Effect of Pre-Germination Laser Treatment on Lettuce Grown in a Controlled Environment

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Abstract

Laser technology has the potential to impact agriculture and contribute to overall sustainability by reducing required inputs. Previous research indicates pre-germination laser treatment of seeds has a positive impact on overall growth and productivity of plants. By laser treating seeds, researchers have shown it is possible to get more yield or a faster growth rate without increasing other inputs. Although these studies suggest the applicability of laser technology to agriculture, more research is required to test the effectiveness of laser treatment before it becomes commercially available on a large-scale. This study tested laser treated lettuce seeds in a controlled environment. Rosalyn, Elizium, Cristabel, and Fairly lettuce varieties were used. Fresh foliage weight and head height were collected at regular intervals. Laser treatment was not found to have a significant effect on lettuce fresh foliage weight or head height.

Keywords: laser technology, controlled environment, seeds, sustainable agriculture, growth rate, productivity, yield, lettuce

Introduction

Laser technology has many uses. From medical, to environmental, to engineering, laser technology has positively impacted multiple sectors. Recently, there has been attempts to apply laser technology to agriculture. Although it is not currently widespread within agriculture, research indicates laser technology could positively influence agriculture.

Organic Path International (OPI) is a local Vancouver company exploring the applications of laser technology to agriculture, specifically to the organic sector. In 2020, they collaborated with the Sustainable Agriculture program at KPU to have a student research their laser technology. Nikita Ershov performed the study with field trials of corn and cucumber that received laser seed treatment with OPI's technology. Ershov's study (2020) observed a positive correlation between laser seed treatment and increased yield of corn and cucumber, suggesting laser seed treatment positively influences plant yield.

CubicFarms is another local company specializing in a dynamic vertical farming system which eliminates the supply chain and produces vegetables year-around. Their growth system is situated in containers and has a precisely controlled environment with constant nutrient supply and ideal lighting. Due to the highly controlled environment, the CubicFarm's growth systems provides ideal growing conditions because there are far fewer uncontrolled variables compared to a field trial.

If laser technology can increase crop yield and growth rate, it would be profoundly important to the agricultural sector. From both an economical and environmental perspective, reducing growth cycle time or increasing yield would decrease environmental and economic resources required to grow a plant from seed to harvest.

Objectives

- Primary objective*:*
    - To test the effects of pre-germination laser treatment on lettuce cultivated in the CubicFarms' growth systems.
- Secondary objective:
    - To test if the effects of laser treatment differ between lettuce varieties.

Literature Review

There are two main hypotheses which seek to explain how pre-germination laser treatment influences plant growth and productivity. The first hypothesis suggests laser treatment has an effect because it stimulates important enzymes in the seed, maximizing growth potential caused by the enzymes (Podleśny et al., 2012). The second hypothesis posits that the laser treatment acts like a fungicide and eliminates pathogens on the seed surface that adversely impact germination and growth (Perez Reyes et al., 2015). Either way, studies of plants that received laser treatment suggest a positive effect of laser seed treatment on growth and productivity.

Laser treatment and enzyme activity

Although the exact effect the laser treatment causes in the seeds is still open to conjecture, Podleśny et al (2012) found laser treatment of white lupine and faba bean seeds influenced the level of amylolytic enzyme activity within the seeds. Initial changes in the seed enzyme activity were notably observed at 48 hours after planting and reached a maximum activity 120 hours after transplanting. Interestingly, Podleśny et al (2012) did not detect a significant amylolytic enzyme activity difference between seeds in different laser treatment groups. Additionally, they found white lupine and faba beans in the laser treatment group had greater root and hypocotyl length, suggesting elevated enzyme activity, resulting from laser treatment, increased plant growth.

. Purveen et al (2021) had similar findings in their study, which involved treating safflower seeds with a laser. They saw an increase in amylolytic enzyme activity when seeds underwent laser irradiation. They postulated laser treatment "awoke" the enzymes responsible for converting stored starch into sugar, meaning treated plants had a more ready food-source than their untreated counterparts. In their study, Purveen et al (2021) witnessed an increase in enthalpy of seeds treated with the laser. This is known as the photoacoustic effect. When the laser hits the seed, the seed receives a large input of energy. The seed rapidly heats up, and as it does, it produces what can be best described as a "mini sonic-boom". It is postulated that it is this photoacoustic effect that leads to increased enzyme activity which primes the seed for growth.

