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Differences in Temperature Responses of Achene Types in *Centaurea melitensis*

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Abstract

*Southern California has a Mediterranean climate with wet winters and hot dry summers. This type of environment is associated with irregular temperature patterns and rainfall, which can be unpredictable. Unpredictable environments can threaten the germination and survival of plants like *Centaurea melitensis*. To ensure germination and survival under unpredictable environmental conditions, some plants may develop characteristic structures like cleistogamous flower heads, and in some cases they will develop heteromorphic achenes. Morphological differences between peripheral and center achenes may indicate variable responses to temperature. Additionally, unpredictable environmental conditions may lead to differences in the number of peripheral achenes to center achenes in each head type. This study was designed to investigate differences in germination of peripheral and center achene's of each head type CH (Chasmogamous), iCL (Initial cleistogamous) and fCL (Final cleistogamous) when subjected to constant temperatures 5°C, 10°C, 15°C, 23.5°C and 30°C. Differences in achene ratios (central to peripheral) and persistence were also investigated. A total of 400 achenes per achene type were used. Twenty achenes were placed in a petri dish filled with 6 ml of distilled water; the plates were incubated at one of five temperatures for seven days. The number of achenes (peripheral to central) remaining in the flower heads three months post dispersal achenes was documented. The results indicate that *Centaurea melitensis* was significantly affected by temperature. A fraction of the achenes remain in the flower head until early fall. Ratio differences in peripheral and center achenes may be a reflection of unstable environmental conditions.*

Centaurea melitensis is a non-native winter annual that resides in areas of disturbance, such as those found along roadsides, open fields and among California coastal sage scrub. It is also known to grow in subnitrophilous pioneer pasture communities (Porrás and Muñoz, 2000ab; Bossard and Randall, 2000). All these plant communities are associated with a Mediterranean type climate, which consists of wet winters and hot dry summers (Di Tomaso, 2001; Porrás and Muñoz, 2000ab; Maddox et al. 1985). Although predictable, this type of climate can result in irregular temperature patterns and rainfall variation (NOAA; Ritter, 2006; Porrás and Muñoz, 2000ab; Roche et al. 1997).

In southern California, where *Centaurea melitensis* is commonly found in open fields and among California coastal sage scrub, irregular temperature patterns and variable rainfall can lead to significant environmental changes threatening germination and survival (Porrás and Muñoz, 2000ab). For instance, in San Bernardino, California, temperatures during winter months can dip

down into the thirties resulting in foliage and ground freezing, which can destroy new seedlings or freeze achenes (seeds) (Gibson et al., 2012). Low temperatures can also slow down the metabolic rate hindering germination. In the summer the temperatures can get over hundred degrees producing near arid conditions, with little to no rainfall, preventing germination (NOAA; <http://www.wrcc@dri.edu>). The winds can also get very strong during the late summer, and fall making conditions even drier (Figure 1) NOAA; <http://www.wrcc@dri.edu>).

