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Testing the Fertility and Allelopathic Abilities of Arctotheca prostrata (Salisb.) Britten (Asteraceae, Arctotideae), a South African Plant Species that has Naturalized in California

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By

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This thesis has been examined and approved by the following members of the student's committee.

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ABSTRACT

Testing the fertility and allelopathic abilities of *Arctotheca prostrata* (Salisb.) Britten (Asteraceae, Arctotideae), a South African plant species that has naturalized in California

By: Jordy Veit, Master of Science in Biology, Minnesota State University, Mankato, MN, 2014.

Arctotheca prostrata is a perennial plant species native to South Africa that reproduces vegetatively by long runners. In South Africa it also reproduces sexually, producing fruits with fertile seeds. The species was brought to California to serve as a ground cover but fruits had not been reported. Historically, it has been said to be infertile. This study tested the fertility of *A. prostrata* in California by crossing plants from California with each other and with plants grown from imported South African seeds. Pollen viability was tested and morphological measures were made on heads, leaves, and pollen. Mean Californian and South African measurements were compared by nested analysis of variance (ANOVA). Earlier research indicated that *A. prostrata* is allelopathic. Lettuce seeds were germinated for 72 hours in extracts containing different amounts of ground leaves, runners, and roots of *A. prostrata* to test for allelopathic abilities. My study found that *A. prostrata* from California is outcrossing and fertile. It does not differ much, if at all, in morphology from South African individuals in the study. It is allelopathic. My results suggested that plants from California have a stronger allelopathic effect on

lettuce seedling growth than plants from South Africa. Currently, naturalized populations of *A. prostrata* in California are rare. However, if populations increase in number and begin to produce seeds, the species has the potential to become invasive.

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I. INTRODUCTION

Invasive species are a worldwide problem. Pimentel (2005) surveyed six nations, (United States, Australia, United Kingdom, South Africa, Brazil and India), and found that more than 120,000 species of plants, animals, and microbes had been introduced. It is estimated that 500,000 non-native species have been introduced to different ecosystems on Earth (Pimentel 2005), many of which have potential to become invasive.

During the first decade of the 21st century in the United States, invasive plants were causing $35 billion in economic loss annually. In addition 50% of all invasive plants and 85% of invasive woody plants were introduced for ornamental and landscape use (Li et al. 2004). Campbell (1999) identified 454 invasive plants and created a list called "plants that hog the garden" of which 292 were still being sold in nurseries at the time of the study.

Not all introduced species become invasive. Some non-natives become incorporated into the biota of a region, reproducing like natives but not profoundly disturbing native communities (van Kleunen et al. 2011). Such species are said to be "naturalized." Invasive species outcompete and replace native species; they may alter and decrease biodiversity (Goodwin et al. 1999). Invasive plant species' competitive strategies vary but include one or more of the following: longer fruiting and flowering times (Lloret et al. 2005), greater seed production (Mason et al. 2008, Knight et al. 2007, Heywood and Brunel 2008), shorter seed germination times (Rejmanek and Richardson 1996, Wildrlechner et al. 2004), higher rates of seedling survival (Grotkoop et al. 2010),

greater and/or more rapid vegetative growth (Lloret et al. 2005, Heywood and Brunel 2008), spreading aggressively by producing clones (Larkin et al. 2012), earlier leaf production in the spring and later leaf retention in the fall (Knight et al. 2007), ability to start on bare ground (Heywood and Brunel 2008), higher tolerance for dry conditions, and production of allelopathic chemicals (Brooker et al. 2011). Understanding how naturalized plant species outcompete natives will help us predict which introduced plants may become invasive.

This research focused on *Arctotheca prostrata* (Salisb.) Britten (prostrate capeweed), which is native to South Africa. *Arctotheca prostrata* was introduced as a ground cover in California. For many years, naturalized populations of *A. prostrata* only appeared to reproduce vegetatively by runners and were misidentified as sterile forms of invasive and fertile *A. calendula* (capeweed) (Clark 1975, Hickman 1993, McClintock 1993, Mahoney 2006, Mahoney and McKenzie 2008 and 2012, McKenzie and Mahoney 2010). *Arctotheca calendula* has naturalized in areas with Mediterranean climates including Spain, Australia, New Zealand, and California. Dana et al. (2012) gave the species a rating of "3 = dangerous (causing ecological damage or alteration) for natural ecosystems." California Invasive Plant Council (Cal-IPC 2014) has given *A. calendula* a moderate invasive rating as of this writing.

Re-examination of herbarium specimens revealed there are two distinct species, *A. calendula* and *A. prostrata* (Mahoney and McKenzie 2008 and 2012, McKenzie and Mahoney 2010). *Arctotheca prostrata* is fertile in South Africa (personal communication,

Dr. Robert McKenzie, Rhodes University, South Africa). As of this writing both "fertile" and "sterile" forms of *A. calendula* are currently listed in the California Invasive Plant Inventory (Cal-IPC). However, *A. prostrata* is now listed as an alternative name for sterile *A. calendula* (Cal-IPC 2014). It is unclear why *A. prostrata* only appears to reproduce vegetatively in California. This research sought to determine if *A. prostrata* in California is able to produce fertile seeds or if it is sterile. In addition other characteristics of the species were examined.

II. OBJECTIVES/RESEARCH QUESTIONS

This project sought to answer the following questions:

1. Is *Arctotheca prostrata* in California sterile?
2. If *A. prostrata* in California is found to be sterile,
    A. is the pollen produced by the plants dysfunctional, and can this be observed by pollen staining techniques?
    B. could California's *A. prostrata* populations be sterile hybrids developed for horticultural purposes?
3. Do vegetative parts of *A. prostrata* release allelopathic chemicals?

III. LITERATURE REVIEW

Invasive species

Early European immigrants to North America introduced a wide variety of species for ornamental and agricultural purposes (Inderjit et al. 2005). John Bartram, an 18th century botanist, first noticed negative effects of introduced plants (Inderjit et al. 2005).

EFFECTS OF INVASIVE SPECIES

The U.S. government defines an invasive species as a non-native species "whose introduction does or is likely to cause economic or environmental harm or harm to human health" (Federal Register – Presidential Documents 1999 in Niemiera and Von Holle 2009). The American Nursery and Landscape Association and the Weed Science Society of America give a similar definition but add that invasive plants develop harmful "self-sustaining populations" in native plant communities and managed plant systems (Niemiera and Von Von Holle 2009). Invasive species are the second leading cause of the loss of biodiversity (Jose et al. 2013). Every year $145 million is spent to control invasive aquatic and wetland plants (Jose et al. 2013).

There are an estimated 50,000 non-native plants, animals and microbes in the U.S. (Pimental 2005). Most non-native plants were deliberately introduced for various purposes including forestry, agriculture, and ornamental plant trade (Pimentel 2005). Ornamental plants make up more than 80% of naturalized species in the United States and 52% of those naturalized in Europe (Grotkoop et al. 2010). Some were accidental introductions (Pemberton and Liu 2009). Some of these are of great benefit, especially

in agriculture where as many as 99% of food crops are non-native in some areas (Pimentel 2005).

Lythrum salicaria (purple loosestrife), brought over during the 19th century as an ornamental, now spreads at a rate of 100,000 ha per year (Pimentel 2005). In the U.S., Florida has the largest number of non-native plants of which 25,000 are for ornamental and agriculture purposes and of these, 900 have escaped into a natural ecosystem (Pimentel 2005). In the Great Smokey Mountain National Park 400 of the 1500 vascular plants are non-native, and in California, *Centaurea solstitialis* (yellow star-thistle has taken over more than 4 million ha in the northern grasslands (Pimentel 2005). In Hawaii about 35% of plants are now nonindigenous (Pimentel 2005).

In some cases the use of invasive plants have been promoted unknowingly by government agencies (Li et al. 2004). *Pueraria montana* (kudzu) was introduced as a ground cover, later it was promoted by the U.S. Soil Conservation Service (SCS) to be used for soil erosion control (Li et a. 2004). *Cytisus scoparius* (Scotch broom) was used first as an ornamental and also later promoted by SCS for erosion control (Li et al. 2004).

The impacts of invasive species can be put into several broad categories. The first is economic. Jose et al. (2013) estimates crop production losses of $24 billion are due to non-native plants. As recently as 1998, 1,343 U.S. nurseries were selling invasive ground covers and 761 U.S. nurseries were selling invasive vines (Li et al. 2004).

Invasive species threaten natural communities by reducing overall biodiversity, changing habitats used by animals, and by competing with rare or threatened native

plants (Goodwin et al. 1999, Jarchow and Cook 2009, Kirk et al. 2011, Larkin et al. 2012).

Invasive species can affect the gene pools of native species, usually though hybridization or introgression (gene flow between species) (Lockwood et al. 2013). Hybrid individuals are often larger and more vigorous than their parents (heterosis or hybrid vigor) and may be able to outcompete native species (Kirk et al. 2011, Larkin et al. 2012, Lockwood et al. 2013). Genetic changes to gene pools of native populations are not well documented or understood and long-term effects are difficult to predict (Lockwood et al. 2013).

WHY INVASIVE SPECIES ARE SO SUCCESSFUL

Innate characteristics of potentially invasive plant species: Taxonomy may provide clues to potentially invasive species. Heywood and Brunel (2008) indicated that 63% of 76 serious invasive species occurred in just six families: Rosaceae (Rose Family), Fabaceae (Pea Family), Myrtaceae (Myrtle Family), Salicaceae (Willow Family), Oleaceae (Olive Family), and Caprifoliaceae (Honeysuckle Family). In a larger study Heywood, (1989) found that invasive species occurred most often in the Apiaceae (Carrot Family), Asteraceae (Sunflower Family), Brassicaceae (Mustard Family), Lamiaceae (Mint Family), Fabaceae, and Poaceae (Grass Family). These families share complex, but highly successful reproductive and dispersal mechanisms (Heywood and Brunel, 2008).

Phenology may play a role in the ability for an introduced species to become invasive. Invasive species often flower earlier in some locations and later in others when

compared to native species (Godoy et al. 2008, Goodwin et al. 1999, and Pyšek et al. 2003). Longer fruiting and flowering times (Lloret et al. 2005) may lead to greater seed production and a greater presence in the seed bank. Introduced species often leaf out earlier in the spring and retain their leaves later in the fall (Knight et al. 2007) when compared to native species. This increases the length of the growing season for invasives. They would have the potential to grow larger and to use more resources than native species with shorter growing seasons.

Many characteristics of invasive plants are attractive to gardeners and the horticulture industry including longer flowering and fruiting times (Lloret et al. 2005), more seed production (Mason et al. 2008, Knight et al. 2007, Heywood and Brunel 2008), shorter seed germination times (Rejmanek and Richardson 1996, Wildrlechner et al. 2004), higher seedling survival rate (Grotkoop et al. 2010), greater vegetative growth (Lloret et al. 2005, Heywood and Brunel 2008), and the ability to germinate and survive in a bare ground landscape (Heywood and Brunel 2008). Many of these characteristics are found in early successional species that we consider “weeds.”

The use of chemicals by invasive plant species to outcompete natives has been called the “novel weapon hypothesis” (Jarchow and Cook 2009, Jose et al. 2013). Some plant species secrete secondary compounds called allelochemicals that can inhibit the germination of seeds and/or growth of other neighboring species (Jarchow and Cook 2009, Brooker et al. 2011). Allelochemicals can stunt growth or kill the roots of other species and may have other functions such as defense (Brooker et al. 2011, Jones et al.

2013). Commonly the chemicals are produced in roots (Jarchow and Cook 2009, Brooker et al. 2011) but may come from other parts of the plant like berries, leaves, and runners (Veit and Proctor 2011, Bechel and Proctor 2012, Gruber and Proctor 2012). *Rhamnus cathartica* (common buckthorn), *Phalaris arundinacea* (reed canary grass), and *Typha angustifolia* (narrow-leaved cattail) are Eurasian species that have become invasive in North America. All three species produce allelochemicals (Knight et al. 2007, Jarchow and Cook 2009, Kirk et al. 2011, Veit and Proctor 2011, Larkin et al. 2012).

Some species can modify their local environments by changing the soil PH and nutrient cycling; a decrease in soil PH releases more nutrients (Holzmueller and Jose 2013). *Typha* X*glauca* is the F_1 hybrid of introduced *T. angustifolia* (narrow-leaved cattail) and native *T. latifolia* (broad-leaved cattail) (Kirk et al. 2011, Larkin et al. 2012). In North America, dense cattail stands are replacing diverse wetland plant communities and degrading habitats for animals, especially waterfowl (Jarchow and Cook 2009, Kirk et al. 2011). Using molecular analyses, Kirk et al. (2011) showed that *T.* X*glauca* dominates cattail stands they studied in Ontario. Larkin et al. (2012) showed that live *T.* X*glauca* plants do not seem to affect other plant species as much as the deep layers of litter they create. This litter degrades slowly and may reach a decimeter thick (Larkin et al. 2012). It can interfere with seed germination of other species by reducing mean soil temperature, reducing temperature fluctuations, and creating a physical barrier (Larkin et al. 2011).

Naturalized species may lack predators or not be susceptible to diseases in new habitats (Larkin et al. 2012, Holzmueller and Jose 2013). This may improve their chances of survival and allow them to outcompete native species (Inderjit et al. 2005).

As human mediated disturbances and plant dispersal increase worldwide, we are becoming more interested in being able to predict what species are likely to become invasive (Goodwin et al. 1997). Goodwin et al. (1997) tested easily obtainable characteristics of plants: habit (annual or perennial; herbaceous or woody), stem height, flowering period, and native geographic range (including environmental conditions) of 110 pairs of European species from 29 plant families. Pairs were congeners (from the same genus) with one naturalized in New Brunswick, Canada, and the other not occurring in North America (Goodwin et al. 1997). (Note that the authors refer to all the naturalized species as "invasive;" they do not distinguish between aggressive non-native species that crowd out native species and those non-native species that have naturalized without doing much harm.) A regression analysis on 55 paired congeners used biological characters only. Results indicated that habit had no effect on the ability to naturalize. However, the naturalized species were both significantly taller and had longer flowering periods than their European congeners (Goodwin et al. 1997). When this model was applied to the second set of 55 pairs of congeners, it was no better able to predict "invasiveness" than random selection (Goodwin et al. 1997). When native range was added to the analysis, only range was retained in the second model. The second model was able to correctly predict about 71% of the 55 species, which was

significantly different than random selection (Goodwin et al. 1999). The results of this study indicate that habit may not be as important as we think and that non-native plants cannot naturalize unless they encounter the right environmental conditions.

All plants respond to local environmental conditions. For instance, plants grown in the shade usually have larger leaves than individuals of the same species grown in ambient light (Raven et al. 2005, Taiz and Zeiger 2010). This kind of response is referred to as phenotypic "plasticity" (Raven et al. 2005, van Kleunen et al. 2011). Van Kleunen et al. (2011) tested whether invasive species have phenotypic plastic responses that pre-adapt them to a wider variety of environmental conditions than non-invasive species. Van Kleunen et al. (2011) looked at phenotypic plastic responses to shading because most introduced species first establish themselves in an open, disturbed habitat. Later, as plants become established, shade usually increase. The authors hypothesized that increases in leaf length, specific leaf area, and shoot to root ratios (indicating more resources allocated to stems and leaves) are adaptive phenotypic plastic responses to shade (van Kleunen et al. 2011). If invasive species show more phenotypic plastic responses to shade than non-invasive species, they could be pre-adapted to a wider variety of environmental conditions making them more successful (van Kleunen et al. 2011).

Van Kleunen et al. (2011) used a common garden experiment and 14 congeneric pairs of herbaceous species from 10 families with treatments of ambient light and shade. All of the species used were native to Europe but are now naturalized in North

America. One of each pair is invasive and the other non-invasive (van Kleunen et al. 2011).

At the end of the growing season van Kleunen et al. (2011) found that, overall, invasive species produced more biomass than non-invasive species. Both groups produced more biomass in ambient light than they did in shade but this response did not differ significantly between the two groups (van Kleunen et al. 2011). The authors found that invasive species had significantly higher shoot-root ratios, which likely enables them to shade out competitors. Leaf lengths and specific leaf areas of invasive and non-invasive species were not significantly different (van Kleunen et al. 2011). Results of the study indicated that high biomass production across the different light treatments pre-adapts species to become invasive in another location (van Kleunen et al. 2011). However, longer leaves and greater specific leaf area are not adaptive phenotypic plastic responses of invasive species. The authors suggested there may be other phenotypic responses to shade they did not test and/or increased biomass of invasive species is due to adaptive physiological plasticity (van Kleunen et al. 2011).

Environmental and physical factors that support invasive species: All invasive species go through 3 establishment stages (Niemiera and Von Holle 2009). Stage 1 is slow initial population expansion when the species first arrives at a new location (Niemiera and Von Holle 2009). During Stage 2, the species experiences rapid exponential population

ngrowth and, finally, in Stage 3, growth levels off and the species becomes established in the new area (Niemiera and Von Holle 2009). Figure 1 summarizes these stages.

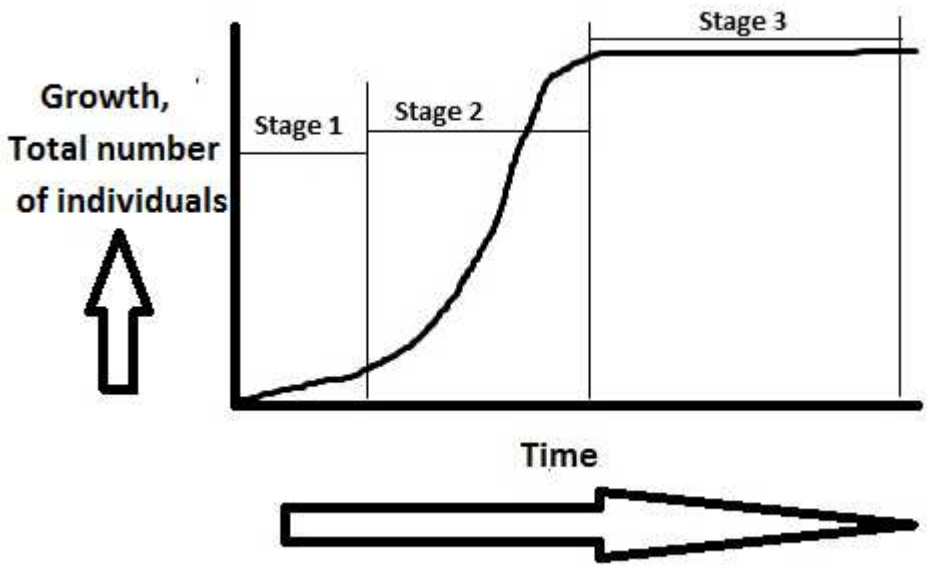

Figure 1. Stages of establishment of an introduced plant species after Niemiera and Von Holle 2009, p. 167-187.

Holzmueller and Jose (2013) propose that empty niches are important factors in an invasive plant's ability to succeed. In species-rich communities, niches are filled and resources are used up by native species, making it difficult for non-natives to become established (Holzueller and Jose 2013). When resources are not used there is an empty niche, which leaves a gap that is susceptible to invasion (Holzmueller and Jose 2013). Since most invasive species show rapid growth and reproduction, they can often produce dense monocultures that at times are more productive than diverse communities of native species (Holzmueller and Jose 2013).

The rate of invasion in the past century has accelerated (Niemiera and Von Holle 2009). This may, in part, be due to changing dispersal methods. Human-mediated dispersal is faster and carries non-natives farther than natural dispersal mechanisms. Human-mediated dispersal tends to deposit individuals into a new area more than once and from multiple populations (Lockwood et al. 2013, Jose et al. 2013).

Natural and human-mediated disturbances may facilitate invasions by giving new plants the chance to take root in empty niches (Vitousek et al. 1997, Jarchow and Cook 2009, Kirk et al. 2011, Lockwood et al. 2013). Many invasive plants are early-successional species that can establish themselves in a disturbed habitat with minimal shading (Goodwin et al., 1999, Brooker et al. 2011, Van Kleunen et al. 2011). As we expand our roads, cities, and agricultural fields, more open ground become available for early-successional and other invasive species (Kirk et al. 2011).

