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Effects of Asphondylia borrichiae, Simulated Herbivory, and Nutritional Status on Survival, Flowering, and Seed Viability in Sea Oxeye Daisy (Borrichia frutescens)

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Effects of *Asphondylia borrichiae*, simulated herbivory, and nutritional status on survival, flowering, and seed viability in sea oxeye daisy (*Borrichia frutescens*)

By

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Abstract

Although herbivory and other types of plant damage typically are viewed as detrimental to plant survival and performance, vigorous regrowth, greater seed set, and fitness benefits may be possible when damage to the apical meristem, or actively growing stem terminal, is involved. Such damage releases apical dominance, or the hormonal suppression of lateral buds, activates dormant lateral buds, and enables lateral shoots to grow. Since in plants with terminal flowers, each stem may bear a flower, removal of the apical meristem may result in stem bifurcation and ultimately increase the number of flowers and seeds, thereby increasing potential fitness. In the current study, possible overcompensation in response to apical meristem damage caused by simulated herbivory (clipping) and the gall midge *Asphondylia borrichiae* Rossi and Strong (Diptera: Cecidomyiidae) (galling) was investigated in the native coastal halophyte, sea oxeye daisy *Borrichia frutescens* (L.) DC. (Asteraceae), in relation to nutrient supplementation. Results suggest a strong correlation between stem count and gall count at the study site; moreover, apical dominance was relatively weak early in the growing season and stronger in short plants that were shaded by taller neighbors later in the season. Results also indicate that overcompensation or even full compensation is an unlikely response to apical meristem damage in *B. frutescens*. Stem count was similar across all stem treatments, but increased significantly with nutrient supplementation, which all supports weak apical dominance in sea oxeye daisy. Nearly all measures of fitness also were either slightly or significantly lower when clipped and galled compared to plants with stems intact, while seed count responded positively to nutrient supplementation.

Chapter 1: Introduction

Two exogenous factors impact a plant's survival, growth, and reproductive output (fitness): (1) resources such as nutrients, water, and light, and (2) damage incurred by herbivores, parasites, pathogens, environmental events (e.g. storms, fire), and other biotic and abiotic disturbances. Plant response to herbivory and other damage often depends on the health of the plant, its nutritional status, and resource availability. Herbivory is one of many types of damage that may be inflicted on plants, but it has the added characteristic of involving often complex interactions between plants and herbivores that may lead to adaptations in one or the other, or both (coevolution). Herbivores may consume all or only part of a plant and, if the plant is not killed in the process, it may respond with a change in morphology (e.g. architecture, growth pattern, or amount or quality of regrowth) or physiology (e.g. physical or chemical defenses). Under some conditions, plant responses may lead to higher potential fitness. In many cases, the plant's response elicits a particular response from the herbivore, such as complete or partial avoidance, subsequent herbivory on only certain parts of the plant, or, conversely, even an increase in herbivory (Crawley 1983; Strong *et al.* 1984).

Types and Preferences of Herbivores

Although most terrestrial communities or ecosystems are dominated by plants, their nutritional quality may be suboptimal from the perspective of herbivores. Plants that are adapted to their normal habitat often have limited amounts of nutrients in available or assimilable forms or indigestible or unpalatable tissues, resulting in the necessity of herbivores to consume large quantities of plant tissue to meet their nutritional needs (McNeil and

Southwood 1978; Crawley 1983; White 1984; Taylor 1989; Weis and Berenbaum 1989). Insect herbivores develop more slowly and produce fewer eggs when food quality is poor, and insect growth rate is strongly correlated with feeding rate (Crawley 1983; Awmack and Leather 2002). Growth of larvae of the aphid *Rhopalosiphum padi* was reduced when free amino acids in the phloem sap of oat and barley declined seasonally (Weibull 1987). The salvinia moth *Samea multiplicalis* experienced lower growth on *Salvinia molesta* host plants containing low nitrogen, even when larvae started on high nitrogen and were switched to low nitrogen only during the last instar stage (Taylor 1989). Poor nutrient and water conditions for piñon pine *Pinus edulis* caused significantly lower cocoon mass, proportion of females, and individual reproductive potential in piñon sawfly *Neodiprion edulicolis* (Mopper and Whitham 1992). Preszler and Price (1988) manipulated stress level and health of potted *Salix lasiolepis* with varying water-level treatments and found that the willow-galling sawfly *Euura lasiolepis* suffered a three-fold decrease in gall initiation and egg release and a four-fold increase in first instar mortality on low-water *Salix*. In gall-inducing insects, larval performance and fecundity is increased when healthy host plants are utilized, as evidenced by the presence of large galls. The galling cynipid *Belonocnema treatae* formed larger galls on and preferentially galled healthy *Quercus fusiformis* that already had higher-gall-density (Egan and Ott 2007). *Asphondylia borrichiae* had higher galling rates and produced significantly larger galls on and preferentially attacked large, vigorously growing *Borrichia frutescens* (Rossi *et al*. 1992).

Optimal Foraging Theory predicts that given a variety of equally palatable foods, a species should pursue only those foods that will return the highest reward in terms of energy, nutrition, abundance, or resulting performance with the least cost in terms of time or energy spent obtaining them (Pyke *et al.* 1977; Rhoades 1979). Indeed, herbivores often benefit most from and are often most attracted to the most highly nutritious plants or plant parts in any

given plant community (Price *et al.* 1987a, b; Price 1991). However, Optimal Foraging Theory may be more applicable to predator-prey and less to herbivore-plant relationships, since the herbivore diet often includes a mixture of plant foods including suboptimal ones, and plant food selectivity may be based on factors other than optimal foraging (Pyke *et al.* 1977; Crawley 1983). Such factors may include high hunger level; costs of discrimination outweigh benefits; foods do not differ significantly from each other; density of optimal foods is too low, distribution too spaced, or abundance changes over time; optimal foods lack in some nutrients and must supplemented with others; competition for optimal foods is high; food quality changes over time (e.g. phenology-related or age-related); and nutritious foods are toxic and must be eaten in limited quantities or avoided (Crawley 1983). In particular, levels of secondary defensive chemicals in plant foods may play a greater role in food preference than nutrient concentration, particularly for generalist or polyphagous herbivores (those that eat many plant species). A review of the literature by Bryant and Kuropat (1980) involving the winter browsing habits of various species of ptarmigan (*Lagopus lagopus*, *L. mutus*, *L. leucurus*), grouse (*Bonasa umbellus*, *Canachites canadensis*, *Dendragapus obscurus*), capercaillie (*Tetrao urogallus*), hare (*Lepus americanus, L. timidu*), and moose (*Alces alces*) found that these subarctic vertebrates place greater importance on relative concentrations of secondary defensive chemicals like terpenes and phenolic resins and less importance on energy and nutrient content. In choice experiments, the generalist squareback marsh crab *Armases cinereum* based its feeding preferences primarily on plant toughness, with salt content, silica content, and protein playing lesser or no roles (Pennings *et al.* 1998). In a study on food preferences of over three dozen insect herbivores in relation to nitrogen and secondary compounds, all nine polyphages preferred mature leaves containing low levels of nitrogen and compounds, while all 26 monophages and oligophages preferred young leaves containing high levels of nitrogen and compounds (Cates 1980). This

dramatic result suggests that monophagous and oligophagous insects (those utilizing one or few food plants, respectively), as specialists, are more adapted to the high levels of toxins present in young leaves and may even use the presence of secondary plant compounds for host recognition, while polyphagous herbivores, being generalists, are limited to lower-quality foods with lower toxin levels.