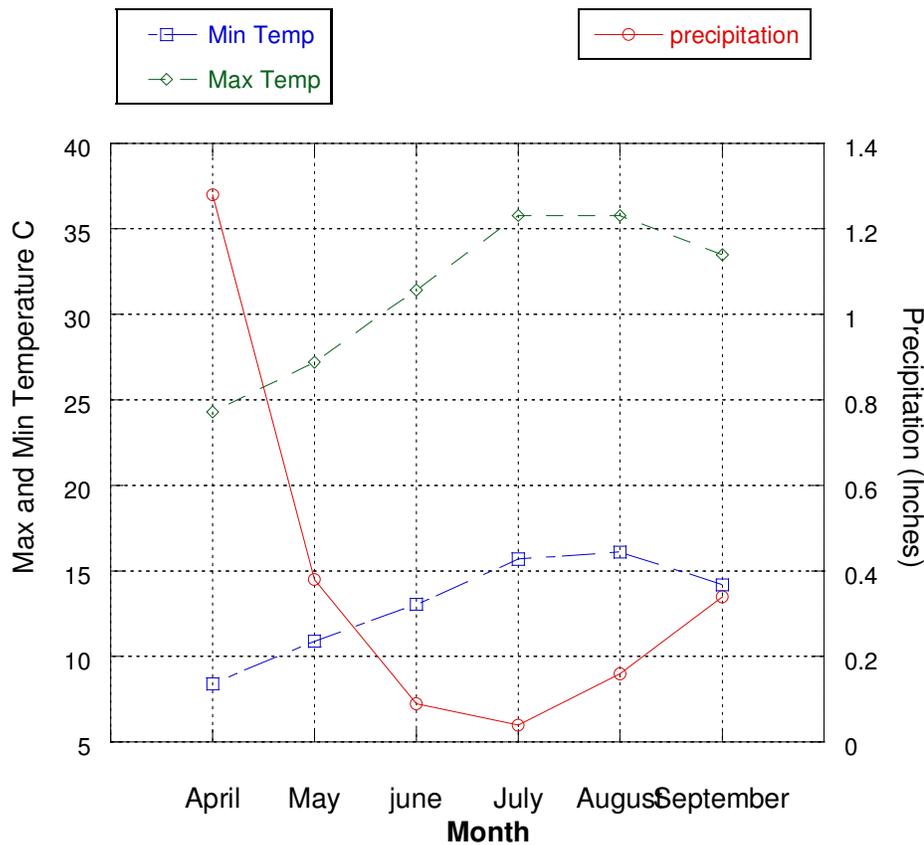


Figure. 1 Displays the average max temperature, average minimum temperature and average precipitation for San Bernardino, CA recorded by Western Regional Climate Center. The months April through September cover flower head and achene development and dispersal for *Centaurea melitensis*.

Despite the predictability of southern California's, Mediterranean climate, variation in temperature and rainfall can occur from season to season. Rainfall amounts can range between 12-24 inches (Tyrrel, 1981). During El Nino years the winter and spring months can be very wet, and during La Nina years the winter and spring months can produce little rain leading to dry conditions, which can compromise germination (NOAA, Larson and Kiemnec, 1997). These variations can also be seen in areas where the species reside such as San Bernardino, California.

Centaurea melitensis can be found in disturbed and undisturbed (chaparral) areas of San Bernardino, California. Irregular temperature patterns, variable rainfall and strong winds are common in this area. Garrett and Dutcher (1963) reported the annual rainfall between 1952 an

and 1971 was on average (426.7mm). Between 1981 and 2010 the mean daily temperature for San Bernardino, during March and April were 21.1°C and 24.3°C.

In order to deal with environmental variation in temperature and moisture, species of plants like *Centaurea melitensis* can develop morphologically different flower heads and morphologically different achenes that develop at different times during the growing season (Porrás and Muñoz, 2000ab; Di Tomaso, 2001; DiTomaso, 1996). Achenes are seeds or one seeded fruits with a thin wall as in a Sunflower seed (<http://waynesword.palomar.edu/termfr2.htm>). The growing season ranges from March to late June. The CH (Chasmogamous) flower heads and achenes develop in the middle of the growing season April through May. The iCL (initial cleistogamous) flower heads develop at the beginning of the growing season March through April. The fCL (final cleistogamous) flower head develop at the end of the growing season May through June. Each flower head also contains a peripheral and center achene that vary morphologically. Furthermore, not only do they vary morphologically but, they develop at different positions within the same flower head and disperse at different times throughout the growing season (Joley et al., 1997; Porrás and Muñoz, 2000ab; Robbins and Bellue et al., 1951; Di Tomaso, 2001).

Achene Position and Temperature

Morphologically different achenes that develop at different times and different locations within the flower head may respond differently to variable temperatures and moisture produced with changing environmental conditions found during the growing season.

In the two yellow starthistles found in California *Centaurea solstitialis* (northern California) and *Centaurea melitensis* (southern California), the peripheral achenes are located near the bracts and the center achenes are located in the center (Young and Clements et al., Di Tomaso, 2001, Di Tomaso 1996).

In the closely related congener of *Centaurea melitensis*, *Centaurea solstitialis*, the peripheral achenes are pappus-less and develop first. They also remain in the flower head until winter, until they are forced out through constant drying and wetting action that occurs with fall rains (Roche, 1992). The center achenes have a pappus and are located in the middle of the flower head are released first when the flower head senescence, usually during the summer (Di Tomaso, 2001; Di Tomaso 1996; Roche, 1992; Joley et al., 1997). In *Centaurea melitensis* the peripheral and center achenes both have a pappus. The center achenes release upon maturity and during flower head senescence following their developmental period. Peripheral achenes tend to remain in the flower for *Centaurea solstitialis* however; the persistence of peripheral and center achenes in *Centaurea melitensis* until fall is not known at this time and is currently being investigation.

Temperature and Yellow Starthistle (*Centaurea solstitialis*)

Several studies have examined the effects of temperature on *Centaurea solstitialis* achenes. Young et al. (2005) investigated the germination of *Centaurea solstitialis* under constant and alternating temperatures. They used temperatures ranging from very cold, cold fluctuating, fluctuating moderate and warmer. They found that the overall germination in the peripheral achenes was significantly lower than the center achenes. At 0°C no germination differences were observed between the achene types. The peripheral achenes were found to have lower

germination at alternating temperatures of 0°C/2°C, 0°C/5°C and a constant temperature of 2°C compared to the center achenes (Young and Clements et al., 2005). Young et al. (2005) attributed these differences to the position of the achenes in the flower head and dispersal time. In this study they were unable to find an optimum germination temperature regime. However, they did find that peripheral non-pappus bearing achenes preferred warmer temperatures and center pappus bearing achenes prefer colder temperatures (Young et al., 2005).

