THESIS

A Study of the Pollination System of Steershead (*Dicentra uniflora*)

Submitted by:

Julia S. Rayl

In partial fulfillment of the requirements for the

Thesis in Zoology

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Abstract

A two-year pollination study was conducted on a small population of *Dicentra uniflora* at Monte Cristo, Weber County, UT. Treatments were limited to bagged and nonbagged unmodified flowers because the flowers were cleistogamous with anthers dehiscing in the early stages of development. Analysis of seed set obtained from flowers in bagged treatments indicates that cross-pollination is not required; however, it could not be determined from this study if seed set in bagged treatments was due to self-pollination or apomixis. Observations for potential pollinators indicated no activity in either year. In 2000, the ambient temperature was significantly warmer than 2001, with nonbagged treatments producing more seeds than flowers in bagged treatments. The reverse was true in 2001 where bagged treatments produced more seeds than nonbagged treatments. A significant interaction effect between year and bagging appears to have been correlated with the differences in ambient temperature between years. It is uncertain if this interaction is due to temperature differences caused by bagging or to differences in pollination activity between years.

Introduction

Plants, unlike most animals, lack the ability to move about their environment in search of food and shelter or in seeking potential mates. To overcome these limitations, plants have evolved a variety of adaptations to satisfy these needs, such as growth responses and also by the structures that they produce. Prime examples of these structures are ways that plants have modified their leaves (Crane et al., 1995; Stewart and Rothwell, 1993). For example, many plants have evolved different shapes and sizes of leaves to meet their energy requirements through photosynthesis. Other plants such as cacti, have replaced leaves with spines. The spines protect the trunk from predators and also reduce the loss of moisture. Leaf modification has also produced a set of features that has allowed many angiosperms directed mobility in seeking a mate. This set of adaptations is captivatingly embodied in the flower (Raven et al., 1992).

Under the pressure of competition, flowers have employed many devices to be more attractive than their neighbors so that pollinating insects will help them reproduce (Barth, 1991). These changes are also reflected in the specialized characteristics that have coevolved in the visiting insects (Raven et al., 1992), and in the development of the bisexual flower in their plant partners. The placement of pollen-producing and pollen-receiving organs within the same flower allows insects to deposit pollen from neighboring flowers while gathering pollen with each visit (Baker and Hurd, 1968). Although insects are by no means the only way that plants are pollinated, their interaction has been a major influence in plant diversification and has played a significant role in propelling angiosperms into their position of dominance within the plant kingdom.

The prerequisite of survival in an environment of increasingly sophisticated competitors and neighbors requires characteristics that allow an organism to live long enough to successfully

reproduce many well-adapted offspring (Burger, 1981). Characteristics that allow individual plants within populations a greater advantage at reproducing are measured as an individual plant's fitness (Ridley, 1996). Reproductive characteristics that affect plant fitness are: mode of seed dispersal, mode of protection from seed predators, seed set efficiency, phenology of flowering, and breeding systems (Smith et al., 1986). Some plants are capable of reproducing both sexually and asexually (vegetative growth) and use one method over the other depending on the conditions of their environment (Ridley, 1996). Cross-pollination, which is one form of sexual reproduction, increases genetic variability, but outcrossing at great distances may reduce fitness when populations of plants exhibit spatially defined variation over small scales of distance (Ricklefs, 1997).

Another method of sexual reproduction that is available to some plants is the ability to self-pollinate. This is particularly common in plants that complete their life cycle within a few weeks and in plants living in environments with cold and wet weather during their flowering season that lack access to adequate numbers of pollinators (Proctor et al., 1996). Cross-pollination relies on an external agent to transfer pollen from one individual to another whereas self-pollination does not. If individuals are widely spaced or if habitats lack suitable pollinators, then having the ability to self-pollinate is an advantage. Although inbreeding can create genetic problems, the ability to produce seed is better than producing no seed at all (Ricklefs, 1997).