Studies in California show that fire affects different invasive species in different ways (Lockwood et al. 2013, Holzmueller and Jose 2013). *Arundo donax* (giant reed), fire promotes population density. *Carpobrotus edulis* (highway iceplant) invasion is usually slow without fire but more seeds germinate and plants spread more quickly after fire; a last example is *Bromus rubens* (reed brome) which only invades slowly without fire but stands can thicken and dominate an area with fire (Lockwood et al. 2013).

Global climate change has the potential to create more disturbances at both local and regional levels (Dale et al. 2011). Changes in temperature and precipitation patterns can lead to droughts, floods, fires, even changes to cloud cover (Dale et al.

2011). The occurrence, timing, frequency, duration, and intensity of disturbances will change, which may increase the number of invasive species that become established in an area (Dale et al. 2011).

The ranges of both native and invasive plant species are mostly determined by climate (Dale et al. 2011). There are many models to help us predict what climate change will bring but the effects are complex and vary from model to model (Dale et al. 2011). We know that enhanced CO_2 levels will benefit plants that use a C3 pathway for photosynthesis (Vitousek et al. 1997, Dale et al. 2011). The effect of rising CO_2 levels on plants that use the C4 photosynthesis pathway is less predictable (Dale et al. 2011). While we would like to know what specific effects climate change may have on disturbance and invasive species, it is difficult to predict what is going to happen (Dale et al. 2011).

Taxonomy of *Arctotheca prostrata*

Arctotheca prostrata is a member of the Asteraceae (Sunflower Family) and native to South Africa. Figure 2 shows its classification within the family.

Family: Asteraceae (Compositae)
Tribe: Arctotideae
Subtribe: Arctotidinae
Genus: *Arctotheca*
Species: *Arctotheca prostrata*

Figure 2. Classification of *Arctotheca prostrata* within the Asteraceae.

ASTERACEAE

Asteraceae is the most recently-evolved vascular plant family and may also be the largest with about 23,600 species in 1,590 to 1,620 genera (Funk et al. 2009). Most species in Asteraceae are herbaceous annuals and perennials; some trees, shrubs and vines occur in tropical areas (Simpson 2010). Members of Asteraceae are found worldwide, commonly in arid and semi-arid regions in lower to middle latitude (Funk et al. 2009). Asteraceae has relatively few economically important plants; artichoke and sunflower are the most important (Simpson 2010).

An older name for the family, Compositae, refers to the head, the distinguishing feature of the family. The head or capitulum is a compact arrangement of many small flowers called “florets.” One or more heads may be arranged in secondary inflorescences (Simpson 2010). There are three kinds of heads, which are made up of three kinds of florets. Disk florets are radially symmetrical with fused corollas; they are almost always perfect with functional stamens (male reproductive structures) and pistil (female reproductive structure). Ray florets are bilaterally symmetrical with an elongate, strap-shaped “ray” that imitates the petal of a typical flower; they are usually imperfect, often with the stamens absent. Ligulate florets resemble ray florets but are perfect and fertile.

Radiate heads consist of a central disk consisting of disk florets surrounded by one or more series of ray florets. Discoid heads consist only of disk florets; ligulate heads consist only of ligulate florets (Simpson 2010). Florets are mounted on a

receptacle, which is surrounded by an involucre made up of one or more series of bracts called phyllaries or involucre bracts (Funk et al. 2009). All members of the family produce fruits called achenes, which are dry, indehiscent, single-seeded fruits. Each achene functions like a seed. Herein, I will use the term “achene” when specifically referring to fruit production but will use “seed” when discussing germination.

TRIBE ARCTOTIDEAE

Tribe Arctotideae consists of 215 species in 17 genera. All but three species are native to southern Africa (McKenzie and Barker 2008). The only established members of tribe Arctotideae in North America are found in California, New Mexico, (Mahoney 2006), and Hawaii (Starr and Starr 2011). The species were introduced for horticultural purposes (Mahoney and McKenzie 2008). Of species in Arctotidinae only *Arctotheca calendula* is considered invasive (Dana et al. 2012, Mahoney and McKenzie 2012, Cal-IPC 2014).

GENUS *ARCTOTHECA*

Some species in genus *Arctotheca* have naturalized in Australia, Spain, Portugal, North America, and other places with Mediterranean climates (Brickell 1997, Mahoney 2006, Mahoney and McKenzie 2008 and 2012). *Arctotheca* species are annuals and perennials with creeping to erect stems; leaves are basal and cauline, blades are mostly obovate with the margins pinnatified to pinnatisect with lower leaf surfaces +/- white-woolly pubescent and upper leaf surfaces sparsely pubescent to glabrate; heads are

radiate with flat receptacles, ray florets are yellow to bluish; disk florets are bisexual and fertile with the corollas yellow or purplish to brownish (McClintock 1993, Brickell 1997, Mahoney 2006, Mahoney and McKenzie 2012).

There has been a considerable amount of taxonomic confusion about *Arctotheca prostrata* and *A. calendula*. In California both species have been identified as *A. calendula* (McClintock 1993, Mahoney and McKenzie 2008). A recent publication from Hawaii indicates this identification error continues to be made (Starr and Starr 2011).

Arctotheca prostrata is native to the Western Cape, Eastern Cape and KwaZulu-Natal provinces of South Africa (R. McKenzie, personal communication). It is a perennial, rosette-forming herb with creeping stems that root at nodes to form new rosettes. Ray florets are yellow on both faces and disk florets are completely yellow (McKenzie and Mahoney 2010, Mahoney and McKenzie 2012). *Arctotheca calendula* is a tap-rooted annual with ray florets yellow above and reddish purple below and disk florets are dark greenish-purple to purple-brown. *Arctotheca prostrata* can be found in disturbed areas and roadsides in California's north coast, south coast, central west, and western Transverse Ranges (Mahoney and McKenzie 2012). Reports indicate that *A. prostrata* is sterile (McClintock 1993). Mahoney (personal communication) examined nine herbarium specimens and did not find mature achenes on any of them. However, the absence of achenes could be due to the small number of specimens available, immaturity of the individuals, or other factors (McKenzie and Mahoney 2010).

Life History Characteristics of Flowering Plants

REPRODUCTION

Plants can reproduce, both sexually and asexually (Raven et al. 2005). Sexual reproductive success depends on the uniting of sperm with eggs in reproductive structures (Raven et al. 2005). Flowering plants require that pollen be placed on the stigma of a flower (pollination) and fertilization requires the pollen to grow a tube to transport sperm to eggs within ovules within the ovary of a flower (Raven et al. 2005). Fertilization of an egg results in a zygote that develops into an embryo. Embryos are enclosed within seeds (mature ovules) within fruits (mature ovaries). Seeds are surrounded by protective seed coats.

Flowering plant species may be self-compatible, outcrossing (self-incompatible), or both. A self-compatible individual uses its own pollen to fertilize its eggs, either within the same flower or on other flowers on the same plant or on a clone (Raven et al. 2005). In temperate regions, more than half of the flowering plant species are self-compatible (Raven et al. 2005). Outcrossing species require pollen from genetically different individuals to produce seeds (Raven et al. 2005).

There are a number of ways that pollen can be transferred from one flower to another. The most common vectors are bees (Raven et al. 2005). Bees will locate a flower by odor and orient themselves by color and texture (Raven et al. 2005). Bees are most often attracted by blue or yellow flowers (Raven et al. 2005). Other vectors include beetles, wasps, flies, birds, bats, wind, and water.

Asexual reproduction produces a clone of the parent plant as only mitotic cell division takes place (Raven et al. 2005). One type of asexual reproduction is vegetative reproduction where new plants are produced from non-reproductive tissues (Raven et al. 2005, Simpson 2010). Runners or stolons are above-ground stems that can root at tips or nodes to produce new plants that are clones (Raven et al. 2005). Other vegetative plant parts that can produce new plants include rhizomes, roots, stems, and leaves. When new shoots form on roots or leaves they are referred to as adventitious structures (Raven et al. 2005).

Vegetative reproduction has advantages for species that tend to have individuals widely-spaced or rare in their habitats (Raven et al. 2005). Pollen usually doesn't travel very far (Raven et al. 2005). A gap of 300 m is usually sufficient to isolate two populations of the same insect-pollinated species in temperate regions (Raven et al. 2005). A trait of asexually-producing plants is that overall genetic variability within populations is reduced (Simpson 2010). This is beneficial for species that are well-adapted to their habitats. However, reduced genetic variability can increase a species' susceptibility to disease or reduce its ability to adapt to changing environmental conditions (Raven et al. 2005). Humans use asexual reproduction frequently in agriculture and horticulture to produce plants with desired characteristics (Raven et al. 2005).

HYBRIDS

Hybridization among different plant species occurs naturally (Raven et al. 2005, Simpson 2010). It can only take place if the two individuals are genetically similar, usually species in the same genus (congeners) (Simpson 2010). Hybrid offspring may be sterile or partially- to fully-fertile (Simpson 2010). Sterile hybrids can persist or create new "individuals" (clones) if they are able to do so via vegetative asexual reproduction (Raven et al. 2005). Introgression, which is when hybrid individuals backcross to one or both of the parents, is also common in nature. This can promote gene flow between two populations of different species (Raven et al. 2005, Simpson 2010).

Many hybrid individuals in the first filial (F_1) generation are bigger and produce more, larger fruits and flowers than their parents (Jones et al. 2013). This is referred to as heterosis or hybrid-vigor (Raven et al. 2005, Jones et al. 2013). Humans have taken advantage of this phenomenon by creating inbred lines of economically important plants and crossing them to produce F_1 hybrids (Jones et al. 2013). Creating and crossing inbred lines was first developed in the early 20th century in *Zea mays* (corn or maize). F_1 hybrids were 25% taller and produced larger cobs than their parents (Raven et al. 2005, Jones et al. 2013). In the U.S. 95% of corn is of hybrid origin (Jones et al. 2013). However a downside of this practice is that it is necessary to recreate the hybrid seeds every season (Jones et al. 2013)

Invasive *Typha* X*glauca* is the F_1 hybrid of introduced *T. angustifolia* (narrow-leaved cattail) and native *T. latifolia* (broad-leaved cattail) (Kirk et al. 2011, Larkin et al.

2012). A molecular study found up to 70% of the cattails in stands tested in Ontario were T. X*glauca* (Kirk et al. 2011). Historic observations suggested T. X*glauca* was sterile but recent evidence indicates it is at least partially fertile and can backcross with its parents (Kirk et al. 2011). Heterosis may play a role in the hybrid's ability to dominate stands; clumps of T. X*glauca* are larger, on average, than those of its parents (Kirk et al. 2011).

Plant species may spontaneously double their sets of chromosomes producing polyploids (Raven et al. 2005, Brooker et al. 2011). Sterile or partially-sterile diploid (having two sets of chromosomes) hybrids can double their chromosomes to produce tetraploids (having four sets of chromosomes), which restores fertility (Raven et al. 2005). Kowal (1975) found that the pollen grains of tetraploid individuals are larger than those of diploid individuals in *Packera* (Asteraceae). Comparing pollen diameters may provide evidence that an individual in question is a polyploid.

POLLINATION

Pollination in flowering plants is the transfer of pollen that produces sperm from one flower to another. Successful pollination results in sperm fusing with eggs to create zygotes that will develop into embryos within seeds.

Pollination failure: There are several steps that must take place for successful pollination in flowering plants. First pollen must be released, then transported, and

finally, deposited on a stigma of a flower (Raven et al. 2005, Wilcock and Neiland 2002). Most plants, at least at times, experience varying rates of pollination failure (Wilcock and Neiland 2002). Wilcock and Neiland (2002) identified general factors that may cause pollen failure, including pre-dispersal failure, dispersal failure, and post-dispersal failure. Pre-dispersal failure may be due to loss of pollen to pollen feeders, losses to the environment, a lack of resources including water and nutrients, or because pollen is not removed from anthers (Wilcock and Neiland 2002).

Dispersal failure under natural conditions is typically due to pollinator limitation (Wilcock and Neiland 2002), which may be due to pollinator losses because of environmental factors. For example, if a flower is wind-pollinated, failure may be due to low pollen density and/or unfavorable weather conditions (Wilcock and Neiland 2002). A positive correlation between success and the floral display size has been found among a variety of plant species (Willson and Price 1977, Campbell 1989). Wilcock and Neiland (2002) note that reductions in petal size will frequently reduce pollinator visitation. Anderson (1991) studied pollination success in *Achillea ptarmica* (Asteraceae), a perennial herb found in damp grassland. The radiate heads are typically visited by syrphid flies. Anderson (1991) studied 40 patches of *A. ptarmica* that were chosen at random. Rays were removed from heads in some patches during the experiment (Anderson 1991). Anderson calculated the visitor rate on three consecutive days finding that the average visitation rate declined by 51% following the removal of the rays. Anderson (1991) also observed a slow increase in visitation rate with increased

inflorescence size. Thus the larger the inflorescence the more pollen it should receive (Anderson 1991). Removal of rays only slightly reduced seed set (Anderson 1991).

There are four categories of post-dispersal failure: (1) pollen reaching a stigma is not or no longer viable; (2) low pollen densities, (3) poor pollen quality, and (4) the presence of too much self or heterospecific pollen on the stigma (Wilcock and Neiland 2002). Pollen may be inviable due to chromosomal imbalance or environmental influences or because it is immature or unable to produce a pollen tube (Wilcock and Neiland 2002). Pollen may become inviable when it takes too long to reach a stigma (Wilcock and Neiland 2002). Poor pollen quality is commonly due to damaging environmental influences during its development (Wilcock and Neiland 2002).

Pollen viability: Stone et al. (1995) and Firmage (2001) stress the importance of assessing the viability of pollen that will be used in hand-pollination studies. Stone et al. (1995) indicated that in papers they reviewed, pollen viability was not always noted.

There are several ways to test pollen viability. Pollen may be stained using acetocarmine, aniline blue, lactophenol cotton blue, propione carmine, Tetrazolium Chlorine (TTC), Iodine-Potassium-Iodide (IKI), safranin, and fluorescein diacetate (Ockendon and Gates 1976, Bolat and Pirlak 1999). Germination tests incubate pollen grains in sucrose solutions, either in droplets (“hanging drop method”) or in a solid medium (Ockendon and Gates 1976). Results of these tests are variable (Ockendon and Gates 1976, Bolat and Pirlak 1999). Stain tests are faster and much less complicated to

perform than germination tests. However, germination tests are thought to give the most accurate results (Bolat and Pirlak 1999).

Acetocarmine or lactophenol cotton blue staining allows for clear distinctions between pollen grains with cytoplasm and those without a cytoplasm, which are sterile (Ockendon and Gates 1976). Stucky et al. (2012) studied pollination in an endangered perennial in the Asteraceae using the lactophenol cotton blue staining method. Pollen that is round, plump, and stained dark blue is positively correlated with pollen viability (Kearns and Inuoye, 1993). Stucky et al. (2012) collected anthers from 4 disk flowers from randomly selected heads (they used eight heads from each of six populations), and prepared slides with macerated anthers and stain. They viewed slides at 100x and sorted the pollen into two groups: (1) not shriveled and stained dark blue and (2) shriveled and/or not stained or only stained light blue. More than 93% of the pollen, over all six populations, stained dark blue and was considered viable (Stucky et al. 2012). This method has been widely used to assess pollen viability in cultivated and wild *Helianthus* (sunflower) species and in some interspecific hybrids (Atlagić et al. 2012). Atlagić et al. (2012) recommend increasing staining time to several hours because sunflower pollen is thick-walled with a spiky external coat.

Agbagwa et al. (2007) used lactophenol cotton blue to stain pollen of three different varieties of *Cucurbita moschata* (Cucurbitaceae) from Nigeria. Agbagwa et al. (2007) found that time of day was an important factor in pollen viability. Pollen harvested in the morning was 96% viable but was reduced to 78%-62% by early

afternoon to only 8% by the next day. Pollen was dipped into a solution of lactophenol cotton blue in a glass Petri dish and allowed to sit and soak for 5 minutes when it was mounted on slides (Agbagwa et al. 2007).

The fluorochromatic reaction (FCR) method of Heslop-Harrison and Heslop-Harrison (1970) is a widely-accepted pollen viability technique (Pinilos and Cuevas, 2007). Several sources show correlations between FCR results and in-vitro germination of pollen grains of many different species (Ockendon and Gates 1976, Shivanna and Heslop-Harrison 1981, Heslop-Harrison et al. 1984.)

FCR tests the integrity of the plasmalemma and the activity of nonspecific esterases in the cytoplasm in living pollen grains. (Pinilos and Cuevas, 2007). Fatty acid esters in fluorescein easily enter the cell where they are hydrolyzed by esters to release fluorescein, which is fluorescent. This process will work only if the cell is intact so fluorescein only accumulates in living cells (Pinilos and Cuevas, 2007). Sunflower pollen contains a sugar (calose-beta-1, 3-glucon) that gives a fluorescent reaction and facilitates fluorescent microscopy methods (Atlagić et al. 2012).

The FCR procedure is a more expensive and exacting method that requires special equipment. Substrate concentrations must be consistent if comparisons are to be made (Pinilos and Cuevas, 2007). Fluorescein slowly abandons pollen grains making it difficult to estimate the results as its concentration decreases in cells and increases in the medium. This limits the evaluation time of the procedure to only 15 minutes (Pinilos and Cuevas 2007, Dafni et. Al (2005). However, FCR tests have an advantage over in-

vitro germination tests because they are faster and easier to conduct.

In-vitro pollen germination is the most common way of testing pollen viability (Shivanna and Johri 1985). This process is based on the assumption that if pollen will germinate and produce a pollen tube in vitro it will do the same under natural conditions (Pinilos and Cuevas 2007). Accurate results require that the germination medium, temperature, and humidity levels must be optimal for each species (Pinilos and Cuevas 2007).

Bolat and Pirlak (1997) compared three stain tests (TTC, IKI, and safranin) to actual germination rates of pollen grains in sucrose solutions (using hanging drop and solid medium techniques) for fruit trees. They found only IKI stain test results differed significantly from TTC and saffranin tests. In all comparisons, stain tests predicted higher pollen viability than was observed in the germination tests but found that the results were correlated (Bolat and Pirlak 1999). Although Bolat and Pirlak (1999) indicated that similar studies have concluded that these stains are not reliable, consistent, or correlated with germination results, they felt staining pollen provided a faster, easier alternative to germination methods.

Ockendon and Gates (1976) used onion pollen to compare lactophenol cotton blue stain, FCR, and two germination methods: hanging drop and solid medium. The authors found the hanging drop method underestimated pollen germination because cells often burst during the procedure. FCR and solid medium germination techniques gave similar results. Cotton blue stain appeared to overestimate pollen viability

(Ockendon and Gates 1976). Ockendon and Gates (1976) indicated that the two halves of an anther can produce pollen of different viabilities and that different techniques present different technical problems. Although pollen germination techniques are thought to be the best test of pollen fertility, the authors point out that not all pollen that germinates is fertile (Ockendon and Gates 1976).

Staining with lactophenol cotton blue is a fast, inexpensive and commonly-used method to test the viability of pollen. Although it does assess loss of viability over time (Heslop-Harrison et al. 1984, Knox 1984) and tends to overestimate viability (Ockendon and Gates 1976, Bolat and Pirlak 1999), it will indicate if an individual has the potential to fertilize an egg (Atlagić et al. 2012).

Hand-pollination: Hand-pollination is used in studies that compare the performance of pollen donors, test inbreeding effects and incompatibility systems, and investigate sexual selection in plants (Stone et al. 1995). Hand pollination may be used to supplement natural pollination in crops in a case where resources are limited (Stone et al. 1995). Hand-pollination is the most common method for producing pure seeds (Ashworth 2002) or to increase fruit and seed production (Bierzychudek 1981, Willmer 2011).