A more extensive study was conducted by Joley et al. (1997) comparing light and temperature responses on germination of *Centaurea solstitialis* achenes. For the purpose of this paper only the temperature aspects will be addressed. This study found that germination was greatest when both peripheral and central achenes were exposed to moderate temperatures compared to germination when exposed to extreme temperatures. They also observed a plateau of 100% germination in the middle three temperatures which ranged between 10°C and 25°C. They also tested fresh and stored achenes and found achenes freshly collected in July germinated best at 25°C:15°C and germination for stored achenes decreased at 25°C:15°C and 30°C:20°C (Joley et al., 1997).

Other Members of Asteraceae

Asteraceae is known for producing morphologically different achenes (Forsythe and Brown 1982). While they did not focus specifically on temperature they did address a small aspect of how temperature affected the morphologically different achenes from different positions of the flower head in the species *Bidens pilosa* L. In *Bidens pilosa* L. elongated achenes are narrow and located in the center of the flower head and shorter brown achenes are located in the periphery. Germination of both types of achenes occurred at 20°C, 25°C, and 30°C. They found optimum germination for the center and peripheral achenes was 25°C. Temperatures of 32°C did not yield and germinants for either achene type (Forsythe and Brown, 1982). They also discovered that longer achenes germinated over a larger range of conditions (Forsyth and Brown, 1982).

***Centaurea melitensis* and Temperature**

There has been a lot of research done on the closely related congener of *Centaurea melitensis*, *Centaurea solstitialis*, but very little has examined *Centaurea melitensis* response to temperature or other environmental cues. Most of what has been done has been conducted in Spain, which has a similar Mediterranean type climate. The average temperatures during *Centaurea melitensis*'s growing season in Cordoba Spain: March, April, May and June 21°C, 22°C, 26°C and 32°C (<http://climatedata.eu>). Spanish researchers Porras and Munoz (2000ab) looked for differences in germination capacity in achenes of the three head types CH (chasmogamous open flower head) and cleistogamous (iCL and fCL closed flower heads) in populations of *Centaurea melitensis* in Spain. They discovered the germination rate for CH achenes was greatest at 12°C, 25°C/15°C and 19°C. Germination was greatest for iCL achenes at 12°C. However the iCL achenes were adversely affected alternating temperatures 15°C/5°C and 25°C/15°C. They observed no adverse effects of any temperature in the fCL achenes (Porras and Munoz, 2000ab). Porras and Munoz (2000ab) touched on the effects of temperature between the three head types but they did not identify differences between peripheral and center achenes. Differences in temperature responses between peripheral achene and center achenes may indicate

different requirements for germination under variable environmental conditions in areas of disturbance.

Temperature plays a critical role in germination studies. The proper temperature can break achene dormancy, spark germination, and help determine the timing of emergence in the field for better biological control. Morphologically different achenes that develop and different position within the flower heads and disperse at different times throughout the growing season makes *Centaurea melitensis* an ideal model for temperature investigations. These differences have prompted the following questions; 1) Do peripheral and center achenes in *Centaurea melitensis* respond differently? 2) Is there an optimum temperature which they will germinate? 3) Is there a difference between the numbers of center achenes compared to peripheral achenes for each head type?

Purpose of the Study

The purpose of the study was to investigate the temperature responses of peripheral and center achenes of *Centaurea melitensis* achenes found in the three head types CH (chasmogamous), iCL (initial cleistogamous) and fCL (final cleistogamous). It was also designed to determine an optimum temperature for all achenes to use in future experiments. Additionally, this study examined the proportion of peripheral achenes to center achenes and its implications to temperature and moisture and looked at achene persistence.

Methods

The Response of *Centaurea melitensis* Achenes to Temperature

To investigate whether there are differences in the germination response of peripheral and center achenes of three head types CH, iCL and fCL of *Centaurea melitensis* (tocalote) to temperature a factorial experiment was performed.

The achenes were previously gathered in the morphology experiment and stored at room temperature 23.5°C in coin envelopes. Whole plants were removed from an area of coastal sage scrub located on the back side of the California State University San Bernardino campus. Each type of flower head was isolated and achenes were removed by hand.

A total of 400 achenes per achene type were used in the experiment (CHc, CHp, iCLc, iCLp, fCLc, and fclp). There were a total of 4 replicates. The peripheral and center achenes for all three head types (CH, iCL and fCL) were divided into batches of twenty achenes per replicate. Five temperatures were selected after reviewing literature on *Centaurea melitensis* and *Centaurea solstitialis* germination responses to temperature temperatures experienced during the fall in southern California and warmer temperatures of early summer time. They were 5°C, 10°C, 15°C, 23.5°C and 30°C).

A room temperature growth chamber was constructed using a cardboard box. The size of the box was consistent with size of the four incubators. The incubators had an outside dimension of 38.8cm wide x 28cm tall x 48cm deep. Inside dimensions were 31.7cm wide x 19cm tall x 31.7cm deep. The incubators were Revolutionary Science RS-IF-202 incubators. To obtain the same lightening that the incubator would achieve during the experimental process a clear plastic trash bag was placed over the opening and the light intensity was measured at all angles of the box. The back of the box was 0.24WM² (watts/per meter squared) the front end measured

.093WM². The incubators were labeled A, B, C, and D. The light intensity was measured in each incubator prior to placing experimental dishes in them (Table 1).

