12-2015

VARIATION BETWEEN PERIPHERAL AND CENTER ACHENE MORPHOLOGY AND ATTRIBUTES OF ACHENE GERMINATION IN THE INVASIVE SPECIES, CENTAUREA MELITENSIS

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VARIATION BETWEEN PERIPHERAL AND CENTER ACHENE MORPHOLOGY AND ATTRIBUTES OF ACHENE GERMINATION IN THE INVASIVE SPECIES, *CENTAUREA MELITENSIS*

A Thesis
Presented to the
Faculty of
California State University,
San Bernardino

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
in
Biology

by
Kandee Lea Bain
December 2015
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Approved by:

Kimberlyn Williams, Committee Chair, Biology

John Skillman, Committee Member

Anthony Metcalf, Committee Member
ABSTRACT

Invasive species are often successful and problematic because of their ability to persist in disturbed and undisturbed environments despite weed management practices. Understanding reproduction and dispersal strategies in these species can aid in developing management approaches to help control their spread. *Centaurea melitensis*, a nonnative invasive with European origins, is found in disturbed areas of southern California. It produces three different types of flower heads that develop at different times and at different locations on the plant during the growing season. The chasmogamous (CH) flower heads are located at the top of the plant, the initial cleistogamous (iCL) heads are located at the base of the plant and at some branch and axillary points, and the final cleistogamous (fCL) heads are located along the stem and at some branch points. This pattern differs from that in *Centaurea solstitialis*, its most closely related congener, which develops one type of flower head with two morphologically distinct achenes within each flower head: peripheral and center achenes. The overall objective of this study was to examine potential differences between peripheral and center achenes of *Centaurea melitensis*, including morphological differences in dispersal features, potential differences in response to temperature, tendency to disperse, dormancy and viability. The approach involved selecting whole plants from the field and separating peripheral and center achenes from each of the three head types. Morphological differences were assessed by measuring mass, fruit length, fruit width, pappus length,
pappus width, and elaiosome features. Temperature response patterns were evaluated by exposing peripheral and center achenes to temperatures ranging from 5 ºC to 30 ºC and measuring germination. Tendency of different achene types to remain in the flower heads was assessed by comparing ratios of peripheral to center achenes in heads immediately after maturation (March – June) to the ratios remaining in heads in early fall (September). Viability and dormancy testing was performed using a cut test and tetrazolium chloride tests in conjunction with germination tests. Results of these studies indicate that peripheral achenes of *Centaurea melitensis* were lighter and narrower, with shorter pappi and smaller elaiosomes than center achenes. Peripheral achenes responded similarly to their center counterparts in germination response to temperature, but the pattern differed among head types. Broad temperature optima were observed within the fCL and CH heads and a narrow optimum was observed in iCL. There was no evidence that the peripheral achenes remained in the heads longer than the center achenes. Ratios of peripheral to center achenes were 3.8:1 in CH heads, 2.23:1 in iCL heads, and 1.94:1 in fCL heads. Peripheral achenes were more likely to be dormant while maintaining viability than center achenes were. The results of this study, therefore, indicate that peripheral and center achenes of *Centaurea melitensis* differ morphologically. Dispersal features, such as pappi and elaiosomes, were more highly developed in center achenes than in peripheral achenes, but these differences were not reflected in differences in behavior to the extent we could measure it (i.e., the tendency to
remain in heads or the germination responses to temperature). Differences between peripheral and center achenes of Centaurea melitensis trended in the same direction as differences seen in its closely related congener, Centaurea solstitialis, which has center achenes that disperse more readily than peripheral achenes. However, the differences observed in Centaurea melitensis were not as pronounced as those seen in Centaurea solstitialis.
ACKNOWLEDGEMENTS

I would like to thank my P.I. Dr. Kimberlyn Williams, whose plant expertise was a continuous pool of knowledge and help on my study subject, *Centaurea melitensis*. Also for her patience and understanding throughout my research. She has been a great influence in my undergraduate interest in plants and graduate research interest in the field of invasive plant research and ecology. She also has been a great help in the laboratory and in the field during my study. Her knowledge and skills on native and non-native plants of Southern California have enhanced my understanding of the nature and behavior of plants in the field and recognition of many species found in and among our California coastal sage scrub and the effects of fire on the plant community. With gratitude I thank her for her help in writing skills on my thesis, scholarships and grants. I would like to thank my committee, Dr. John Skillman for his knowledge on ecology and plant populations as well as the use of his lab. I thank want to thank Dr. Tony Metcalf for his feedback on my research and for his knowledge on ecology, conservation and plant populations. I would like to thank Dr. Kevin Middleton for his training and expertise on the statistical program R as well as his statistical advising on analyzing the data during the my research project.

I owe a special thanks to Dr. Gerald Thrush, whose professional and profound enthusiasm quarter after quarter in his teaching and understandings of students influenced my desire to pursue graduate school and obtain a master in Biology and further my studies in a PhD program and become a university
instructor. His compassion for students and teaching has rubbed off on me and my dedication to the laboratory teaching as a graduate student. He also taught me to persevere in what I seek to do and give it all that I have, and that reaching a goal is not how long the road is but the end result and that is to continue my research and teach at the university level.

I would also like to thank Dr. Michael Wall from Rancho Santa Ana Botanic Garden seed lab for the opportunity to do an internship where I learned multiple skills that I used in conducting my research (germination studies, preserving seeds, collecting seeds, viability testing and dormancy testing).

I would also thank my family and extended family and friends and network of people for their continuous support through my many academic endeavors and nights of staying awake studying.

In conclusion I would like to thank Dr. Williams and the University for the use of her lab and equipment during my research and the many academic scholarships that I received for materials through my research.
DEDICATION

Kandee Bain passed away in October of 2015, shortly before receiving her master’s degree. Kandee’s colleagues, friends, and family dedicate this thesis to her courage, tenacity, and optimism, and submit it on her behalf.
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CHAPTER ONE

INTRODUCTION

The Study Species: *Centaurea melitensis* (Tocalote)

There are more than twenty taxa and sub-taxa of *Centaurea* that exist in Spain (Riba et al. 2002). Here in California there are twelve species. All of these are nonnative to California and nine have purple or white flowers. There are three yellow-flowered species that reside in California: *Centaurea solstitialis* L., *Centaurea sulphurea* Willd., and *Centaurea melitensis* L. All three of the yellow-flowered species have sharp needle-like spines on their flower heads (DiTomaso 2001).

*Centaurea melitensis* (tocalote), a member of the Asteraceae family, is one of the yellow starthistles. It is classified as an annual or biennial herb, which is accustomed to changing environments (Halvorson and Guertin 2003). *Centaurea melitensis* is known to reside in areas that are created by disturbance such open fields, roadsides and fire-prone communities like California coastal sage scrub (Riba et al. 2002). Like many other nonnative species it has European origins that include Southern Europe, North and West Africa as well as Macaronesia (Porras and Muñoz 2000a). The flowers are brilliant yellow, hence the name yellow starthistle. The leaves are arranged alternately. The inflorescences are discoid and are 10 – 15 mm in size (Keil and Turner 1993). The central spines on the flower head are about 5 – 10 mm long (DiTomaso 2001).
Seed Production and Variation in *Centaurea melitensis* (Cleistogamy)

*Centaurea melitensis* is a cleistogamous species, which means it has smaller flower heads as well as regular size flower heads on the plant (Porras and Muñoz 2000a). More specifically, cleistogamy is defined as “the state of bearing small flowers as well as fully developed flowers as in a pansy, in which the small ones do not open but are pollinated by their own anthers” (Ologies & Isms 2008). There are generally two types of flowers in cleistogamous species, small cleistogamous flowers, which lack an anthesis, and chasmogamous flowers, which are open and potentially allogamous (reproduce by cross fertilization) (Porras and Muñoz 2000a). *Centaurea melitensis* is the only member of the Asteraceae family that possesses chasmogamous and two types of cleistogamous flower heads (iCL and fCL) on the same plant (Porras and Muñoz 2000a). In contrast to traditional cleistogamy, which involves individual flowers remaining closed at anthesis, the head of flowers (or capitulum) is the functional reproductive unit in *Centaurea melitensis*, with the involucre of the capitulum enclosing the florets and preventing access to pollinators (Porras and Muñoz 2000a, 2000b, 2000c). The flower heads are characterized by their functional and morphological characteristics and location on the plant. Both the flower heads and the fruits are heteromorphic (Porras and Muñoz 2000b). According to Porras and Muñoz (2000a, 2000b) cleistogamy includes structural as well as functional variability of the plants reproductive system that requires a balance.
Variation among flower heads in *Centaurea melitensis* was described by Porras and Muñoz (2000a). The first flower head to develop is the iCL (initial cleistogamous) head, which is located at or near the base of the plant. The number of flower heads produced for iCL range between 1 and 6. The number of florets in these heads ranges from 15 to 25 (Porras and Muñoz, 2000a). The function of these flower heads is to produce seeds at the beginning of the flowering period to assure some seeds are available. The iCL flower heads produce achenes (single-seeded fruits) that are fairly large and have shorter pappi compared to the other two types. They are approximately 1.33 mm wide with a pappus diameter of 3.60 mm (Porras and Muñoz 2000b). The second flower head to develop is the largest and is called CH (chasmogamous), which grows in the middle of the flowering period and is located near the apex of the plant. The number of CH flower heads can range from 45-100. Their function is to attract pollinators (Porras and Muñoz 2000a). The CH flower heads produce much smaller achenes than fCL and iCL, but the CH achene has a more developed pappus. The width of this achene is approximately 1.06 mm with a pappus diameter of 5.18 mm (Porras and Muñoz 2000b; Figure 1). The last flower head to develop is called fCL (final cleistogamous). These flower heads range in number from 3 to 13 and may function as a reserve by offering a low cost source of seeds when most of the other seeds have been dispersed (Porras and Muñoz 2000a, 2000b, 2000c). The fCL flower head produces achenes that are similar in size to those of the CH heads, but their pappi are not as developed...
(Porras and Muñoz 2000b). The average width of fCL achenes is 1.13 mm and its pappus diameter averages 2.94 mm.

Figure 1. Three achene types of *Centaurea melitensis*, CH, iCL and fCL.

**Phenological Development of *Centaurea melitensis***

Optimal environmental conditions lead to germination of the various achenes during the rainy part of the season: October through March. Achenes begin to emerge at various times throughout the season producing a seedling with two cotyledons. This is followed by formation of several sets of leaves. The leaves are lobed and unlobed; the edges of the leaf blades can have both teeth and lobes (New England Wild Flower Society 2011). Development of 8 - 15 leaves above ground leads to formation of a rosette, which lies flat on the ground and measures 5 – 15 cm in diameter (Figure 2). The plant remains in the rosette
stage until early spring. The leaves are a grayish green. The iCL flower head emerges from the basil rosette. The main shoot develops about the same time, along with a poorly developed branch. The vegetative system is both sympodial and basilpetal (the main axis doesn’t grow but another iCL flower head emerges at the top of it). First order branches emerge from axillary buds, followed by terminal bud development that are located at the base of the branch and emerge from axillary buds also. This is followed by terminal bud development that also arises from the base of the branch and emerges underneath these. Another iCL develop at the tip or a CH flower head (Porras and Muñoz 2000a; DiTomaso 2001; Halvorson and Guertin 2003).

Figure 2. *Centaurea melitensis* development.
Main shoot development from rosette with iCL flower head (late April 2013).
There is a balance between the chasmogamous and cleistogamous flower heads in this particular species. Chasmogamous heads primarily function to perpetuate the species through colonization of new habitats and are adapted to pollinators. These flower heads function in response to the environmental conditions. In other words, when growth conditions are favorable the plant will increase in size, and the number of chasmogamous flower heads will increase. Porras and Muñoz (2000a) found that production of chasmogamous heads (CH) require the plant to be at least a 35 cm tall. However, when conditions are less favorable and the plant is smaller, more cleistogamous flower heads will be favored (Porras and Muñoz, 2000a). The first cleistogamous flower heads (iCL) develop with no regard to height as they enable the production of achenes immediately, securing a seed bank for future progeny (Porras and Muñoz 2000a). The fCL flower heads however, depend on shoot development since they develop at the end of the growing season and ensure a low cost back-up plan to produce achenes at the end of the season (Porras and Muñoz 2000a, 2000b, 2000c). In the case of *Centaurea melitensis*, changes in environment can also affect the number of achenes and flower heads produced through the growing season. This is contrasted with the closely related congener *Centaurea solstitialis*, which produces only chasmogamous heads (DiTomaso 2001).

Development is not rapid or synchronous. There are lags between flower head development and achene development. The iCL flower heads take about two weeks to develop and reach maturity. The CH heads take a full month to
develop and mature and reach anthesis. This leads to overlaps between development of other flower heads with achenes like the fCL which takes about two weeks to develop (Porras and Muñoz, 2000a)

Patterns of Achene Variation in *Centaurea* Species

According to Porras and Muñoz (2000b) the phenomena of achene heteromorphism in the Asteraceae family is associated with intracapitular variation. In other words there can be two types of achenes in one flower head that are different in shape and size. This type of achene variation has been identified in *Centaurea solstitialis*, which is the sister species of *Centaurea melitensis* (Garcia-Jacas et al. 2006). *Centaurea solstitialis* has two types of achenes: those that are pappus-bearing and those that have no pappus or a poorly developed pappus (DiTomaso 2001). All the achenes of *Centaurea melitensis* possess a pappus. Similar to *Centaurea solstitialis*, *Centaurea melitensis* has achenes located in the periphery of the flower head and in the center of the flower head in a ring. According to Roche (1992), the achenes located in the center of the flower head of *Centaurea solstitialis* are dispersed first, and the achenes in the periphery tend to remain in the flower until early winter. Therefore, it reasonable to assume that the achenes in the periphery of the flower head *Centaurea melitensis* may exhibit the same behavior. Not only are the achenes different in shape and size in *Centaurea solstitialis*, but they may also exhibit different germination patterns. Although some researchers have found little evidence of dormancy in seeds of *Centaurea solstitialis* (e.g.,
Benefield et al. 2001; Riba 2002), several researchers have found that peripheral achenes of *Centaurea solstitialis* exhibit slightly lower germination rates than center achenes when collected shortly after maturation (e.g., Joley et al. 1997; Larson and Kiemnec 1997). In the field, these peripheral achenes tend to delay germination until the spring, when temperatures warm, a pattern that may be caused by after-ripening requirements (Joley et al. 1997) or by differences in temperature responses of germination (DiTomaso 2001) or by a combination of these factors (Young et al. 2005). Seed dormancy has not yet been investigated in *Centaurea melitensis*. Although a possible secondary dormancy was noted in the seeds of iCL achenes by Riba et. al. (2002), the differences in achene shape and size make *Centaurea melitensis* and interesting nonnative for study all in its self.

**Invasiveness of Centaurea Species**

*Centaurea* and other nonnative species can inhibit germination of natives by out-competing other plants for nutrients, space or inhibiting other plants from growing through allelopathy (DiTomaso 2001). For example, *Bromus madritensis* has been found at high densities following fire. This is possibly due to its early uniform germination and low moisture requirement, which allows it to spread at higher densities. It may also act as fuel that increases the extent and intensity of fire in areas of the south western US (Salo 2004). This species has also been noted to compete with natives for nitrogen and nutrients following fire (Brooks and Pyke 2002). *Brassica nigra* has also been considered an opportunistic
species or a noxious weed and has been found to be widespread following repeated fires (Haidinger & Keeley 1993), possibly due its persistent seed bank. Species of yellow starthistle like *Centaurea solstitialis* and *Centaurea melitensis* often compete for space and nutrients and moisture, which aid in their spread in areas of disturbance (DiTomaso 2001; Brooks and Pyke 2002; Halvorson and Guertin 2003). Fires and disturbance favor opportunistic plants such as *Centaurea melitensis*, *Brassica nigra* and *Bromus madritensis* (DiTomaso 2001; Keeley 2005). Habitat degradation by frequent fire is one of the biggest threats to rare and endangered plants, because many of these plants may not have time to recover (Halsey 2005; Keeley 2005). Colonization by invasive plants further prevents native plants recovery. Weedy species produce an abundance of seeds and can become more aggressive in new and disturbed environments leading to rapid colonization, further reducing recovery and establishment of natives and decreasing biodiversity (Keeley 2005; Moroney 2013).

Both *Centaurea melitensis* and *Centaurea solstitialis* produce abundant seed, contributing to their invasive ability. Yellow starthistle in general can produce a large amount of seeds in the range of 35 to 80 seeds per seed head (DiTomaso 2001). The seed production of *Centaurea melitensis* is considered to be highly variable, according to Halvorson and Guertin (2003). Some plants can produce as many as 100 flower heads that contain anywhere from 1 to 60 seeds per head.
Nonnative or invasive species can alter soil stability by prompting erosion (Brooks et al. 2004) and can hinder the native species from growing, ultimately decreasing biodiversity (DiTomaso, 2001; Keeley 2005). For many conservationists nonnative weedy species are a serious threat to plant communities (Sakai et al., 2001, Brooks and Pyke 2002; Keeley 2005). Infestations of species yellow starthistle like *Centaurea melitensis* and *Centaurea solstitialis* can threaten native species because they use deep soil moisture reserves. *Centaurea solstitialis* can use up to 50% of the annual soil moisture (DiTomaso 2001). This can be detrimental to native plants, which rely on summer soil moisture. Moreover, *Centaurea melitensis* can compete for space of agricultural crops as well (Halvorson and Guertin 2003).