Hand-pollination techniques transfer pollen from an anther to a receptive stigma and can be used in both wind and insect pollinated flowering plants (Ashworth 2002). Flowers that serve as females must be isolated before and after pollination to prevent

contamination by unwanted pollen. The bagging technique is common though other methods, such as spatial separation, may be used (Ashworth 2002). In the bagging method polyester cloth or other lightweight fabric or paper is used to cover the flowers, or in the case of members of the Asteraceae, the entire head, to prevent insects or wind from transferring unwanted pollen (Kowal 1975, Ashworth 2002). Clear plastic bags or glossy envelopes should never be used as these might damage the flower by overheating it (Ashworth 2002).

There are three outcomes for tests that assess natural pollination success: 1) an increase in reproductive success, 2) a decrease in success, or 3) no change in success. In a review of 99 cases of hand-pollination, Young and Young (1992) found 42.4% had an increase in female reproductive success, 40.4% had no effect, and 17.2% were found to have a reduction in reproductive success. Bierzychudek (1981) compared hand-pollinated and naturally-pollinated individuals in a natural population of *Arisaema triphyllum* (Jack-in-the-pulpit). Results showed that hand-pollinated individuals produced an average of 43.2 mature seeds compared to only 1 mature seed in naturally-pollinated individuals (Bierzychudek 1981). Martínez-Pallé and Aronne (2000) found similar results in the percent of fruit set in *Ruscus aculeatus* (butcher's broom), a Mediterranean shrub. Only 3% of flowers set fruit when plants were open-pollinated while nearly 80% of flowers set fruit when hand pollinated (Martínez-Pallé and Aronne 2000).

Young and Young (1992) list factors that could account for reduced fruit or seed set in hand-pollination efforts. These include (1) pollen density on a stigma is so great that pollen tubes interfere with each other, (2) stigmas may be damaged by pollen thieves, pollinators, or during the hand-pollination process, (3) low genetic diversity of pollen because only one donor is used, (4) the bagging process may reduce seed set, (5) hand pollination is performed before or after the stigma is receptive, (6) pollen may be inviable or too little pollen is applied (Young and Young 1992). Pollen age is also a factor. Stone et al. (1995) indicate that the average half-life of six species in Asteraceae is just 2.45 hours and any slight change in conditions can alter this. Hand-pollination methods assume that pollen is of good quality but a review of 283 articles concerning hand pollination indicated 70% of authors did not mention pollen freshness or age (Stone et al. 1995).

Seed Dormancy

Each flowering plant seed contains an embryo and endosperm, a nutrition and hormone source for the embryo (Raven et al. 2005). Seeds are surrounded by a protective seed coat (Hopkins and Hüner 2009). Fruits containing seeds are adapted for dispersal by a variety of agents including animals, wind, and water (Raven et al. 2005). Many seeds germinate when water, oxygen, and appropriate temperatures are present (Jones et al. 2013). The seeds of wild plants are subjected to highly variable environmental conditions especially the timing and amount of rainfall (Chrispeels and

Sadava 2003). Seeds of some species have developed a variety of mechanisms to cope with unpredictable conditions including seed dormancy to delay germination (Chrispeels and Sadava 2003; Taiz and Zeiger 2010). Seeds may remain dormant for variable periods allowing wild plants to build up a seed bank in the soil (Chrispeels and Sadava 2003; Taiz and Zeiger 2010). Seeds germinate when conditions are optimal (Chrispeels and Sadava 2003).

Seeds are usually dessiccated when they are shed (Taiz and Zeiger 2010). Germination starts with imbibition (uptake of water) by the dry seed followed by embryo expansion. Germination is complete when the embryo breaks through the seed coat (Finch-Savage and Leubner-Metzger 2006, Taiz and Zeiger 2010).

Some viable seeds do not germinate even under favorable conditions (Jones et al. 2013). These dormant seeds require additional stimuli to germinate (Finch-Savage and Leubner-Metzger 2006, Jones et al 2013). There are two types of seed dormancy: primary and secondary (Jones et al. 2013). Primary dormancy occurs when the seeds are dormant at the time they are shed (Taiz and Zeiger 2010, Jones et al. 2013). Secondary dormancy is initiated by environmental conditions after seeds have been shed (Jones et al. 2013). Secondary dormancy can be induced by extreme temperatures and/or the presence or absence of light (Finch-Savage and Leubner-Metzger 2006, Jones et al. 2013). The function of secondary dormancy is to delay germination until environmental conditions favor growth (Jones et al. 2013).

Dormancy is controlled by either the seed coat or from within the embryo (Jones et al. 2013, Finch-Savage and Leubner-Metzger 2006). The seed coat can be very complex and has several functions including suppressing germination by restricting imbibition and oxygen and limiting the expansion of the embryo (Hopkins and Hüner 2009). Dormant seeds of some species under this control can endure hundreds of years and still germinate (Raven et al. 2005). Seed coat dormancy is common in annuals that must avoid germination during the dry season (Raven et al. 2005). Seed coat dormancy can be found in a wide variety of species including *Arabidopsis*, barley, lettuce, rice and oats (Jones et al. 2013).

Seed coat dormancy is controlled by four mechanisms (Taiz and Zeiger 2010). The seed coat can (1) prevent imbibition, (2) act as a mechanical constraint to the embryo, preventing it from breaking out, (3). interfere with the exchange of gasses, especially oxygen, and (4) block germination-inhibiting compounds, such as abscisic acid (ABA), from leaching out of the seed (Taiz and Zeiger 2010).

Embryo dormancy occurs when the embryo does not grow even when the seed coat has been removed (Jones et al. 2013). Embryo growth can sometimes be stimulated by cutting off the embryo's cotyledon(s) (first leaf or pair of leaves), which may contain inhibiting compounds, such as ABA (Taiz and Zeigler 2010). Seed dormancy in onions is controlled by its embryo (Jones et al. 2013).

Seed dormancy can be broken in a number of ways. Environmental cues such as temperature may stimulate germination in some species (Finch-Savage and Leubner-

Metzger 2006, Jones et al. 2013). Both alternating temperatures (with fluctuations as little as 5-10°C) and/or low temperatures are known to break seed dormancy, but most seeds will not germinate until consistent temperatures are present (Hopkins and Hüner 2009). Some species' seeds need to be stratified, which is going through a period of cool moist conditions (Jones et al. 2013, Hopkins and Hüner 2009). Seed stratification is often required by species in temperate regions (Jones et al. 2013). Light can also be in important trigger; species require a specific wavelength to break dormancy (Jones et al. 2013). Sometimes the seed coat must be abraded or removed by sand, microbes, or the passing of the seed through an animal gut (Hopkins and Hüner 2009). In Asteraceae, Solanaceae (Tomato Family), and Rubiaceae (Coffee Family) endosperm rupture is required before an embryo can emerge (Kucera et al. 2005).

Plant hormones like abscisic acid (ABA) and gibberellins (GA) play a role in seed dormancy (Jones et al. 2013, Taiz and Zeiger 2010). Plant hormones are thought to play more active roles in embryo-mediated dormancies than in seed coat-mediated dormancies (Taiz and Zeiger 2010). However, the seed coat can block hormones from leaching out of a seed (Taiz and Zeiger 2010). ABA is a positive regulator for dormancy induction, which means it can delay germination (Kucera et al. 2005). When ABA is deficient, primary dormancy is absent in most cases (Kucera et al. 2005). GA stimulates growth (Taiz and Zeiger 2010). Increasing levels of GA can counteract the effects of ABA (Kucera et al. 2005). Another compound that can promote seed germination is ethylene (Kucera et al. 2005). Ethylene can promote germination of non-dormant seeds and in

some species like *Arachis* (peanut) and *Helianthus* (sunflower) ethylene can release the seed from dormancy (Kucera et al. 2013). However in most species ethylene alone cannot induce germination (Kucera et al. 2005).

A study by Schultz et al. (2002) studied seed dormancy in a Mediterranean climate, which is characterized by having a prolonged dry period and a moderately cool, rainy winter. This climate occurs around the Mediterranean Sea and along the west coasts of continents at about 45 degrees north and south latitude, including parts of California, Chile, Africa, and Australia (Raven et al. 2005). Most seeds adapted to this climate are dispersed at the end of the rainy season and must remain dormant until the next rainy season (Bell 1999, Schultz et al. 2002). The length of dormancy may coincide with the length of the unfavorable season (Schultz et al. 2002). Rare summer or early fall rainfall can stimulate germination (Ellery and Chapman 2000). However the effects of unseasonable rain are short-lived creating unsustainable conditions for seedling development. Such events are called “false breaks of season” (Ellery and Chapman 2000). False breaks can cause large numbers of seeds to germinate. When dry conditions return, seedlings die, leaving an inadequate seed bank for later favorable conditions (Ellery and Chapman 2000).

Schultz et al. (2002) studied four annual Asteraceae species in southwestern Australia finding that seeds of all four were strongly dormant at maturity and that dormancy was alleviated very slowly. As the number of days of the trials increased so did the number of seeds that germinated with the average length of the dormancy

coinciding with the length of the unfavorable period (Schultz et al. 2002). Schultz et al. (2002) also concluded that there is little carryover of seeds from the first season to the next season, so not much of a seed bank is established.

Ellery and Chapman (2000) studied seed dormancy in *Arctotheca calendula* (capeweed), a very close relative of *A. prostrata*. Both species are adapted to Mediterranean climates. Initially it was though that capeweed dominated other species in pastures because its seedlings were able to tolerate early season drought. More recently, studies have shown this may not be the most important factor (Ellery and Chapman 2000). Ellery and Chapman (2000) propose that capeweed's success is due to deep seed dormancy mechanisms that protect its seeds from germinating during false breaks.

Ellery and Chapman (2000) performed three experiments that assessed seasonal effects and physiological effects of embryo and seed coat dormancy on germinability (percent of viable seeds) of capeweed seeds. Seeds were stored at constant temperature in the lab or placed outdoors on the soil surface where they were exposed to natural conditions, including seasonal temperature fluctuations (Ellery and Chapman 2000). Treatments to test embryo dormancy included leaching the seeds and cotyledon removal by bisection; both treatments would remove compounds that might inhibit embryo growth. Treatments to test seed coat dormancy included leaving seed coats intact, removing them entirely, and rupturing them (Ellery and Chapman 2000).

Results indicated that at constant room temperature germinability of intact

seeds did not differ significantly from 0 at any time over the 10 month testing period (Ellery and Chapman 2000). Intact seeds on the soil surface in natural conditions showed primary seed dormancy lasted about six weeks. From February to September germinability on the soil surface increased to a maximum of about 14% in May. The time period corresponds to late fall, winter, and early spring in the southern hemisphere. Germinability of embryos without seed coats increased linearly to about 60% under constant room temperature. Under natural conditions, embryos without seed coats germinated immediately, rising to a maximum germinability of 70% in March. Both treatments showed a dip in germinability that corresponded to minimum temperatures falling below 25 degrees C in midwinter (Ellery and Chapman 2000).

Tests for embryo dormancy showed intact seeds did not germinate following 24 hours of leaching or imbibition in Petri dishes. Seeds with coats that were bisected, a process that removes cotyledons from embryos, showed about 10% germinability. When seed coats were removed for all three classes, control embryos showed 21.4% germinability, leached embryo germinability increased to 73%, while bisected embryos showed 83% germinability, indicating that compounds in seeds and/or cotyledons inhibit germination (Ellery and Chapman 2000).

Ellery and Chapman's (2000) tests for seed coat dormancy showed that after 12 months only 1.3% of intact seeds germinated. Removing seed coats increased germinability to 77% (Ellery and Chapman 2000). Although seed coat dormancy is a factor, it is unclear exactly how it functions. Capeweed seed coats did not appear to

prevent embryos from growing nor did they prevent imbibition or gas exchange (Ellery and Chapman 2000). It is possible that seed coats contain a germination inhibitor but this was not tested (Ellery and Chapman 2000).

Ellery and Chapman's (2000) study found primary and secondary forms of seed dormancy in capeweed. Seed coat and embryo dormancy combine to create a very high level of dormancy; only 14% of viable seeds germinated under natural conditions in the first year after they were produced (Ellery and Chapman 2000). Embryo dormancy shows a seasonal cycle, which is typical of annual species. This type of dormancy can benefit the population by restricting germination to a season when a seedling is most likely to survive (Ellery and Chapman 2000). The deep embryo dormancy that Ellery and Chapman (2000) observed would prevent seed germination during false breaks in early spring but would not be effective during false breaks later in the season. However, seed coat dormancy might protect seeds from late season germination. This high level of seed coat dormancy can provide an additional benefit of ensuring the seeds maintain a dormant state for germination in future years (Ellery and Chapman 2000).

Studies indicate that capeweed seed dormancy may be broken by a variety of stimuli including reduction of germination inhibitors over time by leaching or decreased production in cotyledons, high temperatures, large fluctuations in minimum and maximum daily temperatures, and the presence of light (Ellery and Chapman 2000). Rupture of the seed coat was not an important mechanism for breaking dormancy (Ellery and Chapman 2000).

IV. METHODS

Data and Statistical tests

Data measured for this study are nested at the following levels from most-inclusive to least-inclusive.

LEVEL 1. POPULATION (designated by two capital letters)

There are four populations from California and one from South Africa; F_1 progeny generated by crosses may also be considered at the population level. Table 1 gives names and abbreviations for populations:

Table 1. Origins, population names and abbreviations for *Arctotheca prostrata* plants used in this study.

Origin	Population Name	Abbreviation
California	Gualala Bluff	GB
California	Rodeo Valley	RV
California	Tiburon	TI
California	Tennessee Valley	TV
South Africa	South Africa	SF
Crosses	First filial progeny	CX

LEVEL 2. CHUNKS (California) or INDIVIDUALS (South Africa and F_1 progeny) (designated by numbers).

"Chunks" of plants were dug from populations in California at some distance from each other. Each chunk was made up of rosettes, either attached to each other by runners or not. Attached rosettes were noted when they were potted up. All plants

from South Africa and F_1 progeny were genetically unique individuals because each was generated by a seed.

LEVEL 3. ROSETTES – California only (designated by capital letters)

Within each chunk from California I designated another level, the rosette. These were both connected and unconnected plants within chunks. It is not possible to confirm whether unconnected rosettes were genetically unique from other rosettes in the same chunk. Individuals from South Africa and F_1 progeny do not have any plants at this level.

LEVEL 4. CLONES (designated by lower-case letters set off by hyphens.)

These are plants that were created by rooting rosettes on runners either from rosettes (CA) or individuals (SF or CX). Note: for consistency all plants received a designator at this level whether they were cloned or not.

LEVEL 5. REPLICATES (numbers set off by hyphens at the ends of labels)

If more than one measurement was made on a plant, I refer to them as replicates.

All distributions of measurements were analyzed by Shapiro-Wilk tests for normality. Normally-distributed data were tested by one-way analysis of variance

(ANOVA). Data that failed Shapiro-Wilk tests were analyzed by Kruskal-Wallis one-way analysis of variance on ranks (SigmaPlot for Windows).

All measurements were analyzed from least-inclusive (lowest) to most inclusive (highest) nested levels to determine whether variability among means at any level were significantly different. It is not advisable to combine and analyze data that differ significantly at lower levels because these differences may be hidden by upper-level analyses (Kowal 1975). Measurements that were not significantly different within lower levels were pooled and analyzed at the next level up.

Live Plant Collection in California

Dr. Alison Mahoney and two volunteers, Mary Sue Ittner and Fraser Muirhead collected living plants of *Arctotheca prostrata* from four populations in California. Volunteer collectors were given specific directions on how to identify, harvest, label, and package plants for shipment.

It was my goal to have five to six well-rooted chunks (putative "individuals") of *Arctotheca prostrata* growing "a good distance" from each other from each population. Any rosettes attached along a runner were labeled as clones of the same rosette.

Collectors were requested to carefully wash the soil off plant roots, wrap them in wet paper towels, seal into plastic bags with each plant chunk in a different bag. Using a sharpie marker, collectors labeled the outside of the bag with a number or letter to

identify each chunk. A note inside each bag also identified each specimen. Bags were stored in a cooler or refrigerator until ready for shipping.

Collectors were asked to provide the following information: 1) date plants were collected, 2) detailed directions from an intersection that appears on a map to the collection site, 3) description of habitat, and 4) other ephemeral characters such as ray and disk color, insects present, scents, etc. Plants were shipped overnight to ensure the least amount of stress. No permit was required to ship this species within the U.S.

Once plants were received on campus, they were potted as soon as possible. Labels were assigned based on the system described above. Each plant was put in a labeled 4.25-inch clay pot in sterilized soil containing 1 part perlite, 1 part black soil, 1.5 parts peat moss, 1 part sand. Clay pots were put into large plastic trays (22 inch by 11 inch by 2.5 inch) with two to three inches of water in them and allowed to sit in water to assure proper moisture for a couple days and kept out of direct sunlight. After a few days, plants were put in a greenhouse and watered as needed. During summer months (mid-May through August) the plants were kept outside on a deck in New Ulm, MN. Plants received direct sunlight about 60% of the day from morning into early afternoon. During this time plants were only watered as needed and pots were allowed to drain freely. Plants were kept in a greenhouse during late fall, winter and early spring in trays that did not allow free drainage. A list of all the plants and their roles in this study is given in Appendix I, Table A-2.

Seed Germination

Dr. Robert McKenzie (Rhodes University, South Africa) collected seeds from *Arctotheca prostrata* in Grahamstown, South Africa and shipped them to MSU under a U.S. Department of Agriculture permit to import small lots of seeds, no. p37-11-00316.

After unsuccessful attempts to germinate South African seeds in a soil and sand mix, I attempted to germinate them in Petri dishes. A new plastic 2-inch diameter Petri dish was lined with a standard household paper towel cut to fit inside the dish. Seeds were then added (one to six seeds per dish) and DISTILLED water was added until the paper towel and seeds were wet. The dish was then put in a south/southeast-facing window where it received sunlight and warmth with an average temperature of 68°F. DISTILLED water was added as needed to keep the seeds moist. When a seed germinated it was kept in the dish until it was large enough to be transplanted into a small plastic starter pot (two inch diameter). After about two weeks it was transplanted into a 4.25 inch clay pot. Dates that seeds were started, germinated, and moved from pot to pot were recorded.

Testing Pollen Viability

Lactophenol cotton blue is a fast, inexpensive test for pollen viability. For these reasons, I tested the viability of pollen of *Arctotheca prostrata* after the method of Agbagwa et al. (2007). Pollen from a mature head of *A. prostrata* was collected by holding the head over a clean glass slide (3 by 1 inch) and tapping the head. One to two

drops of lactophenol cotton blue was added and allowed to sit for 30 minutes. A cover slip was then added and pollen was observed under a compound microscope at 100x. The cover slide was sealed with clear nail polish to preserve the slide for a short time.

I counted all pollen grains within one randomly-chosen field of view at 100x. Pollen grains were considered "viable" if they stained dark blue, looked plump, round, and uniform in size. Grains were considered "inviable" if they did not stain or were clearly smaller and misshaped. I measured and recorded the diameter of 25 pollen grains in micrometers on each slide using the inter-ocular measuring device. Measurements are given in Appendix II, Table A-2.

Crossing Experiments (Hand Pollination)

To test the fertility of *Arctotheca prostrata,* multiple crosses were attempted using the method of Kowal (1975). During the first season crosses, were attempted between clones, rosettes from different chunks within the same California populations, between rosettes from different California populations, between different South African Individuals, and between Californian rosettes and South African Individuals. After the germination of F_1 seeds, individuals that survived and flowered were also back-crossed to Californians and South Africans and crossed with each other. The availability of heads in anthesis (disc florets open and releasing pollen or with stigmas exposed) dictated what crosses were attempted. Plants produce more than one head and I numbered them in order of their appearance.

Heads were bagged using lens paper (Distributed by VWR Scientific products # 52846-001) when heads were near opening or immediately after opening before anthesis after the method of Kowal (1975). Lens paper was cut to size to cover the head (about 4 inches by 3 inches), labeled with a head number, and tied shut with very thin light-weight wire just under the involucre (Figure 3). Bags were opened periodically and inspected with a hand lens to look for signs of anthesis (the presence of pollen and/or extended stigmas). Heads that had not reached anthesis were rebagged. If the cross proceeded the two heads were touched to each other to transfer pollen, the head would be carefully moved several times and touched to give multiple chances for the transfer of pollen. Both heads were carefully rebagged after the cross and kept bagged to prevent contamination from other pollen. Plant and head numbers were recorded with the date of the cross, along with any other observations. Bagged heads were left on the plants until they matured when they were removed and dissected to look for mature achenes.