Table 1. Light intensity measurement inside incubators.

Chamber	Back or front	Doors open/closed	Light reading Watts/M ²
A	Back Front	open :closed open : closed	.006WM ² ,.006WM ² .130WM ² ,.030WM ²
B	Back Front	open :closed open :closed	.007WM ² ,.005WM ² .030WM ² ,.019WM ²
C	Back Front	Open :closed Open :closed	.010WM ² ,.006WM ² .176WM ² ,.050WM ²
D	Back Front	Open :closed Open :closed	.010WM ² ,.006WM ² .176WM ² ,.050WM ²

Experimental Setup

Twenty achenes for each achene type for each of five temperatures were sown in Fisherbrand sterile 100mm x 15mm polystyrene Petri Dishes with Fisherbrand glass filter paper (9.0cm). The achenes were moistened with 5ml of distilled water and the Petri Dishes were sealed with saran wrap to prevent moisture loss during incubation. The replicates were divided into 1-4. Only one set of replicates were placed in the incubators at a time due to size limitations. The temperatures were also changed to one of the designated temperatures each time a replicate set was placed in them. The set up was as follows replicate #1 incubator A) 5°C, B)10°C, C)15° and D)30°C and room temperature(Box). Replicate# incubators A) 15°C, B) 30°C, C) 5°C and D) 10°C. Replicate #3 A) 30°C, B) 15°C, C) 10°C and D) 5°C. Replicate #4 A) 10°C, B) 5°C, C) 15°C and D) 30°C. The achenes were incubated at one of five temperatures for seven days. Germination results were then scored and analyzed.

Methods to Determine Achene Ratios and Persistence

To determine the number of center achenes to peripheral achenes in all three head types (CH, iCL and fCL) 20 plants were randomly chosen from an area of coastal sage scrub located on the campus of California State University San Bernardino. The area is found north, north east of the Biology building. This area was approximately 34.188° North (latitude) and 117.324° west (longitude). Collection took place at the end of each developmental time following flower head senesce but prior to dispersal. The times end of April 2012 for iCL, end of May 2012 for CH and end of June 2012 for fCL. Approximately 11.04 hectares was included in the collection area. A total of 100 heads per head type were collected. The head type were brought back to the lab and

wrapped in saran wrap individually to prevent and achenes from spilling out should they open. The numbers of filled center, peripheral and aborted achenes were recorded.

Statistical analysis

All data values were collected and analyzed using the R project statistical program version i386 2.15.1. Several two-way ANOVA's were performed to examine the effect of temperature and position on germination for individual head types as well as and overall two ANOVA. A two-way ANOVA testing the effect of temperature and head type on germination was also performed.

Results

Examination of the effect of temperature (5°C, 10°C, 15°C, 23.5°C, 30°C) on *Centaurea melitensis* peripheral and center achenes for each head type (CH, iCL, and fCL) showed broad temperature optimums. There was a lack of germination at 5°C for both peripheral and center achenes for the CH and fCL head types (Figure 2, 3, 4). On average there was more germination in the center achenes compared to the peripheral achenes for all temperatures 10°C, 15°C, 23.5°C and 30°C. In the CH head type there was a significant decline in germination at 15°C (M Chc = 19.0, SD = 0, M = CHp 16.0, SD = 2.00) $t(6) = 3.00$, $p = 0.024$. However, the pattern of response was similar to the center achenes for each temperature (Figure 2). In the iCL head type both peripheral and center achenes showed a significant drop in temperature beyond 10°C, suggesting they prefer cooler temperatures. For both peripheral and center achenes the pattern of response to temperatures was similar in iCL achenes (Figure 3). In the fCL head type both peripheral and center achenes showed a similar response to temperature. The peripheral achenes showed a significant drop in germination at 15°C (M fCLc = 18.0, SD = 1.83, M fCLp = 12.5, SD = 1.29) $t(6) = 4.92$, $p = 0.002$.

Two way ANOVA- examining the effect of temperature and position (peripheral vs. center) on germination showed significant effect of temperature ($F = 13.28$, $DF = 1$), ($p = 0.0004$) and a significant effect position on germination ($F = 87.2$, $DF = 2$), ($p = 2.2 \times 10^{-16}$) with no interaction.

Individual two-way ANOVA's for examination of the effect of temperature and position on germination showed the following results. **CH** - there was a significant effect of temperature and position on germination of achenes within the CH head type. There was no interaction found (Table 2). **ICL** - There was a significant effect of temperature and position and germination of iCL achenes. There was no interaction (Table 3). **FCL** - there was a significant effect of temperature and position on germination of iCL achenes. There was no interaction between temperature and position (Table 4).

