007		
	Total	67652.235	33			
Week 5 Height (mm)	Between Groups	13647.304	8	1705.913	.726	.667
	Within Groups	58703.167	25	2348.127		
	Total	72350.471	33			

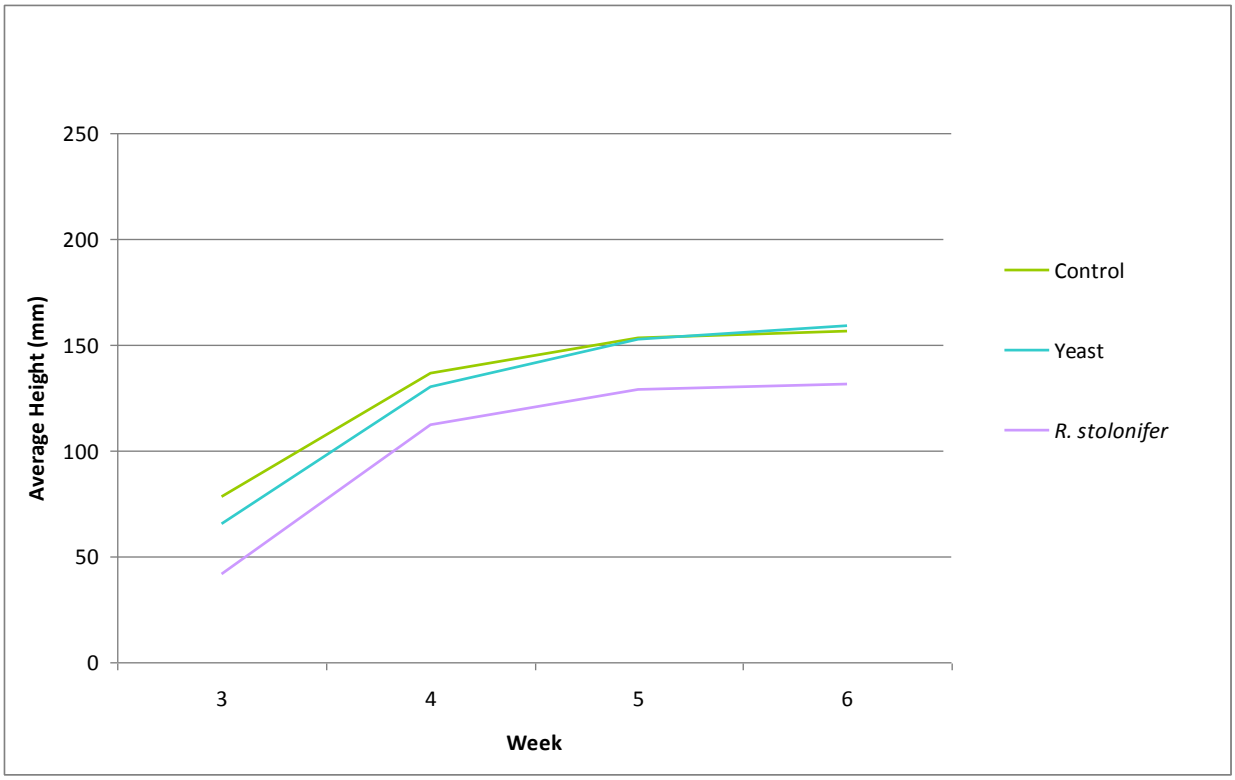
Week 6 Height (mm)	Between Groups	15290.196	8	1911.275	.836	.580
	Within Groups	57145.833	25	2285.833		
	Total	72436.029	33			

Figure 8



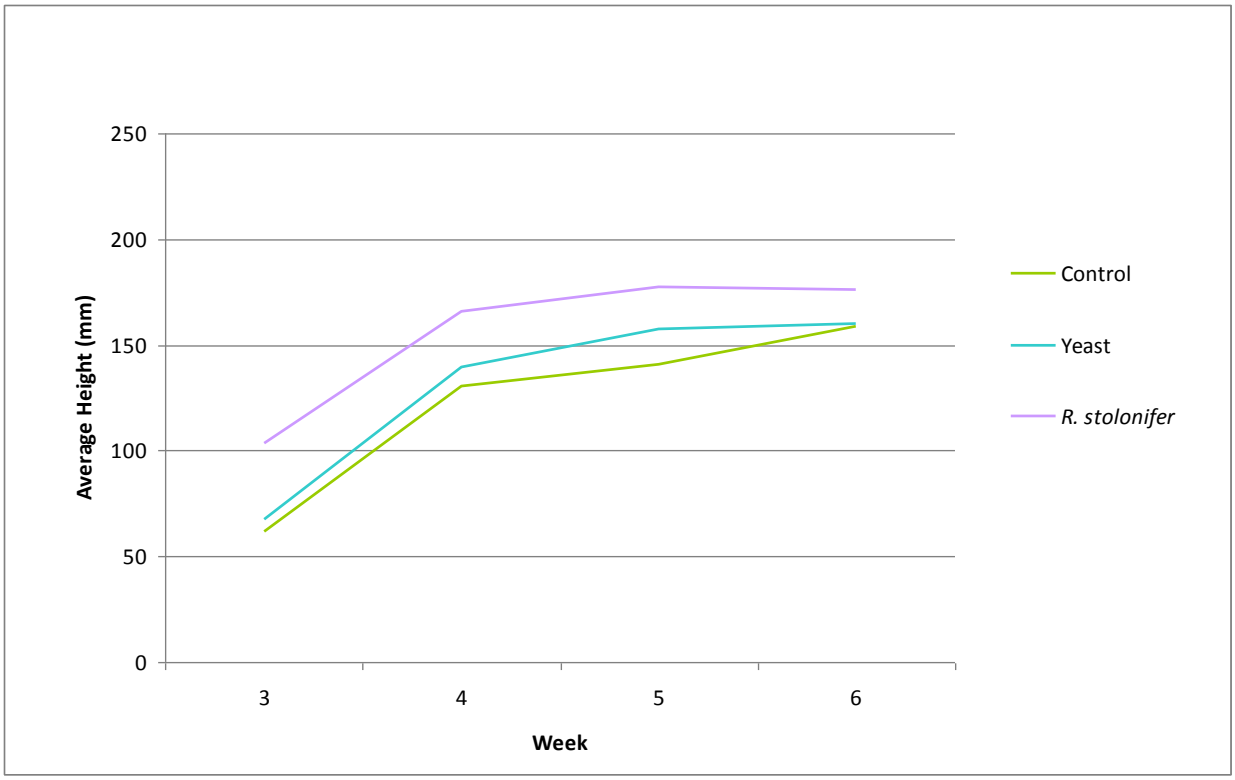
Growth Rates of *B. rapa* Conditions Never Sprayed

Figure 9



Growth Rates of *B. rapa* Conditions Sprayed Before and After Germination

Figure 10



Growth Rates of *B. rapa* Conditions Sprayed After Germination Only