To serve as controls some heads were bagged but never crossed. This tested for self-compatibility. During the summer when plants were outdoors, some heads were left open to allow for natural pollination by bees and other insects.

Allelopathic Abilities Testing

Table 2 lists the plants used and what parts (leaves, roots, or runners) were harvested to test for allelopathic effects of *Arctotheca prostrata*.

Figure 3. *Arctotheca prostrata* heads were bagged using lens paper and very fine wire.

Table 2. Plants used in experiments to test for allelopathic effects on lettuce seedlings. Parts harvested from plants: L = leaves, Rt = roots, and Run = runners.

Gualala Bluff (GB)		Rodeo Valley (RV)		Tiburon (TI)		Tennessee Valley (TV)		South Africa (SF)	
4E-a	L	5A-f	L	1A-c	L	3A-b	L	1-d	L
4E-a	Rt	5A-g	L	1A-c	Rt	3A-c	L	5-a	L
		5A-f	Rt	1A-a	Run	3A-b	Rt	1-d	Rt
		5A-g	Rt			3A-c	Rt	5-a	Rt

After plants were removed from pots the soil was carefully removed from the roots by hand to insure as little damage as possible to the roots. The plants were also washed with a minimum amount of water to remove any remaining soil. Figure 3 shows what plants looked like after being cleaned. Each plant was then placed in a labeled plastic Ziploc bag and stored in a refrigerator until further processing took place.

Figure 4. A single plant after removal from its pot and minimal rinsing.

Next each plant was separated into three parts, 1) runners, 2) leaves, cut just above the crown, and 3) roots, cut just below the crown. The fresh material was ground in a ball grinder for 5 minutes and then kept in a new, labeled glass bottle. Samples were kept in the refrigerator for two weeks until further processing took place. The ground material was weighed to produce aliquots of approximately 0.5, 1, 2, and 4 grams and placed in centrifuge tubes. Several replicates at each weight were created for each plant part. Some fresh material from each sample was saved to calculate percent water for all the samples. Ten mL of HPLC-grade water was put in each measured sample, vortexed for 5 minutes, and then centrifuged at 2500 RPM for 10 minutes.

Filter paper was put into clean, heat-sterilized (550°C, 1 hour) glass Petri dishes. Each Petri dish was labeled on the bottom with the plant's name and sample parts and

weight. Ten lettuce seeds were placed in each labeled Petri dish (Burpee, Black lettuce seed packaged for 2013) and liquid from each centrifuged sample was pipetted off and placed in the appropriate dishes. Controls were created by placing ten lettuce seeds in a Petri dish with filter paper and HPLC-grade water. The dishes were incubated at 26°C with 10 hours light and 14 hours of dark. Dishes were rotated vertically on the shelves every day, with samples from the bottom moved to the top and samples on shelves rotated to the shelves below. The number of seeds germinated was recorded after 24 hours to assess germination rates. After 72 hours the experiment was terminated (Figure 4). Lettuce seedlings were removed from dishes and their roots measured.

To calculate percent water, the remaining fresh (wet) material from each sample was divided into as many as four aliquots for replication. The number of replicates depended on the amount of material left over in each sample. Fresh material was put in pre-weighed Petri dishes, weighed and heated in a 103-105°C oven for 24 hours. Samples were placed in a desiccator and reweighed after cooling. Data are provided in the Appendix, Table A-16. Percent water was calculated and used to convert the wet weights into dry weights using the following steps:

1. Percent water = (wet weight - dry weight) / wet weight x 100
2. Percent dry material = (100 - percent water)
3. Dry weight = wet weight x percent dry material (number determined in #2)

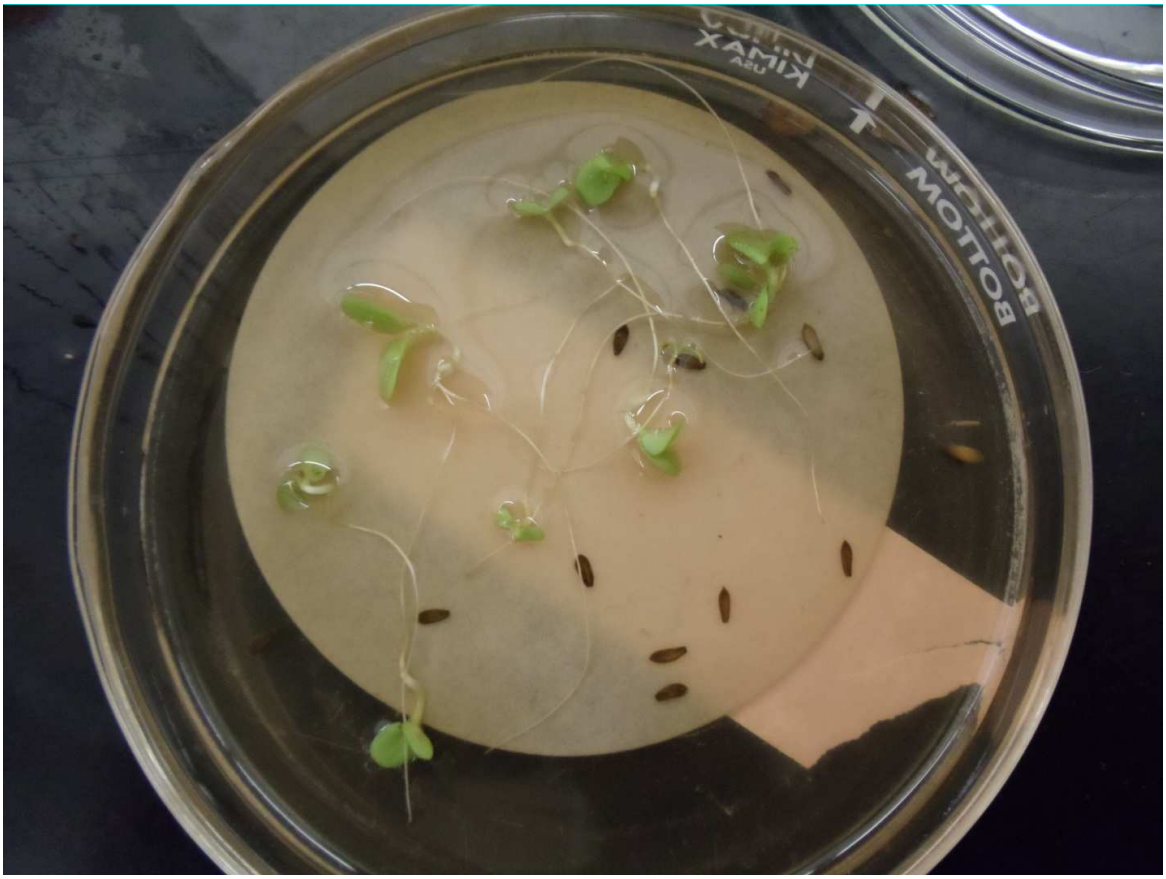

Figure 5. Lettuce seedings and ungerminated seeds in a Petri dish after 72 hours.

Morphological Measurements

Measurements are given in the Appendix, Tables A-4, A-5, and A-6. Plants were measured when the first head on an individual opened. Measurements to the head included disk width measured across center of the disk (Figure 5). Ray length was measured by removing a ray floret, flattening it on a ruler, and measuring it from the base to the tip (Figure 6). Up to the first four heads on a plant were measured.

Figure 6. Disk width was measured on up to four heads on each plant across the center of the disk in cm when the head was fully open.

Figure 7. Ray length was measured on up to four heads on each plant. Rays were flattened on a ruler and measured from base to tip in cm.

Two leaf measurements (leaf length and leaf width) were made on the three largest leaves at the time the first head was measured. The length of each leaf was measured from the center of the rosette to the tip of the leaf. Leaf width was measured at the widest part (Figure 8).

Figure 8. Leaf length and width were measured in cm on three leaves on each plant at the time head measurements were made.

V. RESULTS

Seed Germination

Seed germination time varied among and within populations from a minimum of 5 days to a maximum of 296 days. Seeds received from South Africa took an average of 86.3 days to germinate (n = 6, SD 49.0). Seeds generated from my artificial crosses on South African plants took an average of 97.3 days (n = 10, SD 70.9); seeds generated on Californian plants took an average of 194.4 days (n = 4, SD 100.2). Table 3 summarizes results for the germination trials.

Pollen Viability

Pollen viability was tested by staining pollen with lactophenol cotton blue. Pollen grains were considered "viable" if they were uniform in size, round, and stained dark blue. Pollen grains that were either unstained or small and shriveled were considered "inviable." The total number of grains counted per slide ranged from 75 to 412. Data are given in the Appendix, Table A-3. Counts on seven clones from three populations indicated plants from California had, an average pollen grain viability of 94.4% (2200 pollen grains counted; SD 3.6). Five counts on two South African plants had an average viability of 96.5% (1270 pollen grains counted; SD 1.9). Table 4 gives results of ANOVAs comparing pollen viability among populations within California and pollen viabilities of Californian and South African plants.

Table 3. Origin of seeds (achenes), number of seeds in each trial, number of seeds that germinated, days to germination, number of days ungerminated seeds were kept moist in their plates before the attempt was terminated, and offspring produced.

Origin/Female (H = head no.)	Male	Number of seeds/trial	Days to germination	Length of trial (days)	Offspring
South Africa		2	0	455	
South Africa		1	92	-	SF 1-a
South Africa		1	140	-	SF 2-a
South Africa		1	0	435	
South Africa		1	92	-	SF 3-a
South Africa		1	0	489	
South Africa		1	21	-	SF 4-a
South Africa		1	21	-	SF 5-a
TV 1A-a H:2	Open	7	0	195	
TV 1A-a H:2	Open	1	83	-	CX 6
TV 1A-a H:2	Open	1	95	-	
TV 1A-a H: 2	Open	4	0	315	
TV 1A-a H: 2	Open	1	296	-	CX 14
SF 2-a H:1	GB 1A-a	4	0	245	
SF 2-a H:1	GB 1A-a	1	170	-	CX 9
SF 2-a H:1	GB 1A-a	1	170	-	CX 10
SF 2-a H:1	GB 1A-a	3	0	195	
SF 2-a H:1	GB 1A-a	1	195	-	CX 11
SF 1-a H:4	Open	4	0	325	
SF 1-a H:4	Open	5	0	392	
SF 1-a H:4	Open	10	0	210	
SF 1-a H:4	Open	1	0	209	
SF 1-a H:4	Open	3	0	324	
SF 1-a H:4	Open	6	0	195	
SF 1-a H:4	Open	1	5	-	CX 1
SF 1-a H:4	Open	1	7	-	CX 2
SF 1-a H:4	Open	1	153	-	CX 3
SF 1-a H:4	Open	1	41	-	CX 4
SF 1-a H:4	Open	1	54	-	CX 5
SF 1-a H:4	Open	1	81	-	CX 7
SF 1-a H:4	Open	1	97	-	CX 8
GB 5A-e H:1	GB 5A-b	5	0	330	
GB 5A-e H:1	GB 5A-b	3	0	209	
GB 4A-f H:1	TI 1A-e	3	0	209	
GB 4A-f H:1	TI 1A-e	2	0	293	
GB 4A-f H:1	TI 1A-e	1	221	-	CX12
GB 4A-f H:1	TI 1A-e	1	277	-	CX 13
GB 1A-a H:2	SF 2-a	6	0	259	
GB 1A-a H:2	SF 2-a	5	0	209	
GB 1A-a H:1	RV 4A-a	5	0	307	
GB 1A-a H:1	RV 4A-a	9	0	209	

Table 4. Summary of results of one-way ANOVAs on percent pollen viability for *Arctotheca prostrata*. N-values given in respective order. Significance level of $p<0.05$.

Level:	Among CA Populations
Entities:	**GB, RV, TI**
Values of X:	pooled means* and unreplicated
n:	3, 2, 1
p-value:	0.506

Level:	Between CA and South Africa	
Entities:	**CA Populations**	**SF 1**
Values of X:	pooled means*	replicates
n:	6	5
p-value:	0.183	

* Mean values for replicates for any single plant were calculated and pooled with unreplicated values to prevent weighting problems. Raw data are given in the Appendix, Table A-3.

Crossing Experiments

An important component of this study was performing artificial crosses to determine if *Arctotheca prostrata* plants from California are fertile. Artificial crosses were made as heads in anthesis were available simultaneously. If only one head was available it was either bagged as a control (self-cross) or left unbagged (open). Out of 104 total attempts, 25 heads produced mature achenes/seeds (Table 5). Of 36 open pollination attempts, nine produced achenes. The average ratio of mature achenes to potential achenes in artificial crosses was similar to that of openly-pollinated heads (Table 6).

Achenes from intentional crosses or openly-pollinated heads were germinated resulting in 14 F_1 individuals designated CX 1 through CX 14. Some of these individuals

were backcrossed to Californian and South African plants. Table 5 lists successfully crossed individuals, number of mature achenes, partially- or undeveloped achenes, total number of potential achenes (number of disk florets), and the ratios of mature to potential achenes for all successful artificial crosses and openly pollinated heads, and F_1 offspring generated. A summary of successful crosses is given in Table 6. Complete crossing data are given in the Appendix, Table A-7.

In all but one of 24 attempts, uncrossed bagged heads (self-crosses) or crosses between known clones or assumed clones of the same individual failed to produce any achenes. The exception was a cross between Gualala Bluff (GB) clones 5A-b and 5A-e, which resulted in 12 achenes out of a potential 50. (See discussion.) Six attempts were made to cross rosettes from different chunks within the Rodeo Valley (RV) California population. All failed to produce achenes. No other within-California population/among chunk crosses were attempted because heads in anthesis were not simultaneously available.

Four attempts were made to cross different South African individuals. A pair of reciprocal crosses produced viable achenes. Nineteen attempts were made to cross rosettes from different California populations. Two crosses with Gualala Bluff heads serving as females were successful. Four attempts were made to cross South Africans serving as females with Californians. Two crosses were successful. Five attempts were made to cross Californians serving as females with South Africans. Three crosses were successful. Five crosses between F_1-generation individuals were attempted. Four were

Table 5. Successful artificial crosses and open pollinations of *Arctotheca prostrata* plants. Population/group abbreviations are as follows: GB, RV, and TV = California populations; SF = South African individuals; CX = F-1 generation individuals. Arrows indicate reciprocal crosses. Individuals within populations or groups with the same numbers are or were considered likely to be clones.

					Achenes			
Female	**Head**	**Male**	**Head**	**CX**	**Mature**	**Partial + Undevel.**	**Total**	**Ratio Mat./Total**
GB 1A-a	1	RV 4A-a	3		21	8	29	0.72
GB 1A-a	2	SF 2-a	2		30	21	51	0.59
SF 2-a	2	GB 1A-a	2	9*, 10, 11	33	39	72	0.46
GB 4A-f	1	TI 1A-e	1	12, 13	10	45	55	0.18
GB 5A-e	1	GB 5A-b	2		12	34	46	0.26
TV 1A-a	2	Open		6, 14*	19	48	67	0.28
TV 1A-a	1	Open			8	78	86	0.09
SF 1-a	1	Open			2	58	60	0.03
SF 1-a	9	Open		1, 2, 3,* 4, 5, 7, 8	65	0	65	1.00
SF 1-a	7	Open			1	57	58	0.02
SF 1-a	10	Open			40	93	133	0.30
SF 1-a	15	RV 1A-a	1		4	86	90	0.04
RV 1A-a	1	SF 1-a	15		11	28	39	0.28
SF 1-b	5	CX 5	2		32	62	94	0.34
CX 5	2	SF 1-b	5		47	100	147	0.32
SF 1-ce	1	SF 4-c	1		22	63	85	0.26
SF 4-c	1	SF 1-ce	1		48	81	129	0.37
CX 2	1	CX 5	1		12	106	118	0.10
CX 5	1	CX 2	1		8	113	121	0.07
CX 4	2	CX 6	2		26	73	99	0.26
CX 5	3	CX 6	1		68	53	121	0.56
CX 6	1	CX 5	3		77	13	90	0.86
CX 7	1	Open			46	72	118	0.39
CX 8	2	Open			18	69	87	0.21
CX 2	2	Open			17	53	70	0.24

Table 6. Mean ratios of mature achenes to potential achenes produced by heads serving as females in crossing attempts by population. Att. = number of crossing attempts; n = number of successful crosses; Ratio = mean of the ratios of mature achenes/total number of disk florets (potential achenes) in successful crosses; Open = heads that were not bagged or deliberately crossed.

	Level of analysis and male parents																
Population	**Self-crosses**			**Among chunks (CA only)**			**Among pops. in CA or x CA**			**x S. Africa**			**x CX**			**Open**		
Female parent	**Att.**	**n**	**Ratio**	**Att.**	**n**	**Ratio**	**Att.**	**n**	**Ratio**	**Att.**	**n**	**Ratio**	**Att.**	**n**	**Ratio**	**Att.**	**n**	**Ratio**
CX series (F-1s)	2									2	1	0.32	10	5	0.32	7	3	0.28
GB	4	1	0.24	0			6	2	0.31	2	1	0.54	0			1		
RV	2			6			8			1	1	0.28	0			7		
TI	0			0			2			0			0			2		
TV	0			0			3			0			0			3	2	0.17
S. Africa	12						4	2	0.20	2	2	0.32	2	1	0.34	16	4	0.31

successful. Two crosses between South African individuals and F_1-generation individuals were attempted. One was successful.

The average ratio of the numbers of mature achenes to potential achenes (total number of disk florets) in a head was the same (0.29) for both plants from California (n = 13; SD 0.15) and from South Africa (n = 17; SD 0.279). The maximum ratio among Californians was 0.54 versus 0.93 for the South Africans.

Allelopathic Abilities

Table 7 lists the plants used to test allelopathic abilities of *Arctotheca prostrata*. A complete list of plants, their fresh (wet) and dry weights, and information concerning germination times for lettuce seeds are summarized in the Appendix, Table A-8. Raw data are given in the Appendix, Tables A-12 through A-15. Measurements made on fresh (wet) and dried samples for calculations of percent water and percent dry weight are given in the Appendix, Table A-16.

My results indicate that aqueous solutions of ground up leaves, runners, and roots of *Arctotheca prostrata* had an overall negative effect on the growth of lettuce seedling roots. Figure 9 compares six lettuce seedlings germinated in two extracts containing 2.0 g and 3.98 g fresh ground roots of *A. prostrata* plant TV 3-c. In general the higher the concentration of ground material, the greater its effect on limiting growth of the lettuce seedlings' roots. Ground root and leaf material with the same dry

Figure 9. Lettuce seedlings after 72 hours in extracts containing 2 g (left) and 3.98 g (right) fresh ground roots of *Arctotheca prostrata.*

weight from Californians appeared to have a greater effect on lettuce seedling root lengths than material from South Africans (Figures 10 and 11). Runners appeared to have a greater effect on limiting growth than roots and leaves (Figure 12).

One-way analysis of variance (ANOVA) tests indicated that there was high variability among some replicates and clones. Summaries of results of ANOVAs are given in the Appendix, Tables A-9, A-10, A-11. It was difficult to measure root lengths of less than 5 mm accurately. Statistical analyses were run with complete data sets and with values of less than 5 mm omitted. There were two changes in statistical significance among tests run with and without low values (Tables A-9 and A-11).

Figures 10 and 11 show mean lettuce seedling root lengths when grown in aqueous solutions of various concentrations derived from leaves and roots of *Arctotheca prostrata* plants. Low values were removed from data in these figures.

Table 7. *Arctotheca prostrata* plants contributing leaves (L), roots (Rt), and runners (Run) to test their allelopathic effects on the growth of lettuce seedling roots.