Steershead (*Dicentra uniflora*) is a low-growing perennial herb belonging to the Papaveraceae. In Utah, it is found on sagebrush plains to open subalpine ridges. It first appears just after snowmelt, produces a single bilateral flower (Stern, 1961), then times its retreat to dormancy when neighboring vegetation begins to take over. The common name derives from the flower's mature corolla morphology in which the distal ends of the two outer lateral petals

flex back on themselves similar to steers horns, and the two inner petals form what looks like the skull of a steer. Several characteristics of this plant, such as its early and rapid flowering period, production of a single flower, which appears to be cleistogamous (remain closed), and its (six) anthers that are close to the same height as the stigma (Uphof, 1938), suggest that *D. uniflora* may be self-pollinating. However, the flowers are bisexual, have nectaries, and are bilateral, suggesting that at some point in this group's history an association with specialized, cross-pollinating insect visitors has occurred (Baker and Hurd, 1968; Barth, 1991; Stebbins, 1957). Little is known about its pollination ecology; therefore, the primary objective of this study was to determine if *D. uniflora* is self-pollinated (autogamous), cross-pollinated (xenogamous) or both.

Materials and Methods

Dichogamy, having the reproductive organs mature at different times, is a mechanism used by some plants to prevent self-pollination (Barth 1991; Proctor et al., 1996). Since *D. uniflora* exhibits cleistogamy, flowers were checked while all petals were in the closed state to see if they exhibited protandry (maturation of anthers first) or protogyny (maturation of stigma first). Exhibiting these features eases testing capabilities for which mating system is used for I could then modify the flowers by emasculating immature anthers and cross-pollinate by hand in conjunction with having unmodified flowers in different bagging treatments to see which set fruit. Seed set in nonbagged flowers with emasculated anthers would potentially indicate that cross-pollination had occurred, and seed set in bagged unmodified flowers would potentially indicate self-pollination. Asexual reproduction is shown in some plants as either vegetative growth or as apomixis, which is the production of seeds without fertilization (analogous to parthenogenesis in animals). Apomixis can be tested at the same time using bagged flowers with emasculated anthers to see if there is any seed set (Kearns and Inouye, 1993; Wyatt et al., 1992).

The preceding two tests would be confirmed if no seed set occurred in the apomixis test. It was not possible to use all of these bagging treatments because the anthers and stigma had already reached maturity in the flower's unopened state. Therefore, I was limited to testing between self-pollination/apomixis and cross-pollination using unmodified bagged and nonbagged flowers.

Three populations of steershead were considered in Monte Cristo (elevation 2697 m), Weber County, Utah. Because of the unpredictability in the timing of snowmelt and the difficulty of finding flowers that had just crowned the surface with all four petals still in the closed formation, only one population with a later cycle could be used. All field studies were done on this population (located off of Utah Route 39 west of the Monte Cristo campground) between the last week of May and the middle of June in 2000 and 2001.

A total of 41 flowers were used in 2000, which were randomly assigned to treatments. Nineteen of the flowers were isolated from potential pollinating insects using bags made with bridal veil (10 x 10 threads/cm). This material was used because it causes less severe changes in temperature and humidity than using bagging material such as paper or plastic (Barrett and Helenurm, 1987; Bertin and Willson, 1980; Kerns and Inouye, 1993, Wyatt et al., 1992). Bags were closed around the stem with a sewn-in drawstring. Inside the bags, the stems of the flowers rested on a 5.08 cm x 3.81 cm x 2.54 cm platform made of Popsicle sticks so that the flowers were suspended away from the bagging material. The remaining 22 flowers were left nonbagged. Soil temperature and soil humidity were initially recorded for all plants that were used in the experiment. Flowers could not be checked every two days as planned because of snow and rain, but were checked when the weather permitted, which was every three to five days.

Fifty-one plants were used in the 2001 experiment. Twenty of the flowers were bagged using the same techniques as in the 2000 experiment. The remaining 31 flowers were left nonbagged. Weather conditions permitted soil temperature, soil humidity, ambient temperature, and bag temperature to be recorded every two to three days. Because of the potential for loss by predation, more flowers were purposefully placed in the nonbagged treatment in both years. Flowers were collected once fruits had matured.