Laser treatment as a fungicide

Besides increasing enzyme activity, it has been posited that laser treatment increases plant growth and yield by killing pathogens present on the seed which would curtail germination and seedling growth. Perez Reyes et al (2015) saw a reduction of up to 50% in mycobiota on the seed surface, negating the need for chemical fungicides. However, there are some conflicting studies on this hypothesis. Klimek-Kopyra et al (2020) found laser irradiated soybean seeds had a reduced occurrence in only certain types of pathogenic fungi, not all pathogenic fungi. Additionally, they found laser seed treatment reduced the population of certain non-pathogenic fungi on the seed surface. This study confirms that irradiating seeds with lasers does indeed reduce the mycoflora on the seed surface but not necessarily in a desirable or effective way.

Laser treatment and plant growth

Whether laser treatment increases plant growth and productivity due to stimulating enzyme activity or acting as a fungicide, Samiya et al (2020) found that pre-germination laser treatment causes notable effects on “number of roots, number of shoots, germination percentage, dry and fresh weight of shoot length, dry and fresh weight of root length, [and] protein percentage.” Podleśna et al (2015) tested the effects of laser irradiation on pea plants and found that it resulted in increased amylolytic enzyme activity. Additionally, their study observed an increase in leaf size and number of flowers between treatment and control groups with the pea plants in the laser treatment group producing between 11.2% - 11.9% more yield in pea seeds due to an increased number of pea pods.

Sacala et al (2012) studied the effects of laser treatment on 2 different beet varieties and found that one variety had a more significant response than the other variety. This is not the only study suggesting that certain plant varieties are more responsive to laser treatment. Ri et al (2019) observed comparable results when testing laser treatment on 2 varieties of rice. They found the laser treatment increased the overall growth and productivity of both rice varieties by 7.7% and 21%, suggesting certain plant varieties are more reactive to laser treatment.

Materials and Methods

The study took place at CubicFarm located in Pitt Meadows, British Columbia. Plants were grown inside the CubicFarm's propagator and cultivator growth machines. Raw seeds were laser treated in California using OPI's laser technology. The study was constructed as a completely randomized factorial design. The primary factor was laser treatment, and the secondary factor was lettuce variety. Collected data was analyzed using multiple means ANOVA in the jamovi interface of R-code.

Seeds were supplied by Enza Zaden and consisted of 4 lettuce varieties already known to grow well in the CubicFarm growth systems: Cristabel, Elizium, Fairly, and Rosalyn. The laser treatment had five levels consisting of laser exposure for 0(control), 15, 30, 60, and 120 seconds. In total, there were 20 treatment groups consisting of 4 plant varieties and 5 laser treatments. Each treatment was replicated twice.

Seeds germinated in the CubicFarm propagator with 80 plugs per tray and 2 trays per spine. Seedlings were transplanted to the cultivator at approximately 2 1/2 weeks. 120 trays, each holding 20 lettuce plants, were used in the cultivator. Seedlings were then grown for approximately 3 weeks in the cultivator to complete the growth cycle. In total, the experiment took approximately 5 weeks.

Data Collection

Treatment replicates were placed into plots of 60 plant with 30 treatment plants per plot. There were 40 plots total. Each plot had 3 trays with 10 treatment plants per tray. 10 plants from 1 tray were used for data collection on the 3 different days of data collection. The treatment plants were randomly assigned to start on the first or second plug and then every other plug from there. The intervening plugs were filled with non-treatment lettuce plants. Table 1 shows the dependent variables, and the days data was collected on them. Fresh foliage weight was measured by cutting the lettuce plant at the base of the stem and weighing it on a scale. Head diameter and height were measured by laying the lettuce head on top of a 1x1 inch grid. Data was entered into an excel sheet in flat table format. For analysis, data was converted to pivot table and exported to jamovi.

Dependent variable	Day 7 post-transplant	Day 14 post-transplant	Day 18 post-transplant
Fresh foliage weight (g)			
Head diameter (in)			
Head height (in)			

Table 1. Table showing when data on each dependent variable was collected. Green boxes indicate which variables were measured on which days post-transplant to the propagator.