In many plant-herbivore systems, plants that are stressed due to damage or atypically low resource levels, such as nutrient deficiency, lack of sunlight, or dehydration, may be more attractive to herbivores than unstressed plants (Plant Stress Hypothesis) (White 1974; Mattson and Addy 1975; White 1984). In particular, sustained (long-term) stress may be particularly beneficial to phytophagous insects by inducing metabolic changes in plants such as hydrolysis and proteolysis and reducing secondary compounds (Mopper and Whitham 1992). Under stressful or suboptimal conditions or when damaged, plants often recycle nitrogen in senescing or stressed tissues by breaking it down to free amino acids (proteolysis) and transporting them to other plant tissues or organs (Crawley 1983; White 1984). Free amino acids are soluble and more assimilable by insect herbivores, making the plant, particularly the parts of the plant receiving those nutrients, to become more susceptible to attack (White 1984). Other possible chemical changes from stress include increased levels of soluble carbohydrate (sugars) (Mattson and Addy 1975; Mattson and Haack 1987a, b), which also improve palatability, particularly for leaf-chewers and miners (Louda and Collinge 1992). Sap-sucking and stem-galling insects appear to respond to increased nitrogen availability (McNeil and Southwood 1978; Danell and Huss-Danell 1985; Louda and Collinge 1992). In a field experiment involving stress-induced palatability changes in bittercress (*Cardamine cordifolia*) to over 28 species of herbivorous insects that feed on it, no significant changes in total nitrogen, free amino nitrogen, and total amino acid between treatments were detected in leaf tissues, but sugar content doubled and the amino

acid isoleucine also increased (Louda and Collinge 1992). Furthermore, attack by leaf-chewing and mining insects significantly increased, but attack by sap-sucking insects did not change. The grasshopper *Melanoplus differentialis* preferentially feeds on leaves of the sunflower *Helianthus annuus* that have been damaged by other insects, infected with rust fungus *Puccinia helianthii*, and wilted (Lewis 1979). Two gall-inducing dipterans, *Lipara lucens* (De Bruyn 1995) and *Giraudiella inclusa* (Tscharntke 1989), were more common on thin stressed shoots of *Phragmites australis* compared to thick healthy shoots. The psyllid *Acizzia russellae* reached epidemic levels on pruned *Acacia karroo* which was attributed to an increase in high-quality total or soluble organic nitrogen in leaves (Webb and Moran 1978).

Drought also may cause stress in plants, lead to proteolysis, decrease plant defenses, and cause devastating forest insect epidemics (Climatic Release Hypothesis) (White 1974; Mattson and Haack 1987a, b). Water stress caused by drought was associated with outbreaks of the psyllid *Cardiaspina densitexta* on *Eucalyptus fasciculosa* in Australia (White 1969) and the lepidopteran *Selidosema suavis* on *Pinus radiata* in New Zealand (White 1974). Stress-inducing events such as drought, excessive rainfall, and other climatic disturbances to which tree species may not be adapted have been associated with epidemics of *Dendroctonus ponderosae* and other tree-killing bark beetles in coniferous forests of the western United States (Berryman 1976; Christiansen *et al.* 1987). Outbreaks of the forest tent caterpillar *Malacosoma disstria* have been documented in North America following warm, dry weather (Mattson and Addy 1975; Martinat 1987; Mattson and Haack 1987a, b). Such large-scale waves of defoliation may impact woody plants more than herbaceous ones, since trees and shrubs normally produce one flush of leaves per year which, if removed by a defoliating outbreak, may not be replaced until the following spring (Crawley 1983). As a result of time lags, Myers (1988) noted that correlating

outbreaks with meteorological events or other causes of stress may be logistically and statistically difficult.

In some plant-herbivore systems, herbivores preferentially feed not on stressed plants, but on healthy, vigorously growing plants or plant parts, which are more likely to be well-resourced (Plant Vigor Hypothesis; Price *et al.* 1987a, b; Roininen *et al.* 1988; Price 1991). Price (1991) uses the term "vigor" to describe the relative performance of an individual plant or parts of a plant that outpaces that in the rest of the population, specifically, rapid growth that exceeds mean growth rate for the population and leads to larger-than-average size. Plants adapted to growing in well-resourced environments with plentiful nutrients, water, and light often experience more herbivory, particularly fast-growing, poorly defended early successional plants such as those in canopy openings in tropical forests and riparian (riverbank) habitats (Coley 1983; Crawley 1983; Coley *et al*. 1985; Coley and Aide 1991; Price 1991). Young, rapidly growing shoots, leaves, and other plant modules are nutritious, tender (low in fiber, tannins, and lignin), and poorly defended, making them a target for both insect and mammalian herbivores (Owen 1980; Coley 1983; Crawley 1983; Price *et al.* 1987a, b; Roininen *et al.* 1988; Weis and Berenbaum 1989; Coley and Aide 1991; Fernandes and Price 1991; Price 1991; Whitham *et al.* 1991). Larvae of the salvinia moth *Samea multiplicalis* fed preferentially on the young, nitrogen-rich leaves of *Salvinia molesta*, regardless of whether the plants on the whole were high or low in nitrogen (Taylor 1989). Similarly, many mammalian herbivores are also more likely to attack young plants or plant parts, particularly in disturbed or early successional areas such as birch (*Betula pendula* and *B. pubescens*) that are browsed by moose (*Alces alces*) (Danell *et al*. 1985; Danell and Bergström 1989). Additionally, females of many gall-inducing and shoot-boring insect species are highly selective for healthy, vigorous, and well-resourced plants or plant parts for oviposition (Price *et al.* 1987a, b; Roininen *et al.* 1988; Price 1991). Various species of gall-

inducing sawflies (*Euura* spp., Hymenoptera: Tenthredinidae) preferentially attack young, rapidly growing plant parts of several willow species (*Salix* spp., Salicaceae), including *E. lasiolepis* which prefers tender shoots of arroyo willow, *Salix lasiolepis* for oviposition (Craig *et al*. 1986; Waring and Price 1988; Craig *et al*. 1989), as well as *E. exiguae* which attacks sandbar willow, *S. exigua* (Price 1989). *Euura mucronata* attacks and experiences better larval performance on the large buds of long, young, vigorously growing shoots of *S. cinerea* (Price *et al*. 1987a, b; Roininen *et al*. 1988). Young shoots of other plant species are often preferred by gall-inducing cynipid wasps (Hymenoptera: Cynipidae), such as *Diplolepis spinosa* on *Rosa arizonica* (Rosaceae) (Caouette and Price 1989). Some leaf-galling aphid species (Homoptera: Aphididae) are known to prefer large, immature leaves, such as *Pemphigus betae* on *Populus augustifolia* (Salicaceae), where this insect species experiences higher fitness (Whitham 1978, 1980, 1992). Rossi *et al*. (1992, 1998) similarly found that the apical meristem-galling midge *Asphondylia borrichia* is more likely to attack large, vigorously growing plants, particularly those given nutrition supplementation (e.g. nitrogen fertilizer).

