

The Effects of Climate-Driven Changes in Co-flowering between
Linum lewisii and *Potentilla pulcherrima* on Pollinator Services

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Abstract

Anthropogenic climate change is affecting many ecosystems. Changes in the melting of snowpack have altered flowering phenology in subalpine meadows. The subalpine meadows at the Rocky Mountain Biological Laboratory in Gothic, Colorado are experiencing shifts in co-flowering patterns. Two flowers, *Linum lewisii* and *Potentilla pulcherrima*, which had significant historical co-flowering overlap are now having 25% less overlap. The consequences of this shift are not well known and could be having an effect on plant-pollinator interactions. Although *L. lewisii* is self-compatible, outcrossing results in higher fruit and seed production. This means that pollinator services are important to *L. lewisii*, as it requires pollination to reproduce more effectively. This decrease in overlap may mean that there is less competition for pollinator services between these two species. This short-term study looked at pollinator visitation rates and pollen deposition on *L. lewisii* by applying different treatments to plots that decreased flowering overlap even further. Synchrony was manipulated by removing 100% and 50% of *P. pulcherrima* flowers in different sites. I expect that pollinator visitation rates to *L. lewisii* are higher and conspecific pollen deposition higher when there is less overlap. This hypothesis was not supported, however. I found that there was no significant difference between the treatments for pollinator visitation rates and conspecific pollen deposition. Assuming the experiment was conducted at a scale to which pollinators respond, this result suggests that climate-induced changes in co-flowering overlap will not have consequences for reproduction in this plant species.

Introduction

Anthropogenic climate change is altering many ecosystems worldwide (Uchida, Muraoka, & Nakatsubo, 2015; Martel-Mailhot, Brissette, & Caya, 2018; Parmesan & Yohe, 2003). These abiotic changes are altering ecosystems in terms of their snowmelt, precipitation, and temperatures (Parmesan & Yohe, 2003). This includes alterations in plant and animal phenology (Parmesan & Yohe, 2003). Phenology can determine the survival and reproductive success of organisms and, therefore, it is important to understand the ecological effects of these changes in phenology because this will ultimately provide critical information for the preservation of native plant and animal communities.

Flowering dates are changing in response to climate change because of earlier snow pack melt in subalpine environments (Inouye, 2000). These shifts in flowering phenology have led to altered co-flowering patterns (CaraDonna et al. 2014). A long-term flowering phenology study examining changes in co-flowering patterns in subalpine meadows around the Rocky Mountain Biological Laboratory (RMBL) showed snowmelt patterns were causing *Linum lewisii* to flower earlier and *Potentilla pulcherrima* to flower later. This resulted in 24% less overlap (CaraDonna, Iler, & Inouye, 2014). The consequences of these shifts are not well known, and could possibly result in changes in biodiversity, biotic interactions, or abiotic ecosystem processes such as soil composition (Wolf et al., 2017)

The change in co-flowering of *L. lewisii* and *P. Pulcherrima* may be affecting plant-pollinator interactions in these subalpine meadows (CaraDonna et al., 2014).

Pollinator services are important to *L. lewisii* because outcrossing is necessary to produce higher seed sets (Kearns & Inouye, 1994). Kearns (1994) observed which pollinators were visiting *L. lewisii*, how often, and how effective they were at pollinating. She found that Diptera was the most common pollinator but was less effective than various bees (1994). A lack of co-flowering overlap or reduced overlap with *P. pulcherrima* could mean less competition for pollinator services with *L. lewisii*.

In this study, I examined how climate-driven shifts in co-flowering can impact pollinator visitation rates and pollen deposition on *L. lewisii* at RMBL in Gothic, Colorado. I investigated the impact of changes in flowering overlap by applying two synchrony treatments, in addition to an unmanipulated control: removal of 50% and 100% of *P. pulcherrima* flowers, in a series of plots in a subalpine meadow. Pollinator visitation and pollen deposition was measured on *L. lewisii* plants.

I hypothesized that pollinator visitation rates would be higher to *L. lewisii* in the 100% removal plots compared to the 50% plots and controls and that the percent conspecific pollen would be higher in the 100% removal plot compared the 50% plot and controls. This study will provide a better understanding of how climate-driven changes in co-flowering are affecting competition for pollinator services.