Gualala Bluff		Rodeo Valley		Tiburon		Tennessee Valley		South Africa	
Plant	**Part**	**Plant**	**Part**	**Plant**	**Part**	**Plant**	**Part**	**Plant**	**Part**
GB 4E-a	L	RV 5A-f	L	TI 1A-c	L	TV 3A-b	L	SF 1-d	L
GB 4E-a	Rt	RV 5A-g	L	TI 1A-c	Rt	TV 3A-c	L	SF 5-a	L
		RV 5A-f	Rt	TI 1A-a	Run	TV 3A-b	Rt	SF 1-d	Rt
		RV 5A-g	Rt			TV 3A-c	Rt	SF 5-a	Rt

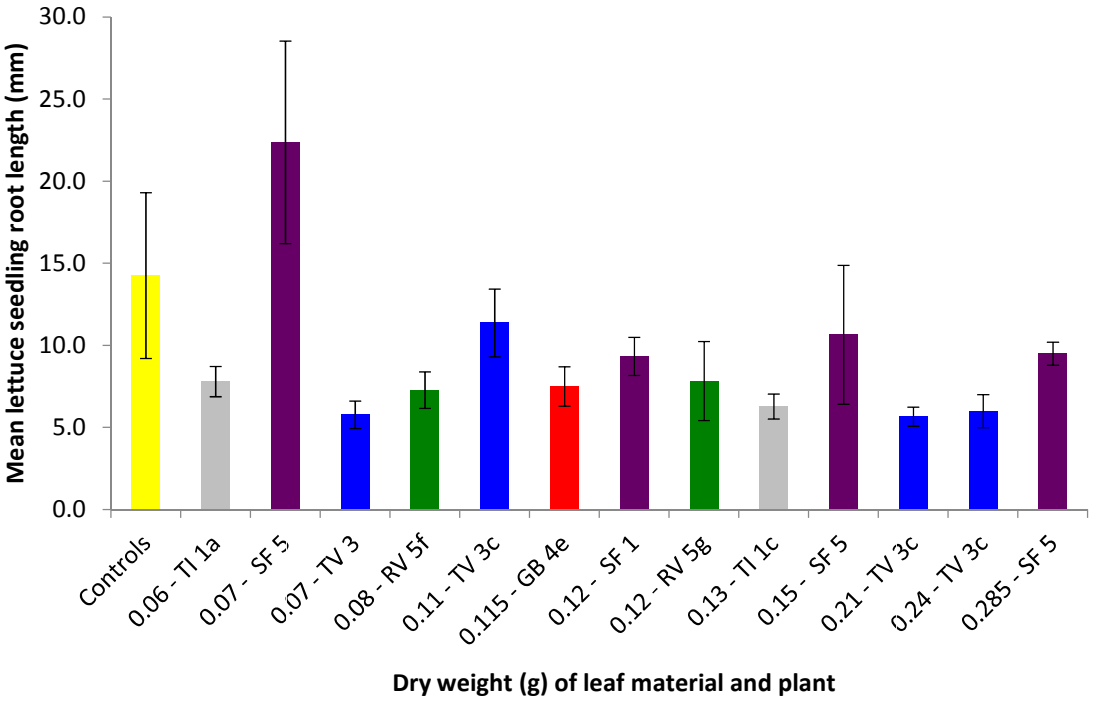

Figure 10. Mean length of lettuce seedling roots (mm) arranged by dry weight (g) of leaf material from *Arctotheca prostrata* in 10 mL of HPLC-grade water. Colors code for populations: yellow = controls, gray = TI, purple = SF, blue = TV, green = RV, and red = GB. Replicates within samples have been combined here. Dry weights of leaf material are shown on the x-axis with the plant name. A * above a bar indicates replicates within samples were significantly different ($p<0.05$). Bars without stars indicate no significant differences among replicates within samples or only one sample was available. Seedling root measurements of less than 5 mm have been omitted.

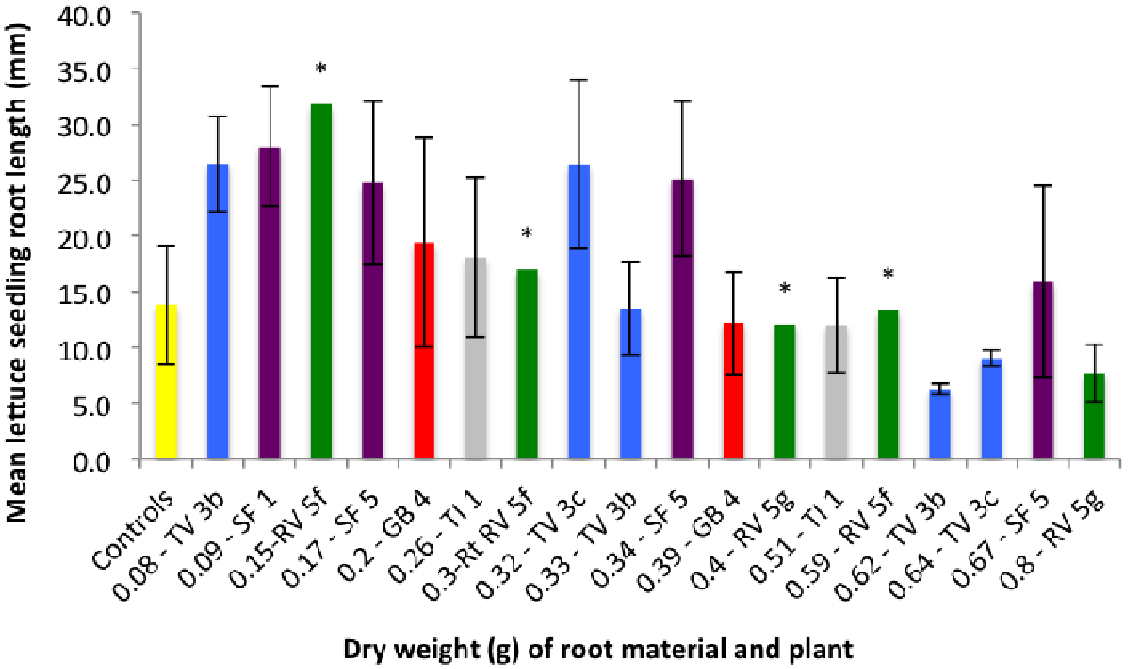

Figure 11. Mean length of lettuce seedling roots (mm) arranged by dry weight (g) of root material from *Arctotheca prostrata* in 10 mL of HPLC-grade water. Colors code for populations: yellow = controls, gray = TI, purple = SF, blue = TV, green = RV, and red = GB. Dry weights of root material are shown on the x-axis with the name of the plant. Replicates within samples have been combined here. A * above a bar indicates replicates within samples were significantly different ($p<0.05$). Bars without stars indicate no significant difference among replicates within samples or only one sample was available. Seedling root measurements of less than 5 mm have been omitted.

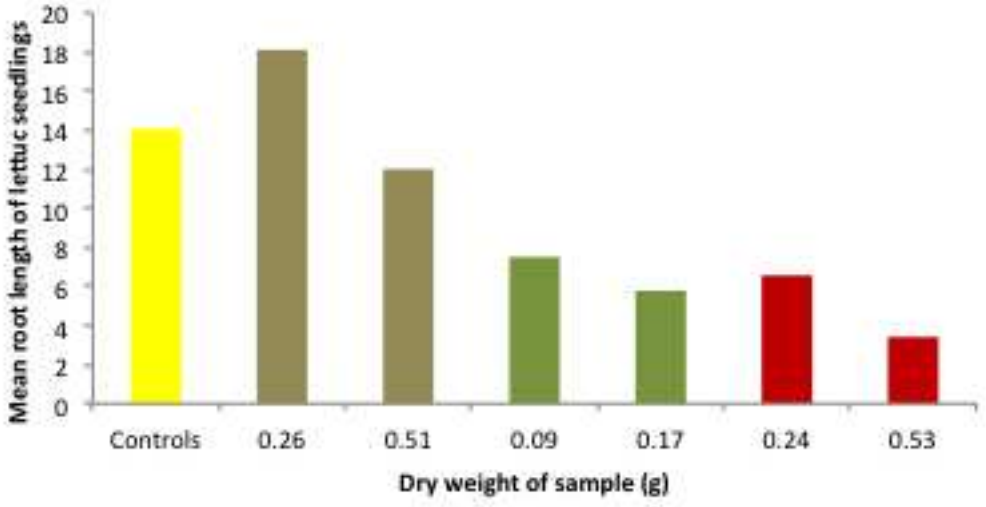

Figure 12. Mean lettuce seedling root lengths measured after 72 hours growth in root, leaf, and runner extracts from the Tiburon (TI) population of *Arctotheca prostrata*. Yellow bar = controls, brown bars = roots, green bars = leaves and red bars = runners.

Morphological measurements

Tables 8 and 9 summarize results from one-way ANOVAs of disk width and ray length, respectively. Analysis of variance found significant differences among replicates at the lowest levels for leaf width and leaf length. No further ANOVAs were carried out on leaf measurements. Raw data sets for disk widths, ray lengths, leaf widths, and leaf lengths are given in the Appendix, Tables A-5 and A-6.

Table 8. Summary of results of one-way ANOVAs on disk width measurements on *Arctotheca prostrata*. N values given in respective order. Significance level of $p<0.05$.

Level:	Within Rosette (among Clones)		
Rosette	**GB 5A**	**RV 5A**	**TI 1A**
Clones	b, d, e, f	e, f, g	a, e
n:	2, 1, 1, 2	2, 3, 5	2, 2
p-value:	0.80	0.40	0.33

Level:	Within Population (among Chunks)		
Population	**GB**	**RV**	**TV**
Chunk	1, 2, 4, 5	2, 3, 4, 5	1, 3
n:	3, 3, 2, 7	2, 3, 5, 8	2, 2
p-value:	0.41	0.40	0.33

Level:	Within Individual (among Clones)	Within Population (among Individuals)	Among Populations
Entity:	**SF 1**	**SF**	
Comparison:	a, b, c	1, 2, 3	GB, RV, SF*, TI, TV
n:	5, 3, 4	10, 3, 2	11, 14,10, 4, 4
p-value:	0.88	0.004	<0.001**

* Individual SF 2 was removed from this analysis because mean disk width measurements were significantly different from disks of SF 1 and SF 3.

** Pairwise comparisons indicate mean disk widths of Individuals SF 1 and 3 differ significantly from mean disk widths of all CA populations. Mean disk widths within CA populations do not differ significantly from each other.

Table 9. Summary of results of one-way ANOVAs on ray length measurements of *Arctotheca prostrata*. N-values given in respective order. Significance level of p<0.05.

Level:	Within Rosette (among Clones)		
Entity:	**GB 5A**	**RV 5A**	**TI 1A**
Comparison:	b, d, e, f	e, f, g	a, e
n:	3, 2, 2, 3	2, 3, 5	2, 2
p-value:	0.47	0.13	0.33

Level:	Within Population (among Chunks)		
Entity:	**GB**	**RV**	**TV**
Comparison:	1, 2, 4, 5	2, 3, 4, 5	1, 3
n:	3, 3, 2, 7	2, 3, 5, 8	2, 2
p-value:	0.38	0.56	1.00

Level:	Within Individual (among Clones)	Within Population (among Individuals)	Among Populations
Entity:	**SF 1**	**SF**	
Comparison:	a, b, c	1, 2, 3	GB*, RV, SF*, TI, TV
n:	5, 3, 4	10, 3, 2	12, 15, 13, 5, 5
p-value:	0.09	0.39	<0.001

* Pairwise comparisons indicate mean ray length of the SF population differ significantly from mean ray lengths of all CA populations except GB. Mean ray lengths within CA populations do not differ significantly from each other.

The average diameter of pollen grains from California plants was 18.8 micrometers (n = 150; SD 1.45). South African pollen had an average diameter of 20.4 micrometers (n = 150; SD 1.65). Table 10 gives results for analysis of variance at various levels for pollen grain diameter.

Table 10. Summary of results of one-way ANOVAs on pollen diameter measurements on *Arctotheca prostrata*. N values given in respective order. Significance level of $p<0.05$.

Level:	Within Clones/Individuals (between Heads)	
Clone:	**GB 1A-a**	**SF 1-a**
Heads:	1 and 2	2, 3,** 6, 7, 8
n:	24 in each*	25 in each
p-value:	0.490	<0.001

Level:	Within Populations (Between Chunks)	
Population:	**GB**	**RV**
Chunks:	1 and 4	4 and 5
n:	48, 25	25 each
p-value:	0.175	0.05

Level:	Within Population (among Individuals)	Within California (among Populations)	Between CA and SF
Entity:	**South Africans**	**Californians**	
Comparison:	1,** 2	**GB, TI, RV**	**CA (pooled) and SF**
n:	100, 25	73, 25, 50	148, 125
p-value:	<0.001	0.149	<0.001

* One value > 2 standard deviations from the mean removed from each data set.

** Pairwise comparisons indicated mean pollen diameter from head #3 was significantly different from mean pollen diameters from other heads, which did not differ from each other. Data from Head #3 were removed from all upper-level tests.

VI. DISCUSSION

Seed Germination

A one-way ANOVA indicated there was no significant difference in the number of days it took for seeds from California, South Africa, or F_1 individuals to germinate. The number of days varied among trials from 5 days to 296 days. This variability suggests seeds require some kind of signal, chemical reaction, or physical reaction to break out of dormancy.

Ellery and Chapman's (2000) study of seed dormancy in capeweed (*Arctotheca calendula*) indicated that species has complex seed dormancy mechanisms that adapt it to a Mediterranean climate with a prolonged dry season and unseasonable rains ("false breaks of season"). *Arctotheca calendula* is a very close relative of *A. prostrata.* Both species are adapted to the same environmental conditions. Strong seed dormancy mechanisms in *A. prostrata* would allow its seeds to germinate when conditions are favorable and to allow a large seed bank to build up.

Pollen Viability

More than 90% of the pollen produced by *Arctotheca prostrata* plants from California and South Africa appeared to be viable using the lactophenol cotton blue staining method. There was no significant difference between Californian and South African viable pollen percentages. Pollen inviability does not appear to be a problem for *Arctotheca prostrata* in California.

Crossing Experiments

Various combinations of *Arctotheca prostrata* plants were artificially crossed to test the fertility of plants from California. Only one of the self-crosses (a cross between two clones, GB 5A-b and GB 5A-e on July 18, 2012) was successful, which is not expected for outcrossing species (Table 6). Between July 16 and July 20, plants RV 4A-a, RV 5A-g, TV 3A-a, and SF 1-a had heads close to anthesis. During the summer, the plants were kept outside where pollinators had access to them. It is possible that contamination occurred prior to GB 5A-b's head being bagged and the cross with GB 5A-e's made.

Attempts to cross plants from different California populations (putative distinct individuals) succeeded (Tables 5 and 6) supporting my hypothesis that plants in California are fertile. Two plants from Gualala Bluff were crossed successfully with a plant from Rodeo Valley and a plant from Tiburon. Two open-pollinations occurred on two heads from a plant from Tennessee Valley. The heads were at anthesis on approximately June 2 and August 17 of 2012, so could not have fertilized each other. Achenes from one of these heads produced two F_1 offspring, although one died (Table 6). Plants from Gualala Bluff and Rodeo Valley were successfully crossed with two individuals from South Africa.

Six attempts to cross rosettes from different chunks within the Rodeo Valley, California population were made. No achenes developed from these crosses. This suggests that at least some of the "populations" in California consist of rosettes cloned from the same individual. If the species is outcrossing, as my results suggest, achenes

would not be expected to develop despite pollen moving from rosette to rosette. It would have been better if other among-chunk/within population crosses had been tested. However the crosses were determined by the availability of heads in anthesis at any given time.

On average, about 29% of the florets in a head produced viable achenes in successful crosses/open pollinations of Californian, South African, and F_1 generation individuals (Table 6). There was no significant difference between the number of achenes produced by South Africans and Californians. However, many crossing attempts failed. Not enough is known about the expected rate of pollination success and achene maturation for this species to say whether this is normal. Poor timing may have contributed to a lower-than-expected fertility rate. If pollen was immature or stigmas unreceptive, crossing attempts would have failed. Over the three seasons these experiments were carried out, I used a hand lens to look for receptive stigmas. I also crossed heads in various stages to help rule out problems with timing.

Arctotheca prostrata plants from California were found to be fertile under the conditions of this study. Controlled, hand-pollinated individuals and uncontrolled open pollinations produced fertile achenes. This species has been considered “sterile” in California. However, Mary Sue Ittner, one of the collectors for this study, noted what she thought were seedlings in the Gualala Bluff population. Naturalized populations are not common. Relatively few herbarium specimens have been made, suggesting the species has not been well studied. The species has been confused with the annual *A.*

calendula, which produces achenes abundantly. As a perennial, *A. prostrata* may rely less on seed production and more on its ability to spread aggressively by runners. My results suggest that *Arctotheca prostrata* is outcrossing, although a single putative crossing between two clones did produce achenes. It is possible that many, if not most, "populations" from California represents single clones. If a distance of 300 m is sufficient to isolate two populations of insect pollinated species (Raven et al. 2005), California's widely-spaced "populations" may almost never receive pollen from genetically distinct individuals.

Allelopathic Abilities

Lettuce seedling root lengths varied from about 3 mm to 44 mm. Often, the roots less than 5 mm long appeared stunted and compressed. They were difficult to measure accurately. While previous experiments did not show extracts of *Arctotheca prostrata* had an effect on lettuce seed germination rates (Gruber and Proctor 2012, Bechel and Proctor 2012) my records indicate that not all lettuce seeds had germinated within 24 hours (Appendix, Table A-7). It seems likely that seedlings with shorter roots germinated later than those with longer roots. However, it was not possible to determine whether this was so from these data.

Because of the difficulty of getting accurate short root measurements, one-way ANOVAs were run with and without lettuce seedling root lengths of less than 5 mm (Appendix, Tables A-9, A-10, and A-11). Running the data two ways resulted in two

different results. When all values were included the effects of 1 g fresh leaf material from the two South African individuals, SF 1 and SF 5, were significantly different but were not different when values less than 5 mm were omitted (Appendix, Table A-9). When all values were included the effects of 4 g fresh root material among three replicates of RV 5A-g were not significantly different but when values less than 5 mm were omitted, they were different (Appendix, Table A-11). In each case, there were many values less than 5 mm.

Observations on germination after 24 hours indicated some of the Petri dishes appeared to be drier than others. Some replicates were lost when dishes became too dry and the seedlings died (Appendix, Table A-8). Some potential causes for this can be ruled out. All of the samples were run at the same time. The same HPLC-grade water from the same container was used for all samples and controls, and the water was measured carefully. All samples were incubated at the same time together in the incubator and were rotated vertically on the shelves every day. However, they were not rotated horizontally. If one side of the incubator was slightly warmer than the other, dishes on that side may have dried faster.

Within sample/among replicate analyses: I did not expected to observe significantly different mean lettuce seedling root lengths at this level. As expected, mean root lengths of lettuce seedlings grown in leaf extracts from GB 4E-a (1 g wet weight) and in root extracts from RV 5A-f and RV 5A-g (4 g wet weight, all values included) and SF 5-a,

TV 3A-b, and TV 3A-c (2 g wet weight), and in runner extracts from TI 1A-a (1 and 2 g wet weight) were not significantly different. However, mean lettuce seedling root lengths from RV 5A-f and RV 5A-g in root extracts (1 and 2 grams of fresh weight) were significantly different at the among-replicate level. These differences may be due to several factors:

First, while all the seeds came from the same lot, there may be natural variation in the germination rates of seeds and the growth of the seedlings. Not all the seeds germinated within the first 24 hours. It is possible that seedling root length was inversely correlated with the time it took for roots to break out of the seed coat. A second possibility is that the Petri dishes did not maintain the same moisture level during the germination period. Limited water may have affected seed coat imbibition, germination rates, and root growth. All of the Petri dishes were washed and sterilized at 550 degrees Celsius for 1 hours and this process may have warped the glass causing some lids to fit tighter than others. Moisture may have been lost from dishes with poor-fitting lids.