Thirty-seven flowers were collected in the 2000 experiment. Four of the nonbagged flowers were eaten by rodents. Three additional nonbagged flowers were found to have been chewed upon; two at the distal end of the inner petals where the anthers and stigma are located and one with a bite out of one side of the inner petals with most of the contents of the ovary gone. Seven of the bagged treatments had bags that were heavily damaged with some of the strings removed though the flowers were untouched. The final total for flowers was 18 nonbagged and 19 bagged flowers. Six nonbagged flowers were lost to predation in 2001, leaving a total of 25 nonbagged and 20 bagged flowers for seed analysis. Undeveloped, partially developed, and mature seeds were counted for each flower. Seed set in bagged and nonbagged treatments for both years were compared using the MGLH ANOVA program of SYSTAT (version 5.2) for unbalanced designs (SYSTAT, 1992). Only numbers for the mature seed counts were included in the two-way ANOVA. The initial analysis of mature seeds counted in 2000 was skewed due to four outlyers (one nonbagged and three bagged) that produced ≥ 50 mature seeds. A new analysis was performed excluding the outlyers. T-tests were conducted for temperature differences between treatments and between years. Observations of flowers being pollinated by insects were also recorded for both years.

Results

A total of 96 hours was spent on visual observations of insect foraging behavior on *Dicentra uniflora* in the two years. Ants (Formicidae) were the only insects observed around the flowers this time of year, with their numbers being far fewer in year 2001 than year 2000. No other potential pollinators were observed either year.

The means for seed count grouped by bagging technique are shown in Figure 1. Nonbagged treatments produced 21.5% more seeds per plant than bagged treatments for combined years. However, total seeds per plant grouped by year (Figures 2, 3 and Table 2), show that 159% more seeds were produced in year 2000 than in 2001. Nonbagged treatments for year 2000 produced 204% more seeds than bagged treatments, but in year 2001 the results were reversed, with bagged treatments producing 275% more seeds than nonbagged treatments, although the overall total for seeds in 2001 was significantly less than year 2000. Significant sources of variation were the main effect of year and the interaction between year and bagging (Table 1). The main effect of bagging treatments was not a significant source of variation. Significant differences in temperature were found between treatments in 2000 (Figure 4), but were not significant for 2001 (Figure 5). Ambient temperature differed between years, with 2000 showing a significantly higher overall temperature than 2001 (Figure 6).

Discussion

Seed set in bagged flowers indicates that *D. uniflora* does not require cross-pollination to produce seeds. However, this result does not distinguish between self-pollination and apomixis, though it has been found that flowers that are cleistogamous produce fruits and seeds as a result of self-pollination (Holsinger, 1985, 1991b; Proctor et al., 1996; Uphof, 1938). Further studies, possibly on a genetic level, would more accurately discern which system is truly used. Even

though potential pollinators were not seen either year, it is impossible to say with certainty that cross-pollination did not occurr in nonbagged treatments. Bumble bees have been seen on steershead flowers in populations at lower altitudes in Logan Canyon, Utah, suggesting that cross-pollination probably occurs (J. Cane, pers. comm.). Thus, cross-pollination may occur more often in populations at lower elevations where there is a higher availability of pollinators. According to Macior (1970, 1978), other species within the Fumariaceae (now reclassified as a subfamily of the Papaveraceae) appear to have an inverse relationship between self-pollination and the frequency of insect activity. *Fumaria officinalis* L. exhibits high self-fertilization whereas *Corydalis cava* and *Dicentra cucullaria* are dependent on insect pollinators.

Temperature appears to be the contributing factor for the significance in variation seen in the interaction between year and bagging (Figures 3 and 4). The mean ambient temperature in year 2000 was 12.1° C degrees warmer (Figure 6) than in 2001. Seed production per plant was also 159% higher in 2000, though most of the production was due to flowers in nonbagged treatments. Mean temperature inside bagged treatments in year 2000 was 31.6° C, which may have been too warm for optimal development and may be one possibility why bagged flowers produced 204% less seeds per flower than nonbagged treatments. Overall seed production was significantly lower in 2001, which had much cooler temperatures than the previous year. Although there were no significant differences in temperatures between the treatments in 2001 (Figure 5), bagged flowers were still kept warmer than nonbagged and may account for why flowers in bagged treatments produced 275% more seeds per flower than plants that were left nonbagged. It is possible that the interaction could still be due to potential pollinators. Seed production for bagged treatments remained relatively constant for both years even though there was a 15.6° C degree mean difference inside the bags between years. Even though potential

pollinators were never seen it does not mean they were not present. There is higher insect activity at higher ambient temperature, which may account for the significant difference seen in the nonbagged treatments from one year to the next.