Methods

Study Site

The study site was in in the RMBL Research Meadow (figure 1). This site is a subalpine meadow about 2,800 meters in elevation with some sloped areas. These sloped areas were found in WRM 1-5 (figure 1). The meadow has rich vegetation cover and both *L. lewisii* and *P. pulcherrima* are abundant. The meadow receives full sunlight,

and the growing season length is dictated by the melting of the snowpack (Inouye et al., 2000).

Study Species

Linum lewisii is a subalpine perennial plant with several simple-leaved stalks (Kearns & Inouye, 1994). It produces multiple blue flowers with shallow corollas containing five stigmas. This flower can self-pollinate but outcrossing results in higher seed set (Kearns & Inouye, 1994). *Potentilla pulcherrima* is also a subalpine, perennial plant (Stinson, 2004). It is iteroparous and produces small, shallow yellow flowers (Stinson, 2004). These plants were chosen because of the 24% decrease in flowering overlap that they share and because they are known to share pollinators (Iler, unpublished data).

Experimental Design

Three synchrony treatments were implemented on *L. lewisii* plants. The control received no manipulation. In the removal treatments, 50% and 100% of the *P. pulcherrima* flowers and buds were cut off. Flowers and buds were removed at these treatments levels throughout the entire experiment for the 100% treatment and once for 50% treatment on July 9, 2018 when they were observed to be peak flowering. The goal of these treatments was to test whether a decrease in overlap was affecting pollinator services.

The three treatments were blocked spatially, with three treatments per block. In early June, prior to flowering of either species, twelve blocks were set up in the research meadow, for a total of 36 plots that were 4m x 4m in size. A randomized block design was used to decrease variability among treatments (i.e., each treatment grouping

occurred in a similar area of the meadow). Each plot was selected by ensuring that there were at least five *L. lewisii* plants in the center of the plot and that the plot was surrounded by *P. pulcherrima*. Then the three plots were randomly assigned a treatment. The twelve experimental blocks were spaced >12 meters apart with three 4x4 meter plots each spaced >5 meters apart. Experimental treatments were applied to each 4x4m plot. Each 4x4m plot also contained a 1x1 meter plot in the center. The 1x1 area in the center was observed for pollinator visits, and *L. lewisii* stigmas were collected from this area. The remaining area of the plot was used to act as a buffer to which pollinators could respond.

Pollinator Visitation

Pollinator visitation was observed at each plot for 5 or 10 minutes. The observations were 5 minutes long until July 3, 2018 then were 10 minutes long. This change occurred because 22% of the plots were not flowering and some plots that were flowering had no visitors. By doubling the time I was able to get visitation rates at more plots. I observed as many *L. lewisii* flowers within the 1x1m plot as possible and recorded the total number of flowers observed during each session. I recorded the number of visits to *L. lewisii* flowers made by each pollinator that entered the plot. Potential pollinators were only included if they were observed to make contact with the reproductive organs of the flower (stigma or anthers). Pollinators were identified to the lowest taxonomic resolution possible based on Kearns and Inouye (1994), the insect collection at the Rocky Mountain Biological Laboratory, and by taking pictures using a Canon EOS Rebel camera.

Pollen Deposition

Five stigmas were collected from naturally pollinated *L. lewisii* flowers within the 1x1m area in the center of the plots to determine what pollen was there and what percentage of it was conspecific. Stigmas were collected from control plants. Pollen deposition was studied during peak flowering season. Stigmas were collected for two weeks which included peak flowering and slightly after peak flowering for a total of around 600 stigmas collected. I collected stigmas after petals had fallen off, so as not to interfere in the reproduction of the flower. Stigmas were placed into Eppendorf tubes prior to analysis. I quantified the percentage of conspecific pollen by dyeing the stigmas with fuchsin gel and mounting them on slides. Pollen grains were classified as *L. lewisii* or other and counted. Pollen grains of *L. lewisii* range from 1.5-2.5mm and *P. pulcherrima* from 0.5-1.0mm, so I was able to distinguish *L. lewisii* from *P. pulcherrima*. However, it was too difficult to categorize *P. pulcherrima* from other pollen grains on the stigmas, so all non-*L. lewisii* pollen was categorized as 'other'.