The attractiveness of stressed versus vigorous plants to herbivores is often complicated and clear predictions may be elusive. In a study of the creosote bush (*Larrea tridentata*), which is adapted to xeric environments, and eight associated *Asphondylia* species that induce galls on its leaves, stems, and flowers, Waring and Price (1990) found that densities of five of the *Asphondylia* species were higher on water-stressed plants compared to relatively more vigorous (e.g. less water-stressed). However, the attraction of stressed plants to these gallers appeared to be not due to biochemical changes (e.g. higher amino acids), but for the higher number of stems inherent in the bushier architecture found in stressed *L. tridentata* (Waring and Price 1990).

In considering the effect of herbivory on plants, the assumption has long been that the interaction between herbivore and plant typically is antagonistic. The published literature abounds with examples of herbivory that significantly reduces plant survival, biomass, growth, and/or reproduction (Jameson 1963; McNaughton 1983; Strong *et al*. 1984; Lindroth 1989). Intense vertebrate or invertebrate herbivory on woody plants may severely impact fitness by reverting or maintaining them in juvenile form (e.g. with a flush of new growth) and delaying reproductive maturity (Bryant *et al.* 1983). Galling by the sawfly *Euura lasiolepis* stimulates *Salix lasiolepis* to produce new, young shoots, which prevents the willow from maturing and reproducing (Craig *et al*. 1986). Height and standing crop of heather (*Calluna vulgaris*) decreased significantly when grazed by mountain hare (*Lepus timidus*) and red deer (*Cervus elaphus*), compared to heather protected from grazing for five years (Moss *et al.* 1981). Under normal levels of aphid infestation by *Staticobium staticis*, only 23% of its host plants *Limonium vulgare* and *L. humile* successfully developed flowers and fruits, compared to 100% of plants under aphid-free conditions, and in season of peak aphid density virtually no *L. vulgare* or *L. humile* produced seed (Foster 1984). The selective grazing of cattle on the highly nutritious ramets and inflorescences of *Yucca elata* jeopardizes the plant's obligate mutualistic relationship with its exclusive pollinator, the yucca moth *Tegeticula yuccasella*, and thus its ability to reproduce sexually (Kerley *et al*. 1993). Populations of *Y. elata* subsequently may become genetically homogeneous over time, rendering them less able to adapt to environmental or biotic changes. Artificial defoliation of *Piper arieianum*, a neotropical shrub, to simulate leaf herbivory by insects negatively impacted growth for the following two years in small and medium-sized (≥50 percent decrease in total growth when ≥30% defoliation), and seed production the following year in all class sizes subjected to ≥30% defoliation also was significantly lower (Marquis 1984). In the

same study, plants experiencing 100% defoliation experienced significant setbacks in growth for two years and set no seed the year following treatment (Marquis 1984).

The degree of antagonism can vary depending on the type of herbivory, with browsing and grazing by mammals and defoliation of forest trees by caterpillars, for example, having what might be called a parasitic effect, since the plants are not normally killed by the herbivory; few herbivores act like predators by killing plants directly (Crawley 1983). An exception is seed predators, which kill embryonic plants (Janzen 1983; Stiles 1989). While sap-sucking insects tax host plants by draining carbohydrate reserves and wood- or bark-boring insects may kill trees by destroying phloem, vascular cambium, or xylem tissues, these two types of herbivores also may cause or hasten plant death indirectly by introducing pathogens such as viruses and fungi into plant tissues (Crawley 1983). For example, in the 20th century, Dutch elm disease caused the death of over 40 million native elms in North America, primarily *Ulmus americana*, and is caused by the fungal species *Ophiostoma ulmi* (formerly *Ceratostomella ulmi* and *Ceratocystis ulmi)* and *O. novo-ulmi*, which are introduced by the wood-boring activities of the lesser European elm bark beetle *Scolytus multistriatus* and the North American native elm bark beetle *Hylurgopinus rufipes* (Kammeraad and Brewer 1963; Coulson and Witter 1984; Dreistadt *et al.* 1990; Brassier 1991). The plant disease-causing bacterium *Xylella fastidiosa* is transmitted to coffee, almond, grape, citrus, peach, and other economically valuable crops by members of the xylem sap-sucking hemipteran subfamily Cicadellinae (sharpshooter leafhoppers) (Almeida *et al.* 2005).

While antagonism is typically assumed to be the default relationship, plant-herbivore interactions may be mutualistic in some systems. Some evidence suggests that grasses and grazers have coevolved to the point that some grasses perform best by being grazed (Owen and Wiegert 1981), to the extent that some grasses may be obligate grazophiles, unable to thrive or even survive without grazers (McNaughton 1979; Owen 1980; McNaughton 1985, 1986).

Evidence suggests that grazer saliva contains substances that promote the growth of grazed grasses, an arrangement that benefits both the plant through assisted regrowth and the grazer through quick replenishment of its food supply (Owen and Wiegert 1981; Dyer *et al.* 1982; McNaughton 1986). Serengeti grass species such as *Andropogon greenwayi* and *Kyllinga nervosa* maintain above-ground productivity and below-ground reserves via regrowth after periods of intense grazing by wild migratory ungulates, with which they have a long history (McNaughton 1979, 1985).

Another mutualistic relationship is found in the *Yucca-Tegeticula* system. All 40 species in the genus *Yucca* have an obligate mutualistic relationship with the species in the yucca moth genus *Tegeticula* (Aker and Udovic 1981; Bertin 1989). *Tegeticula maculata* oviposits inside the flower ovaries of *Y. whipplei* and simultaneously and actively pollinates the flower she has chosen with pollen which she carries against her thorax (Aker and Udovic 1981). In this system, pollination is required not only by the plant, but by the moth, since unpollinated flowers are aborted and kill the larvae developing within. Since flowers may produce thousands of seeds and only some ovaries are oviposited, developing yucca moth larvae feed only on some, but not typically all, of a yucca flower's seeds. The yucca moth-plant relationship generally is mutualistic, but may become antagonistic if oviposition occurs late in the flowering season, when *Y. whipplei* flowers are scarce; the moth may instead oviposit in developing seed pods, which aborts the pods and kills the larvae (Aker and Udovic 1981).

Pollination as a mutualism usually involves a reward provided by the plant, often a food like nectar or pollen, although other types of rewards have been reported. Male euglossine bees obtain fragrance compounds from orchids that may be precursors for their own pheromones and transfer pollinaria between flowers at the same time (Feinsinger 1983). Other pollinating mutualists include bees, ants, beetles, flies, hummingbirds, and some mammals (e.g. bats,

lemurs, rodents, marsupials) (Feinsinger 1983; Bertin 1989). Generally, pollination is considered a plant-animal mutualism, but not all animals that visit flowers are good pollinators; the benefit for plants is realized best if the animal is efficient and successful at transporting pollen from one conspecific flower to another without consuming or damaging flowers (Feinsinger 1983; Bertin 1989).