Within rosettes (and chunks or individuals)/among clones: Variability of mean lettuce seedling root lengths among samples that tested differences among clones (within rosettes/chunks or individuals) was analyzed next. Again I expected that there would be no significant differences within rosettes/chunks from California or within individuals from South Africa. However, when comparing the effect of leaves from two clones, RV

5A-f with RV 5A-g, at 1 gram of wet root material there was a significant difference, with a p-value <0.001. The effect of roots from clones TV 3A-b and TV 3A-c were significantly different with a p-value <0.005. Again, this could be explained by variation in the lettuce seeds, or some samples being drier than others.

The age of the harvested *Arctotheca prostrata* plants varied and could be expected to have different effects on the lettuce seedlings. The ages of the original California plants are unknown. GB 4E-a and TI 1A-a represent original plants from California sent in March of 2011. The South African individuals were at least a year younger than the Californians and were germinated from seeds between April 2012 and September 2012 so their ages varied as much as 5 months. However, age can be ruled out as a factor in differences between the effects of clones RV 5A-g and RV 5A-f and clones TV 3A-b and TV 3A-c. In each case the clones were rooted at the same time, each pair from the same runners.

An important problem with comparing the effects of clones were the differences in dry weights of plant material for each set of clones at any particular wet weight class. While dry weights among replicates did not differ significantly, dry weights of leaf material for clones RV 5A-f and RV 5A-g at the 1 gram wet weight class were 0.12 and 0.19, respectively (Table A-8). (All values for root lengths for RV 5A-f were less than 5 mm so the data are not included in Figure 10.) Dry weights of root material for clones RV 5A-f and RV 5A-g at 4 gram wet weight class were 0.59 and 0.79, respectively (Table A-10, Figure 11). In this comparison, higher dry weight of root material did have a

greater effect on lettuce seedling root length. More difficult to explain are the effects of the very similar dry weights of root material from clones TV 3-b (0.33 g dry weight) and TV 3-c (0.32 g dry weight) at the 2 gram wet weight class. The mean length of lettuce seedling roots was 12.8 mm (SD = 2) when grown in an extract from TV 3-b but was 26.4 mm (SD = 2) when grown in an extract from TV3-c (Figure 11). As mentioned before, there was no age difference in the clones.

Measurements of lettuce seedling root lengths with means that differed significantly between clones were not combined for analysis at higher levels.

Within-population/among-individual analysis: Mean root lengths of lettuce seedlings grown in approximately 1g of fresh ground leaf material from individuals SF 1-d and SF 5-a were significantly different when all measurements were included ($p = .005$) but not significantly different when values of less than 5 mm were omitted ($p = 0.6$). Dry weights for the two samples, respectively were 0.15 and 0.12 grams, which are similar and probably comparable. Mean root lengths of lettuce seedlings grown in approximately 2 grams of fresh ground root material from SF 1-d and SF 5-a were not significantly different (Appendix, Table A-11).

Among Populations: Differences in the dry weights of leaf, runner, and root material in extracts and the high variability in mean lettuce seedling root lengths in among-replicates and among-clones tests prevented me from testing among-population effects.

Finding significant differences at lower levels of analysis, differences in dry weights of leaves, runners, and roots in samples of the same class, and different effects from the same dry weights in extracts, make it difficult to draw firm conclusions about the magnitude of the allopathic ability of *Arctotheca prostrata*. We can, however observe trends. Figures 8 and 9 show that the mean length of lettuce seedling roots generally decreases as the concentration of leaf and root material from *A. prostrata* increases. Populations of *A. prostrata* from California appear to have a greater allopathic effect than do those from South Africa. There is also limited evidence that extracts from leaves had the least effect, roots had a moderate effect, and runners had the most effect (Figure 12). However, only the Tiburon population was tested for all three parts due to the lack of runners from other populations at the time of harvesting.

Morphological Measurements

I considered the possibility that populations of *Arcotheca prostrata* in California were sterile horticultural hybrids or tetraploids. Measuring pollen grain diameters indicated that Californian pollen was, on average, slightly smaller than South African pollen. If *A. prostrata* in California was tetraploid, I would expect it to produce larger pollen. Even at the lowest levels, pollen grain diameters were not consistent. Potential variability in measurements could have occurred when the cover slide was placed on top of the stained pollen, which may have squashed it a bit.

Based on the results of one-way ANOVAs, disk widths and ray lengths

differentiated *Arctotheca prostrata* from South Africa and California. Mean disk widths were not significantly different at lower levels of analysis (among replicates, clones, and for the most part, individuals or chunks) but were different when Californian and South African plants were compared (Table 5). Ray length showed the same pattern with the exception that South African rays did not differ from Gualala Bluff rays. While the longer rays of Californian heads may be a sign that humans have chosen the showiest individuals for cultivation and plants with longer rays were brought to California, there is no evidence that these characters are due to heterosis or tetraploidy. The South African plants in this study came from seeds collected from a single population. This small sample does not allow me to draw any strong conclusions about the variability of disk width and ray lengths.

Leaf width and leaf length varied too much at lower levels of analysis to be combined in upper level analyses. Variability among replicates and clones could be due to several different factors. First this could be due to normal variation on leaf growth within individual rosettes. The varied leaf measurements may also be due to environmental conditions such as amount of nutrients available for growth and the amount of sunlight present. When plants receive less light, they may grow larger leaves to receive more sunlight for photosynthesis. These plants were kept within a relatively small area in the same location, but they were not rotated systematically. If a particular individual plant was short on nutrients this may have caused the plant to divert these nutrients to other parts of the plant or other function instead of leaf growth.

Summary/conclusion

This study supports my primary hypothesis that *Arctotheca prostrata* found in California is fertile and able to produce achenes/seeds that germinate to produce offspring. Currently, each isolated "population" of *A. prostrata* in California may consist of one individual that has spread vegetatively. If these populations become more numerous and occur closer together, pollen from one individual may begin to reach other individuals and fertile seeds may begin to be produced. This may be occurring now. Seed production will enable *A. prostrata* to spread more easily to new locations and to start more genetically-unique populations. California has the perfect climate for this species and if it begins to spread by seed it could begin to cause more ecological and economic harm.

My second hypothesis, that pollen from *Arctotheca prostrata* growing in California has lower pollen viability than pollen from South African plants, was not supported. Staining pollen with lacophenol cotton blue provided no evidence that pollen from California plants have reduced viability.

There is also no evidence that plants in California are sterile hybrid cultivars showing heterosis. While ray lengths of California plants were significantly longer than those of South African plants, without more measurements from South Africans, no conclusions can be drawn. There is also no evidence that Californians are tetraploids. Pollen grain diameters from South African plants are too similar in diameter to those of Californians to suggest differences in ploidy number.

My third hypothesis that *Arctotheca prostrata* may form dense clones due to allopathic abilities was supported. My preliminary results show that extracts of leaves, roots, and runners from *A. prostrata* plants reduced the growth of lettuce seedling roots and could have this effect on other species. If *A. prostrata* begins to spread by seeds in California, its allelopathic abilities may enable it to form very large clones very quickly.

To improve future experiments designed to assess allelopathic abilities, factors that made high variability at the replicate level must be minimized so that meaningful statistical analyses can be carried out.

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Table A-1. Population name, collector, date of collection, voucher specimen housed at the Darlene and William Radichel Herbarium (MANK), nation of origin, state/province, county, location, habitat description, and collection notes for *Arctotheca prostrata* plants used in this study.

Population	Collector	Date	Voucher	Nation	State/Prov.	County	Location	Habitat	Notes
Gualala Bluff	Ittner, M.S.	2011-04-04	Veit, J. 17	United States	California	Mendocino	Gualala Bluff Trail. Accessed from Highway 1 in downtown Gualala from the driveway of the Surf Motel.	Soil: very compacted, high clay content, some gravel. Open, sunny. Beekeeper in area says bees love this plant	Mostly spreads by runners, but seedlings also seen. Five chunks collected at least 5 feet from one another.
Rodeo Valley	Mahoney, A.	2011-07-06	Veit, J. 21	United States	California	Marin	Golden Gate National Recreation Area. Intersection of Bunker Rd and an unpaved gated road (Smith Road on the map), 0.8 miles west of intersection of Bunker Road and McCullough Rd and directly across from a stables	Population is about 8 square meter in a very dense, weedy patch with thistles, mustard, chickwed, etc. Ground very hard	Population will shortly be sprayed to eradicate
Tennessee Valley	Mahoney, A.	2011-07-06	Veit, J. 18	United States	California	Marin	Golden Gate National Recreation Area. Intersection of Tennesse Valley Trail and a short trail to a picnic area to right of trail sign on the north side of trail, less then 1/2 mile west of trail head	Population in a mowed area	With *Rubus* sp., *Equisetum* sp., Ferns, *Geranium* sp. Pop. about 1 m-sq.; will be sprayed to eradicate soon.
Tiburon	Muirhead, F	2011-24-28	Veit, J. 19; Muirhead, F. s.n.	United States	California	Marin	Tiburon, private residence. Along trail on hillside; GPS location: 37.90, 122.47		
South Africa	McKenzie, R.J.	12/15/11	Veit, J. 20	South Africa	Eastern Cape		Grahamstown		Seeds provided.

Table A-2. Names and origins of plants used in this study. An 'x' in columns labeled Cross, Allelo, Morph, and Pollen indicate this plant was used in crossing and allelopathic ability experiments, morphology measurements, and pollen viability assays and measurements.

Plant	Origin	Cross	Allelo	Morph	Pollen
CX 10	SF 2-a H:1 x GB 1A-a				
CX 11	SF 2-a H:1 x GB 1A-a				
CX 12	GB 4A-f H:1 x TI 1A-e				
CX 13	GB 4A-f H:1 x TI 1A-e				
CX 14	TV 1A-a H:2 / open				
CX 9	SF 2-a H:1 x GB 1A-a				
CX 1	SF 1-a H:4 / open	**x**			
CX 2	SF 1-a H:4 / open	**x**			
CX 3	SF 1-a H:4 / open				
CX 4	SF 1-a H:4 / open	**x**			
CX 5	SF 1-a H:4 / open	**x**			
CX 6	TV 1A-a H:2 / open	**x**			
CX 7	SF 1-a H:4 / open	**x**			
CX 8	SF 1-a H:4 / open	**x**			
GB 1A-a	Original	**x**		**x**	**x**
GB 1A-b	Attached to original				
GB 1A-c	Attached to original				
GB 2A-a	Original	**x**		**x**	
GB 2A-b	Attached to original				
GB 2A-c	Attached to original				
GB 3A-a	Original				
GB 3A-b	Attached to original				
GB 3C-a	Original, not attached to 3A and 3B	**x**			
GB 3C-d	GB 3C				
GB 3C-e	GB 3C				
GB 4A-a	Original				**x**
GB 4A-b	Attached to original				
GB 4A-c	Attached to original				
GB 4D-a	Original				
GB 4E-a	Original		**x**		
GB 4A-f	GB 4B	**x**		**x**	
GB 4A-g	GB 4B				
GB 5A-a	Original				
GB 5A-b	Attached to original	**x**		**x**	

Table A-2. *continued.* Names and origins of plants used in this study. An 'x' in columns labeled Cross, Allelo, Morph, and Pollen indicate this plant was used in crossing and allelopathic ability experiments, morphology measurements, and pollen viability assays and measurements.

Plant	Origin	Cross	Allelo	Morph	Pollen
GB 5A-c	voucher				
GB 5A-d	GB 5B			**x**	**x**
GB 5A-e	GB 5B	**x**		**x**	
GB 5A-f	GB 5B	**x**		**x**	
GB 5A-g	GB 5A				
RV 1A-a	Original	**x**			
RV 1A-b	RV 1				
RV 1A-c	RV 1				
RV 2A	Original				
RV 2B-a	Original	**x**		**x**	
RV 2C	Original				
RV 3A-a	Original				
RV 3A-b	RV 3	**x**		**x**	
RV 3A-c	RV 3				
RV 4A-a	Original; herbarium specimen	**x**			**x**
RV 4B-a	Original				
RV 4B-c	RV 4B				
RV 4B-d	RV 4B				
RV 5A-a	Original	**x**		**x**	
RV 5A-b	RV 5A				
RV 5A-c	RV 5A	**x**			
RV 5A-d	RV 5A	**x**			**x**
RV 5A-e	RV 5A	**x**		**x**	
RV 5A-f	RV 5A	**x**	**x**		
RV 5A-g	RV 5A	**x**	**x**		
RV 5A-h	RV 5A				
RV 5A-j	RV 5A				
SF 1-a	Seed	**x**			**x**
SF 1-b	SF 1	**x**			
SF 1-c	SF 1	**x**			
SF 1-d	SF 1		**x**		
SF 1-ce	SF 1C; clone of a clone	**x**			
SF 1-cf	SF 1C; clone of a clone				

Table A-2 *continued*. Names and origins of plants used in this study. An 'x' in columns labeled Cross, Allelo, Morph, and Pollen indicate this plant was used in crossing and allelopathic ability experiments, morphology measurements, and pollen viability assays and measurements.

Plant	Origin	Cross	Allelo	Morph	Pollen
SF 2-a	Seed	**x**			**x**
SF 3-a	Seed	**x**			
SF 4-a	Seed	**x**			
SF 4-b	SF 4A	**x**			
SF 4-c	SF 4A	**x**			
SF 5-a	Seed		**x**		
TI 1A-a	Original	**x**	**x**	**x**	
TI 1A-b	Ti 1A				
TI 1A-c	Ti 1A		**x**		**x**
TI 1A-d	Ti 1A	**x**			**x**
TI 1A-e	Ti 1A	**x**		**x**	**x**
TI 1A-bf	TI 1B; clone of a clone				
TI 1A-bg	TI 1B; clone of a clone				
TV 1A-a	TV 1A	**x**		**x**	
TV 1A-b	TV 1A				
TV 1A-c	TV 1A	**x**			
TV 1A-d	TV 1A				
TV 1A-e	TV 1A	**x**			
TV 1A-f	TV 1A				
TV 3A-a	Original	**x**		**x**	
TV 3A-b	TV 3		**x**		
TV 3A-c	TV 3		**x**		
TV 3A-d	TV 3				
TV 3A-e	TV 3				
TV 3A-ef	TV 3E; clone of a clone				

Table A-3. Viable and inviable pollen grain counts for *Arctotheca prostrata*, with total grains counted, percent viable and percent inviable calculated. Head No. indicates heads (acting as replicates) on the same plant.

Plant	Head No.	Viable	Inviable	Total	% Viable	% Inviable
GB 1A-a	1	366	13	379	96.57	3.43
GB 1A-a	2	72	3	75	96.00	4.00
GB 4A-a	1	158	9	167	94.61	5.39
GB 5A-d	2	73	10	83	87.95	12.05
RV 4A-a	1	124	18	142	87.32	12.68
RV 4A-a	2	114	9	123	92.68	7.32
RV 4A-a	3	180	4	184	97.83	2.17
RV 5A-d	1	270	9	279	96.77	3.23
SF 1-a	2	217	14	231	93.94	6.06
SF 1-a	3	136	6	142	95.77	4.23
SF 1-a	7	328	13	341	96.19	3.81
SF 1-a	8	407	5	412	98.79	1.21
SF 2-a	1	141	3	144	97.92	2.08
TI 1A-c	1	296	11	307	96.42	3.58
TI 1A-d	2	198	10	208	95.19	4.81
TI 1A-e	1	252	8	260	96.92	3.08

Table A-4. Pollen grain diameters in micrometers.

Plant	GB 1A-a	RV 5A-d	GB 4A-a	Ti 1A-c	RV 4A-a	GB 1A-a	SF 1-a	SF 1-a	SF 1-a	SF 1-a	SF 1-a	SF 2-a
Head	1	1	1	1	1	2	3	8	7	6	2	1
	21	23	20	18	20	20	19	20	23	20	20	20
	21	20	19	19	19	20	19	21	22	21	21	17
	18	20	20	20	20	17	20	23	20	20	21	19
	19	20	21	17	18	21	18	23	24	19	21	20
	15	19	15	17	19	20	17	22	23	19	20	20
	18	19	18	18	17	20	20	21	25	21	22	19
	16	20	19	20	18	19	20	20	21	22	22	19
	18	19	18	16	18	19	20	22	20	22	21	19
	19	20	20	18	18	20	19	22	20	21	21	19
	20	19	19	19	16	19	19	21	21	20	20	21
	19	20	18	19	17	21	19	20	20	20	21	20
	20	19	19	20	16	21	20	22	20	23	19	21
	20	18	19	19	17	19	18	22	20	20	23	20
	19	20	19	16	19	21	19	22	22	20	20	18
	18	18	19	19	17	19	19	20	22	22	20	19
	19	19	19	20	20	24	20	21	21	20	20	20
	18	19	20	19	18	21	19	22	21	25	23	18
	20	18	18	20	20	19	19	20	20	20	21	18
	18	18	19	16	20	19	19	22	19	21	21	19
	19	19	16	16	18	20	16	21	20	18	18	18
	18	19	18	19	16	18	19	23	25	19	20	18
	17	19	18	18	18	19	17	20	23	20	20	19
	19	18	18	19	19	18	19	21	24	20	20	18
	16	17	18	19	20	17	18	20	24	20	20	17
	20	18	20	18	19	21	20	21	25	21	20	21
Mean:	**18.6**	**19.1**	**18.7**	**18.4**	**18.3**	**19.7**	**18.9**	**21.3**	**21.8**	**20.6**	**20.6**	**19.1**

Table A-5. Disk width and ray length measurements and means in mm for selected *Arctotheca prostrata* plants.

	Disk Width					Ray Length				
	Measurements				Mean	Measurements				Mean
Plant	**1**	**2**	**2**	**4**		**1**	**2**	**3**	**4**	
GB 1A-a	1.2	0.8			1.0	2.5	2.2			2.4
GB 2A-a	0.9	0.8			0.9	2.0	2.3			2.2
GB 4A-f	1.2				1.2	2.5				2.5
GB 5A-b	0.8	1.3			1.1	2.2	2.2			2.2
GB 5A-d	1.5				1.5	2.4				2.4
GB 5A-e	1.0				1.0	2.3				2.3
GB 5A-f	1.1	0.9			1.0	2.3	2.2			2.3
RV 2B-a	1.0				1.0	2.2				2.2
RV 3A-b	0.9	1.0			1.0	2.2	2.4			2.3
RV 4A-a	0.9	1.2	1.1	1.1	1.1	2.0	2.3	2.3	2.8	2.3
RV 5A-e	1.1				1.1	2.1				2.1
RV 5A-f	0.8	1.1			1.0	2.1	2.6			2.4
RV 5A-g	1.0	1.1	0.9	0.9	1.0	2.5	3.0	2.4	2.7	2.7
SF 1-a	1.5	1.6	1.5	1.6	1.5	1.6	1.1	1.1	1.4	1.3
SF 2-a	1.9	2.0			2.0	1.8	1.1			1.5
SF 1-b	1.7	1.2			1.5	2.5	1.8			2.2
SF 1-c	1.7	1.4	1.2		1.4	2.2	2.0	1.3		1.8
SF 3-a	1.2				1.2	2.0				2.0
TI 1A-a	0.9	1.1			1.0	3.4	3.4			3.4
TI 1A-e	1.1	1.2			1.2	2.4	2.4			2.4
TV 3A-a	1.0	0.9			1.0	2.5	2.6			2.6
TV 1A-a	1.3	1.1			1.2	2.2	2.7			2.5

Table A-6. Leaf width and leaf length measurements and means in mm for selected *Arctotheca prostrata* plants.