Data Analysis

Pollinator visitation rates were analyzed using a linear mixed effects model. Pollinator visitation rate was a continuous response variable, treatment was a categorical predictor, the number of *L. lewisii* flowers per 1x1m plot was a continuous predictor variable, and block was a random intercept term. Visitation rates were averaged across all observation dates (n= # plots). Shapiro Tests were performed to confirm that the response variable was normally distributed, and fitted vs. residual plots were examined for evidence of heterogeneity in variance. From the full model, (vist rate~ treatment*avg flwrs), I performed backwards removal and used a X^2 test to determine

the model of best fit. A one-way ANOVA was performed to compare the percent of conspecific pollen deposition across treatments.

Results

Pollinator Observations

The visitors that were observed to visit *L. lewisii* were various flies, bees, wasps, butterflies, and other insects. Some of these were classified through pictures taken and by using the insect collection. These were *Bombus*, *Bombyliidae*, *Hemiptera*, *Muscidae*, *Syrphidae*, *Hesperidae*, and *Lycaenidae*.

There was no significant effect of treatment or number of *L. lewisii* flowers on pollinator visitation rates (Table 1; Figure 2). The *L. lewisii* flower predictor did not improve model fit, nor did treatment, indicating that the best model was the null model (intercept only). Additionally, there were no additive nor interactive effects to the treatments. The predicted means from our statistical model (intercept only) were 0.23983 with a standard error of 0.02906 which very close to the actual means (figure 4). The actual means were 100% removal=0.2353, 50%=0.2587, control= 0.2270 (Figure 3).

Pollen Deposition

Consistent with pollinator visitation rates, there is no effect of treatment on the percentage of conspecific pollen on *L. lewisii* stigmas (table 3. $F = 0.4587$, $P\text{-value} = 0.4999$). The percent conspecific pollen deposition of *L. lewisii* pollen was 30% in the 100% removal treatment, 43% in the 50% removal treatment, and 34% in the control

(table 2). The predicted model for pollen deposition also aligned well with the actual data, showing no evidence for treatment effects (figure 6).

Discussion

This was the first study that examined the consequences of climate-driven changes in co-flowering phenology, using *L. lewisii* and *P. pulcherrima* as a case study. Although there have been studies on changes in co-flowering on other species, the consequences of these change are unknown, and there is still much to learn about what these could mean for our changing ecosystems (Forrest et al., 2010).

The spatial scale at which this experiment was conducted may not have been large enough to detect a response to the treatments. The blocks of this experiment were all in the RMBL Research Meadow, and the farthest plots from each other (WTH1 C, RCH4 C) were 294 meters apart (figure1). The highest from lowest plot (RCH4 C, WRM1 100%) only differed by 32 meters in elevation. Studies have shown that the spatial and temporal scale of snowpack strongly influence flowering phenology (Stinson, 2004). A larger spatial scale may have been able to detect differences in the synchrony treatments.

It is important to note that the year in which this study was conducted was not a good year for flowering of *L. lewisii* because the snowpack melted very early, and it was a very dry year. As a consequence to the drought and early snowpack melt, 22% of the plots did not flower, nor was there consistency in the flowering of the plots. Therefore, the sample size was reduced from the original set-up of 36 plots. The plots lost were RCH 1-5, WTH 2, WRM 2 and 3 (figure 1). This means that the experiment ended up spanning only 167 meters from the farthest plots WTH1 C and WRM5 C (figure 1).

There were very few plots, WTH1 (all), WRM1 C, WRM 5 (all), where *L. lewisii* flowers had relatively high density (figure 1). Pollinators are able to respond to flower density and tend to stay in more flower dense areas at a spatial scale of about 100 m² (Thomson 1981). Additionally, Sieber et al. (2011) found evidence for facilitation of pollination at larger spatial scales but not local ones. Therefore, it is possible that we did not see an effect of *P. pulcherrima* removals because the removals were too small, and a future study could examine larger removal areas. The lack of density in many of the plots may have kept pollinators more concentrated to certain plots, and they may have avoided venturing out on to the plots with fewer flowers. In addition, although *L. lewisii* and *P. pulcherrima* do share pollinators, most were flies, which are not very selective nor effective pollinators (Kearns & Inouye, 1994).