Vertebrate frugivory is another classic example of a plant-animal mutualism, by which seed dispersal may be accomplished through the consumption of edible fruits near the parent plant and excretion of any intact, surviving seeds in a new location away from the parent that may be suitable for germination (Janzen 1983; Stiles 1989; Fleming 1991). Frugivory is a particularly important form of mutualism in the tropics, where a diverse array of tropical plants produces edible fleshy fruits upon which a variety of seed-dispersing vertebrate generalists feed (Janzen 1983; Stiles 1989; Fleming 1991). The seeds of some plant species require pre-germination processing by digestion in the gut of frugivores (e.g. breaking down of the seed coat by digestive enzymes) (Owen 1980; Crawley 1983; Stiles 1989). However, not all animals that consume fruits and seeds are effective dispersers, since seeds may be destroyed by the process (e.g. broken or ground up by teeth or by bird beaks or gizzards, digested); in this case, these animals may be considered seed predators (Janzen 1983; Stiles 1989). Using an array of behavioral and ecological criteria related to how frugivorous birds handle seeds and how often they visit, Howe (1977) identified effective and ineffective seed dispersers from among the variety of birds that consume the fruits, some as obligate frugivores, of the rain forest tree species *Casearia corymbosa*. Only one of 17 bird species studied, *Tityra semifasciata*, was an effective dispersal agent, while the remaining species were either inefficient dispersers or were inconsistently efficient or not present the entire season (Howe 1977). Frugivory by seed-dispersing vertebrates (e.g. seed-swallowing birds, large mammals) may further benefit plants

by removing seeds as potential food sources from seed predators (Janzen 1978, 1983). Even some seed predators such as squirrels and jays may be beneficial by stashing seeds (e.g. acorns) in caches that may remain hidden until germination (Crawley 1983; Stiles 1989), although the location of caches in places suitable for germination is important (Harper 1977). Generally, seed dispersal, whether by animals or wind, increases the probability that seeds will be deposited in locations with fewer specialist predators normally associated with the parent plant (distance-dependent) and reduces competition between seedlings and between a seedling and its parent plant (density-dependent) (Janzen 1978; Crawley 1983). Chinese elm (*Ulmus parvifolia*) seedlings experienced 580 times the attacks by elm leaf beetles (*Pyrrhalta luteola*) when located directly under parent trees than those not located under parent trees, the latter which were able to escape attacks until they exceeded 2.0×10^5 cm^3 (Lemen 1981). Wind-dispersal allowed seedlings of the tropical canopy tree *Platypodium elegans* to escape infection (damping-off) by fungal pathogens originating from parent trees, with the percentage of damping-off decreasing with increasing distance from the parent and with decreasing seedling density (Augsperger 1983).

Plant-animal interactions may also be mutualistic in the long-term cycles found in some forest and grassland ecosystems, as when herbivory increases the rate of nutrient cycling at the community level (Mattson and Addy 1975; Owen and Wiegert 1976; Owen 1980; Crawley 1983; McNaughton 1985; Weis and Berenbaum 1989). Outbreaks of lepidopteran larvae like forest tent caterpillar *Malacosoma disstria* can recycle large amounts of forest nutrients in the form of excrement, dead larvae, and wasted pieces of leaves, wood particles, and other food parts during summer months, a time of year when significant nutrient cycling via leaf litter is absent (Mattson and Addy 1975; Crawley 1983; Weis and Berenbaum 1989). Mammalian herbivores also can significantly increase nutrient cycling in soil via excretion of nutrients from ingested

plant matter, which in turn supports plant productivity (Owen 1980; Crawley 1983; McNaughton 1985, 1986; Lindroth 1989).

Types of herbivory may include browsing of dicot leaves and twigs or grazing of monocot blades (e.g. grasses and sedges) by mammals and birds; leaf chewing and sap-sucking by arthropods (exposed herbivory); wood and stem boring, leaf mining, and gall-inducing by arthropods (concealed herbivory) (Crawley 1983; Lindroth 1989; Weis and Berenbaum 1989). Specialized herbivores often consume reproductive parts of plants, such as flowers, pollen, nectaries, fruit, and seeds (Crawley 1983; Lindroth 1989; Weis and Berenbaum 1989; Fleming 1991). Galling is a prolonged complex form of biotic damage to plants that can be induced by fungi, bacteria, viruses, and nematodes, as well as arthropods (e.g. insects and mites), although fungi, bacteria, and viruses are considered parasites rather than herbivores (Dreger-Jauffret and Shorthouse 1992; Mani 1992). The degree to which herbivory or similar damage impacts a plant species depends much on the plant's phenology (e.g. seasonal timing of growth and reproduction), its architecture, its developmental stage when damaged, environmental factors, and competitive interactions with neighboring species, as well as the type of herbivory and the plant organ affected (Crawley 1983; McNaughton 1983; Weis and Berenbaum 1989). Herbivory on flowers, fruit, and seeds can severely impact seed production and, therefore, fitness (Janzen 1983; Stiles 1989), although plants often produce many more flowers than will mature into seeds (Stephenson 1981; McNaughton 1983), which may reduce the impact of herbivory. Flower- and fruit-feeders can reduce the number of "sinks" (tissues or organs that use) for photosynthate (carbon) (Crawley 1983). Sap-feeders can rob the plant of carbon and nitrogen. Leaf herbivory can decrease the plant's photosynthetic area, cause premature leaf senescence, and cause carbon to be limiting by depriving the plant of its carbon "source." Herbivory of young leaves and leaf buds is especially detrimental due to the loss of future photosynthate that would

have been produced had they persisted on the plant for their normal life span. Root feeders and xylem borers and suckers can alter water and nutrient uptake or flow, and shoot and stem borers and gallers can alter the plant's architecture (Crawley 1983).

Resistance Against Herbivory

Many plant species have evolved traits that make them repellent, unpalatable, undigestible, toxic, or otherwise unattractive to herbivores. These adaptations are considered part of a plant's "resistance" and generally may be referred to as "plant food quality," although it is prudent to note that traits discouraging herbivory may be incidental and may not have evolved specifically as defensive adaptations against herbivores (Futuyma 1983; Weis and Berenbaum 1989). Plant defenses may include maintaining assimilable nitrogen at low levels in plant tissues when not needed and storing nitrogen in toxic or unpalatable allelochemical or secondary chemical forms (Futuyma 1983). Many secondary defensive chemicals are the precursors, intermediates, byproducts, wastes, or storage forms resulting from primary metabolic processes that are incidentally harmful to herbivores (Rhoades 1979; Futuyma 1983). Some defensive compounds may have originally evolved as toxins against pathogens (Futuyma 1983).

Defensive secondary chemicals include non-protein and toxic amino acids, oxalic acid, alkaloids, terpenoids, saponins, tannins, glucosinolates, cyanogenic glycosides, flavonoids, and other phenolic compounds, among many others (Rhoades 1979; Crawley 1983; Futuyma 1983; Strong *et al.* 1984; Lindroth 1989; Weis and Berenbaum 1989; Coley and Aide 1991). Many defensive secondary chemicals specifically have insecticidal properties. Pyrethrum and other pyrethrins are produced by several members of the Compositae (now Asteraceae), most notably *Chrysanthemum cinerariaefolium*, with highest concentrations found in the disk flowers (Matsui

and Yamamoto 1971). Rotenone and related rotenoids are produced by members of the Leguminosae, and roots of the genus *Derris* have been prepared in East Asia for insecticide (Fukami and Nakajima 1971). The alkaloid nicotine, along with anabasine and nornicotine, is produced by at least 18 species of tobacco (*Nicotiana* spp.) and has been applied as an insecticide on agricultural crops in North America and Europe (Schmeltz 1971).