	Leaf Width				Leaf Length			
	Measurements				Measurements			
Plant	**1**	**2**	**2**	**mean**	**1**	**2**	**3**	**Mean**
CX 10	4.8	4.9	4.4	4.7	20.6	21.3	20.2	20.70
CX 11	4.4	4.8	5.2	4.8	15.9	15.9	19.1	17.0
GB 1A-a	1.5	1.5	1.6	1.5	4.3	6.1	3.7	4.7
GB 2A-a	1.5	1.8	1.6	1.6	4.8	5.7	5.1	5.2
GB 4A-f	2.6	2.4	2.8	2.6	5.6	7.1	7.2	6.6
GB 5A-b	3.0	2.3	1.8	2.4	8.9	7.9	7.9	8.2
GB 5A-d	3.1	3.4	2.8	3.1	8.5	8.7	8.1	8.4
GB 5A-e	2.7	2.4	2.6	2.6	6.1	6.8	6.4	6.4
GB 5A-f	2.7	2.7	2.0	2.5	7.1	6.0	4.0	5.7
RV 2B-a	2.9	2.7	2.0	2.5	8.5	7.2	5.1	6.9
RV 3A-b	2.2	2.7	2.3	2.4	7.7	7.5	5.7	7.0
RV 4A-a	3.1	2.7	3.2	3.0	9.7	9.2	10.6	9.8
RV 5A-e	2.7	2.9	2.2	2.6	6.5	7.7	6.2	6.8
RV 5A-f	2.9	2.2	1.7	2.3	7.2	5.7	4.3	5.7
RV 5A-g	2.6	1.3	1.9	1.9	6.3	5.1	5.2	5.5
SF 1-a	4.6	4.5	4.3	4.5	14.8	14.5	14.7	14.7
SF 2-a	2.4	2.2	2.3	2.3	7.8	6.6	8.3	7.6
SF 1-b	2.9	2.7	2.7	2.8	14.7	13.7	13.9	14.1
SF 1-c	2.6	2.8	3.4	2.9	8.1	8.4	9.4	8.6
SF 3-a	3.6	3.0	3.3	3.3	12.7	12.1	11.1	12.0
TI 1A-a	1.5	1.7	1.9	1.7	7.1	5.2	5.8	6.0
TI 1A-e	3.2	2.7	2.6	2.8	7.8	7.6	6.2	7.2
TV 3A-a	2.6	2.5	2.6	2.6	8.5	8.4	7.3	8.1
TV 1A-a	3.2	3.3	3.1	3.2	10.7	9.9	12.1	10.9

Table A-7. Crossing attempts and open pollinations between/among *Arctotheca prostrata* plants. R = reciprocal crosses, Ach. = achenes produced. Self, among clone, and within population (among chunks) crosses are confirmed or likely crosses between the same individuals (shaded in gray). A 'B' in the Self column indicates a bagged head. Other combinations are confirmed or likely crosses between different individuals. Total outcrosses gives total deliberate outcrossing attempts. Open pollination dates are

Female	Head no.	Male	Head no.	Date	R.	Ach.	Self or among clones	W/in pop. (CA)	CA x CA	CA x SF	CX x CA	CX x CX	CX x SF	SF x CA	SF x CX	SF x SF	Open	Total out-crosses
CX 1	1	CX 4	5	07/06/13	x							x						x
CX 1	2	CX 8	1	07/24/13	x							x						x
CX 2	1	CX 5	1	05/17/13	x	12						x						x
CX 2	2	Open		06/01/13		17											x	
CX 2	3	Open		07/13/13													x	
CX 4	1	SF 1-b	5	05/31/13	x								x					x
CX 4	2	CX 6	2	07/06/13	x	26						x						x
CX 4	3	CX 1	1	07/06/13	x							x						x
CX 4	4	Open		06/30/13													x	
CX 4	5	Open		07/02/13													x	
CX 5	1	CX 2	1	05/17/13	x	8						x						x
CX 5	2	SF 1-b	5	06/26/13	x	47							x					x
CX 5	3	CX 6	1	07/04/13	x	68						x						x
CX 6	1	CX 5	3	07/04/13	x	77						x						x
CX 6	2	CX 4	2	07/06/13	x							x						x
CX 7	1	Open		06/07/13		46											x	
CX 7	2	Open		06/07/13													x	
CX 7	3	CX 7	4	07/08/13	x		x											
CX 7	4	CX 7	3	07/08/13	x		x											
CX 8	1	CX 1	2	07/24/13	x							x						x
CX 8	2	Open		08/01/13		18											x	
GB 1A-a	1	RV 4A-a	3	07/13/12	x	21			x									x
GB 1A-a	2	SF 2-a	2	08/20/12	x	30				x								x

Table A-7 *continued.* Crossing attempts and open pollinations between/among *Arctotheca prostrata* plants. R = reciprocal crosses, Ach. = achenes produced. Self, among clone, and within population (among chunks) crosses are confirmed or likely crosses between the same individuals (shaded in gray). A 'B' in the Self column indicates a bagged head. Other combinations are confirmed or likely crosses between different individuals. Total outcrosses gives total deliberate outcrossing attempts. Open pollination dates are approximate.

Female	Head no.	Male	Head no.	Date	R.	Ach.	Self or among clones	W/in pop. (CA)	CA x CA	CA x SF	CX x CA	CX x CX	CX x SF	SF x CA	SF x CX	SF x SF	Open	Total out-crosses
GB 2A-a	1	SF 1-a	5	08/05/12	x					x								x
GB 2A-a	2	RV 5A-g	3	08/27/12	x				x									x
GB 3C-a	1	Self		08/30/11			B											
GB 3C-a	2	TV 3A-a	1	09/08/11					x									x
GB 4A-f	1	TI 1A-e	1	06/27/12	x	10			x									x
GB 5A-b	1	GB 5A-f	1	07/16/12	x		x											
GB 5A-b	2	GB 5A-e	1	07/18/12			x											
GB 5A-d	1	Open		08/01/12													x	
GB 5A-e	1	GB 5A-b	2	07/18/12		12	x											
GB 5A-f	1	GB 5A-b	1	07/16/12	x		x											
GB 5A-f	2	RV 5A-f	1	07/23/12	x				x									x
GB 5A-f	3	TV 3A-a	6	07/23/12	x				x									x
RV 1A-a	1	SF 1-a	15	01/22/13	x	11				x								x
RV 2B-a	1	RV 3A-b	1	07/24/12	x			x										
RV 3A-b	1	RV 2B-a	1	07/24/12	x			x										
RV 3A-b	2	RV 5A-f	2	08/05/12	x			x										
RV 4A-a	1	TV 3A-a	4	09/22/11					x									x
RV 4A-a	2	TI 1A-c	1	10/26/11					x									x
RV 4A-a	3	GB 1A-a	1	07/13/12	x				x									x
RV 4A-a	4	Open		07/16/12													x	
RV 4A-a	5	TV 3A-a	5	07/16/12	x				x									x

Table A-7 *continued.* Crossing attempts and open pollinations between/among *Arctotheca prostrata* plants. R = reciprocal crosses, Ach. = achenes produced. Self, among clone, and within population (among chunks) crosses are confirmed or likely crosses between the same individuals (shaded in gray). A 'B' in the Self column indicates a bagged head. Other combinations are confirmed or likely crosses between different individuals. Total outcrosses gives total deliberate outcrossing attempts. Open pollination dates are approximate.

Female	Head no.	Male	Head no.	Date	R.	Ach.	Self or among clones	W/in pop. (CA)	CA x CA	CA x SF	CX x CA	CX x CX	CX x SF	SF x CA	SF x CX	SF x SF	Open	Total out-crosses
RV 4A-a	6	RV 5A-e	2	07/24/12	x			x										
RV 5A-c	1	Self		09/06/11			B											
RV 5A-c	2	TV 3A-a	3	09/13/11	x				x									x
RV 5A-c	2	Self		09/15/11			B											
RV 5A-d	1	TI 1A-d	2	10/06/11	x				x									x
RV 5A-e	1	Open		07/12/12													x	
RV 5A-e	2	RV 4A-a	6	07/24/12	x			x										
RV 5A-f	1	GB 5A-f	2	07/23/12	x				x									x
RV 5A-f	2	RV 3A-b	2	08/05/12	x			x										
RV 5A-g	1	Open		07/16/12													x	
RV 5A-g	2	Open		07/23/12													x	
RV 5A-g	3	GB 2A-a	2	08/27/12	x				x									x
RV 5A-g	5	Open		07/25/12													x	
RV 5A-g	6	Open		08/01/12													x	
RV5A-g	4	Open		07/24/14													x	
SF 1-a	1	Open		06/30/12		2											x	
SF 1-a	2	Open		06/30/12													x	
SF 1-a	3	Open		06/30/12													x	
SF 1-a	4	Self		07/11/12			B											

Table A-7 *continued.* Crossing attempts and open pollinations between/among *Arctotheca prostrata* plants. R = reciprocal crosses, Ach. = achenes produced. Self, among clone, and within population (among chunks) crosses are confirmed or likely crosses between the same individuals (shaded in gray). A 'B' in the Self column indicates a bagged head. Other combinations are confirmed or likely crosses between different individuals. Total outcrosses gives total deliberate outcrossing attempts. Open pollination dates are approximate.

Female	Head no.	Male	Head no.	Date	R.	Ach.	Self or among clones	W/in pop. (CA)	CA x CA	CA x SF	CX x CA	CX x CX	CX x SF	SF x CA	SF x CX	SF x SF	Open	Total out-crosses
SF 1-a	5	Open		07/20/12													x	
SF 1-a	6	GB 2A-a	1	08/05/12	x									x				x
SF 1-a	7	Open		08/05/12		1											x	
SF 1-a	8	Self		08/05/12			B											
SF 1-a	9	Open		09/17/12		65											x	
SF 1-a	10	Open		09/18/12		40											x	
SF 1-a	11	Open		09/20/12													x	
SF 1-a	12	Open		12/30/12													x	
SF 1-a	13	SF 1-b	1	12/30/12	x		x											
SF 1-a	14	SF 1-c	8	01/09/13	x		x											
SF 1-a	15	RV 1A-a	1	01/22/13	x	4								x				x
SF 1-b	1	SF 1-a	13	12/30/12	x		x											
SF 1-b	2	SF 1-c	2	01/15/13	x		x											
SF 1-b	3	CX 4	1	05/31/13	x										x			x
SF 1-b	4	Open		06/12/13													x	
SF 1-b	5	CX 5	2	06/26/13	x	32									x			x
SF 1-c	1	Self		07/21/12			B											
SF 1-c	2	Self		08/02/12			B											
SF 1-c	3	Open		08/10/12													x	
SF 1-c	4	Self		09/05/12			B											
SF 1-c	5	Open		09/14/12													x	
SF 1-c	6	Self		09/15/12			B											
SF 1-c	8	SF 1-a	14	01/09/13	x		x											

Table A-7 *continued.* Crossing attempts and open pollinations between/among *Arctotheca prostrata* plants. R = reciprocal crosses, Ach. = achenes produced. Self, among clone, and within population (among chunks) crosses are confirmed or likely crosses between the same individuals (shaded in gray). A 'B' in the Self column indicates a bagged head. Other combinations are confirmed or likely crosses between different individuals. Total outcrosses gives total deliberate outcrossing attempts. Open pollination dates are approximate.

Female	Head no.	Male	Head no.	Date	R.	Ach.	Self or among clones	W/in pop. (CA)	CA x CA	CA x SF	CX x CA	CX x CX	CX x SF	SF x CA	SF x CX	SF x SF	Open	Total out-crosses
SF 1-c	9	SF 1-b	2	01/15/13	x		x											
SF 1-ce	1	SF 4-c	1	06/19/13	x	22										x		x
SF 1-ce	2	Open		06/14/13													x	
SF 2-a	1	Open		07/04/12													x	
SF 2-a	2	GB 1A-a	2	08/20/12	x	33								x				x
SF 4-a	1	Open		08/17/12													x	
SF 4-b	1	Open		06/17/13													x	
SF 4-c	1	SF 1-ce	1	06/19/13	x	48										x		x
TI 1A-d	2	RV 5A-d	1	10/06/11	x				x									x
TI 1A-e	2	GB 4A-f	2	06/27/12	x				x									x
TI 1A-e	3	Open		07/23/12													x	
TI 1A-e	4	Open		07/30/12													x	
TV 1A-a	1	Open		06/02/12		8											x	
TV 1A-a	2	Open		08/17/12		19											x	
TV 1A-a	3	Open		09/10/12													x	
TV 3A-a	3	RV 5A-c	2	09/13/11	x				x									x
TV 3A-a	5	RV 4A-a	5	07/16/12	x				x									x
TV 3A-a	6	GB 5A-f	3	07/23/12	x				x									x

Table A-8. Parts of *Arctotheca prostrata* plants used in allelopathy experiments. Part (L= leaf, Rt = root, Run = runner), Class = approximate fresh weight (g), Wet wt = actual weight (g) of fresh material, mean % water for samples (see Table A-16), dry weight (g) of sample, and numbers of germinated lettuce seeds at 24 hours and 72 hours. The last column gives the number of seedling roots that were less than 5 mm long at the end of the experiment.

Part	Plant	Class (g)	Wet wt (g)	Mean % water	Dry wt (g)	24 hrs	72 hrs	Values <5
L	**RV 5A-f**	0.5	0.45	82.3	0.08	7	8	1
L	**SF 5-a-1**	0.5	0.5	85.7	0.07	7	8	0
L	**TI 1A-a**	0.5	0.51	87.3	0.06	11	10	0
L	**TV 3A-c**	0.5	0.55	80.4	0.11	6	11	3
L	**GB 4E-a-1**	1	0.99	87.9	0.12	0	10	5
L	**GB 4E-a-2**	1	0.94	87.9	0.11	0	8	5
L	**RV 5A-f-1**	1	1.05	82.3	0.19	2	8	8
L	**RV 5A-f-2**	1	1.05	82.3	0.19	3	8	8
L	**RV 5A-g-1**	1	0.99	87.6	0.12	5	9	2
L	**RV 5A-g-2**	1	0.97	87.6	0.12	7	10	1
L	**SF 1-d**	1	1.02	87.8	0.12	2	9	6
L	**SF 5-a-1**	1	1.06	85.7	0.15	3	10	1
L	**SF 5-a-2**	1	1.07	85.7	0.15	2	9	0
L	**SF 5-a-3**	1	1.04	85.7	0.15	1	8	0
L	**TI 1A-c**	1	1.01	87.3	0.13	7	9	2
L	**TV 3A-b**	1	0.85	92.0	0.07	9	9	0
L	**TV 3A-c**	1	1.09	80.4	0.21	2	6	3
L	**SF 5-a-1**	2	2.05	85.7	0.29	0	6	4
L	**SF 5-a-2**	2	1.98	85.7	0.28	0	6	6
L	**TV 3A-c**	2	1.86	87.3	0.24	?	6	3
Rt	**TV 3A-b**	0.5	0.51	84.4	0.08	10	10	0
Rt	**GB 4E-a**	1	1.05	81.4	0.20	7	10	1
Rt	**RV 5A-f-1**	1	0.99	85.3	0.15	9	10	0
Rt	**RV 5A-f-2**	1	0.99	85.3	0.15	10	10	0
Rt	**RV 5A-f-3**	1	0.99	85.3	0.15	9	10	0
Rt	**SF 1-d-2**	1	1.06	91.7	0.09	8	8	0
Rt	**TI 1A-a-1**	1	0.99	74.1	0.26	8	10	0
Rt	**TI 1A-a-2**	1	1.01	74.1	0.26	10	10	0
Rt	**GB 4E-a**	2	2.07	81.4	0.38	3	7	2
Rt	**RV 5A-f-1**	2	2.00	85.3	0.29	5	10	0
Rt	**RV 5A-f-2**	2	1.96	85.3	0.29	7	8	0
Rt	**RV 5A-f-3**	2	2.04	85.3	0.30	7	9	0
Rt	**RV 5A-g-1**	2	2.00	80.4	0.39	9	10	0
Rt	**RV 5A-g-2**	2	2.05	80.4	0.40	8	8	0
Rt	**RV 5A-g-3**	2	2.00	80.4	0.39	8	8	0

Table A-8 *continued*. Parts of *Arctotheca prostrata* plants used in allelopathy experiments. Part (L= leaf, Rt = root, Run = runner), Class = approximate fresh weight (g), Wet wt = actual weight (g) of fresh material, mean % water for samples (see Table A-16), dry weight (g) of sample, and numbers of germinated lettuce seeds at 24 hours and 72 hours. The last column gives the number of seedling roots that were less than 5 mm long at the end of the experiment.

Part	Plant	Class (g)	Wet wt (g)	Mean % water	Dry wt (g)	24 hrs	72 hrs	Values <5
Rt	**SF 1-d-1**	2	2.05	91.7	0.17	8	8	0
Rt	**SF 5-a-1**	2	2.04	83.4	0.34	8	9	0
Rt	**SF 5-a-2**	2	2.03	83.4	0.34	8	8	0
Rt	**SF 5-a-3**	2	2.05	83.4	0.34	7	8	0
Rt	**TI 1A-c**	2	1.95	74.1	0.51	8	10	0
Rt	**TV 3A-b-1**	2	2.08	84.4	0.33	4	7	0
Rt	**TV 3A-b-2**	2	2.05	84.4	0.32	6	8	1
Rt	**TV 3A-c-1**	2	2.00	84.0	0.32	8	8	0
Rt	**TV 3A-c-2**	2	2.04	84.0	0.33	4	9	0
Rt	**GB 4E-a**	4	3.99	81.4	0.74	2	7	7
Rt	**RV 5A-f-1**	4	3.99	85.3	0.59	6	9	0
Rt	**RV 5A-f-2**	4	4.03	85.3	0.59	6	8	0
Rt	**RV 5A-g-1**	4	4.00	80.4	0.78	8	9	4
Rt	**RV 5A-g-2**	4	4.02	80.4	0.79	6	10	6
Rt	**RV 5A-g-3**	4	4.05	80.4	0.79	9	10	3
Rt	**SF 5-a**	4	3.99	83.4	0.66	6	9	0
Rt	**TV 3A-b**	4	3.95	84.4	0.62	1	6	2
Rt	**TV 3A-c**	4	3.98	84.0	0.64	5	10	5
Run	**TI 1A-a-1**	1	1.02	76.5	0.24	8	9	0
Run	**TI 1A-a-2**	1	1.08	76.5	0.25	6	10	4
Run	**TI 1A-a-1**	2	2.06	76.5	0.48	2	8	8
Run	**TI 1A-a-2**	2	1.97	76.5	0.46	3	10	6

Table A-9. Summary of results of analysis of variance tests on the mean length of lettuce seedling roots growing in aqueous solutions of ground leaves of *Arctotheca prostrata* plants from California and South Africa. Approximate wet and dry weights of ground *A. prostrata* leaves, test subjects, n for each subject, p-values and levels tested. Gray shading indicates results that differed with and without root measurements less than 5 mm.

LEAF Weights (g)		Test subjects			All data included		Values < 5mm omitted		Test Level	
Wet	Dry	1	2	3	n	p-value	n	p-value	Within	Among
1	0.12	GB 4E-a-1	GB 4E-a-2		10, 8	0.576			Clone	Replicates
1	0.19 / 0.12	RV 5A-f-1	RV 5A-f-2		8, 8	1			Clone	Replicates
1	0.19	RV 5A-g	RV 5A-f		19, 8	<0.001			Rosette	Clones
1	0.12	RV 5A-g-1	RV 5A-g-2		9, 10	0.112			Clone	Replicates
1	0.15 / 0.12	SF 5	SF 1		27, 9	0.005	26, 3	0.6	Population	Individuals
1	0.15	SF 5-a-1	SF 5-a-2	SF 5-a-3	10, 9, 8	0.254			Individual	Replicates
2	0.29	SF 5-a-1	SF 5-a-2		6, 6	0.156			Individual	Replicates

Table A-10. Summary of results of analysis of variance tests on the mean length of lettuce seedling roots growing in aqueous solutions of ground runners of *Arctotheca prostrata* plants from California and South Africa. Approximate wet and dry weights of ground *A. prostrata* runners, test subjects, n for each subject, p-values and levels tested. Gray shading indicates results that differed with and without root measurements less than 5 mm.