Another reason that no differences were detected across treatments could have been that 1,160 minutes of observation were insufficient to detect a significant difference between the treatments. However, this is likely a sufficient observation effort. Our predicted models agreed with the actual data, suggesting that insufficient observation effort is an unlikely explanation for the lack of treatment effects in this study.

Interestingly, despite the large number of *P. pulcherrima* flowers that were removed from the plots, *L. lewisii* still received a similar number of visits across treatments. Since they did share pollinators this may mean that there are other reasons that pollinators may choose between these flowers, such as wind or possibly aromatics. During observations I noticed that on windy days *P. pulcherrima* was visited more frequently than *L. lewisii*, likely because the flowers are closer to the ground and do not wave around as much or because they protect pollinators from the wind. Unfortunately,

P. pulcherrima visitation rates were not documented since this was not a part of my experimental methods. But I would suggest that this could be an interesting question to be addressed in future studies.

In conclusion, this study suggests that climate-driven changes in co-flowering overlap may not have consequences for pollination of *L. lewisii*.

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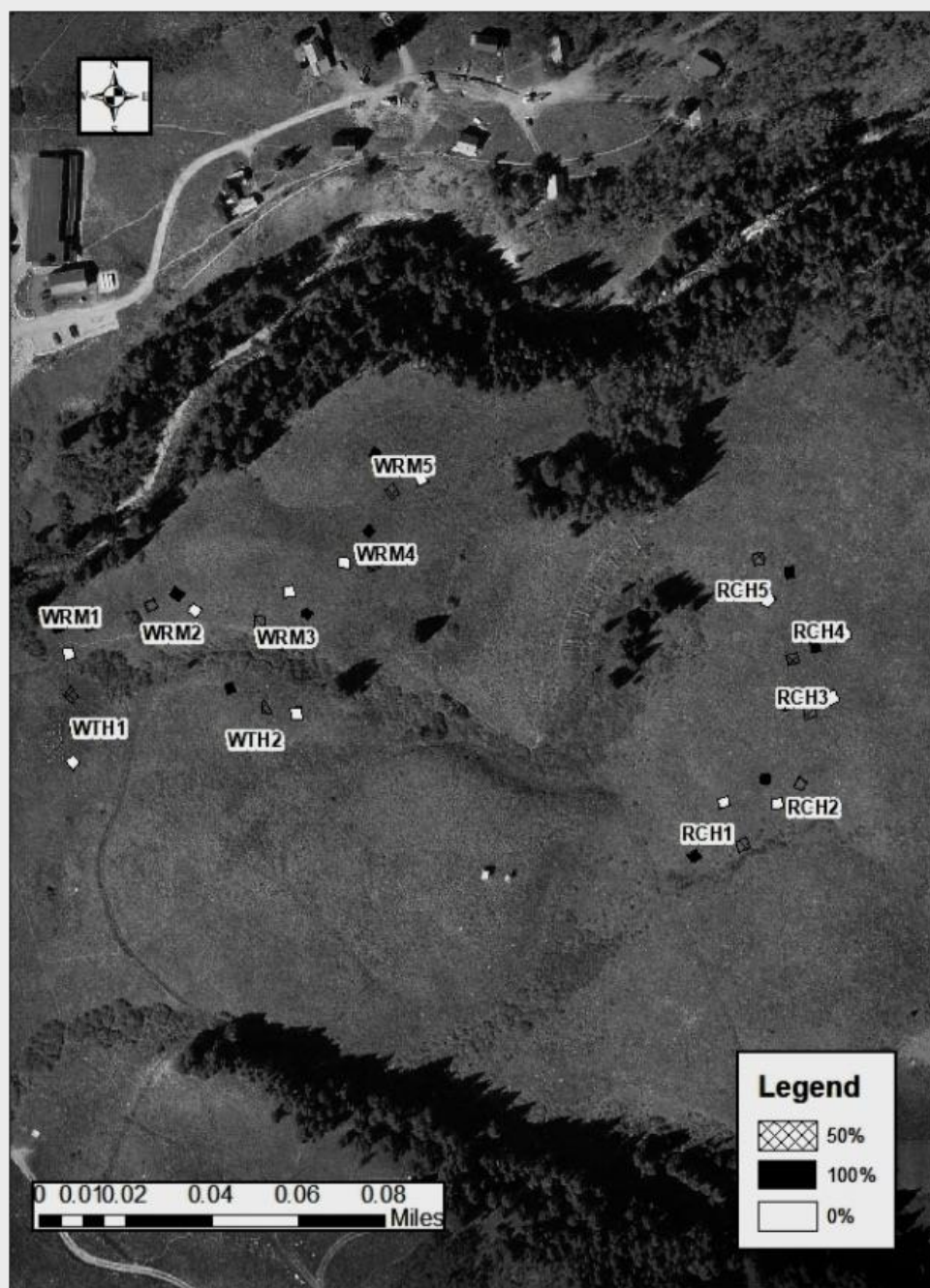