**Germination and Growth Requirements of Centaurea**

In the field most seedlings of *Centaurea solstitialis* and *Centaurea melitensis* germinate in late fall or early winter (Halvorson and Guertin 2003). The flower heads develop fairly slowly and throughout the early spring. The shoots of the plant tend to spring up during early summer. When the plant is mature it produces a waxy grayish coating on the foliage, which helps to reduce the heat load. Yellow starthistle can thrive under direct sunlight in hot and dry conditions (DiTomaso 2001).

Germination is stimulated by a series of environmental and physiological cues such as light, constant temperatures and moisture. According to DiTomaso (2001), maximum germination for yellow starthistle can occur provided there is
adequate moisture, light and constant temperatures ranging between 10, 15, 20 °C or an alternating temperatures of 15:5 or 20:10 °C (DiTomaso 2001). When temperatures rise above 30 °C germination tends to decrease in members of the Centaurea taxa like Centaurea solstitialis (Joley et al. 1997; Roche et al. 1997). DiTomaso (2001) states that yellow starthistle Centaurea solstitialis does not survive well under shaded conditions. Not all yellow starthistle respond the same to varying temperatures. For instance, when Centaurea melitensis was examined alone each of the three seed types responded to differently to temperature and the maximum germination occurred at different temperatures (Porras and Muñoz 2000b). The CH seeds germinated best at temperatures of 12 °C and 19 °C. The highest germination in the iCl seeds occurred at 12 °C. There was no effect on temperature for the fCl seeds (Porras and Muñoz 2000b).

In general, yellow starthistle germination is correlated with winter and spring rainfall (DiTomaso 2001). However, germination can still occur throughout the rainy season. Most yellow starthistle is insensitive to photoperiods, which allows them to germinate continuously. This may not be true for Centaurea melitensis; since the different seed types respond differently to temperature, they may respond differently to light and shading and smoke.

Some nonnative (weedy) species do exhibit dormancy and so do cleistogamous species (McGregor 1990). Dormancy is defined as “the physiological state in which otherwise viable seeds will not germinate even when exposed to growth conductive conditions” (Landis 2000). The seeds of
*Centaurea melitensis* and other *Centaurea* species such as *Centaurea solstitialis* are known to remain viable in the soil for up to ten years (DiTomaso 2001). The seeds in the periphery of *Centaurea solstitialis* flower heads are thought to remain dormant until the time of dispersal (DiTomaso 2001; Halvorson and Guertin 2003). When temperatures increase, as in the summer, seeds in the soil bank may experience a secondary dormancy (DiTomaso 2001). Joley et al. (2003) characterized seeds of *Centaurea solstitialis* as having conditional dormancy, and they identified differences between peripheral and center achenes. Center achenes germinated promptly with the first rains, but peripheral achenes remained in the soil seed bank later into the rainy season (Joley et al. 2003).

Porras and Muñoz (2000b) examined the three seed types of *Centaurea melitensis* and noted the iCL seeds took on average 3.6 days longer to germinate than the CH or the fCL seeds. In addition, they suggested that this seed type may exhibit some type of dormancy, possibly secondary dormancy or a conditional dormancy. It is uncertain at the time of this study whether the seeds are exhibiting any kind of dormancy. It is possible that iCl seeds are exhibiting sluggish germination. Breaking seed dormancy may require an alternative method to stimulate germination such as a chemical cue. This may be the reason for their otherwise sluggish germination time and thick pericarp (Porras and Muñoz 2000b).
The viability of the seed becomes increasingly important in germination studies because underdeveloped or dead seeds will not germinate in response to any cue. The seeds of *Centaurea* do not become fully developed until sometime after late senescence. The seeds are half filled around middle senescence (DiTomaso 2001).

The longevity of yellow starthistle seeds of *Centaurea melitensis* has not been fully investigated. However the longevity of *Centaurea solstitialis* has been addressed in the literature (Callihan et al. 1993). Callihan et al. (1993) looked at the longevity of both the plumed seeds and nonplumed seeds of *Centaurea solstitialis*. What they discovered was the depth at which the seed was buried had no effect on longevity. Other studies reviewed by DiTomaso (2001) conducted on burial depth have found differences in seed longevity at different soil depths. Eighty-eight percent of seeds buried at a depth of 5 cm in California remained viable after a year, whereas only 4% of seeds buried at a depth of 1 cm remained viable (Joley et al. 1992). The longevity of the pappus bearing and nonpappus bearing seeds of *Centaurea solstitialis* was between six and ten years (Callihan et al. 1993). *Centaurea solstitialis* seeds following a six-year period showed 9% germination in the pappus bearing seeds (DiTomaso 2001).

Spira and Wagner (1983) investigated the viability of 40 species of seeds found in 211-year-old adobe bricks. Among the seeds tested was *Centaurea melitensis*. These seeds were estimated to be around 183 years old. They tested
ten of these and found none to be viable. Out all forty species tested only seven were found to be viable and only a few germinated (Spira and Wagner 1983).

Response of *Centaurea melitensis* to Smoke, Heat Shock and Other Post-fire Conditions

Many nonnatives are found around plant communities that experience frequent fires. Species like *Bromus madritensis*, *Brassica nigra*, and *Centaurea melitensis* tend occupy areas of disturbance (DiTomaso 2001; Salo 2004). For many of these species, fire provides an opportunity to spread and germinate (Brookes and Pyke 2002). Several studies on the influence of smoke on weedy species have shown positive germination results (Adkins and Peters 2001). Akins and Peters (2001) tested the effects of smoke and smoke water on arable weeds that were both monocotyledonous and dicotyledonous species. They found that all the monocots responded positively to smoke water. The dicotyledons on the other hand were strongly stimulated, moderately stimulated or unaffected by the treatment (Adkins and Peters 2001). Stevens et al. (2007) further tested the effects of the recently derived butenolide 3-methyl-2H-furo[2,3-c]pyran-2-one on weedy species. This chemical is thought to be a major component found in smoke, that triggers germination (Flematti et al. 2007; Stevens et al. 2007). The results showed that freshly collected seeds treated with butenolide had significantly increased germination. While many species respond positively, other like *Centaurea melitensis* may be suppressed by smoke exposure (Riba et al. 2002; Personal observation and preliminary data). This may be due to different
environmental factors that are acting independently or in combination with smoke.

Riba et al. (2002) examined the effects of fire and postfire conditions on germination and establishment in twenty *Centaurea* taxa. Among the taxa studied were two also found in California: *Centaurea melitensis* and *Centaurea solstitialis*. Early temperature experiments performed by Riba et al. (2002) on *C. calcitrapa ssp. aspera*, *C. aspera*, *C. paniculata ssp. hanryi* and the two seed types of *C. solstitialis* showed temperatures between 115 and 120 ºC were lethal. Riba et al (2002) further examined the effects of short-term heat shock treatments on germination using three heat levels. The twenty taxa *Centaurea* were treated with three temperatures (room temperature, 70 ºC and 110 ºC) using four replicate batches and 20 seeds per species. They took twenty seeds per species and exposed them to one of three temperatures at random for five minutes using a dry air stove, then germinated the seeds at room temperature. This experiment showed that germination percentage was variable among taxa. However there was no significant difference found in regards to temperature treatments. They also discovered that temperature had no effect on T50 (time at which 50 percent of seeds germinate).

In the spring of 1998 they also performed a field experiment to evaluate the effect of post fire conditions on the *Centaurea* taxa. Post-fire field experiments found significant variability in the percent germination among the twenty taxa. The highest germination for all taxa occurred with shade and
unburnt soil (75.8 ± 14%). The lowest germination occurred in burnt soil and full light conditions. Moreover, adding burnt soil to the pots decreased germination in some of the taxa and not in others (Riba et al. 2002). They also noted the rate at which germination occurred was affected by a significant shade X soil type interaction. In other words seeds germinated faster in shade and unburned soil conditions. Germination for Centaurea melitensis appears to increase in shaded and unburned soil conditions. Riba et al. (2002) also examined the effects of smoke on germination in both seed types of Centaurea solstitialis. When exposed to burned soil and shade conditions both seed types were found respond similarly to shading. There were differences seen in the effect of litter type (burned vs unburned soil) in the seed types of Centaurea solstitialis. There was no difference in percent germination in nonplumed seeds when both soil treatments were examined. However the percent germination was less in plumed seeds treated with burned soil compared to unburned soil (Riba et. al, 2002).

When effects of post-fire environmental conditions on seedling growth and establishment were examined, such as nutrient availability and litter type (burned or unburned soil), the results were similar to those above. Addition of mineral nutrients from ash was found to increase seedling growth for most of the taxa (Riba et al. 2002). Shading was found to influence seedling growth and leaf shape. Leaves in plants grown in full sunlight were smaller and there were more of them (Riba et al. 2002). In pots treated with burned soil and in low light conditions, the leaves were longer (Riba et al. 2002). Interestingly, Riba et al.
(2002) data show the number of leaves for *Centaurea melitensis* were greater under shade and burnt soil compared to full sunlight.

The overall conclusion of this study was that fire negatively impacted survival and that temperature greater than 120 °C will ultimately lead to 100% mortality. Under post fire conditions, shading increased percent germination. The authors also alluded to the fact that there may be other factors, alone or in combination, in regards to low regeneration that need to be further explored.

**Preliminary Investigations**

During a preliminary investigation in the spring of 2008 on the effects of aerosolized smoke on germination of six species, three natives, *Salvia mellifera*, *Emmenanthe penduliflora* and *Nicotiana quadrivalvis*, and three nonnatives, *Centaurea melitensis*, *Bromus madritensis* and *Hirschfeldia incana*, I observed achene variation within chasmogamous heads of *Centaurea melitensis*. The achenes appeared to vary in size, shape, pappi and elaiosomes. Within each *Centaurea* flower head some achenes were situated in a ring-like fashion in the center and some achenes were offset to them developing closer to the bracts. The achenes were separated and labeled CH and CM2 (peripheral and possibly dormant). They were sown in either sand or topsoil, exposed to aerosolized smoke or not (a control treatment), then watered with deionized water. Results indicated that there was more germination in sand than soil for both types of *Centaurea* achenes. Average germination in smoke-treated sand and unsmoked sand was not significantly different. However, the germination in unsmoked
topsoil was markedly higher than that in smoked topsoil (10.2% vs. 0.3%). The pattern differed for peripheral achenes. In sand, germination was suppressed by smoke, but in topsoil there was no effect of smoke on germination. These findings prompted further investigation of morphology and positional responses to environmental cues related to germination.

Objectives of the Study

The purpose of these experiments was to investigate differences between peripheral and center achenes within each head type of *Centaurea melitensis*. I investigated morphological differences between peripheral and center achenes and differences in germination responses to temperature. These investigations were aimed at determining whether peripheral and center achenes of *Centaurea melitensis* differed morphology, dispersal potential, and possible dormancy in ways that were similar to those observed *Centaurea solstitialis*, its sister taxon.
CHAPTER TWO

MORPHOLOGICAL VARIATION IN ACHENES OF CENTAUREA MELITENSIS IN SOUTHERN CALIFORNIA

Abstract

*Centaurea melitensis* is a non-native species found in southern California that produces heteromorphic flower heads that develop at different times at different locations on the plant during the growing season. Some members of the Asteraceae family have shown that morphology differences can be found within the flower head, too. The closely related congener, *Centaurea solstitialis*, produces two different achenes: one without a pappus found in the periphery of the flower head and with a pappus which is located in the center of the flower head. Previous studies have shown that the different head types in *Centaurea melitensis* also contain morphologically distinct achenes. This study investigated differences in achenes found in the periphery (located near the bracts) and the center (located in center of flower head) of each of the flower head types in *Centaurea melitensis*. The study was conducted in the summer of 2010 on the campus of California State University San Bernardino. Fifty achenes were selected from the peripheral and central area within each flower head type (CH, iCL and fCL). Morphological differences between central and peripheral achenes within each head type (mass, fruit length, fruit width, pappus length, pappus width, elaiosome height, elaiosome depth and elaiosome volume) were
investigated. Peripheral achenes were significantly lighter than central achenes within each head type. Morphological features varied among all head types. Additionally, there were morphological differences found between peripheral and center achenes within all head types. Peripheral achenes were narrower than central achenes of similar lengths in CH and iCL head types; they were also smaller than center achenes in the fCL heads, but similar in shape. Peripheral achenes had smaller elaiosome volumes than center achenes of similar lengths in all head types. Peripheral achenes had smaller pappi than center achenes only in the chasmogamous head type. Where variation was detected, features that promote animal dispersal (elaiosome) and wind dispersal (pappus size) were less well developed in peripheral achenes than in center achenes. Differences in morphology between peripheral and center achenes of *Centaurea melitensis* are apparent, although they are not as distinct as those seen in its better-studied congener, *Centaurea solstitialis*.

**Introduction**

Many non-native weedy species are annuals and considered invasive. Invasive species tend to dominate areas when given the opportunity and the right environmental conditions. The problem with this is they can threaten the germination and establishment of native species. This decreases biodiversity, affects community structure and food sources for wild life, and disrupts the water cycle (Sakai et al. 2001; Brooks and Pyke 2002). The management of these species can be quite costly (Sakai et al. 2001).
The spread and establishment of non-native weedy annuals depends on seed or achenes (one seeded fruit with a thin wall as in a sunflower) production and dispersal. Whether they produce seeds or achenes depends upon the species itself. However, the life cycle of weedy annuals is usually about one year or less. They can be summer or winter annuals. Summer annuals tend to germinate in late spring and also early summer. They usually set seed in the late summer or early fall. Winter weedy annuals germinate in fall or early spring and set seed in late spring, then die off when it gets hot.

There are also weedy perennials that spread not only through seed dispersal but vegetatively through horizontal shoots, nutlets or rhizomes. They tend to live more than two years.

The Asteraceae family contains many species that are considered weedy in nature. Success in the surrounding environment as well as other environments depends upon achene production, more importantly on effective dispersal and germination. Effective dispersal is often correlated with variation in achene morphology, survival, and germination success in many members of the Asteraceae family (Sheldon 1974; Sheldon and Burrows 1973).

Achene size, shape, and position of the achene in the flower head, seed coat, presence or absence of a pappus (small bristle like protrusion from achene) and presence or absence of an elaiosome (oily lipid rich area on achene that promotes dispersal) are important morphological features that influence dispersal, germination and establishment. For instance, the mass of the achene
can play a role in how far the achene disperses from the plant, how fast it falls and how it responds to changes in environmental conditions in the field, which influences germination (Porras and Muñoz 2000a, 2000b). Small achenes have a greater surface area touching the soil surface therefore; they take up more water and germinate quicker than larger achenes (Sheldon 1974). Small achenes have been shown to be sensitive to temperature fluctuations (Liu et al. 2013). Small achenes are more likely to get buried according to Liu et al. (2013) and must be able to sense changes in temperature through light burial while larger achenes (seeds) can germinate from greater soil depths. A bigger mass can imply a bigger embryo size (Ruiz de Clavijo 2001). Ruiz de Clavijo (2001) suggested that a bigger embryo can allow the achene to emerge from greater depths, increase growth rate and survival. The pappus is important not only for dispersal but it also plays a role in securing the achene to the soil surface (Sheldon 1974). In addition, Sheldon and Burrows (1973) suggest that dispersal efficiency in regards to the pappi depends on the finer details of the pappus like the geometry, which make it aerodynamically fit for dispersal. Moreover, these features can not only indicate the possible dispersal methods by which the achenes are dispersed, they can also infer the type of habitat the species resides in. Both a pappus and elaiosome imply the achenes are dispersed by wind and also by insects. Therefore the habitat they reside in must experience winds. In addition, these features may allow the achenes to escape predation and add to the seed bank via insect dispersal and burial (Baskin and Baskin 2001). If we are to understand
the nature of our many plant species and their persistence we must first understand their ability to succeed.

Achene dispersal morphology can aid in establishment and survival as observed in other members of the Asteraceae family. Forsyth and Brown (1982) investigated differences in germination and dormancy of two morphologically different achenes found in hairy beggartricks (*Bidens pilosa* L.). They discovered that longer (center) achenes germinated over a larger range of conditions while shorter peripheral achenes had a more stringent germination requirement (Forsyth and Brown 1982). In addition to varying responses in germination, they also noted the influence of differing morphology on dispersal (Forsyth and Brown 1982; Callihan et al. 1993). The shorter peripheral achenes of *Bidens pilosa* were heavier than the central achenes leading the authors to speculate that they did not disperse as far from the parent plant like the central achenes (Forsyth and Brown 1982). In contrast, Roche (1965) found that non-pappus-bearing peripheral achenes were lighter than the center pappus bearing achenes in *Centaurea solstitialis* (Callihan et al. 1993). The non-pappus-bearing achenes may not disperse far from the parent plant despite their light weight (Halvorson and Guertin 2003). As previously mentioned, the pappus can act as a wind dispersal unit and help carry achenes to greater distances thereby increasing the chances of survival. How far the achene travels may be a reflection of pappus size and shape and geometry (Sheldon and Burrows 1973). Additionally, the pappus can act as weathervane that can orient the achene to the ground during
dispersal and germination. The orientation can be opposite the wind. On a windy
day the pappus can act as lever allowing the achene to move in the opposite
direction to the wind (Sheldon 1974; Porras and Muñoz 2000b).