Dicentra uniflora appears to be capable of producing many seeds. There were three outliers from the 2000 experiment that had to be excluded from analysis because the number of mature seeds that were produced was not representative of the population and skewed the analysis. Even so, seed production was greater than 50. Of these, the highest production of mature seeds by a single flower was 57. This number does not include the other 19 seeds that were either partially or non-developed. In one of the flowers that were included in the analysis, 104 non-developed seeds were counted. I have not found any literature indicating the potential range in seed production though this would make for an interesting study.

Dissection of fresh inner petals under a microscope revealed that the tissue at the proximal end was fleshy, but toward the distal end the tissue separated into two distinct layers forming a space or cavity inside. This brings up a few hypotheses that I would like to put forth for consideration as to what purposes might be served in having two distinct layers of tissue, especially at the distal end of the petals. First, these plants produce flowers when ambient temperatures are still cool and when pollinator activity is still somewhat low, especially at higher elevations. Under self-fertilization, there is still no guarantee that every flower will produce seeds. Pollen must come into contact with the stigma, but limiting factors such as the distance between the reproductive organs may be great enough, even though the organs are adjacent to prevent placement from occurring. When air is warmed, expansion occurs with increasing pressure. If, for example, air is trapped within the space created by separating the petal into two layers and is then warmed by the afternoon sun enough to cause lateral expansion of these

tissues, then the tissue would in essence mechanically push the anthers into contact with the stigma. This process could act as a mechanism for fertilization, especially when pollinators are scarce and temperatures are cooler than average. Second, the tissue separation may simply be to help accelerate desiccation of the petals to make room for the expanding ovaries since the species has a rapid reproductive cycle.

In conclusion, I have found that *D. uniflora* is capable of producing seeds without being cross-pollinated. Future studies may want to take this study a step further by determining if seed production in bagged treatments is due to self-pollination or apomixis. Further analysis is needed to determine if optimum seed production is contingent upon temperature or whether interaction, as was seen in this study, is really caused by increased pollinator activity because of temperature. Genetic comparisons will definitely provide some insight as to how much pollinator activity is taking place and would also be beneficial in viewing what kind of genetic diversity is present in populations at different elevations.

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Table 1. Two-way ANOVA for mature seed count from bagged and nonbagged treatments for years 2000 and 2001.

Source of variation	df.	SS	MS	F	P
Year	1	634.281	634.281	4.795	0.032
Bagging	1	129.065	129.065	0.976	0.326
Year x Bagging	1	1,050.922	1,050.922	7.945	0.006