Figure 1. Map of experimental blocks (three plots per block) in the Research Meadow at Gothic, CO.

Table 1. Chi-square tests to compare statistical models. A non-significant p-value indicates that the additional term does not improve the model fit. Statistics correspond to a comparison between the model above and below (1 vs. 2, 2 vs. 3, and 3 vs. 4).

model	df	X ²	p-value
1) (avg visitation rate)~1			0.5
2) (avg visitation rate ~ treatment)	2	0.22	0.89
3) (avg visitation rate ~ treatment + avg # <i>L. Lewisii</i> flowers)	1	0.66	0.42
4) (avg visitation rate ~ treatment * avg # <i>L. Lewisii</i> flowers)	2	0.86	0.65

Table 2. Percent conspecific pollen deposited on *L. lewisii* stigmas.

Treatment	% conspecific pollen
100%	29.95%
50%	42.92%
0%	33.68%

Table 3. ANOVA results for conspecific pollen deposited on *L. lewisii* (pdl). Model 1 is the null and model 2 looks at pdl per treatment and shows a P-value of 0.499 which means no significant difference in conspecific pollen deposited per treatment.

model	D.F	F	P-value
1) null (pdl~1)			
2) (pdl~treatment)	0.02884	0.4587	0.4999

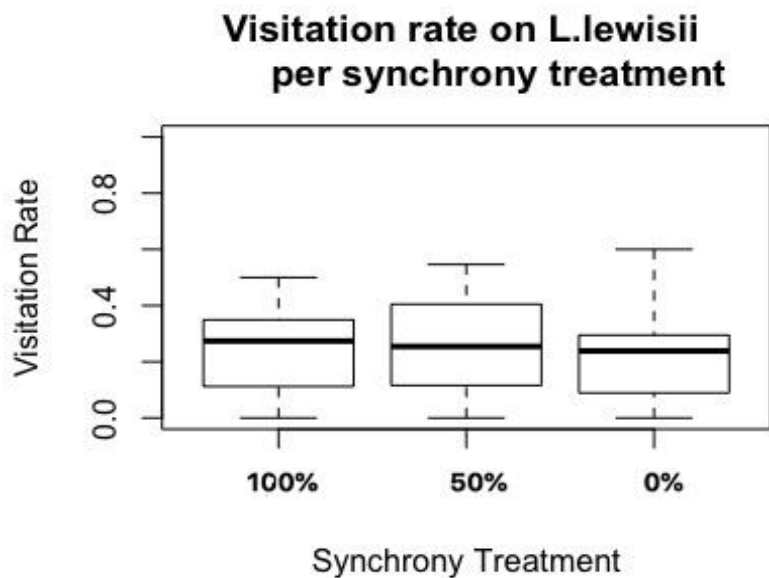


Figure 3. Box and whisker plots of pollinator visitation rate in each treatment. The treatments are 100%, 50%, and 0% removal of *Potentilla Pulcherrima* flowers. Mean of each are 100%= 0.2353, 50%=0.2587, control= 0.2270.