Elaiosomes can be found in many members of the Asteraceae family
(32%) of them to be exact. They differ in color shape and oils (Pemberton and
Irving 1990; Baskin and Baskin 2001; Viegi et al. 2003). They are known to
shrink severely when dried (Pemberton and Irving 1990). They can further aid in
dispersal of achenes that develop all through the flowering season (Mark and
Olesen 1996). They also have other functions such as inhibiting germination and
promoting dormancy, facilitating rehydration and germination by up taking water
slowly, facilitating rehydration by allowing and area for water loss and dispersal
via ants, which can carry the achenes off as far as 100 m (Viegi et al. 2003).
Mark and Olesen (1996) indicate the importance of elaiosome size to achene
removal in *Hepatica noblis* and *Myrmica ruginodis*. The study found that ants
prefer larger achenes with bigger elaiosomes. Insects vary in size and shape as
well as elaiosomes: smaller insects may prefer deeper and smaller elaiosomes;
larger ants may prefer larger elaiosomes (Mark and Olesen 1996). If given a
choice of a larger achene or a bigger elaiosome the ants were more likely to
choose a bigger elaiosome. They also found that achene size correlated with
elaiosome size (Mark and Olesen 1996). *Centaurea melitensis* possesses both a
pappus and elaiosome; these features may be advantages to spread. Having a
larger elaiosome or more variation in elaiosome size may permit more
elaiosome-carrying insects to carry it off before it is consumed by birds or other seed-eating animals.

Yellow starthistles are among several non-native weedy species found in California. They are known to infest more than a million hectares in the Pacific northwest (Maddox et al. 1985). Generally speaking there are two yellow starthistles found in California, *Centaurea solstitialis* and *Centaurea melitensis*. The yellow starthistle most reviewed in the literature is *Centaurea solstitialis*, the sister taxon to *Centaurea melitensis* (Garcia-Jacas et al. 2006). *Centaurea solstitialis* is a non-native erect winter annual and is a native to southern Europe (Halvorson and Guertin 2003). It is found more commonly in northern California than in southern California. It is known to be one of the most widespread weedy invasive species of California grasslands and infest more than 6 million hectares (Young et al. 2011). It produces two distinct types of achenes that develop within the same flower head. There is a peripheral achene that contains no pappus and develops first. Peripheral achenes are defined as those located in the outer ring of the flower head close to the phyllary (bracts or a tiny modified leaf). The second is a pappus-bearing achene that develops in the center of the flower head. The peripheral achenes are lighter than the center achenes (Callihan et al 1993).

The peripheral achenes of *Centaurea solstitialis* contain no pappus or hairs and tend to remain in the flower head into the winter when the flower head deteriorates (Halvorson and Guertin 2003). These achenes fall close to the
parent plant during dispersal. However, they can be catapulted from the plant to a distance equal to the plant height during a windy day according to Roche (1992). The pappi of the pappus-bearing achenes act as wind dispersal units. These achenes have been documented as traveling up to 0.6 m beyond the plant. They can also attach to animal fur, feathers and clothing and travel much farther (Robbins et al. 1951; Roche 1965; DiTomaso and Gerlach 2000). Some authors report that achenes of *Centaurea solstitialis* do not possess elaiosomes (Pemberton et al. 1990), but others have found that center achenes possess elaiosomes and peripheral achenes lack them (Riba et al. 2002; Viegi et al. 2003). The center achenes and the peripheral are produced at ratio of 3:1 but can vary to 9:1 (Callihan et al. 1993; DiTomaso 1996).

The second most common yellow starthistle found in California is *Centaurea melitensis* (tocalote), which is also a non-native winter annual (DiTomaso 2001). It has origins in southern Europe, western Africa and Macronesia (Porras and Muñoz, 2000a). It is found along roadsides and open fields and in areas of disturbance in southern California (Halvorson and Guertin 1993). It is more prominent in southern California compared to its sister taxon *Centaurea solstitialis*. *Centaurea melitensis* possesses various types of flower heads, some that are cleistogamous and some that are chasmogamous (Porras and Muñoz 2000a; 2000b, 2000c). The cleistogamous flower heads remain closed and self-pollinate and the chasmogamous flower heads are open (Porras and Muñoz, 2000a, 2000b). The open flower heads produce bright yellow
inflorescence and are pollinated by insects. In *Centaurea melitensis* the flower heads develop at different locations at different times on the plant and vary in shape and size (Porras and Muñoz, 2000a, 2000b). The chasmogamous (CH) flower heads are found at the apex of the plant and develop during the middle of the flowering season April through May. The initial cleistogamous (iCL) flower heads develops at the beginning of the flowering period March through April. The final cleistogamous (fCL) flower heads develop at the end of the flowering period May through June (Porras and Muñoz, 2000a, 2000b; Halvorson and Guertin 2003). Not only does *Centaurea melitensis* produce different types of flower heads that are considered heteromorphic, but it produces heteromorphic achenes. Porras and Muñoz (2000a, 2000b) discovered three achenes types CH, iCL and fCL that differed morphologically according to head type.

Both species produce different achenes with different morphology, but the pattern of variation described for each differs. *Centaurea solstitialis* has morphologically different peripheral achenes and center achenes in the same flower head. Such differences have not been described for *Centaurea melitensis*, although achenes from different head types have shown to differ in fruit length, fruit width pappus diameter and mass (Porras and Muñoz 2000 a, 2000b). Examination of flower heads during my preliminary study of *Centaurea melitensis* in 2007 suggested some previously undescribed morphological differences between peripheral and center achenes. These observations prompted me to investigate the extent to which the morphology of center and peripheral achenes
differed in *Centaurea melitensis*, especially with respect to achene characteristics that could affect dispersal and invasive success. These morphological differences may play a role in plant dispersal and survival. These differences between peripheral and center achenes of *Centaurea melitensis* in southern California have not yet been identified.

The purpose of this study was to investigate morphological differences in six achene types (CHc, CHp, iCLc, iCLp, fCLc, and fCLp) (p= peripheral and c = center) of *Centaurea melitensis*, by addressing the following questions. 1. Are the peripheral achenes lighter than the center achenes in all three head types CH, iCL and fCL, as they are in *Centaurea solstitialis*? 2. Are there differences between peripheral and center achenes in fruit length, fruit width, pappus length, pappus width, as there are in other members of the Asteraceae family (*Centaurea solstitialis* and *Bidens pilosa*)? 3. Does the elaiosome volume differ in peripheral achenes compared to central achenes? 4. Do the differences lead to shape differences in some morphological characters?

**Methods**

To determine morphological differences between the six achene types of *Centaurea melitensis* (CHc, CHp, iCLc, iCLp, fCLc, fCLp) and to compare peripheral and central achenes from the same type of flower heads, the following characteristics were measured: mass, fruit length, fruit width, pappus length, elaiosome height, and elaiosome depth and elaiosome volume.
The achenes were collected 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 from the late spring of 2009 to the early fall of 2009. Approximately 11 hectares was included in the collection area. Approximately 200 *Centaurea melitensis* (tocalote) plants were clipped or pulled out at the roots, depending on the size, and placed in large open bags. The bags were stored at room temperature in the lab until needed. The whole plant was removed to ensure that all the necessary flower heads containing the various achenes could be located on the plant.

Chasmogamous flower heads containing CHc and CHp achenes are found on the apex of the plant (Porras and Muñoz 2000a). The iCL flower heads containing the iCLc and iCLp achenes are located at the base and in the axil points (where the plant bifurcates). The fCL flower heads containing fCLc and fCLp achenes are found along the main axis from the base up and also on the first and second order branches (Porras and Muñoz 2000a).

Flower heads containing the desired achene types were located and removed one at a time. The achenes were plucked by hand, one flower head at a time, in order to keep the peripheral and central seeds separate. Once removed the achenes were placed in a small paper coin envelope and stored at room temperature. The achenes were removed from approximately 130 plants. Fifty
achenes of each of the six achene-types were removed from batches obtained from the 130-plant sample. A total of 300 achenes were used in the analysis.

**Morphological Measurements and Achene Imagery**

Morphological measurements were taken from a digital image of individual achenes. Digital images were obtained by placing a single achene onto the stage of an Olympus SZX9 dissecting scope (Denver Instrument Company). A ruler for scaling of measurements was placed next to the achene. The dissecting scope was set up with a Nikon Coolpix 5000 digital camera. The image of the achene was adjusted to show 5 mm or more of the scale and the entire achene. Each achene was then photographed.

Each image was uploaded to a computer and saved as a Jpg file. The following measurements were used to determine differences among the seed types: fruit length, fruit width, pappus length, pappus width, elaiosome height, and elaiosome depth and elaiosome volume. The pappus length was not measured directly; instead the fruit length was subtracted from the total fruit length + pappus length. The elaiosome height was measured as the distance between the end of the achene and the beginning of the fruit body (Figure 3). It is the opening of where the elaiosome sits (Figure 3). The depth was measured from the outside surface, the point at which the oily fleshy part begins to the deepest point of the hook in the achene in which it rests.
Each individual image of the achene was brought up on the screen and identifying points for the characteristics were measured (Figure 3). All measurements were made using a 15 cm and 30 cm flat plastic ruler. In addition to the morphological characteristics, the images of the ruler reflecting a total of 5
mm were measured to convert morphological measurements to actual measurements in millimeters.

**Mass Determination of *Centaurea melitensis* (Tocalote) Achene Types**

To determine the average mass of each of the six achene types (CHc, CHp, iCLc, iCLp, fCLc, fClp) of *Centaurea melitensis* and to determine the differences in mass between the peripheral and center achenes for each head type, the achenes used for morphological measurements above were weighed. Fifty achenes of each achene type were separated into five samples of ten achenes. These samples were weighed to the nearest 0.1 mg on a Denver Instrument M220 analytical scale. The 50 achenes of each achene type were weighed in 5 batches of 10 because of the scale’s limitations. The achenes were weighed twice to ensure that repeated measures were within 0.3 mg of each other. These duplicate measurements were averaged.

**Statistical Analysis of Mass Differences**

A two-way ANOVA was performed to examine the effect of head type and position (central vs. peripheral) on mass of *Centaurea melitensis* achenes. Post-hoc Tukey HSD was also performed to identify all pairwise differences between peripheral and center achenes for each head type: CH, iCL and fCL.

**Statistical Analysis of Differences in Morphological Characters**

The R Project i386 2.15.1 statistical program was used to analyze all data.

Six two-way ANOVA’s were used to analyze the effect of position and head type on each morphological character. Post-Hoc Tukey HSD tests were
conducted following two-way ANOVA to identify significant differences between peripheral and center achenes for the following characters: fruit length, fruit width, pappus width, pappus length, elaiosome height and elaiosome depth and elaiosome volume.

As an extension of the two-way ANOVA and to minimize the effects of Type II error, three sets of two-way ANCOVA’s were performed to examine differences between peripheral and center achenes for each head type controlling for differences attributable to overall achene size. The following characters were selected for analysis, fruit width, pappus length, and elaiosome depth and elaiosome height. The first set of ANCOVA’s examined the effect of position (peripheral vs. central) on the relationship between fruit length and fruit width for each head type. The second set of two-way ANCOVA’s was performed to examine the effect of position (peripheral vs. central) on the relationship between pappus length and fruit length. The third set of two-way ANCOVA’s was performed to examine the effect of position (peripheral vs. central) on elaiosome depth on the relationship between elaiosome depth and elaiosome height for each head type. All ANCOVA’s were fitted to the best model. The morphological characters that were chosen for the ANCOVA’s are related to achene dispersal. All ANCOVA’s allow for examination of each character based on position while taking into account fruit length or elaiosome height. Shapiro Wilks test and Bartlett test of homogeneity were run to examine data for normality.
Elaiosome Volume Determination

Further examination of the elaiosome was necessary to determine elaiosome volume in all achene types. To determine elaiosome volume in the previously measured data, where elaiosome height and elaiosome depth were measured from 2 dimensional images, morphological examination of the elaiosome was performed. A total of ten achenes were randomly selected from the previous 50 achenes per achene type. The achenes were placed on a small piece of clay to prevent movement. They were then placed under an Olympus SZX9 dissecting scope (Denver Instrument Company). A ruler for scaling was placed next to the achene. Two images were obtained of each elaiosome to capture three dimensions (height, depth and width). Images were taken using a Coolpix 5000 digital camera. The image of the achene was adjusted to show 2 mm of the scale and the portion of the achene showing the elaiosome.

Images were uploaded to the computer as previously described. Elaiosome depth, height and width were measured using the same methods as above. Elaiosome volume was then estimated as the product of elaiosome depth X elaiosome height X elaiosome width (3Dimensional) and also by elaiosome height X elaiosome height X and elaiosome depth using 2-dimensional measurements (Figure 4A and 4B). The 2-dimensional estimates were regressed using linear regression against the 3-dimensional estimates to determine if the 2-dimensional estimates yielded a good estimate of elaiosome volume (Figure 5).
The estimate of elaiosome volume from 3-dimensional measurements was well predicted by the estimate obtained from 2-dimensional measurements ($y = 1.1852x + 0.0006$). Furthermore, the slope was close to 1 and the intercept was close to zero indicating that that the 2-dimensional methods gave similar estimates. Therefore, elaiosome volume was estimated in the larger data set (300 achenes) from the 2-dimensional measurements using the formula $\text{elaiosome height}^2 \times \text{elaiosome depth}$ (Figure 5). A two way-ANOVA was performed to examine the effect head type and position on elaiosome volume. Further examination of elaiosome volume was done using two-way ANCOVA’s for all three head types to address whether differences in elaiosome volume in peripheral and center achenes were significant if fruit length was held constant.
Figure 5. The predicted elaiosome volume from 2D measurements compared to the elaiosome volume taken from 3D measurements.

Results

The Effect of Position on Mass of Achenes of Centaurea melitensis

Masses of the peripheral achenes and the center achenes within the three head types (CH, iCL and fCL) of Centaurea melitensis are shown in Figure 6. Results of the two-way ANOVA indicate that there was a significant effect of position, and a significant effect of head type, with no interaction (Table 1, Figure 6). On average the peripheral achenes were lighter than the center achenes in all
head types. Post–hoc Tukey HSD comparisons indicated significant differences between the mass of peripheral and center achenes within the iCL and fCL head type but not within the CH head type ($p \leq 0.001$): iCLp–iClc and fCLp–fClc. Tukey HSD found significant differences between head types at ($p \leq 0.001$) in the following pair wise comparisons: iCLp–CHp and iCLp–fCLp.

![Figure 6. Average achene mass of peripheral and center achenes of *Centaurea melitensis* for all three head types: CH, iCL and fCL. Error bars represent one standard error. Asterisks indicate significant differences between peripheral and center achenes within head types ($p \leq 0.05$). See Table 1 for ANOVA results.](image)
Table 1. ANOVA of the effects of position (peripheral vs. central) and head type (CH, iCL and fCL) on achene mass of *Centaurea melitensis*

<table>
<thead>
<tr>
<th>Effect on achene mass of:</th>
<th>df</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position</td>
<td>1</td>
<td>8.40x10^{-5}</td>
<td>8.40x10^{-5}</td>
<td>41.26</td>
<td>1.21x10^{-6} ***</td>
</tr>
<tr>
<td>Head Type</td>
<td>2</td>
<td>1.25x10^{-4}</td>
<td>6.25x10^{-5}</td>
<td>30.72</td>
<td>2.40x10^{-7} ***</td>
</tr>
<tr>
<td>Head Type x Position</td>
<td>2</td>
<td>7.96x10^{-6}</td>
<td>3.98x10^{-6}</td>
<td>1.95</td>
<td>0.16</td>
</tr>
<tr>
<td>Residuals</td>
<td>24</td>
<td>4.88x10^{-5}</td>
<td>2.03x10^{-6}</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Differences in the Morphological Characters of Achenes of *Centaurea melitensis*

**Fruit Length.** Fruit length of peripheral and center achenes of three head types (CH, iCL and fCL) of *Centaurea melitensis* are shown in Figure 7. Results of two–way ANOVA showed a significant effect of head type and a significant effect of position on fruit length, and a significant interaction between head type and position (Table 2). The peripheral achenes were on average significantly shorter than the center achenes in the fCL head type. In the CH head type and the iCL head type the peripheral and center achenes did not differ significantly in fruit length (Figure 7).

Post–hoc Tukey HSD tests on all pair-wise contrasts revealed differences between fruit length of peripheral and center achenes were significant only within the fCL head type (p ≤ 0.05). Tukey HSD found significant differences between peripheral achenes from the different head types in the following pair wise comparisons: iCLp–CHp and iCLp–fCLp (p ≤ 0.05).
Table 2. ANOVA summary for the effects of head type and position on morphological characters of *Centaurea melitensis*.

The statistical significance of each effect is indicated as *** (p ≤ 0.001), ** (p ≤ 0.01), * (p ≤ 0.05), or n.s. (not significant at p ≤ 0.05). The letters in parenthesis indicate that the p-value is for (h) head type, (p) position and (I) interaction. Degrees of freedom for head type = 2, 294. Degrees of freedom for position = 1, 294.