Results

Statistical analysis showed laser treatment did not have a significant effect on lettuce head fresh foliage weight and head height. Day 14 data on lettuce head diameter did not satisfy assumptions of normality ($p>0.05$) so lettuce head diameter was not included in analysis.

Fresh foliage weight required log10 transformation to satisfy Shapiro-Wilk homogeneity test ($p<0.05$). Multiple means ANOVA indicated no effect of laser treatment. The test indicated that plants grew over time, but laser seed treatment did not have a significant effect. P-value for variety*laser exposure effect was 0.722. Figure 1 shows marginal means in which it can be visually observed that values between control and treatment as well as between varieties are not significantly different.

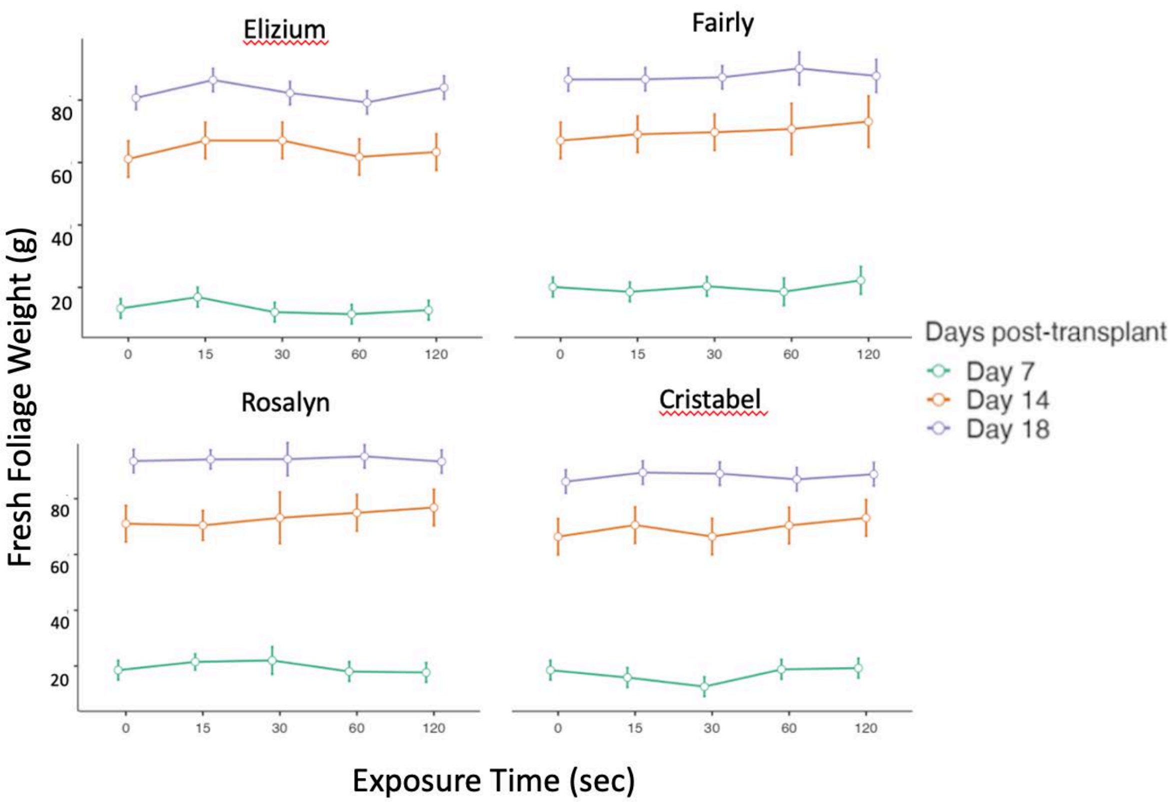

Figure 1. Estimated marginal means showing fresh foliage weight (g) of different lettuce varieties (Elizium, Fairly, Rosalyn, and Cristabel) at different laser treatments (0, 15, 30, 60, 120) on three separate data collection days (days 7, 14, 18 post-transplant).