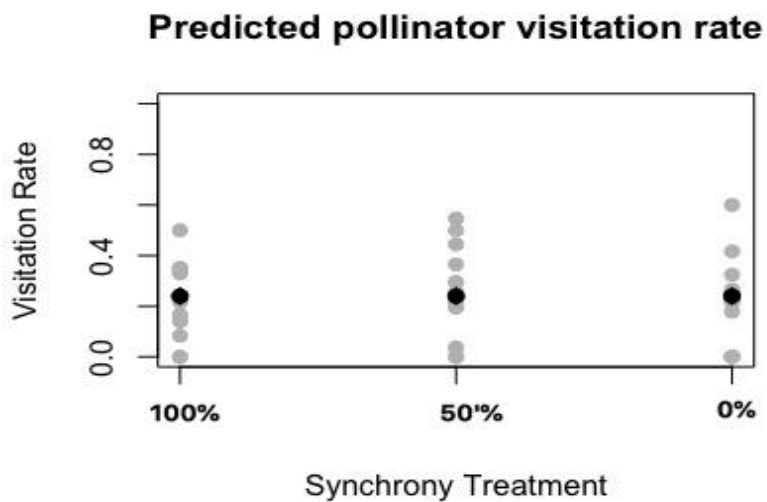


Figure 4. Predicted model of visitation rates for each treatment which strongly agrees with the actual data. The gray dots represent actual data and the black dots represent predicted means. Predicted means are 0.23983 for three treatments and the standard errors are 0.02906.

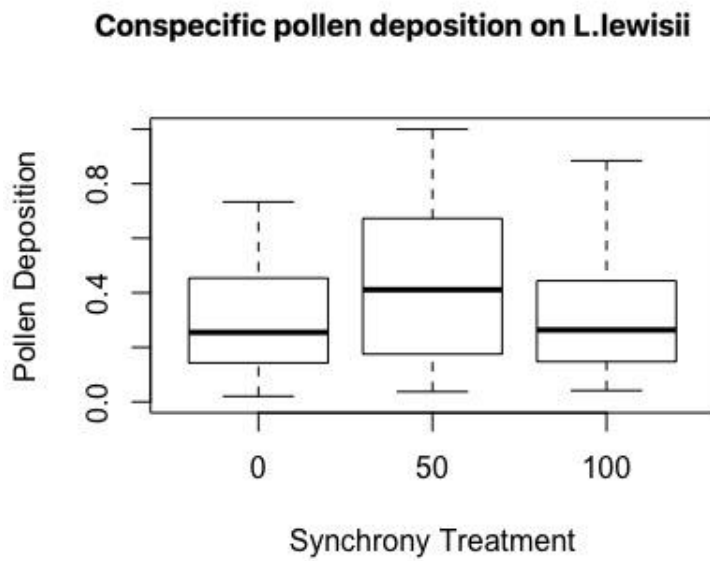


Figure 6 Box and whisker plot of actual conspecific pollen deposition on *L. lewisii*. Means per *P. pulcherrima* removal treatments are 100% = 0.3368, 50% = 0.4291, and 0% = 0.2995.

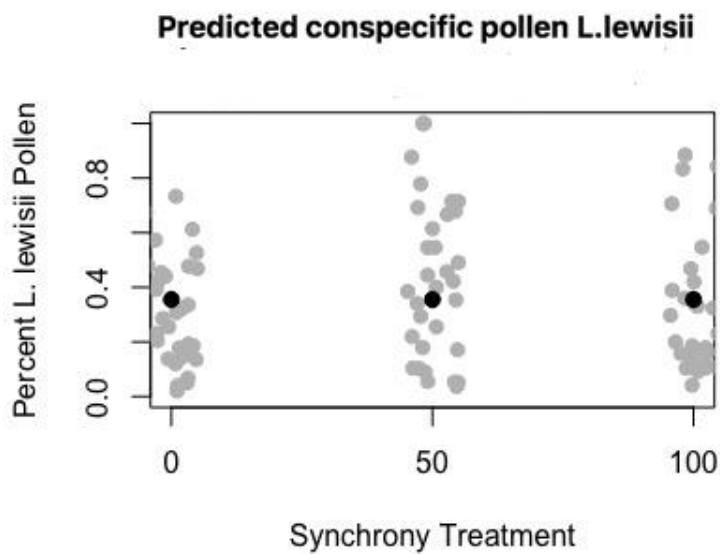


Figure 7. Predicted means for pollen deposition of *L. lewisii* which shows no significant difference between means. Gray dots signify actual data and black dots are predicted means. Predicted means are 0.3553473 for three treatments and the standard errors are 0.02592584.