<table>
<thead>
<tr>
<th>Morphological Character</th>
<th>Effect of Head Type</th>
<th>Effect of Position</th>
<th>Interaction</th>
<th>F -statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit Length</td>
<td>***</td>
<td>*</td>
<td>*</td>
<td>67.85(h)</td>
<td>2.0x10⁻¹⁶(h)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.11(p)</td>
<td>0.013(p)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.45(I)</td>
<td>0.032(I)</td>
</tr>
<tr>
<td>Fruit width</td>
<td>***</td>
<td>***</td>
<td>*</td>
<td>119.93(h)</td>
<td>2.2x10⁻¹⁶(h)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>48.11(p)</td>
<td>2.56x10⁻¹¹(p)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.84(I)</td>
<td>0.022(I)</td>
</tr>
<tr>
<td>Pappus Width</td>
<td>***</td>
<td>n.s.</td>
<td>***</td>
<td>350.73(h)</td>
<td>2.2x10⁻¹⁶(h)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.21(p)</td>
<td>0.648(p)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.96(I)</td>
<td>0.0001(I)</td>
</tr>
<tr>
<td>Pappus Length</td>
<td>***</td>
<td>***</td>
<td>n.s.</td>
<td>678.9(h)</td>
<td>2.2x10⁻¹⁶(h)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13.25(p)</td>
<td>0.0003(p)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.19 (I)</td>
<td>0.305(I)</td>
</tr>
<tr>
<td>Elaiosome Height</td>
<td>***</td>
<td>***</td>
<td>n.s.</td>
<td>13.41(h)</td>
<td>2.7x10⁻⁶(h)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>34.91(p)</td>
<td>9.55x10⁻⁹(p)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.18(I)</td>
<td>0.834(I)</td>
</tr>
<tr>
<td>Elaiosome Depth</td>
<td>***</td>
<td>*</td>
<td>*</td>
<td>164.60(h)</td>
<td>2.0x10⁻¹⁶(h)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.02(p)</td>
<td>0.025(p)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.05(I)</td>
<td>0.048(I)</td>
</tr>
<tr>
<td>Elaiosome volume</td>
<td>***</td>
<td>***</td>
<td>n.s.</td>
<td>50.1(h)</td>
<td>2.2x10⁻¹⁶(h)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14.23(p)</td>
<td>0.0001(p)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.269(I)</td>
<td>0.282(I)</td>
</tr>
</tbody>
</table>
Figure 7. Comparison of average fruit length of peripheral center achenes in *Centaurea melitensis* head types CH, iCL and fCL. Error bars represent one standard error. Asterisk indicates significant difference between peripheral and center achenes within the head type fCL as determined by Tukey HSD (p ≤ 0.05). See Table 2 for ANOVA results.

**Fruit Width.** Fruit widths of peripheral and center achenes in the three head types (CH, iCL and fCL) are shown in Figure 8. Results of two-way ANOVA indicate a significant effect of head type on fruit width, and a significant effect of position on fruit width, and a significant interaction between head type and position on fruit width (Table 2). On average fruit width was greater in center achenes than peripheral achenes. Tukey HSD comparisons indicated all pair
wise contrast between fruit widths of peripheral and center achenes were significant ($p \leq 0.001$): CHp–CHc, iCLc-iCLp and fCLp-fCLc. Tukey HSD found significant differences between peripheral achenes from the different head types were compared to each other ($p \leq 0.05$) in the following pairs: iCLp–CHp and iCLp– fCLp. Significant differences in fruit width between peripheral and center achenes of the CH and fCL head depend on their position ($p \leq 0.001$) (Figure 8).

Figure 8. Comparison of the average fruit width in center and peripheral achenes of different head types of Centaurea melitensis. Error bars represent one standard error. Asterisks indicate significant differences between peripheral and center achenes within head types as determined by Tukey HSD ($p \leq 0.05$). See Table 2 for ANOVA results.
ANCOVA results for differences in fruit width using fruit length as a covariate showed differences between peripheral and center achenes. There was a linear relationship between fruit width and fruit length for all three head types (CH, iCL, and fCL) (Figure 9 and Table 1 Appendix A). The center achenes appeared wider than the peripheral achenes for any given fruit length in the chasmogamous (CH) head type \((t = -5.7, p < 0.001, \text{Figure 9A, Table 1 Appendix A})\). The center achenes were wider than the peripheral achenes in the initial cleistogamous (iCL) head type. These differences were significant, but the differences between center and peripheral achene fruit widths in the iCL head types became insignificant when differences in overall fruit length were taken into account \((t = -1.65, p = 0.102, \text{Figure 9B, Table 2 Appendix A})\). This indicates the shape between the two was not significantly different. The center achenes were significantly wider than the peripheral achenes for any given fruit length in the final cleistogamous (fCL) head type \((t = -4.5, p < 0.001, \text{Figure 9C, Table 3 Appendix A})\). Therefore, when fruit length was taken into account for the CH and fCL head types the differences between peripheral and center achenes were still significant, indicating that they were differed in shape.
Figure 9. ANCOVA data show the relationship between fruit width and fruit length in three head types of *Centaurea melitensis*: (A) CH, (B) iCL, and (C) fCL. All slopes are parallel. For ANCOVA results see Tables 1, 2 and 3 in Appendix A.

Pappus Width. Pappus widths of peripheral and center achenes within the three head types (CH, iCL and fCL) of *Centaurea melitensis* are shown in Figure 10. Results of two-way ANOVA indicate a significant effect of head type on pappus width. There was no effect of position on pappus width. There was a
significant interaction between head type and position on pappus width \( (p \leq 0.001, \text{ Table 2}) \). The center achenes had a wider pappus on average than the peripheral achenes in the CH head type, but not in the other two head types (Figure 10).

Figure 10. Comparison of average pappus width in peripheral vs center achenes in all three head types of *Centaurea melitensis*. Error bars represent one standard error. Asterisk indicates significant difference between peripheral and center achenes as determined by Tukey HSD \( (p \leq 0.05) \). See Table 2 for ANOVA results.
Tukey HSD comparisons indicated significant differences in pappus widths of peripheral and center achenes for the CH head type only (p ≤ 0.001). Tukey HSD found significant differences between peripheral achenes from the different head types in all comparisons: fCLp-CHp, iCLp–CHp, and iCLp-fCLp (p ≤ 0.05).

**Pappus Length.** Pappus lengths of peripheral and center achenes of the three head types (CH, iCL and fCL) of *Centaurea melitensis* are shown in Figure 11. Results of two-way ANOVA indicate a significant effect of head type on pappus length, and a significant effect of position on pappus length and no interaction (Table 2). Center achenes had significantly longer pappi on average compared to peripheral achenes. Post–hoc Tukey HSD tests on all pair-wise contrasts, however, revealed that pappus length differences between peripheral and center achenes were only significant within the CH head type (p ≤ 0.001). Tukey HSD found significant differences between peripheral achenes from the different head types when the following head types were compared: fCLp-CHp and iCLp–CHp (p ≤ 0.05). Tukey HSD also indicated the only achene types affected by position and head type were in the chasmogamous head type (p ≤ 0.05).

ANCOVA results for the examination of pappus length using fruit length as a covariate showed that there was no relationship between pappus length and fruit length in the CH head type or the iCL head type of *Centaurea melitensis* (Figure 12, Tables 4 and 5 in Appendix A). The peripheral and center achenes of the fCL head type showed a linear relationship between pappus length and fruit
length, but when fruit length was taken into account for the fCL head type, the difference in pappus length between peripheral and center achenes was still not significant ($t = -0.990, p = 0.32$; Figure 12, Table 6 Appendix A). The longest pappi were observed in CH achenes compared to those of either of the cleistogamous head types (Figure 11).

![Figure 11](image.png)

Figure 11. Comparison of average pappus length in peripheral and center achenes in *Centaurea melitensis* head types. Error bars represent one standard error. Asterisks indicate significant differences between peripheral and center achenes as determined by Tukey HSD ($p \leq 0.05$). See Table 2 for ANOVA results.
Figure 12. ANCOVA analysis showing the relationship between pappus length and fruit length in peripheral and central achenes of *Centaurea melitensis*. All slopes are parallel A) CH B) iCL C) fCL. For ANCOVA results see Tables 4, 5 and 6 in Appendix A.

**Elaiosome Height.** Elaiosome heights of peripheral and central achenes for the three head types (CH, iCL and fCL) are shown in Figure 13. Results of the two-way ANOVA indicate that there was a significant effect of effect of position and a significant effect head type on elaiosome height, and no interaction.
between head type and position (Table 2). On average, the elaiosome heights were greater in the center achenes in every head type.

Figure 13. Comparison of average elaiosome heights in peripheral vs. central achenes of *Centaurea melitensis* head types. Error bars represent one standard error. Asterisks indicate significant differences between peripheral and center achenes as determined by Tukey HSD ($p \leq 0.05$). See Table 2 for ANOVA results.
Post–hoc Tukey HSD tests on all pair-wise contrasts revealed elaiosome height differences between peripheral and center achenes were significant within all head types: CHp–CHc, iCLc-iCLp and fCLp-fCLc (p ≤ 0.05). Tukey HSD found significant differences between peripheral achenes of the following head types fCLp–CHp and iCLp–fCLp (p ≤ 0.001).

Elaiosome Depth. Elaiosome depths for peripheral and center achenes within the three head types (CH, iCL and fCL) are shown in Figure 14. Results of the two-way ANOVA indicated there were significant effects of position and head type on elaiosome depth, and a significant interaction between head type and position in elaiosome depth (Table 2). On average elaiosome depths were greater on center achenes compared to peripheral achenes (Figure 14).

Post–hoc test Tukey HSD on all pair-wise contrasts revealed elaiosome depth differences between peripheral and center achenes were significant only in the fCL head type (p ≤ 0.05). Tukey HSD found significant differences between peripheral achenes of all head types: fCLp–CHp, iCLp–CHp and iCLp–fCLp (p ≤ 0.001). Tukey indicated the only head type affected by position in regards to elaiosome depth was the final cleistogamous head type (p ≤ 0.05).
Figure 14. Comparison of average elaiosome depth in peripheral vs. central achenes in *Centaurea melitensis* head types. Error bars represent one standard error. Asterisk indicates significant differences between peripheral and center achenes as determined by Tukey HSD ($p \leq 0.05$). See Table 2 for ANOVA results.

**Elaiosome Volume.** Elaiosome volumes for peripheral and center achenes of *Centaurea melitensis* are shown in Figure 15. Results of two-way ANOVA indicate a significant effect of position and a significant effect of head type on elaiosome volume with no interaction (Table 2, Figure 15). Elaiosome volumes were on average largest in the iCL head type and bigger in the center achenes...
than in the peripheral achenes across head types (Figure 15). Post–hoc Tukey HSD tests on all pair-wise contrasts revealed elaiosome volume differences between peripheral and center achenes were significant (p ≤ 0.001): CHp–CHc, iCLc-iCLp and fCLp-fCLc. Tukey HSD found significant differences between peripheral achenes in the following head-type comparisons iCLp-CHp and iCLp–fCLp (p ≤ 0.001).

ANCOVA results examining elaiosome volume differences in peripheral and center achenes after taking into account fruit length were significant. The relationship between elaiosome volume and fruit length also was found to be linear. In the CH head type the peripheral achenes had a significantly lower elaiosome volume than the center achenes (t = -2.076, p < 0.05; Figures 16 and 17A, Appendix A Table 7). In the iCL head type the peripheral achenes were also found to have a significantly lower elaiosome volume (t = -2.84, p < 0.01; Figures 16 and 17B, Appendix A Table 8). In the fCL head type the peripheral achenes were also found to have a significantly lower elaiosome volume than the center achenes (t = -2.86, p < 0.01; Figures 16 and 17C, Appendix A Table 9). Differences in elaiosome volume were still present following post-hoc analysis with Tukey HSD indicating elaiosome volume in the center achenes was greater than in the peripheral achenes for all three head types.
Figure 15. Average elaiosome volume in peripheral and center achenes of three head types of *Centaurea melitensis*. Error bars represent one standard error. Asterisks indicate significant differences between peripheral and center achenes as determined by Tukey HSD (*p* ≤ 0.05). See Table 2 for ANOVA results.
Figure 16. ANCOVA data showing the relationship between elaiosome volume and fruit length in peripheral and center achenes of *Centaurea melitensis*. A) CH B) iCL, C) fCL. For ANCOVA results see Tables 7, 8, and 9 in Appendix A.

Elaiosome Shape. ANCOVAs that examined variation in elaiosome depth, while controlling for elaiosome height revealed differences between peripheral and center achenes in elaiosome shape in chasmogamous heads (CH). A linear relationship was found between elaiosome depth and elaiosome height in all three head types (CH, iCL and fCL) of *Centaurea melitensis* (Figure 17).
elaiosome depth was significantly greater in the peripheral achenes ($t = 2.243$, $p < 0.05$) than the center achenes for a given elaiosome height in the chasmogamous (CH) head type (Figure 15A, Table 10 Appendix A). In the initial cleistogamous (iCL) and in the final cleistogamous (fCL) head types, there were no detectable differences in shapes of elaiosomes of peripheral achenes compared to those of center achenes (Figures 15B and 15C, Tables 11 and 12 in Appendix A). Elaiosomes were both deeper and longer in the center achenes of the iCL and fCL heads types, with no change in shape. In the CH head type, center achenes the elaiosomes were longer but not deeper resulting in a slightly different shape, with more elongated elaiosomes in center achenes.
Figure 17. ANCOVA data showing relationship between elaiosome depth and height in the center and peripheral achenes of *Centaurea melitensis*. All slopes were parallel. A) CH B) iCL C) fCL. For ANCOVA results see Tables 11, 12, and 13 in Appendix A.
Discussion

This study found marked differences in mass between peripheral and central achenes in *Centaurea melitensis*. Spanish researchers, Porras and Muñoz (2000a, 2000b) found achene mass varied with head type. They noted that the iCL achene had the greatest mass compared to the CH and fCL. My study not only found these differences between peripheral and central achenes, but in two of the head types (fCL and iCL) the differences were almost as large as those previously recognized by Porras and Muñoz (2000a, 2000b) among the head types.

Similarly in the closely related congener, *Centaurea solstitialis*, the peripheral achenes were lighter (Callihan et al. 1993). In other species, peripheral achenes can be heavier, as in the case of *Leontodon longirostris* (Ruiz de Clavijo 2001) or lighter, as in the case of *Bidens pilosa* (Rocha 1996). One might think peripheral achenes would be heavier because they develop first as in the sister taxon, *Centaurea solstitialis* (DiTomaso 2001; Young et al. 2005). The question then becomes one of achene maturity. Are the peripheral achenes mature? It appears that achene development occurs in the same fashion in *Centaurea melitensis*, from the peripheral to the center (Personal observation). Studies have found that achenes of *Centaurea melitensis* tend to develop and mature along with the flower head, meaning the months in which they are growing (Porras and Muñoz 2000a). However, there is no information on developmental differences between peripheral and center achenes of *Centaurea*
*Centaurea melitensis*. Would this account for variation in mass related to head type and position? Only plants that were senescent were collected. Achenes were considered mature based on developmental patterns described by Porras and Muñoz (2000a, 2000b) and seen in the sister taxon, *Centaurea solstitialis* (DiTomaso 2001). To ensure full maturity of the plant, the achenes were gathered late spring through late summer.

There may be a number of reasons why peripheral achenes are lighter. They may or may not be filled, the pappi may vary in size and shape, the pericarp may thicker, fruit width may differ and elaiosome size may differ. In this case all achenes upon visualization under the scope appeared plump and filled and healthy free of any fungus on the outside. Another possible reason for mass differences in achenes is the question of viability. If the achene is unfilled that may affect mass. Viability testing was performed in a separate experiment using the cut test and the tetrazolium test, which revealed viable achenes in all achene types. There has been some suggestion that iCL achenes, being the heaviest of the three sets, may be dormant and may have bigger embryo's (Porras and Muñoz, 2000a, 2000b). The peripheral achenes for *Centaurea melitensis* have a pappus, so the differences may lie in their shape and possibly thickness of the bristles. The pappi of the peripheral achenes of the iCL and fCL head type appear thicker than that of the CH. The current study also indicated the pappi in the peripheral achenes in *Centaurea melitensis* appeared not as full as the center achenes, which may have played a small part in the mass differences.
Elaiosomes definitely add mass to the achenes, and differences in elaiosome size probably contributed to mass differences in *Centaurea melitensis*.

There are also implications for being heavier or lighter. Anderson (1993) suggested that plants develop different adaptations for achene dispersal such as heavier achenes. He suggests that heavier achenes don’t disperse well but may have better chances of survival and competitive abilities. Forsyth and Brown (1982) also noted that heavier achene may fall closer to the parent plant. Falling closer to the parent plant doesn’t always mean there less competition, though. If the achene is lighter it may travel further than the heavier achene with the added appendages like the pappus. Forsyth and Brown (1982) also suggested that achenes with thicker seed coats may indicate a period of burial. A period of burial can indicate some degree of dormancy.

Many members of the Asteraceae family produce achenes that vary in shape and size (Forsyth and Brown 1982; Callihan et al. 1993; Ruiz de Clavijo 2001). These differences are believed to be important for dispersal and survival. Being able disperse beyond the parent plant to a more favorable environment provides an opportunity for establishment and continuation of the species. Differences in size and shape of the achenes may be an investment that allows for more efficient dispersal (Ruiz de Clavijo 2001).