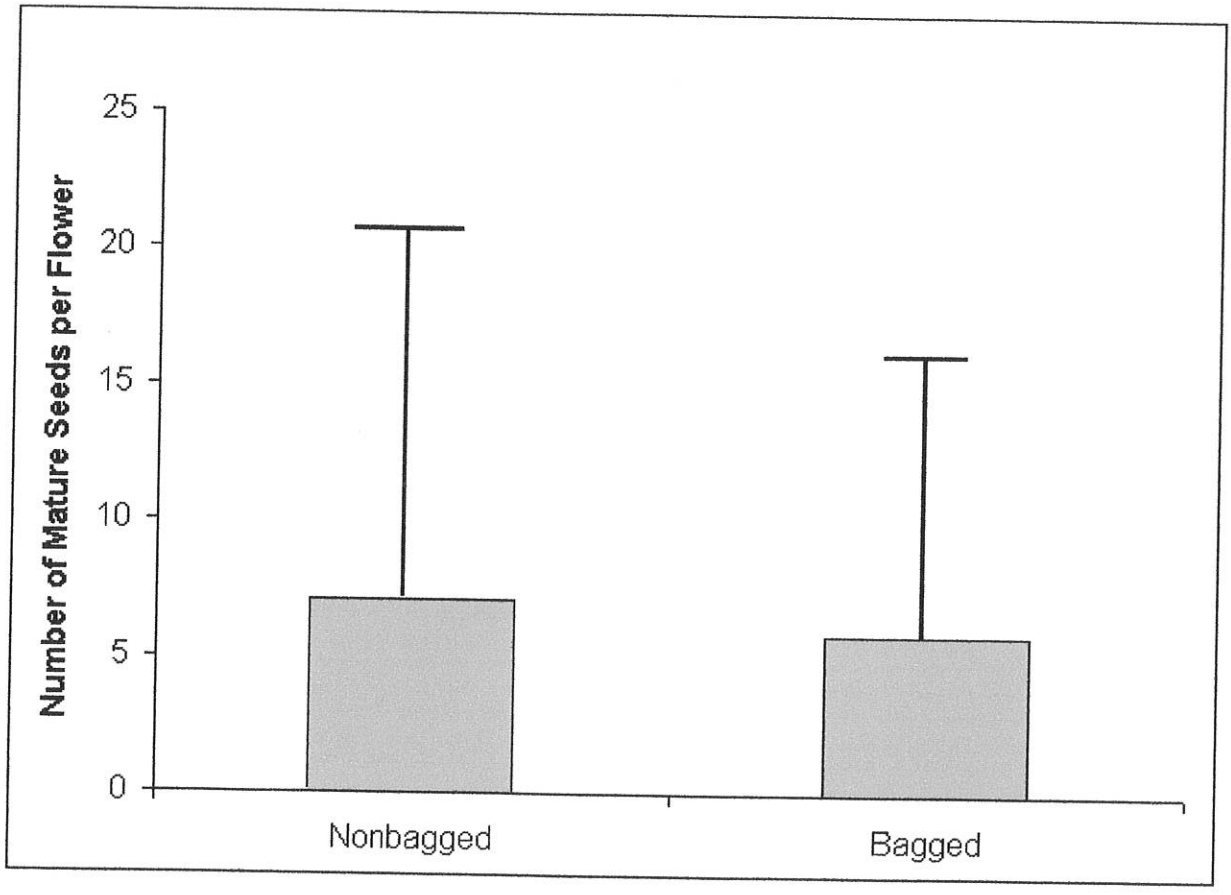

Figure 1. Mean difference (+ 1 S.D.) of seeds between treatments over two years (nonbagged: N=42, Bagged: N=36, p>.05), refer to Table 1.