Two way ANOVA- examination of the effect of head type and temperature on germination showed a significant effect of head type ($F = 40.04$, $DF = 2$), ($p = 1.86 \times 10^{-13}$) and a significant effect of temperature on germination ($F = 151.26$, $DF = 4$), ($p = 2.2 \times 10^{-16}$). There was also a significant interaction between head type and temperature on germination suggesting there are differences among head types in their germination response to temperature ($F = 4.87$, $DF = 8$), ($p = 3.9 \times 10^{-05}$).

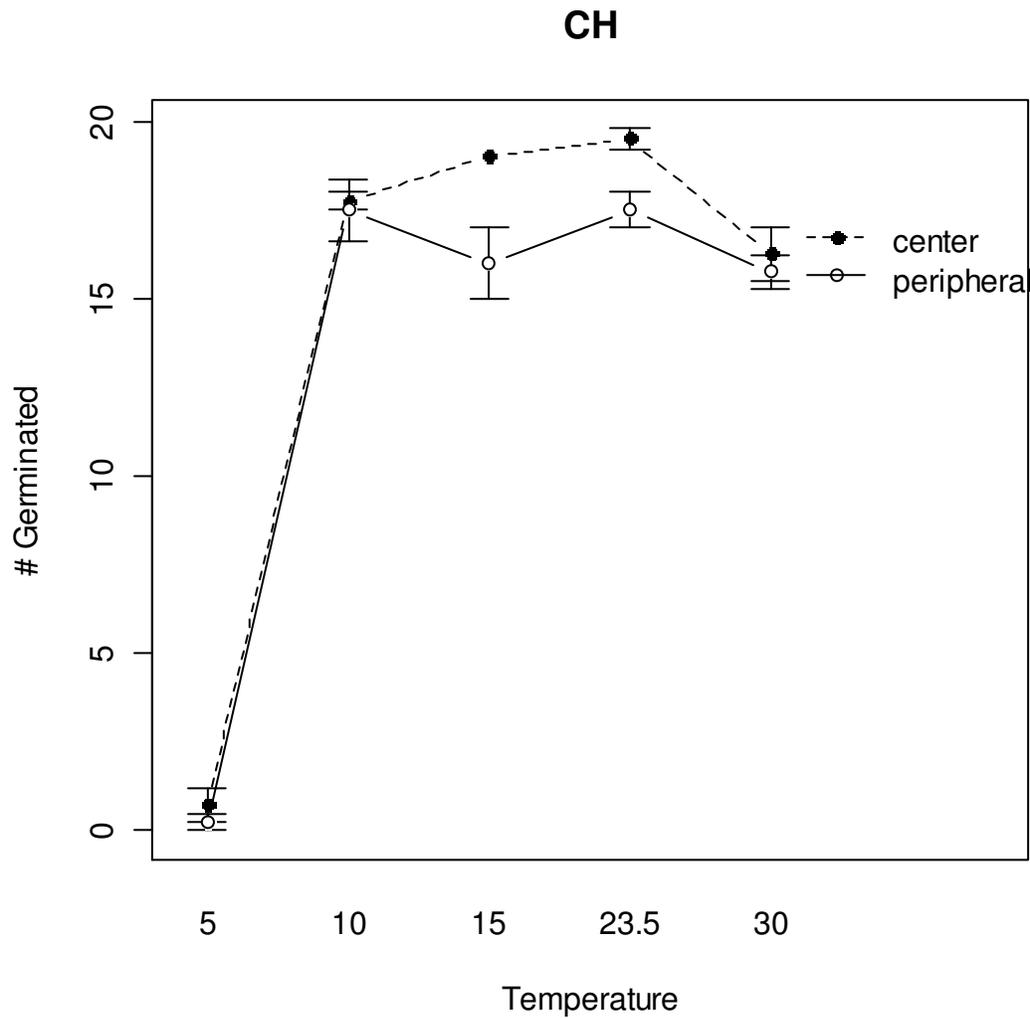


Figure 2. Shows the number germinated plotted against the temperature. The center achenes in the chasmogamous head type had a greater germination than the peripheral at each temperature with the exception of 5 degrees C. The greatest germination occurred at 10°C and 15°C for center and declined at 15°C for the peripheral achenes. The bars represent standard error.

Table 2. Two way ANOVA showing effect of temperature and position on germination of CH achenes from the chasmogamous head type.