*Centaurea melitensis* has peripheral and center achenes that differ morphologically. This study was the first to quantify shape in *Centaurea melitensis* peripheral and center achenes and give a visual graphical image
produced from ANCOVA to display them. My data indicated that there were shape differences between the peripheral and center achenes in the CH and iCL head types, with center achenes being wider for any given length.

In *Bidens pilosa*, the peripheral achenes are shorter and triangular in shape, with a thicker seed coat and fall closer to the parent plant, but were more sensitive to temperature and light and exhibit dormancy while the longer elongated center achenes had a thinner seed coat and germinated much easier and did not exhibit dormancy (Forsyth and Brown 1982). The peripheral and center achenes of *Leontotodon longirrostris*, differ in size and shape and degree of pappus development (Ruiz de Clavijo 2001). The center achenes germinated in higher proportions under a wide range of conditions and the peripheral had more restricted germination and with a thick pericarp and tends to remain in a dormant state for a period of time following dispersal (Ruiz de Clavijo 2001).

The current study showed that fruit lengths in peripheral achenes and center achenes differed significantly only in the fCL head type while in the CH and iCL achenes they were similar. Possible reason for this may be that the fCL head type is the last set of achenes to develop on the plant and tend to have less developmental time. Their flower heads are much smaller as well as most of the plants resources have been depleted (Porras and Muñoz 2000a, 2000b). It may be more important to invest in dispersal features like the pappi and elaiosome.

Porras and Muñoz (2000a) looked at differences in achene length without a pappus and found that CH and fCL achenes to be similar in achene length and
iCL to be the longest of the three. I found a similar trend when comparing all center achenes. Other explanations for varying fruit lengths are the peripheral achenes are very close to the bracts during development. They may not have much room to develop compared to their center counter parts. In *Centaurea solstitialis* the peripheral and center achenes were similar in length ranging from 2 to 3 mm for peripheral achenes and 2 to 4 mm for center achenes.

Fruit width varied with head type and position within the head. The data indicated fruit widths of peripheral achenes within each of the head types (CH, iCL and fCL) were smaller in comparison to the center achenes. In *Centaurea solstitialis* the peripheral achenes have been called asymmetrical, possibly because they during development they are pressed against the bracts (Young et al. 2005). This is a plausible explanation for narrower peripheral achenes in *Centaurea melitensis*. Forsyth and Brown (1982) found the peripherally located achenes to be shorter and wider than the center achenes in *Bidens pilosa*. The peripheral achenes had shorter survival rates and lower and slower germination rate than the narrower center achenes, which had higher germination rates. Achene width may also be related rapid development during periods where environmental conditions are limiting. On the other hand wider achenes can indicate a larger embryo which can increase the probability of emerging from increased burial depths (Ruiz de Clavijo 2001), and a thicker pericarp may add to fruit width which may the achene exhibits some dormancy (Ruiz de Clavijo 2001). Others authors have shown peripheral achenes lighter and narrower but they can
also be heavier and wider in other members of the Asteraceae family (DiTomaso 2001; Ruiz de Clavijo 2001). Being narrow can be beneficial for dispersal; if the peripheral achenes are catapulted from the flower head on a windy day or through constant wetting a drying they may disperse further from the parent plant due to their shape.

Similarities between what was found in my study and Porras and Muñoz (2000a, 2000b) were the iCL achenes were the widest and the CH achenes were the narrowest of the three achene types. The peripheral iCL achenes were still the widest among the peripheral achenes and more similar in width to center achenes in the same head type than to achenes in other head types. The iCL achenes are the first achenes to develop before the plant gains any height; this ensures the achenes are made rapidly to ensure some progeny are in the seed bank (Porras and Muñoz, 2000a, 2000b). The greater fruit width in peripheral and center achenes of the iCL achenes may be due to the fact that they don’t need to be disbursed far from the plant and rely on other methods of dispersal. Other studies have implied that iCL achenes may exhibit some type of dormancy (Porras and Muñoz, 2000a, 2000b). If this is true then these achenes may wider because a larger embryo or thicker pericarp allowing them to remain in the soil longer and be carried off to anthills where they are possibly buried adding to the seed bank. The CH peripheral achenes had the smallest fruit widths compared all other achene types followed by the fCL achenes. Final cleistogamous (fCL) achenes can be thought of a backup plan, meaning at the end of the flowering
period they may add an additional low cost seed source (Porras and Muñoz 2000a). Personal observations indicate that peripheral achenes in the fCL head type are few in number as well.

Pappi are a key feature in this species and a mode for dispersal. Although, I did not examine pappus shape differences directly, differences in shape were observable (Appendix B). The peripheral pappus appeared more underdeveloped than center achenes and the bristles appear thicker in the cleistogamous head types. A parachute like pappus in the CH center achenes indicate they are more likely to be carried off via the wind and the fCL is more likely to be fall next to the parent plant (Porras and Muñoz 2000a, 2000b). A parachute-like pappus is aerodynamically beneficial for dispersal and provides opportunity for dispersal beyond the parent plant and possibly in new environments (Sheldon and Burrows, 1972). The shape differences for some aspects of dispersal became more prominent because I was able to look at a particular character as it related to position, which has not been done before. Other authors have suggested the relative importance of shape to dispersal and establishment (Sheldon 1974).

Pappus length between peripheral and center achenes were not as marked as I thought. The only significant differences in pappus size between peripheral and central achenes were found in achenes of the CH head type; center achenes of the CH head type had pappi that were both longer and wider than peripheral achenes. The purpose of the pappus is to act as a wind dispersal
mechanism. Some authors would reject this, suggesting that the pappus in *Centaurea solstitialis* and *Centaurea melitensis* does not allow for effective wind dispersal and that the falling speed of the CH achenes is too slow to be effective in wind dispersal (Porras and Muñoz, 2000a, 2000b). However, other studies have shown *Centaurea solstitialis* achenes to disperse up to 0.6 m from the parent plant (Roche 1965). According to Sheldon and Burrows (1973) it is not just the presence of the pappus itself that determines its dispersal distance, it is the geometry of the pappus that affects aerodynamics. In addition, if the achenes are released at certain height of the plant and the winds are steady this can increase the trajectory increasing dispersal distance (Sheldon and Burrows, 1973). It is possible that *Centaurea melitensis* achenes can be catapulted from the plant, and under high Santa Ana winds like those experienced in southern California they can be carried some distance away. Porras and Muñoz (2000a, 2000b) examined falling speed in achene types and found that CH the achenes with the longest pappus had the slowest falling speed compared iCL and fCL. This suggests, the CH achenes of *Centaurea melitensis* are more likely to be picked up by the wind due to their shape and length.

My data indicated pappus width varied by head type. As expected the widest pappi were in the CH achenes, specifically the center achenes. Interestingly, the pappi for the peripheral achenes of the iCL and fCL head types tended to be wider than the central achenes, although not significantly so. Porras and Muñoz (2000b) measured the external diameter, which confirmed the CH
achenes had the widest pappi followed by iCL and fCL. As noted earlier the sister
taxon of _Centaurea melitensis_, _Centaurea solstitialis_ has peripheral achenes
without a pappus. In _Centaurea melitensis_, the pappi found in the periphery are
relatively poorly developed but not so much so they couldn’t be useful for
dispersal by orienting the achene on the soil in the direction of the wind. Some
iCL achenes develop at the base of the plant and some are axial, so it’s possible
they can use the wind for dispersal. According to Sheldon and Burrows (1972)
the distance of dispersal depends on the release height, the strength of the wind
and the convection of the air. If the peripheral achenes are axillary located and
remain in the plant until early fall they may need a backup plan that allows them
to disperse further. Pappus width in basil achenes may not be as important for
wind dispersal but may allow for attachment to objects and passersby to reach
other locations. The fCL achenes also develop along the stem, so they do have a
little height on them. Initial dispersal of the peripheral and center achenes may
require the plant to move slightly via a breeze. Peripheral achenes may need that
extra assistance to be picked up by wind and carried off on clothing or fur. It is
also possible that they are dispersed through another form of dispersal called
chame-anemochory where the achene is rolled along the ground under high wind
conditions (Benvenuti 2007). This mode of dispersal occurs in rangelands
pastures and dry environments where species like _Centaurea melitensis_ reside
(Benvenuti, 2007).
Some members of the Asteraceae family have an elaiosome. This study found elaiosomes were present in all the achenes within the three head types of *Centaurea melitensis* as previously noted by Porras and Muñoz (2000b). In my study the smallest elaiosome height was found in fCL achenes, which may indicate smaller ants or insects dispersal. The largest was found in iCL center achenes and intermediate between the two was the CH center achenes. Larger ants or even rodents may prefer iCL achenes (Benvenuti 2007). Other predatory beetles may carry off a heavy elaiosome bearing achene; the trade off for the insect is energy cost of carrying a large achene vs. a large elaiosome but they be more fit than smaller achenes to undergo burial (Pemberton and Irving 1990).

The elaiosome can vary in shape (Pemberton and Irving, 1990; Viegi et al. 2003). This study also found clear differences in elaiosome shape and size among achene types. They were in fact longer and deeper the center fCL achenes compared to peripheral achenes but similarly shaped. Shape differences were visible in the CH achenes and iCL achenes. The elaiosomes were longer in the center achenes for these head types but not deeper resulting in a shape differences. Porras and Muñoz (2000b) did not look at separate measurements’ of the elaiosome, rather they looked at elaiosome thickness. They found the smallest thickness to be in CH and the largest to be in iCL achenes, as did I. There is always a possibility of deterioration while in the flower heads or smaller elaiosomes may the result of selection (Mark and Olesen 1996). Other explanations for varying heights and depth may be the fact that not all ants
are the same size or all insects and therefore may have a preference for elaiosome size (Mark and Oleson 1996). Explanations of CL achenes are the largest achenes with a biggest elaiosome depth in both peripheral and center achenes and are the first to develop so it may be more important to germinate and establish a population right where they lay if the plant doesn’t get any bigger or add to the seed bank. One of the benefits of insect dispersal and removal of the elaiosome may be that it can potentially spark or increase germination (Lobestein and Rockwood 1993).

My data showed that peripheral achenes had smaller elaiosomes compared to the center achenes in all head types. In Centaurea solstitialis, the sister taxon of Centaurea melitensis, some authors have reported that peripheral achenes lack elaiosomes and that center achenes have them (Riba 2002; Viegi et al. 2003). Although my findings do not show such an extreme dimorphism, the size differences in elaiosomes between center and peripheral achenes show a similar trend.

Summary

The morphological differences between peripheral and center achenes of Centaurea melitensis are not as distinct as those in Centaurea solstitialis, but they are different. The peripheral achenes are lighter in both Centaurea solstitialis and Centaurea melitensis. Although lighter in Centaurea melitensis they also exhibited shape differences. The peripheral achenes and the center achenes of Centaurea melitensis both have a pappus, but the peripheral
Achene size, shape, position, presence or absences of an elaiosome are critical features necessary for the effective dispersal and establishment. This is especially true for annuals that depend on achene production for regeneration. Having a combination of dispersal features may allow species like *Centaurea melitensis* to not only disperse effectively, but to respond to various conditions. *Centaurea melitensis* resides in areas where environmental conditions vary. Having center achenes that possess both a larger pappus and elaiosome allow for any type of dispersal. Having variable morphology enables non-native invasives like *Centaurea melitensis* a strategy for effective establishment and may also allow it to respond to variable environmental conditions.
CHAPTER THREE
DIFFERENCES IN TEMPERATURE RESPONSES OF ACHENE TYPES IN
CENTAUREA MELITENSIS (TOCALOTE)

Abstract

The ability to respond various temperatures in areas where irregular temperature patterns and variable rainfall exist, may contribute to the success in weedy species like *Centaurea melitensis*. Temperature responses in achenes that develop and disperse at different times during the growing season may be associated with timing of dispersal, position and morphology. This study was designed to investigate differences in germination of center and peripheral achenes of each of three recognized head types of *Centaurea melitensis* over a range of temperatures. It also examined differences in ratios of peripheral to central achenes produced by different head types, and investigated whether center achenes disperse more readily than peripheral achenes. To investigate differences in response to temperature peripheral and center achenes were subjected to temperatures ranging from 0 °C to 30 °C. To investigate differences between center and peripheral achenes in their ability to disperse, ratios of peripheral to center achenes found in heads prior to dispersal were compared to ratios found late in the season (September). A broad temperature optimum was observed in achenes of both CH and fCL head types but not the iCL head type.
Lower germination was observed in peripheral achenes within all head types especially at 15°C. The iCL achenes were adversely affected by temperatures greater than 10°C. Ratios of center to peripheral achenes in September were similar to those found prior to dispersal, indicating no difference in their tendency to disperse. There were more central achenes than peripheral achenes within each head type. There was no difference in the temperature response pattern between center and peripheral achenes within a head type. Therefore, although differences among head types in temperature response and season of dispersal of achenes may contribute to the success of *Centaurea melitensis* in a variable environment, I found no difference in these features between center and peripheral achenes within any head type. Slightly lower germination rates of peripheral achenes, however, may reflect dormancy, a characteristic favorable in a variable environment.

**Introduction**

Temperature is a critical component of germination in that it affects plant distribution and limits the potential range of expanding or invading populations. It also plays a key role breaking achene dormancy and helps determine timing of emergence in the field for better control of invasions. Understanding how a species deals with variable environmental conditions begins through examination of its response to critical environmental cues such as temperature.

*Centaurea melitensis* is a non-native winter annual that resides in areas of disturbance (areas where temporary changes in environmental conditions occur,
wind storms, flooding and wildfire) such as those found along roadsides, open fields, grasslands and among California coastal sage scrub and fire prone communities (Moroney and Rundel 2012; Porras and Muñoz 2000a, 2000b). It is also known to grow in subnitrophilous pioneer pasture communities (lands slightly enriched by nitrates, grasslands and agricultural areas) (Porras and Muñoz 2000a, 2000b; DiTomaso and Gerlach 2000). Furthermore, *Centaurea melitensis* grows in a Mediterranean climate, which consists of cool wet winters and hot dry summers (DiTomaso 2001; Porras and Muñoz 2000a, 2000b; Maddox et al. 1985). This type of climate can result in irregular temperature patterns and rainfall variation (NOAA; Ritter 2006; Porras and Muñoz 2000a, 2000b; Roche et al. 1997).

Despite the predictability of southern California's Mediterranean climate, variation in temperatures and rainfall can occur from year to year. The amount of rainfall can range between 12-24 inches or 300-600 millimeters (Tyrrel 1982). Southern California can experience wet winters and springs one year, and other years there can be very little rainfall, resulting in a very dry year causing drought. These are often associated with La Niña and El Niño years (NOAA, Larson and Kiemnec 1997). During winter storms high intensity rainfall can occur; between storms, extended dry periods may occur. Limited moisture during the season and summer months leads to dry conditions and relative humidities that fall below 5%, which compromise germination (Tyrrel 1982).
Exposure to irregular temperature patterns and variable rainfall and strong winds can lead to significant environmental changes, which threaten or delay germination and survival. For instance, temperatures during winter can drop down into the thirties destroying new seedlings. Plant cells in leaves can freeze leading to seedling death. In seeds, low temperatures tend to slow metabolic rates ceasing the onset of germination (Forsyth and Brown 1982). Low temperatures can also induce dormancy or break dormancy (Liu et al. 2013). Summer temperatures in southern California can get over hundred degrees, producing arid conditions with little to no rainfall, preventing germination, and leading to dormancy (NOAA 2011; Tyrrel 1982). High temperatures can also denature achene proteins (Forsyth and Brown 1982). These variations are not only visible from year to year, but they can vary from area to area.

Environmental Variation in San Bernardino

_Centaurea melitensis_ (tocalote) is observed in both disturbed and undisturbed (chaparral) areas of San Bernardino California. San Bernardino, California is known to have irregular temperature patterns and variable rainfall and strong winds and is considered a semi-arid. The city is located between the San Gabriel Mountains, the San Bernardino Mountains, and south of Crafton Hills, and the Jurupa Mountains (Dutcher and Garrett 1963). San Bernardino has a long history of variable rainfall. The precipitation can be extremely variable, according to Dutcher and Garrett (1963). The annual rainfall between 1952 and 1971 averaged 16.79 inches (426.7 mm). In some
years precipitation was limited (1898-99: 9.30 inches (236.2 mm)) and in others rainfall in excess of 20.72 inches (526.2 mm) was also documented (Dutcher and Garrett 1963).

Variations in temperatures also impact the San Bernardino area, especially during *Centaurea melitensis*’s growing season. Between 1981-2010, according to the Western Regional Climate center, the mean daily average temperatures and precipitation in March were 21.1 ºC, and the mean nighttime average was 6.5 ºC; rainfall was 2.72 inches (69.1 mm). In April mean daily temperature was 24.3 ºC, and mean nighttime temperature was 8.4ºC; rainfall was 1.28 inches (32.5 mm). May’s mean daily average was 27.2 ºC, and nighttime temperature was 10.9 ºC; rainfall was 0.38 inches (9.65 mm), and for June daily temperatures averaged 31.44ºC, nightly temperatures averaged 13.05 ºC, and precipitation averaged 0.09 inches (2.28 mm) (NOAA 2011; http://www.wrcc@dri.edu). Average precipitation and temperatures during *Centaurea melitensis* growing season in 2011 are visible in Figure 1.