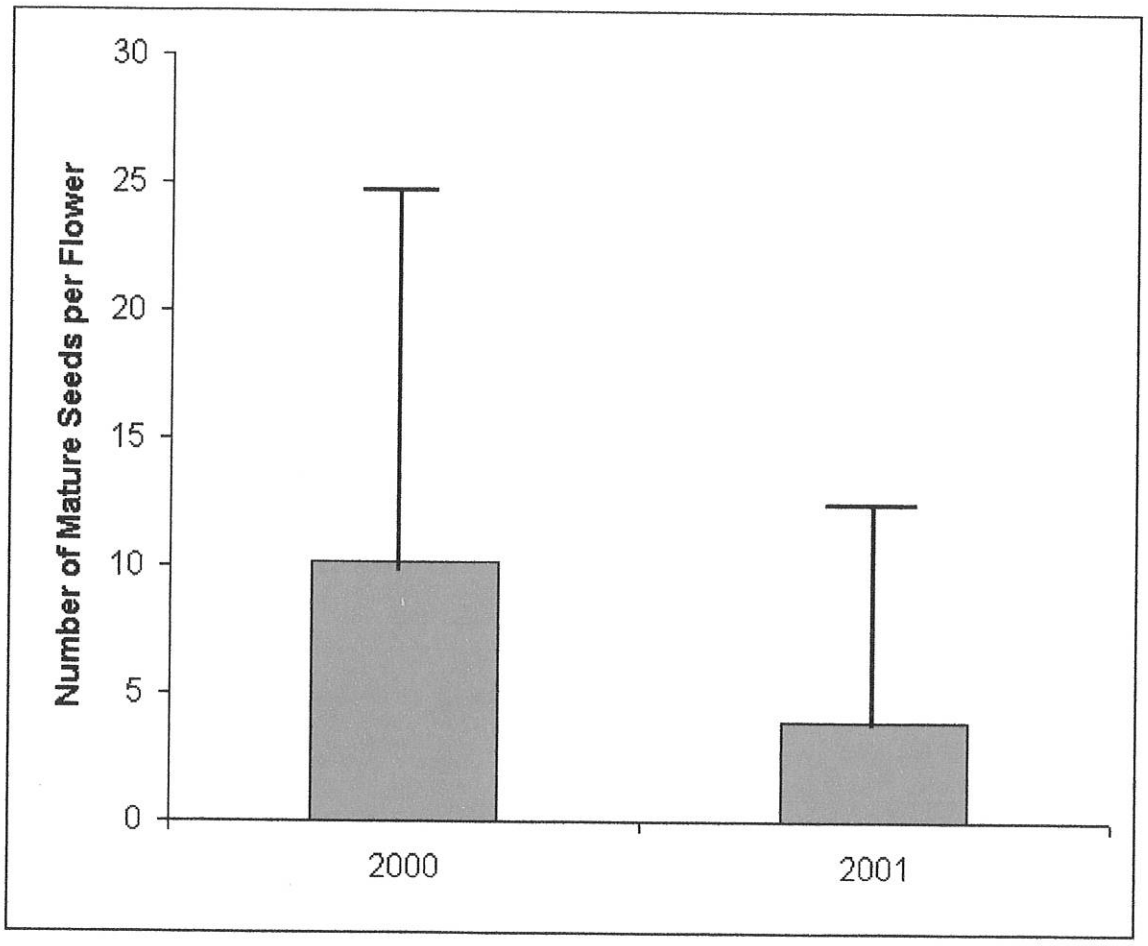

Figure 2. Mean difference (+ 1 S.D.) of total seeds between years (2000: N=33, 2001: N=45, $p<.05$), refer to Table 1.

		Adjusted Least Square Mean	SE	N
Year	= 2000			
Bagging	= Nonbagged	15.000	2.789	17
Year	= 2000			
Bagging	= Bagged	4.938	2.875	16
Year	= 2001			
Bagging	= Nonbagged	1.760	2.300	25
Year	= 2001			
Bagging	= Bagged	6.600	2.572	20

Table 2. Adjusted least squares means for interactions from ANOVA.