Analysis of lettuce head height with multiple means ANOVA did not detect laser treatment as having a significant effect. P-value of variety*laser exposure = 0.508. Figure 2 shows estimated marginal means for lettuce head height, and it is visually confirmable there are no significant differences between treatments.

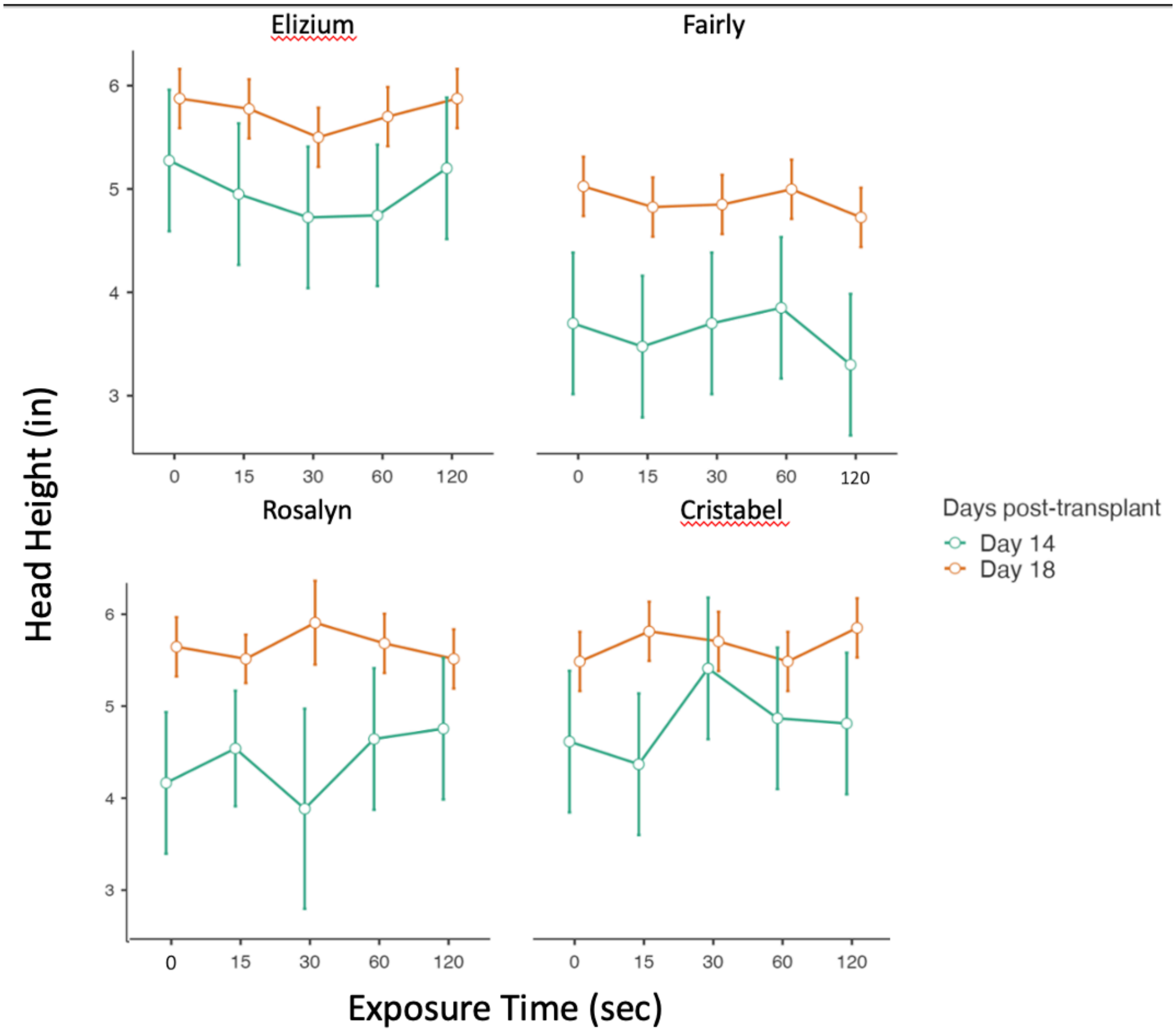

Figure 2. Estimated marginal means plot showing lettuce head height (in) of different lettuce varieties (Elizium, Fairly, Rosalyn, and Cristabel) at different laser treatments (0, 15, 30, 60, 120) on two different days of data collection (day 14, 18 post-transplant).