Warm Santa Ana winds influence the temperatures in the San Bernardino. They flow from the deserts to the sea causing dry conditions to be even drier and can reach 100 mph (Tyrrel 1982; NOAA 2011). The rise in temperature causes temperatures near the soil surface to be really warm. The warm dry air leads to a drop in the relative humidity inhibiting germination (Fovell 2002).
Figure 18. Average maximum 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*.

Dealing with Variable Environmental Conditions

In order to deal with unstable environmental variation in temperature and moisture, species of plants like *Centaurea melitensis* (tocalote) have evolved morphologically different flower heads and morphologically different achenes that
develop and disperse at different times during the growing season (DiTomaso 1996; Porras and Muñoz 2000a, 2000b; DiTomaso 2001). *Centaurea melitensis* has both chasmogamous flower heads and cleistogamous flower heads. Both types of flower heads develop throughout the growing season. Although both types of flower heads are produced in *Centaurea melitensis*, production of chasmogamous flower heads may increase when environmental conditions are optimal (Waller 1988; Porras and Muñoz 2000a, 2000b; Moroney and Rundel, 2012). It has also been suggested that larger more vigorous plants produce more chasmogamous flower heads (Maddox 1981; Waller 1988; Porras and Muñoz 2000a). Moreover, the development of cleistogamous flower heads is often favored in species during times when environmental conditions are sub-optimal and when resources are limited; for example, when precipitation is limited like those experienced during summer months in southern California (Waller 1988; Porras and Muñoz, 2000a, 2000b; Moroney and Rundel, 2012).

The growing season for *Centaurea melitensis* runs from March through 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) (Porras and Muñoz, 2000a, 2000b). However, they have been observed to develop through August (Personal observation).
Achene Position and Environmental Response

Variable temperatures and precipitation in unpredictable environments conditions can also lead to the development of achenes that develop at different positions and disperse at different times during the growing season. Position of the achene within the flower head and timing of dispersal may be indicative of how when they germinate in response to environmental cues. For instance, if peripheral achenes are released in early fall they may require a cold period to break dormancy and germinate the following spring. If the center achenes are released in spring and early summer they may require a warm period to break dormancy and germinate in early fall. This pattern has been observed in the closely related congener Centaurea solstitialis (Joley et al. 1997; Young et al. 2005).

At first glance the position of an achene within a flower head may serve no relevant importance. However, for members of the Asteraceae family position and release may be associated environmental germination cues. In Bidens pilosa, the peripherally located achenes, which are brown, short and slightly triangular, display enhanced germination when subjected to red light, scarification and increased oxygen tensions. Germinability of Bidens pilosa also depends on their position within the flower head and seasonal light cycles (Forsyth and Brown 1982).

Positional development and dispersal has certain ecological implications; in some cases the positional release impacts dormancy cycles (Brandel 2007).
Dormancy cycles are especially important for weedy species since their success depends on timing and emergence, and their control depends on understanding that cycle (Batlla and Benech-Arnold 2007). This is important in reducing the spread of many weedy species.

**Achene Ratio and Temperature**

Variation in temperatures and moisture in unpredictable environments may lead to differences in the number of peripheral and the center achenes in the flower head. This has been observed in other members of Asteraceae like *Bidens pilosa* where the number of peripheral achenes to center achenes depends on changes in temperature: under cold conditions more peripheral achenes are produced than center achenes. Additionally changes in light affects numbers in this species. Long day cycles produce more short (peripheral) achenes per capitulum compared to long (center) achenes (Forsyth and Brown 1982; Waller 1988).

In grasses that are cleistogamous, the flowers are arranged so that there is a constant fraction of flowers on all the plants according to Waller (1988). However, the proportion of flowers and fruits in other cleistogamous species can vary depending on the environmental conditions this is called “environmental cleistogamy” (Waller 1988).

In *Centaurea solstitialis* there are more center achenes than peripheral achenes (3:1; DiTomaso 2001). The ratios of pappus-bearing center achenes to nonpappus-bearing peripheral achenes observed at three locations in California
were 3.4:1, 4.7:1 and 8.7:1 (Joley, et al. 1997). These differences, according to Roche (1965), are the result of plasticity (how effective the plant responds to changes in environmental conditions). Furthermore, it has been suggested that the center pappus-bearing achenes function to perpetuate the species during optimal environmental conditions of adequate light temperature and moisture, while non-pappus bearing peripheral achenes are more persistent and remain in the flower head and serve to perpetuate the species during times when conditions are less optimal (i.e. cold, dry conditions of drought and even shade; Roche 1965; Maddox 1981; Waller 1988). The reason is that cleistogamous flower heads are more energy efficient (Waller 1988). Chasmogamous flower heads are larger and require pollinators thus require more energy from the plant to produce, while cleistogamous flower heads are smaller and located at lower parts of the plant and also self-pollinate requiring less energy (Waller 1988).

In the well-researched yellow starthistle, *Centaurea solstitialis*, found in areas of northern California, the peripheral achenes develop first are located near the bracts and the center achenes are located in the center (DiTomaso 1996; DiTomaso 2001; Young et al. 2005). The peripheral achenes are pappus-less and develop first and are dispersed during the winter. They also remain in the flower head until winter when they are forced out through constant drying and wetting action (Roche 1992). Germination of peripheral pappus–less achenes takes place in the springtime. The center achenes have a pappus, and they are located in the middle of the flower head, and are released first when the flower
head senesces, usually during the summer (Roche 1992; DiTomaso 1996; Joley et al. 1997; DiTomaso 2001). They tend to germinate after first fall rains (DiTomaso 2001).

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 average germination in the peripheral achenes was significantly lower than the center achenes. At 0 °C no significant germination differences were observed between the achene types. The peripheral achenes were found to have 38% lower germination at alternating of temperatures of 0°C/2°C, 0°C/5°C and a constant temperature of 2°C compared to the center achenes (Young 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 a specific temperature for optimum germination. However, they did find that the peripheral achenes prefer 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 effects of light and temperature on germination of *Centaurea solstitialis* achenes. For the purpose of this paper only temperature aspects will be addressed. This study found that germination was greatest in both achenes types (peripheral and
central) when exposed to moderate temperatures. They also observed 100% germination in the middle three temperatures, which ranged between 10 ºC and 25 ºC. They also tested fresh and stored center and peripheral achenes. They found no change in germination in stored achenes when subjected to 25:15ºC and 30:20ºC, but freshly collected germination rates decreased over time (Joley et al. 1997).

Forsyth and Brown (1982) tested the effects of temperature on *Bidens pilosa*. While they did not focus specifically on temperature, they did address a small aspect of how temperature affected the morphologically different achenes of *Bidens pilosa*. Their examinations consisted of exposing the two achene types to temperatures of 20 ºC, 25 ºC, and 30 ºC under constant light. They found optimum temperature for germination of the center and peripheral achenes was 25 ºC. Temperatures of 30 ºC significantly reduced germination (Forsythe and Brown 1982). They also discovered that longer (center) achenes germinated over a larger range of conditions (Forsyth and Brown 1982).

**Species of Interest: *Centaurea melitensis***

Producing two different achene types within the same flower heads that are released at different times relative to position may allow non-native species like *Centaurea melitensis* to survive and persist in areas of disturbance, which makes it an excellent model species for understanding how different achene types in the same plant respond to environmental cues. The achenes may respond to a variety of temperatures, increasing their chances for seedling
survival. Therefore, morphologically different achenes that develop at different times and different positions within the same flower head may respond differently to variable temperatures produced with changing environmental conditions found during the growing season.

Each flower head of *Centaurea melitensis* (tocalote) contains peripheral and center achenes that vary morphologically and develop at different positions within the same flower head. Both peripheral and center achenes of *Centaurea melitensis* have a pappus. The peripheral achenes of *Centaurea melitensis* may persist into the early fall, as do the peripheral achenes of *Centaurea solstitialis*. However, this has not been previously investigated.

**Centaurea melitensis** and Temperature

Minimal investigations on *Centaurea melitensis* (tocalote) and its response to temperature or other environmental cues have been performed. 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 (March, April, May and June) in Cordoba Spain are 21 ºC, 22 ºC, 26 ºC and 32 ºC (http://climatedata.eu). Spanish researchers Porras and Muñoz (2000b) 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 highest at the following temperatures, 12ºC, 25ºC/15ºC, and the greatest germination occurred at 19ºC.
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 positive or negative effects of any temperature regime they tested in the fCL achenes (Porras and Muñoz, 2000b). Although Porras and Muñoz (2000b) compared the effects of temperature on germination of achenes from the three head types, 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.

Germination of peripheral and center achenes can be associated with variations in seasonal temperatures and timing of dispersal in other members of Asteraceae. Position and morphology differences and the number of achenes at each position in the flower head may be associated with environmental conditions as well (Maddox 1981; Joley et al. 1997). Growing seasons, dispersal time and germination in San Bernardino, CA are all associated with variable temperatures’ and rainfall. Therefore, there is reason to believe that temperature plays a critical role in the germination of peripheral and center achenes of Centaurea melitensis. This gives rise to the following questions; 1) Are there differences in season of dispersal (or the ability to disperse) between central and peripheral achenes of Centaurea melitensis? 2) Are there differences in temperature response in peripheral vs. central achenes that may indicate differences in season of germination?
Methods

Germination Responses to Temperature

To investigate whether there are differences in the germination response of peripheral and center achenes of *Centaurea melitensis* to temperature, a factorial experiment was performed. Germination of peripheral and center achenes for all three head types (CH, iCL and fCL) were tested at five temperatures. Temperatures were selected after reviewing literature on *Centaurea melitensis* and *Centaurea solstitialis* germination responses to temperature; they consisted of temperatures experienced during the fall and winter months in southern California. Warmer temperatures were representative of early spring to mid-summer time. They were 5 °C, 10 °C, 15 °C, 23.5 °C and 30 °C.

The achenes were previously gathered in the morphology experiment and stored at room temperature (23.5°C) in paper envelopes. Methods of gathering were reported in the morphology experiment (see Chapter 2).

A total of 400 achenes per achene type (CHc, CHp, iCLc, iCLp, fCLc, and fCLp) were used in the experiment. Twenty achenes for each achene type for each of five temperatures were sown in Fisherbrand sterile 100 mm x 15 mm polystyrene Petri Dishes with Fisherbrand glass filter paper (9.0cm). The achenes were moistened with 5 ml of distilled water, and the Petri Dishes were sealed with plastic wrap to prevent moisture loss during incubation. There were four replicate petri dishes per achene type/temperature combination.
Achenes subjected to 5 °C, 10 °C, 15 °C, and 30 °C temperatures were placed in incubators (Revolutionary Science RS-IF-202). The incubators inside dimensions were 31.7 cm wide x 19 cm tall x 31.7 cm deep. A room-temperature incubator (23.5 °C) was constructed using a cardboard box. The size of the box was consistent with size of the four incubators. Room temperature readings were taken with a (Omega HH-25KC) digital thermometer. To obtain the same lighting that the incubator would achieve during the experimental process a clear plastic trash bag was placed over the opening and the light intensity at all angles of the box were measured. The back of the box was 0.24 Wm⁻²; the front end measured 0.093 Wm⁻². 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 3).

Only one set of replicates was 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 incubators were A) 5 °C, B) 10 °C, C) 15 °C and D) 30 °C and room temperature (box). Replicate# 2 incubators were A) 15 °C, B) 30 °C, C) 5 °C and D) 10 °C. Replicate #3 incubators were A) 30 °C, B) 15 °C, C) 10 °C and D) 5 °C. Replicate #4 incubators were 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. Germination was determined by 3 mm emergence of the radicle.
Table 3. Light intensity measurement inside incubators

<table>
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<tr>
<th>Chamber</th>
<th>Back or front</th>
<th>Doors open/closed</th>
<th>Light reading Watts/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Back</td>
<td>Open : Closed</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>Front</td>
<td>Open</td>
<td>0.130</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Closed</td>
<td>0.030</td>
</tr>
<tr>
<td>B</td>
<td>Back</td>
<td>Open</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>Front</td>
<td>Closed</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Open</td>
<td>0.030</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Closed</td>
<td>0.019</td>
</tr>
<tr>
<td>C</td>
<td>Back</td>
<td>Open</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>Front</td>
<td>Closed</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Open</td>
<td>0.176</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Closed</td>
<td>0.050</td>
</tr>
<tr>
<td>D</td>
<td>Back</td>
<td>Open</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>Front</td>
<td>Closed</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Open</td>
<td>0.176</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Closed</td>
<td>0.050</td>
</tr>
</tbody>
</table>

Statistical Analysis

All data were collected and analyzed using the statistical program R project version i386 2.15.1 (2012-06-22).

Separate two-way ANOVA’s were performed to examine the effect of temperature and position on germination for individual head types, as well as an
overall three-way ANOVA. A two-way ANOVA testing the effect of temperature and head type on germination was also performed.

Production and Persistence of Different Achene Types in Flower Heads of *Centaurea melitensis*

To determine whether peripheral achenes persist in the flower heads longer than center achenes and to determine the relative numbers of peripheral and center achenes produced in each head type, peripheral and center achenes were counted in heads collected prior to achene dispersal and in heads collected in early fall, well after maturation.

The late season heads were collected in September of 2011. Thirty *Centaurea melitensis* plants were removed from the field in an area of coastal sage scrub located on the north side of the 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). Ten of the plants were randomly selected. One flower head of each head type was removed from each of the ten random plants. The peripheral and center achenes were removed as previously described in Chapter 2. The number of peripheral and center achenes were tallied for each flower head and also the number undeveloped achenes were noted. Achenes were considered undeveloped if they were completely flat and shriveled.

The relative number of peripheral and center achenes initially produced in each head type was determined by counting achenes in mature heads collected
prior to dispersal. Flower-head development and achene development was monitored from March through September of 2012 in the field. Flower heads were collected following their senescence but prior to opening all the way in cleistogamous heads and when slightly opened in chasmogamous heads. This ranged from March to the first week of July in 2012. The flower heads containing achenes were selected randomly and 5 of each head type each were removed from each of 20 random plants throughout the study area. The flower heads were then wrapped individually in plastic wrap to prevent the achenes from falling out during storage. They were all stored at 23.5 °C in plastic freezer bags until data collection. The numbers of peripheral achenes, center achenes, filled and undeveloped achenes were recorded for each head type. A total 100 flower heads containing achenes were removed from Centaurea melitensis plants at the end of each growing cycle for a particular head type.

Determination of Achene Dispersal Ease

To determine if center achenes disperse more readily than peripheral achenes and to assess whether the probability of dispersing differed between center achenes and peripheral achenes, three Chi-squared tests were performed (one for each head type). The ratio of the number of peripheral achenes to center achenes was calculated early season and compared to late season ratios in Centaurea melitensis flower heads.

If center and peripheral achenes had similar tendencies to disperse from a flower head, I would expect the ratio of center achenes to peripheral achenes
be similar in heads collected early in the season and late in the season. Because the number of achenes in each head was small for some head types, data for individual head types were pooled within each head type. The late-season expected values were calculated by multiplying the total number of achenes found in late in the season for each head type by the proportions found in center and peripheral positions early in the season. The Pearson Chi-squared test was performed using the online statistic tool by Preacher (2010).

Results

Germination Response of Different Achene Types to Temperature

A two-way ANOVA examining the effect of position and temperature (peripheral vs. center) on germination of Centaurea melitensis achenes showed a significant effect of position and a significant effect temperature on germination with no interaction (Table 4).

Table 4. Two-way ANOVA examining the effect of position and temperature on germination of Centaurea melitensis achenes.

<table>
<thead>
<tr>
<th>Source of the variation</th>
<th>DF</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position</td>
<td>1</td>
<td>151.9</td>
<td>151.87</td>
<td>13.2862</td>
<td>0.0004***</td>
</tr>
<tr>
<td>Temp</td>
<td>4</td>
<td>3991.1</td>
<td>997.78</td>
<td>87.2867</td>
<td>2.2e-16***</td>
</tr>
<tr>
<td>Position:Temp</td>
<td>4</td>
<td>68.9</td>
<td>17.23</td>
<td>1.5072</td>
<td>0.2050220</td>
</tr>
<tr>
<td>Residuals</td>
<td>110</td>
<td>1257.4</td>
<td>11.43</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Germination of peripheral and center achenes of *Centaurea melitensis* showed broad temperature optima in CH and fCL achenes but a narrower optimum in iCL achenes. There was a lack of germination at 5 °C for both peripheral and center achenes for all three head types (Figures 19, 20, 21). On average there was more germination in the center achenes compared to the peripheral achenes for all other temperatures tested. Within the CH head type there was a significant difference in germination at 15 °C between peripheral and center achenes (Figure 19, M CHc = 19.0, SD = 0, M = CHp 16.0, SD = 2.00, t (6) = 3.00, p = 0.024). However, the pattern of temperature response in the peripheral achenes was similar to the center achenes across temperatures. In the iCL head type (Figure 20) both peripheral and center achenes showed a drop in germination above 15 °C, suggesting they prefer cooler temperatures. For both peripheral and center achenes within the iCL head type the pattern of temperature response was similar. In the fCL head type (Figure 21) peripheral and center achenes showed a similar temperature response. Peripheral achenes had significantly lower germination at 15 °C compared to center achenes (M fCLc = 18.0, SD = 1.83, M fCLp = 12.5, SD = 1.29, t (6) = 4.92, p = 0.002).
Figure 19. Germination response to temperatures of different achene types within chasmogamous flower heads. The bars represent standard error. Asterisk indicates significant difference between center and peripheral achene germination at 15 °C.
Figure 20. Germination response to temperature of different achene types within initial cleistogamous flower heads. The bars represent standard error. Asterisk indicates significant difference between center and peripheral achene germination at 15 °C.
Individual two-way ANOVA's for examination of the effect of temperature and position on germination showed the following results. There was a significant effect of temperature and position on germination of achenes within the CH head.
type (Table 5, Figure 19). There was a significant effect of temperature and position and germination of iCL achenes (Table 6, Figure 20). There was a significant effect of temperature and position on germination of fCL achenes (Table 7, Figure 21). There was no interaction between temperature and position for any of the achene types. In each case, the peripheral achenes had lower germination rates than center achenes.