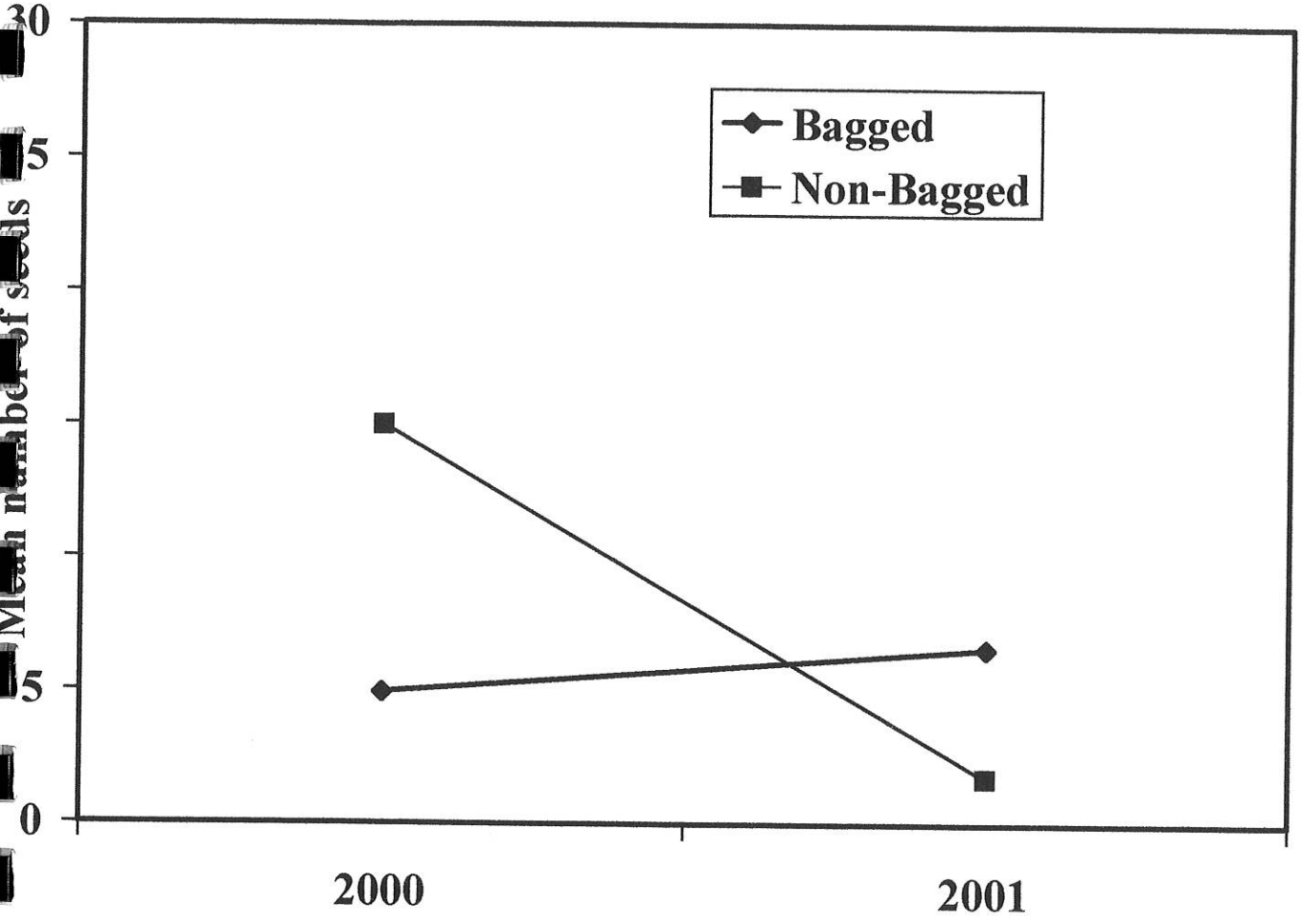

Figure 3. Plot of adjusted least square means for interactions.

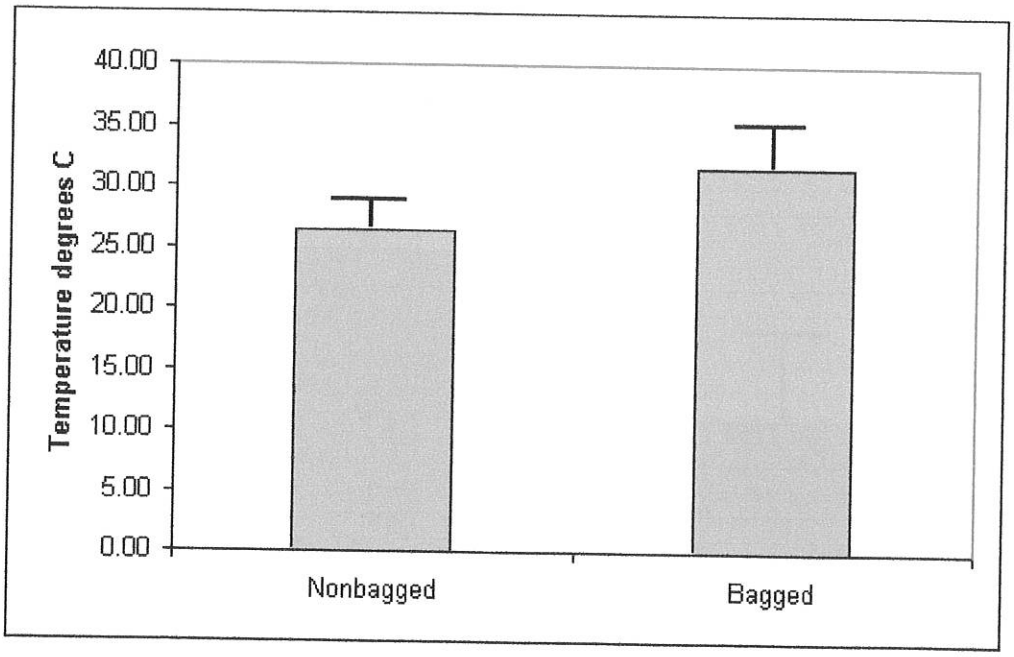

Figure 4. Mean temperature (+ 1 S.D.) of treatments for year 2000, (t=2.05, df=28, p<.001).