Discussion

In this study, the overall effect of laser treatment on lettuce fresh foliage weight and head height was studied. The effect on enzyme activity or fungal occurrence on the seed surface was not looked at. Because neither fungal disease nor poor germination were an issue with lettuce grown in the CubicFarms system, a positive effect from the laser treatment would have been an increased growth rate with foliage weight and head height significantly greater than the control. However, between laser treatments on 4 different lettuce varieties, no significant effect was detected.

Previous studies suggest laser seed treatment affects crop growth and production through either increasing enzyme activity (Podlesny et al., 2012; Purveen et al., 2021) or killing pathogens on the seed surface (Perez Reyes et al., 2015; Klimek-Kopyra et al., 2020). Seeds in this study grew in a highly controlled vertical farm system where there was slight pathogen pressure. Because of this, seeds in this study did not benefit from the laser seed treatment as a fungicide. Although increased enzyme excitation from the laser seed treatment may have occurred, it was not a significant enough effect to be detected by the statistical analysis..

In a previous field study in which the same laser seed treatment was used, laser treatment effect was found to be significant in the lower nutrient environment (Ershov, 2020). The CubicFarms growth system is precisely designed so that all light, gas, and nutrient needs are optimized for the plants. Because conditions were optimal, there was little need for the laser seed treatment. Indirectly, these studies corroborate each other and suggest a trend that laser treatment may not have a significant effect in high nutrient environments.

It is also important to remember that certain plant varieties are more responsive to laser treatment. Sacala et al (2012) and Ri et al (2019) demonstrated this in their research. Sacala et al (2012) saw a significant difference in the effect of laser treatment between 2 beet varieties. Ri et al (2019) looked at laser treatment on two different rice cultivars and found one cultivar had a more significant response to the laser treatment than the other. Both studies showed that laser seed treatment had a differing effect between plant varieties. In this study, 4 different lettuce varieties were used to examine if there was a difference in laser treatment responsiveness between varieties. However, laser treatment effect did not significantly differ between lettuce varieties. It is possible laser seed treatment did not have an effect in this study because the lettuce used is not responsive to laser treatment. This supports the findings of Sacala et al (2012) and Ri et al (2021) who suggest different crops have different levels of response to laser treatment.

During this experiment, BC experienced a heat dome that resulted in above average temperatures. While the CubicFarms growth systems are temperature regulated, they struggled to maintain the programmed temperature. This occurred during the germination and early growth stage of the experiment. Temperatures spiked up to 30° Celsius between June 27-29 away from the programmed temperature of 24°C. Although this uncontrollable variable adversely affected plant growth through stressing the young plants, all experimental units were exposed to the same temperatures. Therefore, because it was an experimental-wide effect rather than just restricted to certain plots, data was not skewed.

Raw lettuce seeds also present a challenge to easily work with due to their fine nature. Some plots had a few seeds from other plots mixed in or were slightly short of seeds. These challenges arose because of the nature of treating such fine seeds and the laser technology itself.

It highlights the need for further development of laser technology if it is to become applicable to agriculture.

There has been no previous research done on laser treatment of lettuce seeds. Most research has either focused on germination and seedling stage, or on maturity in plants where the seeds or reproductive organs are harvested at maturity. This study specifically looked at the effects of laser treatment on lettuce seeds that were grown to maturity in a controlled environment. While no significant effect was detected in this study, future research should continue to explore laser technology and its application as a pre-germination seed treatment within agriculture. Research should focus on determining what crops will respond to and benefit from laser treatment. Additionally, it is recommended to do so in the context of high and low nutrient environments to examine if there is a varying degree of effectiveness between the two environments.

Conclusion

Laser seed treatment did not have a significant effect on lettuce head height or fresh foliage weight in a controlled environment. Future research would benefit from examining the difference in laser seed treatment effect between high versus low nutrient environments. Additionally, studying the difference in effect between various crop types and varieties would ensure laser technology is utilized on crops where it will make a significant difference.

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