Defenses also may be physical (mechanical) or textural such as tissue toughness reinforced by cellulose, lignins, fiber, latex, or silica, or the presence of trichomes (e.g. pubescence, hairs, spines, hooks), wax, or cork on or in the epidermis (Coley 1983; Crawley 1983; Futuyma 1983; Strong *et al.* 1984; Lindroth 1989; Weis and Berenbaum 1989; Coley and Aide 1991). These characteristics may become more prevalent in leaves with age (Crawley 1983) and in shade-tolerant species (Coley 1983). Cellulose is particularly effective at deterring herbivores; very few animals can digest it, and only with the assistance of gut microflora (Crawley 1983; Janzen 1983). Rapid growth to a size beyond the interest or capabilities of herbivores may also be a defensive trait, as in some woody plants like *Betula* spp., where shoots rapidly regrow to greater diameter and the digestibility of bark and wood components decreases as a result (Danell *et al*. 1985). Young leaves, which are tender and often susceptible to herbivores, may mature quickly and increase tissue toughness (Coley 1983; Coley and Aide 1991). Plants may kill endophytic insect herbivores, such as gall-inducers or bark-borers, by exuding excess resin or overgrowing plant tissues into larval chambers (Berryman 1976; Mattson and Addy 1975; Coulson and Witter 1984; Abrahamson and Weis 1987; Christiansen *et al.* 1987). Plants also may evade herbivory with morphological traits such as leaf shapes that mimic plants inedible to insect herbivores (Crawley 1983; Futuyma 1983; Weis and Berenbaum 1989) and prostrate growth forms and basal meristems (e.g. regions of actively dividing and differentiating cells) positioned near the ground and protected by leaf sheaths in some grasses and sedges

from grazing mammals (Owen and Wiegert 1981; Crawley 1983; Lindroth 1989; Lehtilä 2000). Many plants that produce seeds and fruit sought after by herbivores have defenses to protect seeds in the digestive tract and the external environment after excretion, such as seed coats that withstand digestive chemicals and mechanical grinding, slippery fruit pulp that makes it difficult to target the seeds for chewing and encourages swallowing the seed whole, and laxative chemicals that expedite the seed's passage through the gut (Janzen 1983; Stiles 1989).

Plant defenses may be either produced as part of normal tissue development to repel primary herbivory (constitutive) or produced in response to some initial damage or herbivory event to deter subsequent herbivory (induced or facultative) (Crawley 1983; Strong *et al.* 1984; Weis and Berenbaum 1989; Agrawal 1998). As with plant growth and reproduction, the production of defenses requires energy and nutrients, which are typically limited. Given the premise that defenses evolve and are allocated to maximize fitness, the Optimal Defense Theory proposes that defenses increase fitness and are beneficial only to the extent that natural enemies are present (Rhoades 1979). Under conditions lacking enemies, defenses are costly and reduce relative fitness because producing them limits energy and resources that could have been used for reproduction and other vital plant functions (Rhoades 1979; Crawley 1983). Thus, induced defenses may be more beneficial than constitutive defenses because they are produced only when needed (Rhoades 1979; Crawley 1983). Simulated and actual herbivory on leaves induced the wild radish *Raphanus sativus* L. (Brassicaceae) to produce increased concentrations of mustard oil glycosides (glucosinolates) and higher densities of setose trichomes, which reduced subsequent attacks, even by herbivores of other species (Agrawal 1998). Similarly, a change in palatability or quality of *Ipomopsis aggregata*, or scarlet gilia, after initial herbivory was suggested as the factor limiting subsequent ungulate browsing to only the actively growing tips of the plant (Paige 1992). Attack by western tent caterpillars (*Malacosoma californicum*

pluviale) induced red alder trees (*Alnus rubra*) to increase proanthocyanidin levels in remaining leaves (Rhoades 1983). Adventitious shoots sprouted by *Betula papyrifera*, *Populus tremuloides*, *P. balsamifera*, and *Alnus crispa* after browsing by snowshoe hares (*Lepus americanus*) contained unpalatable concentrations of phenolic resins and terpenes and were avoided by hares for subsequent browsing compared to mature twigs (Bryant 1981). Simulated herbivory on *Betula pubescens* leaves induced a change in quality that resulted in lower mass of *Oporinia autumnata* pupae compared to pupae raised on undamaged controls (Haukioja 1980). When 50% of the leaves of tansy ragwort (*Senecio jacobaea*) were removed, a 40-47% increase in alkaloids and nitrogen-oxidases was observed in the remaining leaves (Rhoades 1979). In some coniferous species, a resin-duct system exists as an energy-costly constitutive defensive strategy against bark beetles, while a hypersensitive wound reaction involving impregnating attacked tissues with resinous and phenolic compounds is an acute, induced response (Christiansen *et al.* 1987).

Plant defenses may be quantitative (dosage-dependent) or qualitative (toxic secondary substances) (Rhoades 1979; Crawley 1983). Quantitative defenses include tannins, lignins, resins, silica, physical defenses, and others, are often present in large quantities (Rhoades 1979; Crawley 1983). They are relatively costly to the plant and are employed primarily by plants that are k-strategists (e.g. long-lived, abundant, “apparent” to enemies in time and space) to make themselves less digestible or palatable to herbivores (Rhoades 1979; Crawley 1983). Qualitative defenses include glucosinolates, alkaloids, cyanogenic glycosides, and other substances that are toxic in low concentrations(Stenseth 1978; Rhoades 1979; Crawley 1983). They are most effective against non-adapted generalists, and favored by r-strategist plants (e.g. produce large quantities of seeds, ephemeral, “unapparent”) to interfere with herbivore metabolism (Stenseth 1978; Rhoades 1979; Crawley 1983). Defenses also tend to be relegated primarily to certain

plant organs based on their value to the plant's fitness, such as leaves, flowers, fruits, seeds, young shoots, and buds (Rhoades 1979; Janzen 1979; Bryant and Kuropat 1980). Some woody boreal species grow quickly (e.g. pioneer, gap-colonizing) and have strong chemical defenses only as juveniles, when they must resist browsing and attain light-competitive heights (Bryant *et al.* 1983; Danell *et al*. 1985; Danell and Bergström 1989). Young leaves on mature boreal trees (Dement and Mooney 1974; Cates *et al.* 1983) and tropical tree species (Coley 1983) tend to be lower in quantitative defenses (e.g. tannins, toughness) and higher in qualitative defenses (e.g. cyanogenic glycosides, phenols) compared to their mature counterparts. In an assessment of western spruce budworm (*Choristoneura occidentalis*) success and qualitative defenses, quantitative defenses, and total nitrogen in young needles of the woody boreal species Douglas fir (*Pseudotsuga menziesii*), qualitative defenses like terpenes were found to be most important in reducing *C. occidentalis* success (Cates *et al.* 1983).

Availability of resources and the intensity of interspecific and intraspecific competition can predict the presence or absence of defenses in plants. Poorly resourced plants (e.g. poor light, nutrient, or water status) have slower growth rates, low photosynthetic rates, limited potential to regrow tissue lost to herbivory, low levels of leaf protein, poor below-ground storage reserves, little capacity for growing beyond the browsing height of many herbivorous mammals, and invest in high amounts of constitutive immobile chemical defenses like tannins and polyphenolic compounds (Bryant *et al.* 1983; Coley 1983; Crawley 1983; Coley *et al.* 1985; Danell *et al*. 1985; Chapin and McNaughton 1989; Weis and Berenbaum 1989; Fernandes and Price 1991; Whitham *et al.* 1991). Plants under poorly resourced conditions also often develop physical defenses like trichomes or increased leaf toughness with lignins (Crawley 1983; Price 1991; Coley and Aide 1991; Fernandes and Price 1991) and experience higher longevity and less abscission of leaves and twigs (Coley *et al.* 1985; Lindroth 1989; Fernandes and Price 1991).