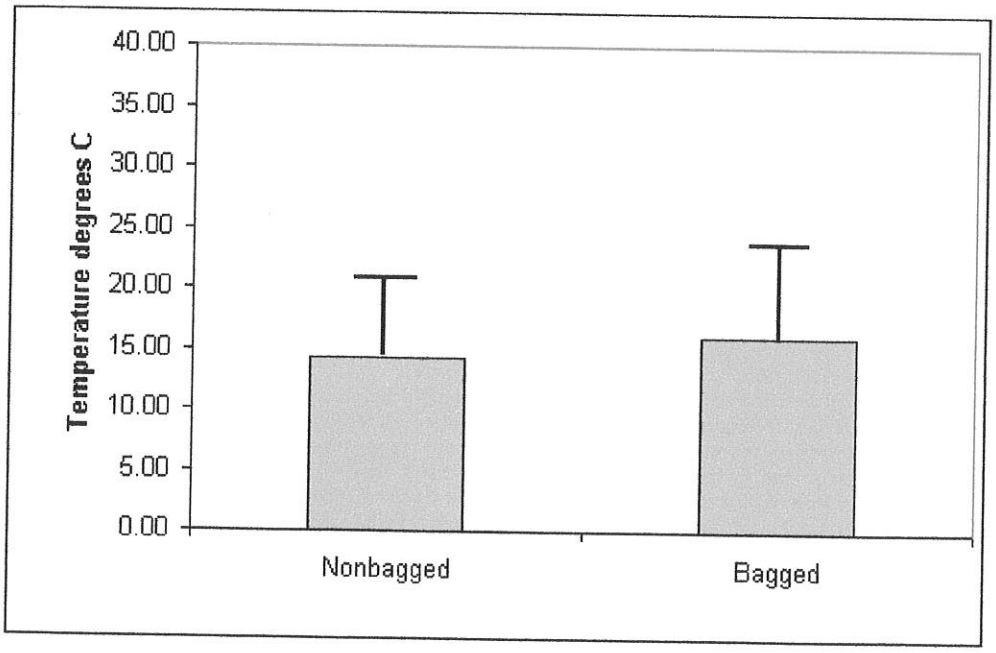

Figure 5. Mean temperature (+ 1 S.D.) of treatments for year 2001, (t=1.97, df=239, p>.05).

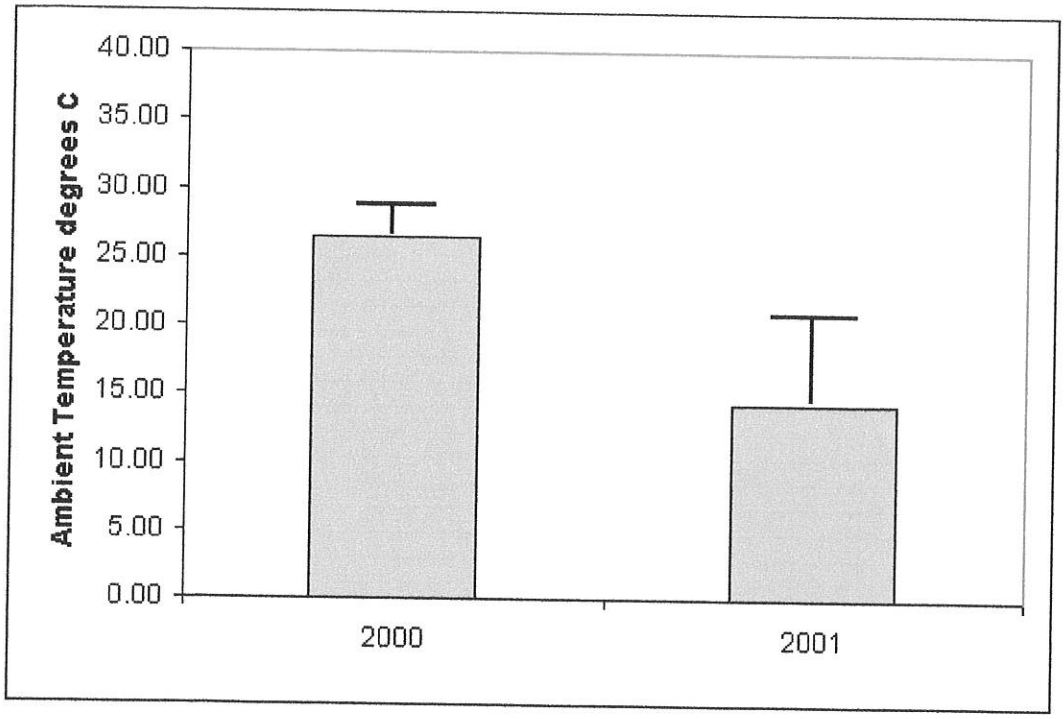

Figure 6. Comparison of mean temperature between years 2000 and 2001. (t=2.00, df=58, p<.001).