### Table 5. Two way ANOVA showing effect of temperature and position on germination of achenes from the chasmogamous (CH) head type.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp</td>
<td>4</td>
<td>1855.10</td>
<td>463.77</td>
<td>359.05</td>
<td>2.2e16***</td>
</tr>
<tr>
<td>Position</td>
<td>1</td>
<td>15.62</td>
<td>15.62</td>
<td>12.096</td>
<td>0.00156**</td>
</tr>
<tr>
<td>Temp:Position</td>
<td>4</td>
<td>11.50</td>
<td>2.87</td>
<td>2.2258</td>
<td>0.089.</td>
</tr>
<tr>
<td>Residuals</td>
<td>30</td>
<td>38.75</td>
<td>1.29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

### Table 6. Two-way ANOVA showing the effect of temperature and position on germination of achenes within the initial cleistogamous (iCL) head type

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp</td>
<td>4</td>
<td>1080.85</td>
<td>270.212</td>
<td>31.3593</td>
<td>2.517e-10 ***</td>
</tr>
<tr>
<td>Position</td>
<td>1</td>
<td>67.60</td>
<td>67.60</td>
<td>67.60</td>
<td>0.008833 **</td>
</tr>
<tr>
<td>Temp:Position</td>
<td>4</td>
<td>34.65</td>
<td>8.662</td>
<td>1.0053</td>
<td>0.420249</td>
</tr>
<tr>
<td>Residuals</td>
<td>30</td>
<td>258.50</td>
<td>8.617</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 7. Two-way ANOVA showing the effects of temperature and position on germination of achenes from the final cleistogamous (fCL) head type.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Residuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp</td>
<td>4</td>
<td>1312.4</td>
<td>328.10</td>
<td>68.1176</td>
<td>1.283e-14 ***</td>
</tr>
<tr>
<td>Position</td>
<td>1</td>
<td>84.1</td>
<td>84.10</td>
<td>17.4602</td>
<td>0.00023***</td>
</tr>
<tr>
<td>Temp:Position</td>
<td>4</td>
<td>37.4</td>
<td>9.35</td>
<td>1.9412</td>
<td>0.1293078</td>
</tr>
<tr>
<td>Residuals</td>
<td>30</td>
<td>144.5</td>
<td>4.82</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Two-way ANOVA examination of the effect of head type and temperature on germination showed a significant effect of head type ($p = 1.86 \times 10^{-13}$) and a significant effect of temperature ($p = 2.2 \times 10^{-16}$) on germination. 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 ($p = 3.9 \times 10^{-05}$) (Table 8).

Table 8. Two-way ANOVA showing the effect of head type and temperature on germination of *Centaurea melitensis* achenes.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AchTYP</td>
<td>2</td>
<td>528.3</td>
<td>264.17</td>
<td>40.0482</td>
<td>1.186e-13 ***</td>
</tr>
<tr>
<td>Temp</td>
<td>4</td>
<td>3991.1</td>
<td>997.78</td>
<td>151.2605</td>
<td>2.2e-16 ***</td>
</tr>
<tr>
<td>AchTYP:Temp</td>
<td>8</td>
<td>257.2</td>
<td>32.15</td>
<td>4.8745</td>
<td>3.9e-05***</td>
</tr>
<tr>
<td>Residuals</td>
<td>105</td>
<td>692.6</td>
<td>6.60</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Production and Persistence of Different Achene Types in Flower Heads of *Centaurea melitensis*

Differences in ratios of center to peripheral achenes were found among the 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); the ratio in iCL was intermediate (2.23:1) (Table 9).

Table 9. Ranges and ratios for the number of center achenes to peripheral achenes in three head types of *Centaurea melitensis*.

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

‘n’ Stands for the number of heads containing achenes.

The fraction of total achenes remaining in the flower heads by the beginning of fall (September) varied by head type. The chasmogamous head type had the lowest fraction (15.8%) of achenes remaining in the flower heads. The initial cleistogamous had the largest fraction 96.4%, and the final cleistogamous was intermediate between the two with 88.5% remaining in the
flower head. The average number of late season achenes was similar to early season achenes for iCL and fCL (Tables 10 and 11).

Table 10. Number of center and peripheral achenes per head before dispersal and after full senescence in Centaurea melitensis.

The mean number achenes per head in CH heads (collection end May 2012), iCL heads (collected end April 2012), and fCL heads (collected end of June 2012), are shown.

<table>
<thead>
<tr>
<th>Head Types</th>
<th>Early Season Samples</th>
<th>Late Season Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Filled Center achenes</td>
<td>Filled Peripheral achenes</td>
</tr>
<tr>
<td>CH head type (n=100)</td>
<td>25.5 79%</td>
<td>6.7 21%</td>
</tr>
<tr>
<td>iCL head type (n=83)</td>
<td>2.6 69%</td>
<td>1.2 31%</td>
</tr>
<tr>
<td>fCL head type (n=95)</td>
<td>1.7 66%</td>
<td>0.9 34%</td>
</tr>
</tbody>
</table>

Table 11. Mean number of center and peripheral achenes per head in head types of Centaurea melitensis collected in fall following head formation (September 2011).

<table>
<thead>
<tr>
<th>Head Types</th>
<th>Filled Center achenes</th>
<th>Filled Peripheral achenes</th>
<th>Total Aborted achenes</th>
<th>Total filled achenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH head type (n=10)</td>
<td>4.5 88%</td>
<td>0.6 12%</td>
<td>14</td>
<td>51</td>
</tr>
<tr>
<td>iCL head type (n=10)</td>
<td>2.8 78%</td>
<td>0.8 22%</td>
<td>1</td>
<td>36</td>
</tr>
<tr>
<td>fCL head type (n=10)</td>
<td>1.4 61%</td>
<td>0.9 39%</td>
<td>3</td>
<td>23</td>
</tr>
</tbody>
</table>
Both achene types (peripheral and center achenes) were present into early fall (September) for all three head types, indicating delayed dispersal. However the CH had very little center achenes left compared to the overall number for that head type. However, the number of center achenes in CH head type was twice the number of center achenes remaining compared to the other two head types iCL and fCL (Table 10, Table 11).

No evidence was found for differential dispersal between center and peripheral achenes in any of the three head types. The probability of achenes remaining in the head was similar for peripheral and center achenes within each head type, despite differences in the likelihood of dispersal between head types (Table 12).

Table 12. Relative persistence of center and peripheral achenes in head types of Centaurea melitensis through the summer (estimated).

The degrees of freedom = 1 for each head type. Expected values were calculated as the number of achenes found in late-season heads multiplied by the fraction found in center and peripheral at the beginning of the season.

<table>
<thead>
<tr>
<th>Head Type</th>
<th>Number of achenes in 10 heads at the end of the season</th>
<th>Observed</th>
<th>Expected</th>
<th>Chi-square value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Center</td>
<td>Peripheral</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH</td>
<td>45</td>
<td>6</td>
<td>40.4</td>
<td>10.6</td>
<td>2.504</td>
</tr>
<tr>
<td>iCL</td>
<td>28</td>
<td>8</td>
<td>24.8</td>
<td>11.1</td>
<td>1.288</td>
</tr>
<tr>
<td>fCL</td>
<td>14</td>
<td>9</td>
<td>15.2</td>
<td>7.8</td>
<td>0.269</td>
</tr>
</tbody>
</table>
Discussion

Although differences in timing of dispersal, position, and morphology may be linked to differences in temperature response, my data did not indicate this. I found that morphology differences in peripheral and center achenes of *Centaurea melitensis* were not reflected in either their tendency to disperse from the flower head or their germination response to temperature. While morphology did not affect the tendency to disperse or temperature response in this study, it is noteworthy to mention that size (mass) may in fact play a role in temperature sensitivity.

The largest of the achenes responded negatively to temperatures greater than 15 °C. Other authors suggest that small achenes or seeds are more sensitive to changes in temperature compared to larger ones and that seed size is positively correlated with seedling size (Ruiz de Clavijo 2001; Liu et al. 2013). Larger seedlings have a better chance of survival and establishment especially where there is a lot of competition. *Centaurea melitensis* is found among coastal sage scrub where there is a lot of competition.

*Centaurea melitensis* achenes germinated over a range of temperatures between 10 °C and 30 °C. In the present study achenes from each of the different head types (CH, iCL, and fCL) germinated over a wide range of temperatures. My study found germination rates were highest between 10 °C and 23.5 °C in achenes of the CH head type. Over this temperature range,
germination was 75% or greater for both center and peripheral achenes (Figure 19). Porras and Muñoz’s (2000b) investigations showed highest germination rates for this head type at 12°C, 19°C and 25/15°C. My investigations on the iCL head type in the present study showed germination rates for this head type were highest between 10º and 15ºC. Temperatures beyond this adversely affected germination rates (Figure 20). Porras and Muñoz (2000b) recorded the highest germination rates for achenes of the iCL head type at 12 ºC. My study also showed that achenes in the fCL head type highest germination rates occurred between 10 ºC and 23.5 ºC, while Porras and Muñoz’s (2000b) data indicated that germination of achenes of the fCL head type did not vary much across the temperature range tested (12 – 25 ºC). These results were comparable to Porras and Muñoz (2000b). There were slight differences found in achenes of the fCL head type, but this may the result of variation in populations and species plasticity. I expanded the range and found at 5ºC there was little to no germination. At the other end of the spectrum, at 30ºC, the achenes from the different head types as well as position diverged. In other words as temperatures increased their degree of similarity diminished.

Temperature response differences between head types of Centaurea melitensis produced different response patterns to germination. Broad temperature response patterns were observed in two of the head types: CH and fCL. These response differences may be associated with developmental timing and season of germination. Peak development in CH head type occurs between
April and May (Porras and Muñoz, 2000a). Daily high temperatures in San Bernardino during these months average 25.8 °C and daily lows average 9.7 °C (Western Regional Climate Center 2015). In the final cleistogamous (fCL) head type peak development occurs between May and June (Porras and Muñoz, 2000a). Daily high temperatures in San Bernardino during these months average 29.4 °C and daily lows average 12 °C. The daily high temperatures fall within the broad temperature optima for germination of these two head types. A limited temperature response pattern was observed in the iCL head type. Peak development occurs in this head type between March and April (Porras and Muñoz 2000a, 2000b). Daily high temperatures during these months average 22.9 °C, which is higher than the peak response temperature observed for iCL, but daily low temperatures average only 7.5 °C. Joley et al. (1997) suggest that a broad temperature range can give species like *Centaurea* a greater flexibility for germination in variable habitats. These temperature response patterns indicate that achenes of *Centaurea melitensis* are well adapted to variations in temperature and some cases like the iCL a cold spell may be required to spark germination.

Although, differences in temperature response were observed between head types, no differences were found in the pattern of response within each head type between peripheral and center achenes. This is consistent with the fact that I found no differences in the tendency to disperse. Both peripheral and center achenes within each head type tended to display the same germination
response to temperature indicating that the achenes within each head type, whether peripheral or central, are capable of responding to their preferred temperatures. In this case, peripheral and center achenes within the CH and fCL head type prefer moderate temperatures, while peripheral and center achenes within the iCL head prefer cooler. In contrast, Joley et al. (1997) found peripheral achenes of the closely related congener preferred warmer temperatures, while the center achenes preferred cooler temperatures.

My data also indicated that there was no difference in the tendency to disperse between peripheral and center achenes despite their morphology differences. I expected a difference due to the morphological differences between peripheral and center achenes. Even though the prospects of being dispersed following seasonal development are similar, both achene types can be found in the flower head in early September. This is not necessarily a negative thing it suggests equal opportunity to disperse, which provides more opportunities for germination. However, many of the center achenes from the chasmogamous head type will have dispersed reducing the fraction remaining in the flower ahead, due to location on the plant by early fall (personal observation). Other studies have shown that the parachute-like pappus in chasmogamous achenes allows them to be picked up by wind more easily (Sheldon and Burrows 1972; Waller 1988). Santa Ana winds can be strong in the San Bernardino area forcing the achenes at the top of the plant to disperse by being catapulted from the plant. I expected peripheral achenes to be more likely found into the late season than
the center achenes for various reasons, they are close to the bracts and can get caught up on the flower heads more easily, where the center achenes are centrally located and can be dispersed with ease. This difference however, had not appeared by early September, but Santa Ana winds often occur mid-September. *Centaurea melitensis* may also be showing a bet-hedging strategy where only some of the achenes are released at the end of each developmental period allowing for a second chance germination should no initial seedlings be established.

Despite the similar temperature responses of center and peripheral achenes, peripheral achenes exhibited lower germination rates, especially at 15 °C. Small differences in germination between peripheral and center achenes may indicate that some peripheral achenes exhibit dormancy. Others members of Asteraceae have peripheral achenes that exhibit dormancy. *Centaurea solstitialis* peripheral achenes exhibit dormancy and both achene types for this species have been viable following several years of burial. *Leontodon longirostris* peripheral achenes remain dormant following dispersal (Joley et al. 1997; DiTomaso 2001; Ruiz de Clavijo 2001).

Delays in dispersal and lags in germination further indicate the presence of dormancy in peripheral achenes. It is highly possible that changes in field conditions like temperature and moisture control dormancy break in the peripheral achenes and some center achenes (Batlla and Benech-Arnold 2007). Porras and Muñoz (2000a, 2000b) investigations lead them to believe that
achenes from the iCL head type were exhibiting dormancy. These achenes took 3.6 days longer to germinate, and their germination rates were lower compared to achenes from the CH and fCL head type. The morphology of the achene suggests that it would be able to withstand a period of burial also, which can affect its response temperature and the requirement for breaking dormancy (Porras and Muñoz 2000b). My investigations indicate that the peripheral achenes for all the head types may be exhibiting dormancy. For some of the achenes environmental conditions such as irregular temperature patterns and limited rainfall may be inducing a secondary dormancy. Delayed dispersal in some achenes may also lead to dormancy. This is the first study to look at differences between peripheral and center achenes and their response to environmental cues, and results suggest that some peripheral achenes may exhibit dormancy.

Differences in the number of peripheral achenes to center achenes within each flower head may be linked variations in environmental conditions as well as seasonal changes. Therefore, irregular temperature patterns and rainfall patterns may allow the number of achenes peripheral to center to be selected for. My data confirmed ratio differences in the number of peripheral to center achenes within each head type (CH 3.82:1, iCL 2.23:1 and fCL 1.94:1). Ratios for Centaurea solstitialis peripheral to central achenes have been recorded as 3:1 (Callihan et al. 1993). Variations in the number of peripheral to center achenes have also been associated with environmental conditions in different areas of California.
(ARR 1981) and other members have shown more peripheral achenes to be produced during long day cycles and cold spells (Forsyth and Brown 1982). Ratios in the CH head type appear to be similar to other authors’ data; however this study is the first comparison of the number of peripheral to center achene for Centaurea melitensis. There is some suggestion of selection playing a role in the numbers of peripheral to center achenes, which I can’t rule out. With variations in the temperature and moisture in areas like San Bernardino, it makes sense to make adjustments in the number of achenes that are produced. Weedy seeds need a continuous seed bank to ensure survival and establishment especially when conditions are not optimal.

Summary

This study has shown the importance of temperature in germination of Centaurea melitensis. Different temperature response patterns associated with the achenes from different head types appear to be related to developmental temperatures during the time of development and temperatures in the local area of San Bernardino. The broad temperature optima found in CH and fCL achenes indicate Centaurea melitensis achenes are adapted to irregular temperature patterns associated with the area. The narrow response pattern found in the iCL achenes indicates these achenes may need a cold spell to germinate and may be dormant for a time. Lower germination rates in the peripheral achenes suggest dormancy in at least some of the achenes. Ratio differences indicate that there are always more peripheral achenes than center achenes. These
ratios may be related to environmental conditions of limited moisture and variable temperatures.

Ecological Implications

It is often difficult to control the spread of invasive species like *Centaurea melitensis* because it has the ability to germinate under various environmental conditions. By understanding the biological responses to various germination cues new tools for the management of *Centaurea melitensis* can be developed. It is also possible for *Centaurea melitensis* to contribute to the seed bank to greater extent than first thought. Achenes in which germination is delayed either by dispersal or dormancy may make it difficult for weed management to control the spread.
CHAPTER FOUR
CONCLUSIONS

The nature of nonnative weedy species is to spread and establish in new environments when opportunity arises. The invasiveness of a species, like *Centaurea melitensis*, is driven by its ability to spread rapidly and survive under a wide range of environmental conditions. Furthermore, rapid spread is often associated with short generation times, large seed production, production of small seeds, prolonged seed viability and transport via wind, all of which *Centaurea melitensis* displays (Alpert et al. 2000). Morphological variation in their achenes (dispersal morphology), broad germination responses to environmental cues, and the ability of the seeds to sit dormant in the seed bank until the right environmental conditions are present all contribute to a plant species’ ability to spread and persist in environments. Many aspects of achene morphology, such as seed shape, size (mass), the presence and shape and length of the pappus and the elaiosome, affect dispersal.