Source of Variation	DF	Sum Sq	Mean Sq	F value	Pr(>F)
Temp	4	1855.10	463.77	359.05	2.2e16***
Position	1	15.62	15.62	12.096	0.00156**
Temp:Position	4	11.50	2.87	2.2258	0.089 .
Residuals	30	38.75	1.29		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

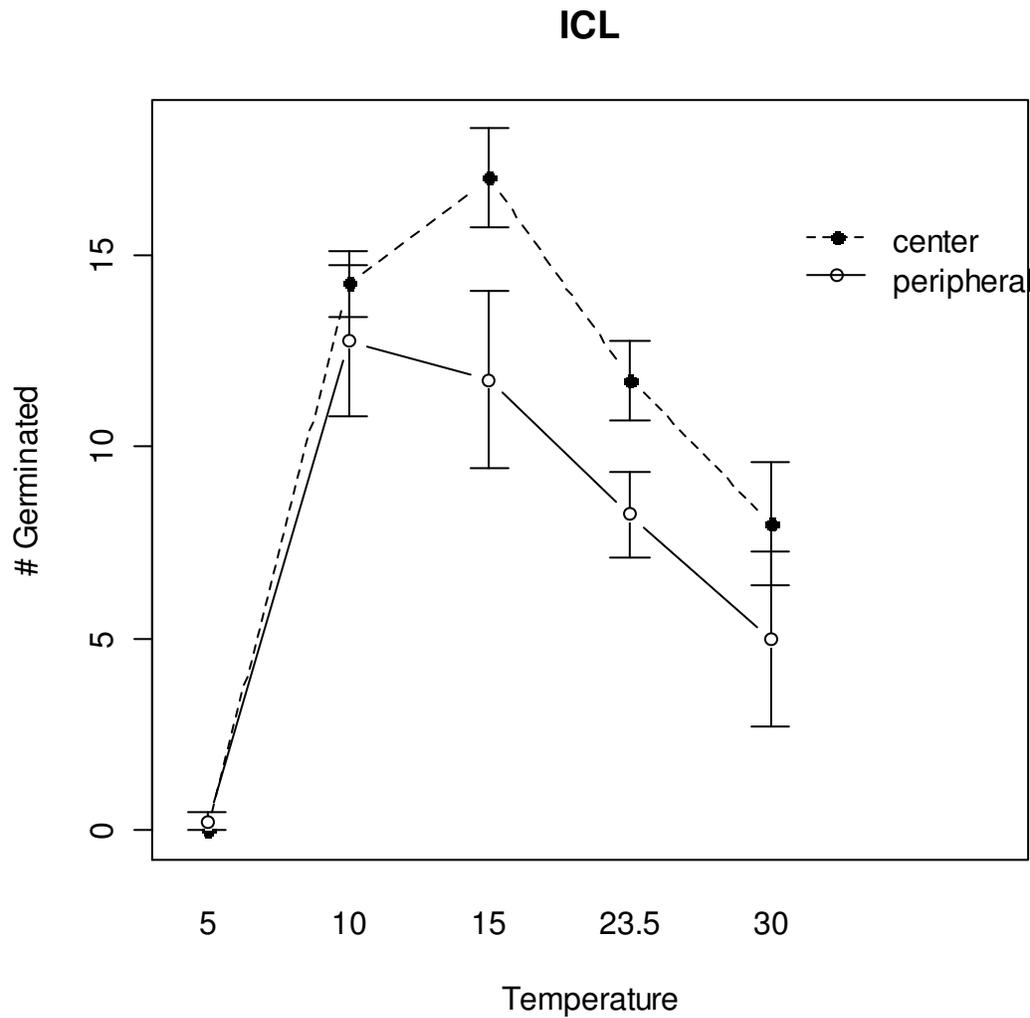


Figure 3. Shows the number germinated plotted against temperature for the initial cleistogamous achenes. There was more germination in the center achenes than the peripheral achenes. The number germinated increased at 10°C and 15°C in both the peripheral and center achenes and decline at 23.5°C and 30°C.

Table 3. Two- way ANOVA showing the effect of temperature and position on the iCL achenes within the initial cleistogamous head type.

Source of variation	DF	Sum Sq Pr(>F)	Mean Sq	F value	Pr(>F)
Temp	4	1080.85	270.212	31.3593	2.517e-10 ***
Position	1	67.60	67.600	67.600	0.008833 **
Temp:Position	4	34.65	8.662	1.0053	0.420249
Residuals	30	258.50	8.617		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