Longer-lived plants tend to be highly defended against herbivory, chemically and mechanically (Stenseth 1978). Since resin-related strategies utilized by many coniferous species can strain the tree's carbon budget, Christiansen *et al.* (1987) proposed that carbon limitations caused by any environmental factor that impedes photosynthesis or the transport of photosynthate may be the primary underlying factor predicting a conifer's ability to defend itself against bark beetle outbreaks.

Plant defenses may act by repelling herbivores, preventing feeding, causing infertility (in vertebrates), inhibiting oviposition or growth (in insects), interfering with digestion, or poisoning (Crawley 1983; Futuyma 1983; Weis and Berenbaum 1989). Pyrethrins (Matsui and Yamamoto 1971) and nicotine (Schmeltz 1971) have a paralytic effect on insects, while rotenone interferes with oxygen uptake of mitochondria (Fukami and Nakajima 1971). Plant defenses may have profound negative effects on some types of herbivores and not on others, with some herbivores even benefiting from them (Crawley 1983). Some chemical defenses may be nutrients at low concentrations and toxins at high concentrations, even for the same herbivore (Crawley 1983). Other defenses may simultaneously repel unwanted herbivores and attract or stimulating feeding by beneficial ones. The bud-galling sawfly *Euura mucronata* may be attracted to favorable *Salix cinerea* by secondary phenolic glycosides produced a result of previous damage, but which are unpalatable to mammalian ungulates (Roininen *et al*. 1988). Similarly, unripe fruits may produce secondary compounds that deter consumption, and ripe fruits may produce ones that attract and benefit seed dispersers and/or repel unwanted animals like seed predators (Janzen 1978; Owen 1980; Crawley 1983; Janzen 1983). The immature fruit pulp of *Heteromeles arbutifolia* was found to harbor high concentrations of tannins and cyanogenic glycosides, the latter which are reallocated to seeds with ripening, making the fruits palatable for birds (Dement and Mooney 1974).

Herbivores, especially insects, that have become specialized to their host plants or are sedentary generalists often adapt physiologically and the plant's secondary compounds attract or stimulate feeding in the herbivore (Crawley 1983; Futuyma 1983). Since grass-grazer systems appear to have coevolved (McNaughton 1979; Owen 1980; Owen and Wiegert 1981; McNaughton 1986), what are often assumed to be defenses in grasses may actually serve more to regulate grazing (e.g. attract grazers but deflect overgrazing), rather than prevent grazing completely (Owen and Wiegert 1981). Other herbivore species simply adapt to or circumvent plant defenses, rendering them ineffective. In small numbers, bark beetles may be killed by resin flooding their feeding and larval chambers, but their presence attracts more bark beetles (via pheromones), which can mass attack the tree and overwhelm its defenses ("social facilitation") (Berryman 1976; Coulson and Witter 1984; Christiansen *et al.* 1987). Galling insects also may be able to circumvent constitutive allelochemical defenses by feeding on nutritive gall tissues that are relatively free of phenolic compounds and other chemical defenses (Fernandes and Price 1991). The shoot-galling sawfly *Euura lasiolepis* appears uninhibited by any induced plant defenses against other herbivores in *Salix lasiolepis*, such as phenolic glycosides (Craig *et al.* 1986). Galling insects may also accumulate plant compounds in gall tissues for their own protection from browsing herbivores (Janzen 1977), fungal infection (Taper *et al.* 1986), or natural enemies (Fernandes and Price 1991). The oak-galling cynipid wasp *Dryocosmus dubiosus* sequesters tannins in gall tissues to prevent fungi from invading larval chambers and consuming larvae (Taper *et al.* 1986).

Many insect herbivores sequester secondary defensive chemicals in specialized body tissues or glands that isolate them and either detoxify them or accumulate them and utilize them against the insect's own predators and parasites (Crawley 1983; Futuyma 1983; Weis and Berenbaum 1989; Brown *et al.* 1991). These insects also may exhibit aposematic signals (colors,

markings, and sometimes sounds or odors) that warn their natural enemies of the possessor's unpalatability (Futuyma 1983; Brower 1988; Weis and Berenbaum 1989; Brown *et al.* 1991). A classic example involves the aposematic coloration of the monarch butterfly (*Danaus plexippus*), which, as larvae, accumulate cardiac glycosides from their primary larval food plant *Asclepias* spp. (milkweed) that act on natural enemies such as birds even when ingested at sublethal doses (Futuyma 1983; Brower 1988; Weis and Berenbaum 1989; Brown *et al.* 1991; Nishida 2002). Similar sequestration of secondary phytochemicals from host plants occurs among numerous other lepidopterans, including but not limited to members of the Danainae, Nymphalinae, and Ithomiinae (family Nymphalidae), Papilionidae, Sphingidae, and Pieridae (*Pieris* spp.) , the latter which sequester mustard glucosinolates from leaves of the Brassicaceae (cabbage family) (Brown *et al.* 1991; Nishida 2002).

Some evidence exists suggesting that induced plant responses may be transferred to neighbors within the population. The Talking Trees Hypothesis proposes that some plant species biochemically "communicate" with each other via air-borne pheromones, so that herbivory occurring on one individual will induce the production of chemical defenses in neighboring individuals (Rhoades 1983; Lindroth 1989; Weis and Berenbaum 1989). One of the earliest documented examples of this phenomenon was reported by Rhoades (1983), who discovered that attack of Sitka willow (*Salix sitchensis*) by western tent caterpillars (*Malacosoma californicum pluviale*) induced a quality change in leaves of both attacked and nearby unattacked willows (controls), thereby increasing mortality of tent caterpillars in subsequent attacks on both treatment groups. With no evidence of root connections between these willows, Rhoades (1983) tentatively suggested that unattacked willows may have been responding to airborne pheromones from attacked willows. In another field experiment involving sagebrush (*Artemisia tridentata*) and neighboring wild tobacco (*Nicotiana attenuata*),

clipping of sagebrush prompted the release of an epimer of methyl jasmonate, which induced increased levels of the defensive compound polyphenol oxidase in neighboring wild tobacco which significantly reduced herbivory by six species of grasshoppers (*Cratypedes neglectus*, *C. lateritius*, *Trimerotropis fontana*, *Conozoa sulcifrons*, *Melanoplus sanguinipes*, and *Cordillacris occipitalis*), and two species of noctuid cutworms (*Peridroma saucia* and *Agrotis ypsilon*) (Karban *et al.* 2000). Blockage of soil contact did not alter this effect, while blockage of air contact did alter it, suggesting that chemical cues from *A. tridentata* were airborne (Karban *et al.* 2000). In both field and laboratory experiments, simulated and actual defoliation of alder (*Alnus glutinosa*) by alder leaf beetles (*Agelastica alni*) resulted in a significant reduction of subsequent *A. alni* feeding and oviposition both on damaged alder and undamaged neighbors, with these beetle activities increasing with distance from defoliated alder (Dolch and Tscharntke 2000).