This study found that differences in morphology, especially dispersal morphology, between peripheral and center achenes were present in heads of *Centaurea melitensis*. The peripheral and center achenes differed morphologically among head types (CH (chasmogamous), iCL (initial cleistogamous), and fCL (final cleistogamous)) and within head types in dispersal features such as mass (size) fruit length, fruit width, pappus length, pappus width
and elaiosome features. Porras and Muñoz (2000a, 2000b, 2000c) identified some of the same differences I found among head types (i.e., differences in achene mass, achene width, pappus length and pappus width), but they did not recognize morphological differences between center and peripheral achenes.

I found peripheral achenes were indeed lighter and narrower than the center achenes. This is what I initially expected, and is consistent with what is known about *Centaurea solstitialis*, the closely related congener: its peripheral achenes are lighter (Roche 1965; Calihan et al. 1993). Several potential factors that could contribute to an achene being lighter are immaturity and underdevelopment of the embryo, size and shape differences (being narrower or shorter), and having a smaller pappus. Immaturity did not appear to explain why peripheral achenes were lighter. The peripheral achenes of *Centaurea solstitialis* develop first, and there is no reason to believe that *Centaurea melitensis* would not follow the same pattern, but further investigations are needed to confirm this. Furthermore, most *Centaurea melitensis* achenes, including peripheral achenes, were plump and filled upon collection and were determined to be viable based on a positive tetrazolium test. Presence of the embryo was confirmed through cut tests that showed the seed was filled from end to end. These findings were parallel to findings for *Centaurea solstitialis*, in which peripheral achenes were also lighter and viable upon collection (Callihan et al. 1993; Joley et al. 2003).

My studies found that peripheral achenes within each head type were narrower and had the shorter pappi and smaller elaiosomes than center
achenes. In two head types (the CH an iCL head types) peripheral achenes were
narrower but similar in length to center achenes. The fCL heads, on the other
hand, had peripheral achenes that were significantly shorter than the center
achenes, in addition to being narrower. The narrowness of the peripheral
achenes may simply result from their proximity to the bracts of the involucre.
Developing close to the bracts can compress achenes into a narrower shape,
and make them asymmetrical. This is consistent with what is known about
Centaurea solstitialis peripheral achene development (Young et al. 2005). Being
asymmetrical actually improves dispersal capacity of the achene according to
Sheldon and Burrows (1972). There is a lot of information that suggests being
lighter and narrower is more efficient for dispersal than being wider and heavier
(Roche 1965; Sheldon and Burrows 1973; DiTomaso 2001; Young et al 2005).

However, characteristics of two additional features, the pappus and the
e liaosome, suggest that the center achenes may disperse more effectively than
the narrow, asymmetric peripheral achenes. The center achenes had better
developed, longer pappi than peripheral achenes. Longer pappi allow for more
efficient wind dispersal and dispersal beyond the parent plant, thereby increasing
its chances to spread to new environments. Of the six achene-types studied, the
center achenes of the CH head types had the longest pappi. Since they are
produced in heads that are higher on the plant than other head types, these
center achenes are likely to disperse furthest. Although the peripheral achenes
have shorter pappi, they are still able to travel along the ground by using the
wind. The peripheral achenes’ pappi can act as weather vanes once the achene is on the ground, orienting them in the direction of the wind and allowing them to blow across the ground (Porras and Muños 2000b). Pappi can have other functions as well, helping the achene adhere the ground and absorb moisture, possibly to key germination (Sheldon 1974). Long-distance dispersal through the air, however, is more likely to be achieved by center achenes, with their longer pappi, than by peripheral achenes.

I also found the center achenes in my study to have larger elaiosomes than the peripheral achenes within each head type. Whether they are big or small, elaiosomes have important ecological functions like facilitating dispersal by insects and birds, preventing germination leading to dormancy, and facilitating seed dehydration and rehydration (Viegi et al. 2003). Rather than finding a trade-off, whereby peripheral achenes had larger elaiosomes to promote insect dispersal in compensation for their less developed pappi, I found that center achenes had both larger elaiosomes and larger pappi, potentially promoting both better wind dispersal and better animal dispersal than peripheral achenes achieve.

Although differences were found between center and peripheral achenes of *Centaurea melitensis*, they were not as pronounced as the differences that have been reported for *Centaurea solstitialis*. Peripheral achenes of *Centaurea solstitialis* lack a pappus, display some dormancy, and germinate at warmer temperatures than center achenes, which have well developed pappi and do not
display dormancy (Roche 1965; DiTomaso 2001, Joley et al. 1997, 2003). I found similar morphological trends in *Centaurea melitensis*, with peripheral achenes having more poorly developed dispersal structures than center achenes, but these differences were less pronounced than those found in *Centaurea solstitialis*. Physiological differences between center achenes and peripheral achenes that influence germination ecology (i.e., temperature responses of germination and dormancy) either were not found or were very minor. Germination of center and peripheral achenes from the same head type had similar temperature responses and appeared to have similar probabilities of being retained in the head over the hot summer. There was a suggestion that a larger fraction of peripheral achenes may initially be dormant, similar to the pattern described for *Centaurea solstitialis* (Joley et al. 2003). The proportion of apparently dormant achenes was very small, but dormancy of even a small fraction of seeds in a weedy species may have large ecological consequences, and this pattern requires further investigation. Overall, achene variation that affects differences in timing of seed production and germination was dominated by differences among head types, as previously reported by others (Porras and Muñoz 2000a, 2000b, 2000c). Differences that affect dispersal distance, however, were found between center and peripheral achenes within a head. The morphological differences that I found between center and peripheral achenes of *Centaurea melitensis* mirrored the better-developed and widely recognized differences between center and peripheral achenes of *Centaurea solstitialis*, in
which center achenes have better-developed dispersal structures than peripheral achenes.
APPENDIX A

ANOVA TABLES
Table 1. Summary of ANCOVA results for fruit width and fruit length in head type CH of *Centaurea melitensis*.

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>Estimate</th>
<th>Std. Error</th>
<th>t-value</th>
<th>Pr(&gt; t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>0.34942</td>
<td>0.11318</td>
<td>3.087</td>
<td>0.00263**</td>
</tr>
<tr>
<td>PositionPeripheral</td>
<td>-0.08860</td>
<td>0.01553</td>
<td>-5.703</td>
<td>1.27e07***</td>
</tr>
<tr>
<td>Fruit.length</td>
<td>0.32651</td>
<td>0.04606</td>
<td>7.089</td>
<td>2.19e-10***</td>
</tr>
</tbody>
</table>

Residual standard error: 0.0773 on 97 degrees of freedom
Multiple R-squared: 0.4856, Adjusted R-squared: 0.475
F-statistic: 45.79 on 2 and 97 DF, p-value: 9.933e-15

Table 2. Summary of ANCOVA results for fruit width and fruit length in head type iCL of *Centaurea melitensis*.

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>Estimate</th>
<th>Std. Error</th>
<th>t-value</th>
<th>Pr(&gt; t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>1.00775</td>
<td>0.21637</td>
<td>4.657</td>
<td>1.02e05***</td>
</tr>
<tr>
<td>PositionPeripheral</td>
<td>-0.03906</td>
<td>0.02368</td>
<td>-1.650</td>
<td>0.102</td>
</tr>
<tr>
<td>Fruit.length</td>
<td>0.11953</td>
<td>0.08003</td>
<td>1.494</td>
<td>0.139</td>
</tr>
</tbody>
</table>

Residual standard error: 0.1184 on 97 degrees of freedom
Multiple R-squared: 0.04778, Adjusted R-squared: 0.02815
F-statistic: 2.434 on 2 and 97 DF, p-value: 0.09304

Table 3. Summary of ANCOVA results for fruit width and fruit length in head type fCL of *Centaurea melitensis*.

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>Estimate</th>
<th>Std. Error</th>
<th>t-value</th>
<th>Pr(&gt; t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>0.64771</td>
<td>0.12823</td>
<td>5.051</td>
<td>20.6e-06***</td>
</tr>
<tr>
<td>PositionPeripheral</td>
<td>-0.09359</td>
<td>0.02034</td>
<td>-4.601</td>
<td>1.27e-05***</td>
</tr>
<tr>
<td>Fruit.length</td>
<td>0.20899</td>
<td>0.05012</td>
<td>4.170</td>
<td>6.64e-05***</td>
</tr>
</tbody>
</table>

Residual standard error: 0.09716 on 97 degrees of freedom
Multiple R-squared: 0.3605, Adjusted R-squared: 0.3473
F-statistic: 27.34 on 2 and 97 DF, p-value: 3.827e-10

Table 4. Summary of ANCOVA results for pappus length and fruit length in head type CH of *Centaurea melitensis*.

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>Estimate</th>
<th>Std. Error</th>
<th>t-value</th>
<th>Pr(&gt; t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>3.44650</td>
<td>0.54871</td>
<td>6.281</td>
<td>9.52e-09***</td>
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<tr>
<td>PositionPeripheral</td>
<td>-0.18748</td>
<td>0.07531</td>
<td>-2.490</td>
<td>0.0145*</td>
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<tr>
<td>Fruit.length</td>
<td>-0.03101</td>
<td>0.22330</td>
<td>-0.139</td>
<td>0.8898</td>
</tr>
</tbody>
</table>

Residual standard error: 0.3747 on 97 degrees of freedom
Multiple R-squared: 0.06016, Adjusted R-squared: 0.04078
F-statistic: 3.104 on 2 and 97 DF, p-value: 0.04934
Table 5. Summary of ANCOVA results for pappus length and fruit length in head type iCL of *Centaurea melitensis*.

| Coefficients      | Estimate | Std. Error | t-value | Pr(> |t |)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>1.78759</td>
<td>0.35157</td>
<td>5.085</td>
<td>1.79e-06***</td>
<td></td>
</tr>
<tr>
<td>PositionPeripheral</td>
<td>-0.08552</td>
<td>0.3848</td>
<td>-2.223</td>
<td>0.3537</td>
<td></td>
</tr>
<tr>
<td>Fruit.length</td>
<td>0.12117</td>
<td>0.13003</td>
<td>0.932</td>
<td>0.3537</td>
<td></td>
</tr>
</tbody>
</table>

Residual standard error: 0.1924 on 97 degrees of freedom
Multiple R-squared: 0.05585, Adjusted R-squared: 0.03639
F-statistic: 2.869 on 2 and 97 DF, p-value: 0.06158

Table 6. Summary of ANCOVA results for pappus length and fruit length in head type fCL of *Centaurea melitensis*.

| Coefficients      | Estimate | Std. Error | t-value | Pr(> |t |)
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>1.36830</td>
<td>0.30370</td>
<td>4.505</td>
<td>1.85e-05***</td>
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<tr>
<td>PositionPeripheral</td>
<td>-0.04770</td>
<td>0.04817</td>
<td>-0.990</td>
<td>0.3246</td>
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<tr>
<td>Fruit.length</td>
<td>0.25727</td>
<td>0.11870</td>
<td>2.167</td>
<td>0.0327*</td>
<td></td>
</tr>
</tbody>
</table>

Residual standard error: 0.2301 on 97 degrees of freedom
Multiple R-squared: 0.07277, Adjusted R-squared: 0.05365
F-statistic: 3.806 on 2 and 97 DF, p-value: 0.02562

Table 7. Summary of ANCOVA results for elaiosome volume and fruit length in head type CH of *Centaurea melitensis*.

| Coefficients      | Estimate | Std. Error | t-value | Pr(> |t |)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-12.6505</td>
<td>1.1283</td>
<td>-11.212</td>
<td>2e-16***</td>
<td></td>
</tr>
<tr>
<td>Log.fruit.length</td>
<td>8.7225</td>
<td>1.2601</td>
<td>6.922</td>
<td>5.63e-10***</td>
<td></td>
</tr>
<tr>
<td>PositionPeripheral</td>
<td>-0.3660</td>
<td>0.1763</td>
<td>-2.076</td>
<td>0.0406*</td>
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</tbody>
</table>

Residual standard error: 0.8614 on 93 degrees of freedom
Multiple R-squared: 0.3693, Adjusted R-squared: 0.3558
F-statistic: 27.23 on 2 and 93 DF, p-value: 4.904e-10

Table 8. Summary of ANCOVA results for elaiosome volume and fruit length in head type iCL of *Centaurea melitensis*.

| Coefficients      | Estimate | Std. Error | t-value | Pr(> |t |)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-9.6833</td>
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<td>2e-16***</td>
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<tr>
<td>Log.fruit.length</td>
<td>6.0220</td>
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<td>6.584</td>
<td>2.36e-09***</td>
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<tr>
<td>PositionPeripheral</td>
<td>-0.2907</td>
<td>0.1022</td>
<td>-2.844</td>
<td>0.00544**</td>
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</table>

Residual standard error: 0.5111 on 97 degrees of freedom
Multiple R-squared: 0.344, Adjusted R-squared: 0.3305
F-statistic: 25.43 on 2 and 97 DF, p-value: 1.318e-09
Table 9. Summary of ANCOVA results for elaiosome volume and fruit length in head type fCL of *Centaurea melitensis*.

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>Estimate</th>
<th>Std. Error</th>
<th>t-value</th>
<th>Pr(&gt; t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-11.3378</td>
<td>0.9347</td>
<td>-12.130</td>
<td>2e-16***</td>
</tr>
<tr>
<td>Log.fruit.length</td>
<td>7.5859</td>
<td>0.9959</td>
<td>7.617</td>
<td>1.82e-11***</td>
</tr>
<tr>
<td>PositionPeripheral</td>
<td>-0.4629</td>
<td>0.1617</td>
<td>-2.863</td>
<td>0.00516**</td>
</tr>
</tbody>
</table>

Residual standard error: 0.7694 on 96 degrees of freedom
Multiple R-squared: 0.4734, Adjusted R-squared: 0.4624
F-statistic: 43.15 on 2 and 96 DF, p-value: 4.279e-09

Table 10. Summary of ANCOVA results for elaiosome depth and height for CH head type of *Centaurea melitensis*.

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>Estimate</th>
<th>Std. Error</th>
<th>t-value</th>
<th>Pr(&gt; t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-0.09740</td>
<td>0.02474</td>
<td>-3.937</td>
<td>0.000155***</td>
</tr>
<tr>
<td>PositionPeripheral</td>
<td>0.02517</td>
<td>0.01122</td>
<td>2.243</td>
<td>0.027204*</td>
</tr>
<tr>
<td>Elaiosome.ht</td>
<td>0.80298</td>
<td>0.08704</td>
<td>9.226</td>
<td>6.3e-15***</td>
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</tbody>
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Residual standard error: 0.05347 on 97 degrees of freedom
Multiple R-squared: 0.4684, Adjusted R-squared: 0.4574
F-statistic: 42.73 on 2 and 97 DF, p-value: 4.914e-14

Table 11. Summary of ANCOVA results for elaiosome depth and height for ICL head type of *Centaurea melitensis*.

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>Estimate</th>
<th>Std. Error</th>
<th>t-value</th>
<th>Pr(&gt; t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.14301</td>
<td>0.03156</td>
<td>4.531</td>
<td>1.68e-05***</td>
</tr>
<tr>
<td>PositionPeripheral</td>
<td>0.01971</td>
<td>0.01267</td>
<td>1.556</td>
<td>0.123</td>
</tr>
<tr>
<td>Elaiosome.ht</td>
<td>0.58611</td>
<td>0.10504</td>
<td>5.580</td>
<td>2.18e-07***</td>
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</tbody>
</table>

Residual standard error: 0.06019 on 97 degrees of freedom
Multiple R-squared: 0.2432, Adjusted R-squared: 0.2276
F-statistic: 15.59 on 2 and 97 DF, p-value: 1.351e-06

Table 12. Summary of ANCOVA results for elaiosome depth and height for fCL head type of *Centaurea melitensis*.

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>Estimate</th>
<th>Std. Error</th>
<th>t-value</th>
<th>Pr(&gt; t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.05605</td>
<td>0.03083</td>
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<td>0.0722</td>
</tr>
<tr>
<td>PositionPeripheral</td>
<td>-0.01486</td>
<td>0.01535</td>
<td>-0.968</td>
<td>0.3355</td>
</tr>
<tr>
<td>Elaiosome.ht</td>
<td>0.74708</td>
<td>0.11657</td>
<td>6.409</td>
<td>5.3e-09</td>
</tr>
</tbody>
</table>

Residual standard error: 0.07161 on 97 degrees of freedom
Multiple R-squared: 0.3549, Adjusted R-squared: 0.3416
F-statistic: 26.68 on 2 and 97 DF, p-value: 5.855e-10
APPENDIX B

IMAGES OF REPRESENTATIVE ACHENES
<table>
<thead>
<tr>
<th>Center achenes</th>
<th>Peripheral achenes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td>CH</td>
</tr>
<tr>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
<td>iCL</td>
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<tr>
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<td><img src="image6.png" alt="Image" /></td>
<td>fCL</td>
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REFERENCES


Western Regional Climate Center. 2015. Available at http://www.wrcc.dri.edu/.