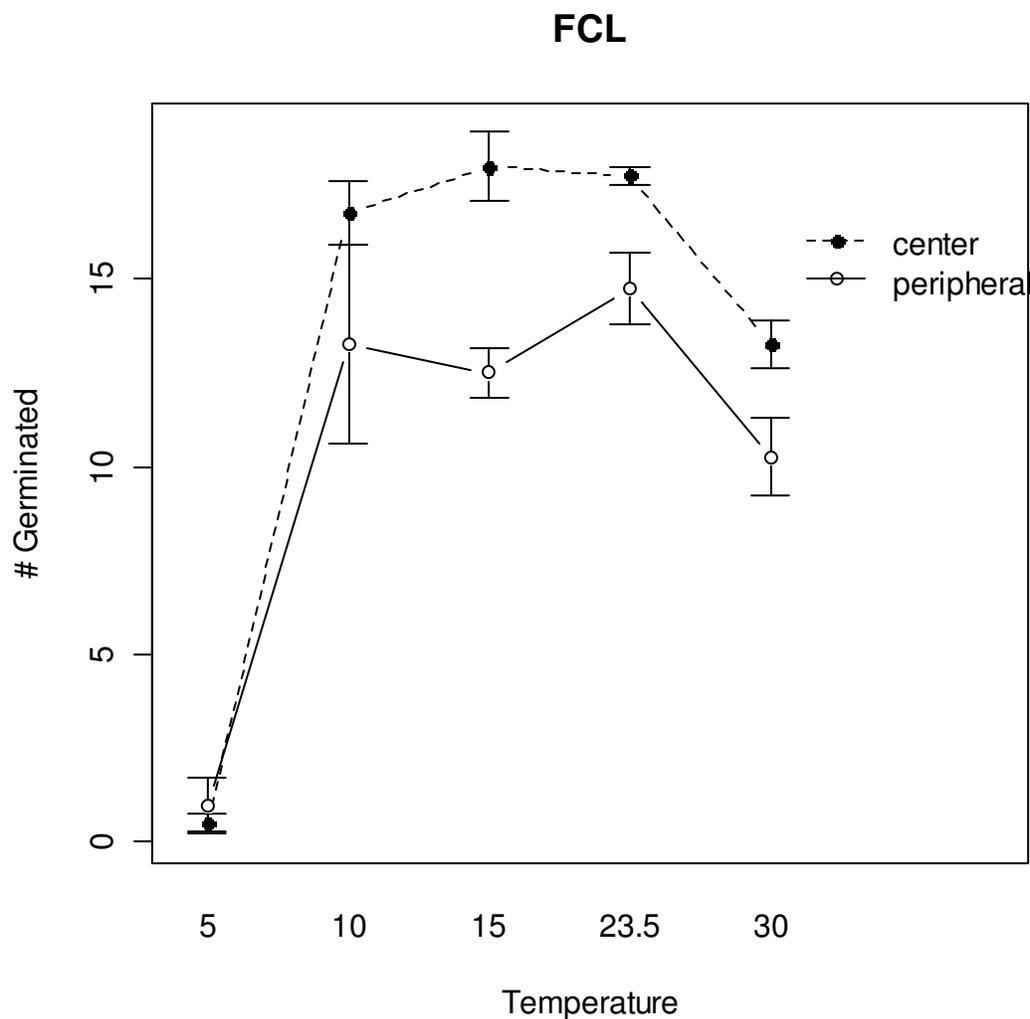


Figure 4. The graph shows the number germinated plotted against temperature for the fCL achenes. There is more germination the center achenes compared to peripheral achenes. There is an increase in germination at 10°C and 15°C for the center, it decreases at 15°C for the peripheral and increases for fcl at 23.5°C.

Table 4. Two-way ANOVA showing the effects of temperature and position on the fCL achenes from the final cleistogamous headtype.

Source of variation	DF	Sum Sq	Mean Sq	F value	Residuals
Temp	4	1312.4	328.10	68.1176	1.283e-14 ***
Position	1	84.1	84.10	17.4602	0.00023***
Temp:Position	4	37.4	9.35	1.9412	0.1293078
Residuals	30	144.5	4.82		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Achene Persistence and Ratio Differences

Differences in achene ratios were found in all three head types of *Centaurea melitensis*. There were more center achenes than peripheral achenes for each head type (CH, iCL and fCL). The largest ratio of center achenes to peripheral achenes occurred in CH achenes (3:82:1) and the smallest ratio of center to peripheral achenes occurred in fCL (1.94:1) and iCL was in the middle (2.23:1) (Table 5).

Persistence investigations included data collected in September of 2011. This was three months post dispersal, a comparison of early counts pre-dispersal and post-dispersal showed a fraction of the achenes still remain in the flower head (data not shown).

Table 5. The number of center achenes to peripheral achenes in three head types of *Centaurea melitensis*.

Head Type	Number of filled center achenes (mean and range)	Number of filled peripheral achenes (mean and range)	Ratio of center to peripheral achenes
CH (n= 100)	25.5 (0-47)	6.7 (0-16)	3.82:1
iCL (n=83)	2.6 (0-10)	1.2 (0-3)	2.23:1
fCL (n=95)	1.7 (0-7)	0.9 (0-4)	1.94:1

(n) Stands for the number of heads containing achenes.

Discussion

Temperature plays a critical role in germination of fairly short lived annuals like *Centaurea melitensis* that reside in areas of disturbance. As previously mentioned in southern California these areas are associated with irregular temperature patterns and moisture due to the Mediterranean climate (ARR, 1981; Di Tomaso, 2001; Porras and Munoz, 2000). *Centaurea melitensis* ability to produce morphologically different achenes that disperse from different locations within same flower is an ecologic benefit to the species since germination can occur

over a range of temperatures. My data showed that *Centaurea melitensis* achenes germinated over a range of temperatures and had a broad temperature optimum. This was in two head types CH, and fCL. The iCL achenes were more restricted to cooler temperatures. Germination over a variety of temperatures is consistent in Asteraceae as seen by the studies conducted on its closely related congener *Centaurea solstitialis* (Joley et al 1997; Young et al. 2005). My data also showed that achene of *Centaurea melitensis* had little to no germination at temperature of 5°C and 30°C. These were indicative of a very cold winter day or a very hot summer day. This data is also consistent with other studies like the one performed by Young et al. (2005) which showed that very little germination occurred in either center or peripheral at temperatures of 0°C and 2°C or for *Bidens pilosa* at 32°C (Forsyth and Brown, 1982).