RUNNER Weights (g)		Test subjects			All data included		Values < 5mm omitted		Test Level	
Wet	Dry	1	2	3	n	p-value	n	p-value	Within	Among
1	0.25	TI 1A-a-1	TI 1A-a-2		8, 10	0.13			Clone	Replicates
2	0.47	TI 1A-a-1	TI 1A-a-2		9, 9	0.24			Clone	Replicates

Table A-11. Summary of results of analysis of variance tests on the mean length of lettuce seedling roots growing in aqueous solutions of ground roots of *Arctotheca prostrata* plants from California and South Africa. Approximate wet and dry weights of ground *A. prostrata* roots, test subjects, n for each subject, p-values and levels tested. Gray shading indicates results that differed with and without root measurements less than 5 mm.

ROOT Weights (g)		Test subjects			All data included		Values < 5mm omitted		Test Level	
Wet	Dry	1	2	3	n	p-value	n	p-value	Within	Among
1	0.15	RV 5A-f-1	RV 5A-f-2	RV 5A-f-3	12, 11, 12,	0.008			Clone	Replicates
1	0.26	TI 1A-a-1	TI 1A-a-2				10, 10	0.812	Clone	Replicates
2	0.29	RV 5A-f-1	RV 5A-f-2	RV 5A-f-3	11, 10, 9	<0.001	7, 10, 9	0.009	Clone	Replicates
2	0.4	RV 5A-g-1	RV 5A-g-2	RV 5A-g-3	10, 8, 10	0.007			Clone	Replicates
2	0.34 / 0.17	SF 5	SF 1		26, 9	0.809			Population	Individuals
2	0.34	SF 5-a-1	SF 5-a-2	SF 5-a-3	9, 8, 8	0.107			Individual	Replicates
2	0.32	TV 3A-b-1	TV 3A-b-2		8, 9	0.338	7, 7	0.084	Clone	Replicates
2	0.32	TV 3A-c	TV 3A-b		16, 15	<0.001	16, 14	<0.001	Rosette	Clones
2	0.32	TV 3A-c-1	TV 3A-c-2		8, 8	0.161			Clone	Replicates
4	0.59	RV 5A-f-1	RV 5A-f-2		9, 8	0.403			Clone	Replicates
4	0.79 / 0.59	RV 5A-g	RV 5A-f		29, 17	<0.001	12, 17	0.007	Rosette	Clones
4	0.79	RV 5A-g-1	RV 5A-g-2	RV 5A-g-3	9, 10, 10	0.182	5, 4, 7	0.004	Clone	Replicates
4	0.63	TV 3A-b	TV 3A-c		7, 10	0.35			Rosette	Clones

Table A-12. Measurements on 72-hour-old lettuce seedling roots growing in aqueous solutions containing various amounts of ground leaf material from *Arctotheca prostrata* plants. Class indicates approximate fresh (wet) weights, Plant names indicate Population, Chunk or Individual, Clone, and Replicate number. Means and sample standard deviations were calculated by Excel; Wet Wt. gives the exact weight of fresh leaf material; dry weights (Dry Wt.) were calculated using mean percent water content of leaves (see Table A-16).

Population	**South Africa**						
Class	**0.5 g**	**1 g**	**1 g**	**1 g**	**2 g**	**2 g**	**1 g**
Plant	**SF 5-a-1**	**SF 5-a-1**	**SF 5-a-2**	**SF 5-a-3**	**SF 5-a-1**	**SF 5-a-2**	**SF 1-d-1**
	29	18	14	18	10	4	10
	27	17	12	14	9	4	10
	26	15	12	14	4	4	8
	25	14	11	6	4	3	4
	24	13	11	5	3	3	4
	22	12	9	5	3	3	4
	14	11	6	5			3
	12	10	6	5			3
		8	6				3
		4					
Mean	22.4	12.2	9.7	9.0	5.5	3.5	5.4
St Dev-s	6.2	4.2	3.0	5.4	3.1	0.5	3.0
Wet Wt. (g)	0.50	1.06	1.07	1.04	2.05	1.98	1.02
Dry Wt. (g)	0.19	0.15	0.15	0.15	0.29	0.28	0.12

Population	**Gualala Bluff**	
Class	**1 g**	**1 g**
Plant	**GB 4E-a-1**	**GB 4E-a-2**
	9	9
	8	7
	8	6
	7	4
	6	4
	4	4
	4	3
	4	3
	3	
	3	
Mean	5.6	5.0
St Dev-s	2.3	2.1
Wet Wt. (g)	0.99	0.94
Dry Wt. (g)	0.12	0.11

Tiburon	
0.5 g	**1 g**
TI 1A-a	**TI 1A-c**
9	7
9	7
8	7
8	6
8	6
8	6
8	5
7	4
7	4
6	
7.8	5.8
0.9	1.2
0.5	1.01
0.06	0.13

Table A-12 *continued*. Measurements on 72-hour-old lettuce seedling roots growing in aqueous solutions containing various amounts of ground leaf material from *Arctotheca prostrata* plants. Class indicates approximate fresh (wet) weights, Plant names indicate Population, Chunk or Individual, Clone, and Replicate number. Means and sample standard deviations were calculated by Excel; Wet Wt. gives the exact weight of fresh leaf material; dry weights (Dry Wt.) were calculated using mean percent water content of leaves (see Table A-16).

Population	**Rodeo Valley**				
Class	**0.5 g**	**1 g**	**1 g**	**1 g**	**1 g**
Plant	**RV 5A-f-1**	**RV 5A-f-1**	**RV 5A-f-2**	**RV 5A-g-1**	**RV 5A-g-2**
	9	4	4	9	12
	8	4	4	8	12
	8	4	4	8	11
	7	4	4	8	9
	7	3	3	7	9
	6	3	3	7	8
	6	3	3	6	8
	3	3	3	4	6
				3	6
					4
Mean	6.8	3.5	3.5	6.7	8.5
St Dev-s	1.8	0.5	0.5	2.0	2.7
Wet Wt. (g)	0.45	1.05	1.05	0.99	0.97
Dry Wt. (g)	0.08	0.19	0.15	0.12	0.12

Popluation	**Tennessee Valley**			
Class	**0.5 g**	**1 g**	**2 g**	**2 g**
Plant	**TV 3A-c-1**	**TV 3A-c-1**	**TV 3A-c-1**	**TV 3A-c-1**
	14	6	7	7
	13	6	6	7
	12	5	5	6
	12	3	4	6
	11	3	3	6
	11	2	2	5
	11			5
	7			5
	4			5
	4			
	3			
Mean	9.3	4.2	4.5	5.8
St Dev-s	4.0	1.7	1.9	0.8
Wet Wt. (g)	0.55	1.09	1.86	0.85
Dry Wt. (g)	0.11	0.21	0.24	0.07

Table A-13. Measurements on 72-hour-old lettuce seedling roots growing in aqueous solutions containing various amounts of ground runner material from *Arctotheca prostrata* plants. Class indicates approximate fresh (wet) weights, Plant names indicate Population, Chunk or Individual, Clone, and Replicate number. Means and sample standard deviations were calculated by Excel; Wet Wt. gives the exact weight of fresh runner material; dry weights (Dry Wt.) were calculated using mean percent water content of runners (see Table A-16).

Population	**Tiburon**			
Class	**1 g**	**1 g**	**2 g**	**2 g**
Plant	**TI 1A-a-1**	**TI 1A-a-2**	**TI 1A-a-1**	**TI 1A-a-2**
	12	9	4	6
	9	8	4	5
	9	8	3	5
	8	6	3	5
	8	6	3	4
	7	5	3	4
	6	5	3	3
	6	5	3	3
	5	4	2	2
		4		2
Mean	7.8	6.0	3.1	3.9
St Dev-s	2.1	1.8	0.6	1.4
Wet Wt. (g)	1.02	1.08	2.06	1.97
Dry Wt. (g)	0.24	0.25	0.48	0.46

Table A-14. Measurements on 72-hour-old lettuce seedling roots growing in aqueous solutions containing various amounts of ground root material from *Arctotheca prostrata* plants. Class indicates approximate fresh (wet) weights, Plant names indicate Population, Chunk or Individual, Clone, and Replicate number. Means and sample standard deviations were calculated by Excel; Wet Wt. gives the exact weight of fresh root material; dry weights (Dry Wt.) were calculated using mean percent water content of roots (see Table A-16).

Population	**South Africa**					
Class	**2 g**	**2 g**	**2 g**	**4 g**	**1 g**	**2 g**
Plant	**SF 5-a-1**	**SF 5-a-2**	**SF 5-a-3**	**SF 5-a**	**SF 1-d**	**SF 1-d**
	37	26	34	26	35	35
	36	25	33	25	34	31
	35	23	31	24	30	30
	32	22	30	23	28	27
	30	21	30	23	27	25
	25	20	27	16	26	18
	22	19	24	12	26	17
	17	12	16	11	18	15
	10			5		
				5		
				5		
Mean	27.1	21.0	28.1	15.9	28.0	24.8
St Dev-s	9.3	4.3	5.8	8.6	5.3	7.3
Wet Wt. (g)	2.04	2.03	2.05	3.99	1.06	2.05
Dry Wt. (g)	0.34	0.34	0.34	0.66	0.09	0.17

Population	**Gualala Bluff**			**Tiburon**		
Class	**1 g**	**2 g**	**4 g**	**1 g**	**1 g**	**2 g**
Plant	**GB 4E-a**	**GB 4E-a**	**GB 4E-a**	**TI 1A-a-1**	**TI 1A-a-2**	**TI 1A-c**
	34	18	4	27	27	18
	32	15	4	25	24	17
	26	12	4	25	23	17
	19	10	3	23	23	12
	18	6	3	22	21	12
	16	4	3	18	20	11
	12	3	3	12	18	11
	11			10	16	10
	7			8	8	6
	3			7	5	6
Mean	17.8	9.7	3.4	17.7	18.5	12.0
St Dev-s	10.3	5.7	0.5	7.7	7.1	4.3
Wet Wt. (g)	1.05	2.07	3.99	0.99	1.01	1.95
Dry Wt. (g)	0.2	0.38	0.74	0.26	0.26	0.51

Table A-14 *continued.* Measurements on 72-hour-old lettuce seedling roots growing in aqueous solutions containing various amounts of ground root material from Arctotheca prostrata plants. Class indicates approximate fresh (wet) weights, Plant names indicate Population, Chunk or Individual, Clone, and Replicate number. Means and sample standard deviations were calculated by Excel; Wet Wt. gives the exact weight of fresh root material; dry weights (Dry Wt.) were calculated using mean percent water content of roots (see Table A-16).

Population	**Rodeo Valley**							
Class	**1 g**	**1 g**	**1 g**	**2 g**	**2 g**	**2 g**	**4 g**	**4 g**
Plant	**RV 5A-f-1**	**RV 5A-f-2**	**RV 5A-f-3**	**RV 5A-f-1**	**RV 5A-f-2**	**RV 5A-f-3**	**RV 5A-f-1**	**RV 5A-f-2**
	44	42	34	20	22	35	19	18
	42	38	32	19	20	30	18	17
	40	36	30	15	18	29	17	16
	40	35	30	13	16	27	17	15
	39	32	29	12	15	24	10	14
	38	31	29	9	15	19	9	13
	37	30	27	8	14	17	8	13
	37	30	27	6	8	16	7	8
	33	27	25	4	7	8	7	
	28	20	9	3				
	15			3				
				3				
Mean	35.7	32.1	27.2	9.6	15.0	22.8	12.4	14.3
St Dev-s	8.1	5.0	7.0	4.5	4.2	7.3	4.4	2.8
Wet Wt. (g)	0.99	0.99	0.99	2.00	1.96	2.04	3.99	4.03
Dry Wt. (g)	0.15	0.15	0.15	0.29	0.29	0.30	0.59	0.59

Class	**2 g**	**2 g**	**2 g**	**4 g**	**4 g**	**4 g**
Plant	**RV 5A-g-1**	**RV 5A-g-2**	**RV 5A-g-3**	**RV 5A-g-1**	**RV 5A-g-2**	**RV 5A-g-3**
	16	10	21	12	6	9
	16	10	17	11	**6**	9
	15	9	16	11	5	8
	15	9	15	10	5	6
	15	9	14	10	4	5
	14	9	14	4	4	5
	14	8	13	3	4	5
	9	7	12	3	3	4
	6		10	3	3	4
	5		10		3	4
Mean	12.5	8.9	14.2	7.4	4.3	5.9
St Dev-s	4.2	1.0	3.3	4.0	1.2	2.0
Wet Wt. (g)	2.00	2.05	2.00	4.00	4.02	4.05
Dry Wt. (g)	0.39	0.40	0.39	0.78	0.79	0.79

Table A-14 *continued.* Measurements on 72-hour-old lettuce seedling roots growing in aqueous solutions containing various amounts of ground root material from *Arctotheca prostrata* plants. Class indicates approximate fresh (wet) weights, Plant names indicate Population, Chunk or Individual, Clone, and Replicate number. Means and sample standard deviations were calculated by Excel; Wet Wt. gives the exact weight of fresh root material; dry weights (Dry Wt.) were calculated using mean percent water content of roots (see Table A-16).

Population	**Tennessee Valley**						
Class	**0.5 g**	**2 g**	**2 g**	**4 g**	**2 g**	**2 g**	**4 g**
Plant	**TV 3A-b**	**TV 3A-b-1**	**TV 3A-b-2**	**TV 3A-b**	**TV 3A-c-1**	**TV 3A-c-2**	**TV 3A-c**
	34	19	21	7	37	30	10
	31	15	18	6	36	28	9
	30	12	16	6	35	26	9
	28	11	15	6	34	26	9
	27	10	13	4	30	25	8
	26	8	13	3	25	24	4
	23	6	12		23	21	4
	23		4		12	11	4
	22						3
	21						3
Mean	26.5	11.6	14.0	5.3	29.0	23.9	6.3
St Dev-s	4.3	4.4	5.0	1.5	8.6	5.8	2.9
Wet Wt. (g)	0.51	2.08	2.05	3.95	2.00	2.04	3.98
Dry Wt. (g)	0.08	0.33	0.32	0.62	0.32	0.33	0.64

Table A-15. Measurements on 72-hour-old lettuce seedling roots growing in HPLC water only. These are controls for tests on the effects of *Arctotheca prostrata* plant parts on the growth of lettuce seedlings.

Control	**C-1**	**C-2**	**C-3**	**C-4**	**C-5**	**C-6**
	21	20	21	20	18	21
	20	19	20	18	17	19
	18	18	20	15	16	18
	17	16	19	15	16	16
	17	15	17	11	15	15
	16	11	14	10	14	15
	14	8	5	7	13	13
	11	6	5	5	6	8
	7	6	3			
	5					
Mean	14.6	13.2	13.8	12.6	14.4	15.6
St Dev-s	5.4	5.6	7.4	5.3	3.7	4.0

Table 16a. Fresh (wet) and dry weights (g) of leaves and runners of *Arctotheca prostrata* plants used to test allelopathic effects on lettuce seedling root lengths. Numbers at ends of plant names indicate replicates for that sample. (Some samples were too small to replicate.) Calculations for percent water and percent dry weight are given in the Methods section on p. 46. Mean percent water of replicated samples and percent water of unreplicated samples were used to calculate dry weights of samples used in each experiment (see Table A-8).

LEAVES

Californian plants

Name	**GB 4E-a-1**	**GB 4E-a-2**	**GB 4E-a-3**	**RV 5A-f**	**RV 5A-g**	**TI 1A-c**	**TV 3A-b**	**TV 3A-c**
Empty Petri dish	29.87	27.91	30.35	30.54	31.62	31.24	31.38	29.90
Petri dish + wet wt.	30.01	28.44	30.67	30.78	32.63	31.43	31.50	30.17
Petri dish + dry wt.	29.88	27.99	30.40	30.58	31.75	31.27	31.39	29.96
Wt of wet sample	0.14	0.54	0.33	0.24	1.01	0.18	0.12	0.27
Wt of dry sample	0.01	0.08	0.05	0.04	0.12	0.02	0.01	0.05
Water wt in sample	0.13	0.45	0.28	0.20	0.88	0.16	0.11	0.22
Percent water	94.35	84.82	84.47	82.32	87.62	87.34	91.99	80.44
Percent dry wt	5.65	15.18	15.53	17.68	12.38	12.66	8.01	19.56

LEAVES

South African plants

Name	**SF 1-d-1**	**SF 1-d-2**	**SF 5-a-1**	**SF 5-a-2**
Empty Petri dish	30.90	31.03	48.36	31.10
Petri dish + wet wt.	31.23	31.68	49.07	31.60
Petri dish + dry wt.	30.94	31.11	48.46	31.17
Wt of wet sample	0.33	0.65	0.71	0.49
Wt of dry sample	0.04	0.08	0.10	0.07
Water wt in sample	0.29	0.57	0.61	0.42
Percent water	88.33	87.22	85.77	85.60
Percent dry wt	11.67	12.78	14.23	14.40

RUNNERS

Californian plants

TI 1A-a-1	**TI 1A-a-2**
29.81	31.35
30.37	32.06
29.94	31.52
0.56	0.71
0.13	0.16
0.43	0.55
76.14	76.87
23.86	23.13

Table 16b. Fresh (wet) and dry weights (g) of roots of *Arctotheca prostrata* plants used to test allelopathic effects on lettuce seedling root lengths. Numbers at ends of plant names indicate replicates for that sample. (Some samples were too small to replicate.) Calculations for percent water and percent dry weight are given in the Methods section on p. 46. Mean percent water of replicated samples and percent water of unreplicated samples were used to calculate dry weights of samples used in each experiment (see Table A-8).

ROOTS

Californian plants

Name	GB 4E-a-1	GB 4E-a-2	RV 5A-f-1	RV 5A-f-2	RV 5A-f-3	RV 5A-g-1	RV 5A-g-2	RV 5A-g-3
Empty Petri dish	30.83	30.19	31.35	31.24	30.00	27.80	30.41	27.78
Petri dish + wet wt.	31.16	31.25	32.34	32.17	30.48	28.76	31.20	29.19
Petri dish + dry wt.	30.89	30.40	31.51	31.37	30.07	27.99	30.56	28.05
Wt of wet sample	0.33	1.06	0.99	0.94	0.47	0.95	0.79	1.41
Wt of dry sample	0.06	0.20	0.15	0.14	0.07	0.19	0.16	0.27
Water wt in sample	0.27	0.86	0.84	0.80	0.41	0.77	0.64	1.13
Percent water	82.02	80.83	84.63	85.25	85.92	80.33	80.40	80.46
Percent dry wt	17.98	19.17	15.37	14.75	14.08	19.67	19.60	19.54

Name	RV 5A-g-4	TI 1A-c-1	TI 1A-c-2	TV 3A-b-1	TV 3A-b-2	TV 3A-c-1	TV 3A-c-2	TV 3A-c-3
Empty Petri dish	31.46	28.83	31.06	30.98	30.82	30.30	31.15	29.55
Petri dish + wet wt.	32.29	29.64	31.76	31.44	31.53	30.92	31.67	29.83
Petri dish + dry wt.	31.63	29.05	31.24	31.05	30.94	30.40	31.23	29.59
Wt of wet sample	0.83	0.81	0.69	0.46	0.71	0.62	0.52	0.29
Wt of dry sample	0.16	0.22	0.17	0.07	0.12	0.10	0.08	0.04
Water wt in sample	0.67	0.59	0.52	0.39	0.59	0.52	0.44	0.25
Percent water	80.36	73.09	75.04	84.17	82.87	83.74	84.20	86.02
Percent dry wt	19.64	26.91	24.96	15.83	17.13	16.26	15.80	13.98

South African plants

Name	SF 1-d-1	SF 1-d-2	SF 1-d-3	SF 5-a-1	SF 5-a-2	SF 5-a-3
Empty Petri dish	30.07	21.22	31.30	29.09	30.94	28.98
Petri dish + wet wt.	30.78	22.51	33.17	29.64	31.95	30.73
Petri dish + dry wt.	30.12	21.33	31.46	29.18	31.11	29.28
Wt of wet sample	0.71	1.29	1.87	0.55	1.02	1.75
Wt of dry sample	0.06	0.12	0.16	0.09	0.17	0.30
Water wt in sample	0.66	1.18	1.71	0.47	0.84	1.45
Percent water	92.29	91.10	91.69	84.56	82.88	82.71
Percent dry wt	7.71	8.90	8.31	15.44	17.12	17.29