Compensation as a Response to Herbivory

Some plant species respond to certain types of low to moderate herbivory-induced damage by compensating in terms of growth, biomass, or even fitness, particularly as a result of removal or damage of the apical meristem (Owen 1980; Crawley 1983; McNaughton 1983; Craig *et al*. 1986; Paige and Whitham 1987; Benner 1988; Lindroth 1989; Maschinski and Whitham 1989; Weis and Berenbaum 1989; Whitham *et al.* 1991; Paige 1992; Aarssen 1995; Huhta *et al.* 2000; Fornoni *et al.* 2003; Wise and Abrahamson 2005, 2008). Grazing by mammals may stimulate propagation via tillering of monocots (e.g. grasses and sedges family Gramineae), which have protected basal meristems, or active growing regions at the base of leaves (Owen 1980; McNaughton 1983; Lindroth 1989; Lehtilä 2000). Scarlet gilia (*Ipomopsis aggregata*), a biennial herbaceous plant that flowers once and then dies (semelparous), experienced heavy

browsing early in the growing season (before flowering) by mule deer (*Odocoileus hemionus*) and elk (*Cervus elaphus*), which removed the stem tips, released apical dominance, and induced the plant to produce multiple new inflorescences that overcompensated for the tissue lost to herbivory (Paige and Whitham 1987). Unbrowsed plants, by contrast, produced only a single inflorescence during the season. Having eliminated other mechanisms for this overcompensation and having found no difference between browsed and unbrowsed plants in other indicators of fitness such as number of seeds per inflorescence, seed mass, germination success, and seedling survival, Paige and Whitham (1987) concluded that browsed plants in this study system experienced a 2.4-fold increase in relative fitness simply by producing more flowering stems. Thistle (*Jurinea mollis*) responds to herbivory by lepidopteran larvae on its basal rosette of leaves by producing multiple rosettes that may each produce a flowering stalk, resulting in up to three times as many seeds produced as in plants with a single rosette and flowering stalk (Inouye 1982). However, herbivory on *J. mollis* flowers results in negative response: When terminal flower heads are attacked by small mammals, the plant produces axillary flower heads that are less successful at seed production than terminal heads, while attack on flower head receptacles by moth and tephritid fly larvae disrupts seed development and may result in no viable seed produced by the plant (Inouye 1982).

Thus, plant responses to herbivory (e.g. negative or positive) may be considered less in opposition or contradiction to each other and more as a range of responses along a continuum from undercompensation to full or equal compensation to overcompensation, all of which may be expressed within a species, a population, and even an individual depending on plant condition and age, nutrient reserve levels, history with previous herbivory, the plant organ or tissue being consumed and its age, resources and other environmental factors (e.g. soil nutrients, water, light, intraspecific or interspecific competition), and the timing, intensity, and

type of herbivory (Crawley 1983; Maschinski and Whitham 1989; Whitham *et al.* 1991). Maschinski and Whitham (1989) coined the hypothesis for this spectrum of responses Compensatory Continuum Hypothesis. In full or equal compensation, the plant compensates for tissue lost to herbivory by producing an equal amount of new tissue, resulting in no net change in biomass, seed production, or fitness, thus representing a net neutral effect on the plant (Crawley 1983; McNaughton 1983; Maschinski and Whitham 1989; Whitham *et al.* 1991). In an experiment, full compensation would be evidenced by no significant net differences in biomass, seed set, or potential fitness between experimentally manipulated plants (e.g. browsed or clipped plants) and control plants. In overcompensation, the plant produces more tissue than was lost, resulting in a net increase in biomass and/or possibly higher fitness and a net benefit to the plant. Experimentally manipulated plants would have significantly more tissue or biomass, higher seed production, and/or higher fitness compared to controls(Crawley 1983; McNaughton 1983; Maschinski and Whitham 1989; Whitham *et al.* 1991; Marquis 1996; Lehtilä 2000). In undercompensation, the plant produces significantly less tissue than was lost, which would be evident when compared to control plants in experiments (Crawley 1983; McNaughton 1983; Maschinski and Whitham 1989; Whitham *et al.* 1991; Marquis 1996). Overcompensation is not infinite and may become full compensation or undercompensation if the level of herbivory surpasses a critical threshold, with positive or neutral effects more likely when herbivory is low or moderate (Figure 1) (Crawley 1983; McNaughton 1983; Marquis 1984; Fornoni *et al.* 2003). Full or equal compensation also may be possible only at low or moderate levels of herbivory, becoming undercompensation at higher levels (McNaughton 1983). Neither full or overcompensation are likely when herbivores are specialists, food-limited, and feed continuously, with no downtime during which the plant may recover, regrow, and reproduce (Crawley 1983; Islam and Crawley 1983; Van Der Meijden 1990).

Plant species associated with full or overcompensation appear to share several characteristics. They tend to be herbaceous, as opposed to woody, and tend to be short-lived annuals or biennials and r-strategists, as opposed to longer-lived perennials and K-strategists (Stenseth 1978; Coley *et al.* 1985; Whitham *et al.* 1991). They often grow under relatively highly resourced conditions (Coley *et al.* 1985; Whitham *et al.* 1991). These plants usually have high growth and photosynthetic rates, large storage reserves, well-integrated vascular connections between "source" tissues (e.g. leaves, roots) and "sink" tissues (e.g. active meristems, flowers), and flexible and indeterminate growth (Coley *et al.* 1985; Whitham *et al.* 1991; Marquis 1996). They have substantial reserves of dormant buds or meristems (bud bank) from which they respond to high rates of herbivory with rapid regrowth (Coley *et al.* 1985; Whitham *et al.* 1991). They also often have an increased tendency after apical meristem damage to retain stems and leaves and replenish the bud bank through lateral buds on new stems (Marquis 1996). Plants that compensate for herbivory often produce low reversible levels of mobile qualitative defensive chemicals such as alkaloids, phenolic glycosides, and cyanogenic glycosides (Coley *et al.* 1985; Whitham *et al.* 1991). Thus, the mechanisms identified as being responsible for full or overcompensation include: increased photosynthetic rates in remaining plant tissue; large storage reserves; increased allocation of and altered distribution patterns of new and stored nutrients to growing tissue; shedding or removal of senescing tissue with low photosynthetic activity; increased light intensity to remaining plant tissues that are no longer shaded by tissues that have abscised or been removed; a large reserve of meristems (bud bank) that sprout new shoots and stems when apical dominance is released as a result of herbivory (Crawley 1983; McNaughton 1979; McNaughton 1983; Paige and Whitham 1987; Whitham *et al.* 1991; Paige 1992; Fornoni *et al*. 2003).

The release of apical dominance and the activation of lateral buds have been implicated in nearly all documented cases of regrowth and overcompensation after apical meristem damage from herbivory (Inouye 1982; Paige and Whitham 1987; Aarssen and Irwin 1991; Whitham *et al.* 1991; Irwin and Aarssen 1996; Marquis 1996; Lehtilä 2000; Fornoni *et al.* 2003; Wise and Abrahamson 2005, 2008). Disruption of apical dominance and subsequent branching can alter plant growth patterns in ways that benefit the plant more than when patterns are undisturbed by herbivory, such as improved support, better positioning of leaves for photosynthesis, and an increased number of terminals for flowering and, thus, seeds (Owen 1980; Inouye 1982; Paige and Whitham 1987). Apical dominance is the inhibition of lateral bud growth by plant hormones produced by actively growing apical tissues, or tissues at the tip of a shoot (Jameson 1963; Leopold 1967; Phillips 1971; Crawley 1983; Cline 1991; Salisbury and Ross 1991; Lehtilä 2000; Domagalska and Leyser 2011; Gallavotti 2013). Apical dominance is adaptive in many plant species for producing tall, spindly plant growth patterns that confer a competitive advantage over neighboring plants in structurally complex communities, or even over other parts of the same plant for light resources (Crawley 1983; Cline 1991; Aarssen and Irwin 1991; Aarssen 1995; Irwin and Aarssen 1996). It also may hold some lateral buds "in reserve" (bud bank) to produce new shoots in the event that the apex of the plant is damaged by herbivory or weather (Cline 1991; Salisbury and Ross 1991; Aarssen 1995; Irwin and Aarssen 1996; Marquis 1996; Lehtilä 2000).