My experiment looked closer into the effects of temperature in *Centaurea melitensis* achenes from the three different head types. Porras and Munoz (2000ab) examined differences in germination response to temperatures within the three head types, however, they did not recognize the presence of peripheral achenes or study difference related to them. In a previous study I discovered the presence of peripheral achenes and differences in their morphology, which inspired this study. This study is the first to examine the effects of temperature on peripheral and center achenes using constant temperatures. Porras and Munoz (2000ab) data indicated that the highest germination for the CH achenes was at 12°C and 25°C/15°C and 19°C. My data showed the highest germination occurred for CH achenes both peripheral and central occurred at 23.5°C, which is somewhere in between. Porras and Munoz (2000ab) data showed that iCL achenes achieved their highest germination at 12°C and were adversely affected by 15°C/5°C and 25°C/15°C. My data indicated the highest germination occurred between 10°C and 15°C and beyond that there was a significant decline in germination for both peripheral and center achenes. The iCL achenes are the first to develop; they usually begin developing in March through April, which are still fairly cool months. It's also possible that these achenes may be exhibiting some sort of dormancy and are harder to germinate under laboratory conditions. These achenes also have the largest elaiosome which is an oily body located at the base of the achene that attracts ants. They can be carried off to ant hills where they are possibly buried and their exposure to direct sunlight is less by soil burial where cooler temperatures exist (Porras and Munoz, 2000ab). Interestingly, their data also showed that the fCL achenes germinated at all temperatures and were not adversely affected by any temperature regime. My data indicated that germination was highest between 15°C and 23.5°C and there was a decline in germination with higher temperatures. My data also showed that peripheral achenes had a drop in germination at 15°C. I am not sure what to make of this but these achenes develop last in the season and usually are the plants last effort to produce achenes before full senescence. They develop in May through June so; they may just prefer warmer temperatures. It is also possible, that these achenes are responding to limits rainfall and increase temperatures and only germinate when conditions are acceptable for seedling survival. Having the ability to respond to variable temperatures in areas of disturbance where environmental conditions are unpredictable can be beneficial because when seasons change there always a potential for germination and long term survival of the species. Some of the response differences could be related to continental changes in landscape or genetics or in climate or location like areas of coastal sage scrub in southern California which experience high winds. These areas are also covered with other species, which may influence germination. *Centaurea melitensis* is found among many other species such as *Salvia mellifera*, *Bromus madritensis* and *Emmenanthe* which all compete for space and nutrients in the soil.

Achene Ratio Differences and Persistence

Differences in achene ratios are thought to be the result of residing in areas where environmental conditions vary. My data indicated differences between peripheral achenes and center achenes for all three head types. In *Centaurea solstitialis* the peripheral achenes preferred warmer temperatures and center prefer cooler. However, I cannot state with certainty that these differences are related to temperature fluctuations. It may be more of a mechanism of survival. Some species are known to release achenes at various times during the growing season to optimize fitness in unpredictable environments (Oloffson, Ripa et al., 2009). The question becomes then do peripheral achenes persist in the flower head until fall as they do in *Centaurea solstitialis*? My data indicates a fraction of both the peripheral achenes and center achenes persist into the early fall however, more research is needed to confirm this.

Summary

Centaurea melitensis germination was significantly affected by temperature. Both peripheral and center achenes germinate over broad range of temperatures between 10°C – 30°C. The most optimum temperature for germination of all achenes is 15°C. Of all the achenes the iCL achenes prefer cooler temperatures. Having the ability to respond to wide range of temperatures may not only increase the chances of germination success in areas where there are irregular temperate patterns of rainfall, but may prevent premature germination when there is lack of rainfall ensuring survival.

Residing in areas of disturbance that have irregular temperature and moisture patterns may lead to the development of achenes at different positions within the same flower head that disperse at different times. These differences may allow a fraction of peripheral and center achenes to remain in the flower head several months post dispersal to assure the possibility of a future generation. The remaining achenes both peripheral and central may be able to withstand higher temperatures however, more testing is indicated.

Ecological Implications

From an ecological standpoint, the peripheral achenes may contribute more to the seed bank than the center achenes, because they are more likely to be caught up in the bracts and dispersed when the flower head fully senescence. They also germinate more slowly. The iCL achenes prefer colder temperatures and are more likely to germinate in winter months. They may also add to the seed bank. Having achenes that develop at different positions with the same flower and persist in the flower head for several months ensures a second chance at dispersal and germination. Ratio differences peripheral vs. central implicate a strategy for survival in unpredictable environments. This strategy increases the chance of germination many months after dispersal.

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