The exact causes of apical dominance are complex, although the plant hormone auxin (e.g. indole-3-acetic acid or IAA) has long been suspected as a primary regulator of apical dominance (Jameson 1963; Leopold 1967; Phillips 1971; Crawley 1983; Cline 1991; Salisbury and Ross 1991; Domagalska and Leyser 2011; Finet and Jaillais 2012). Auxins constitute a class of growth-promoting hormones produced primarily in young leaves, leaf primordia, and activated

meristematic tissues and they play a major role in plant growth and cell elongation (Jameson 1963; Leopold 1967; Phillips 1971; Crawley 1983; Cline 1991; Salisbury and Ross 1991; Finet and Jaillais 2012; Domagalska and Leyser 2011). Auxin transport is unidirectional or polar, that is, along the axis of the plant or plant organ; in shoots, from tips to base (basipetal) (Phillips 1971; Cline 1991; Salisbury and Ross 1991; Domagalska and Leyser 2011; Finet and Jaillais 2012). Auxin transport may occur via active cell-to-cell transporters over short or long distances or via passive diffusion through vascular tissues (Domagalska and Leyser 2011; Finet and Jaillais 2012; Gallavotti 2013).

Recent research indicates that complex interactions among several hormone pathways have been implicated in apical dominance, including auxin, strigolactones, and cytokinins (Domagalska and Leyser 2011; Gallavotti 2013). Strigolactones are a new class of carotenoid-derived, bud-repressing hormones that are upwardly mobile (acropetal, or from base to tip) and are produced mostly in roots but also in shoots (Hayward *et al.* 2009; Domagalska and Leyser 2011). Cytokinins are another class of plant growth hormones that regulate cell division and lateral shoot elongation and promote bud activation (Cline 1991; Salisbury and Ross 1991). Like strigolactones, they are produced primarily in roots, but also in shoots, and are transported acropetally (Domagalska and Leyser 2011).

The Auxin Transport Canalization-based Model (Sachs 1981) proposes that, as a necessary step in bud (meristem) activation, axillary buds must export auxin to the stem, which acts as a strong sink for auxin when auxin flow in the polar auxin transport (PAT) system to the roots is initially low (Domagalska and Leyser 2011; Gallavotti 2013). Auxin produced in an activated axillary bud is exported to the stem and establishes apical dominance over any remaining axillary buds before they can activate and export their own auxin to the stem (Gallavotti 2013). Auxin's inhibitory action on axillary buds is indirect, since it does not actually

enter neighboring buds; its export from activated meristematic tissue reduces the sink strength of the stem, which inhibits the export of auxin from remaining buds (Domagalska and Leyser 2011). Strigolactones play a role in inhibiting shoot branching by reducing the accumulation of transport proteins involved in PAT on cell membranes (e.g. PIN1), decreasing the strength of the shared polar auxin transport system, and increasing competition among axillary buds for exporting auxin to the stem, thereby promoting apical dominance (Domagalska and Leyser 2011; Gallavotti 2013). Sink strength in the stem increases and multiple buds may be activated when the active (apical) meristem is removed or when strigolactone biosynthesis or signaling is impaired (Domagalska and Leyser 2011).

Another model, the Second Messenger Model, proposes that auxin regulates the synthesis of a second messenger, which then migrates into and regulates activity in buds (Chatfield *et al.* 2000; Hayward *et al.* 2009; Domagalska and Leyser 2011). Cytokinin (Chatfield *et al.* 2000; Domagalska and Leyser 2011) and strigolactones (Hayward *et al.* 2009; Domagalska and Leyser 2011) currently are the most promising candidates as this second messenger. Strigolactones inhibit bud activity and are upregulated (synthesis is stimulated) by auxin (Hayward *et al.* 2009; Domagalska and Leyser 2011), while cytokinin promotes bud activity and is downregulated (synthesis is inhibited) by auxin (Chatfield *et al.* 2000; Domagalska and Leyser 2011). Strigolactones also interact with auxin in feedback loops that also regulate auxin's synthesis and activity (Hayward *et al.* 2009). Cytokinin and auxin also regulate each other: A high ratio of cytokinin to auxin promotes bud elongation and shoot growth and inhibits root growth, while a low ratio inhibits shoots and promotes apical dominance and root growth (Salisbury and Ross 1991; Chatfield *et al.* 2000). The Second Messenger Model and the Auxin Transport Canalization-based Model are not mutually exclusively and may both be at work under different conditions (Domagalska and Leyser 2011).

Environmental factors such as light also may play a role in regulating auxin and other plant hormones involved in apical dominance (Franklin 2008; Domagalska and Leyser 2011). Apical dominance is strongest in shaded or crowded conditions (Smith and Whitelam 1997), conditions which may stimulate gene expression associated with auxin signaling (Franklin 2008). Furthermore, disruption of apical dominance due to removal of the apical meristem is more likely to result in bushier growth forms when light competition is low (e.g. in open rangeland) and less likely when light competition is intense (e.g. beneath forest canopy) (Crawley 1983). Nutrients may interact with plant hormones in establishing or overriding apical dominance (Domagalska and Leyser 2011). Thus, the strength of apical dominance and the capacity for full or overcompensation can vary among plant species and even among and within populations of the same plant species depending on timing of apical meristem damage and local resource conditions such as nutrient availability, light direction and intensity, proximity to neighbors, and the age of the plant (Phillips 1971; McIntyre 1977; Benner 1988; Cline 1991; Aarssen 1995).

Apical dominance tends to weaken with increasing distance from the apical meristem, which may be explained by hormonal gradients (e.g. diminution of auxin in concentration as it diffuses away from its source) (Jameson 1963; Cline 1991). Thus, the likelihood of lateral shoots breaking bud and elongating may increase with increasing distance from the apical meristem in many species, with more basally located lateral shoots often breaking bud earlier and being longer than shoots closer to the apex (Cline 1991). Apical dominance is also known to decrease with plant age (Cline 1991; Aarssen 1995). Finally, bud size or age may cause differential responses to apical dominance among lateral buds (Cline 1991). Chatfield *et al.* (2000) found that small buds responded to apical auxin, while large buds were unresponsive.

Apical dominance controls plant architecture (e.g. growth patterns, the absence or presence and extent branching) (Cline 1991; Gallavotti 2013). Plant species that produce high

concentrations of auxin exhibit stronger apical dominance and have a taller, less branched growth form, whereas species with relatively low concentrations of auxin exhibit weaker apical dominance, grow lateral branches readily, and have bushier architecture (Leopold 1967; Cline 1991). Apical dominance can be completely disrupted by damage to or removal of the apical meristem due to various biotic and abiotic causes, including weather events such as storms and frost, accidental or intentional breakage by the activities of animals and humans, and some types of herbivory (Jameson 1963; Cline 1991; Salisbury and Ross 1991). However, the disruption is temporary, since the growing tip of each new shoot eventually exerts its own apical dominance over lateral buds on the same shoot (Cline 1991).

The capacity to compensate for herbivore-related damage includes the type of herbivory, the plant organ attacked, and plant architecture and sectoriality (Marquis 1996; Stowe *et al.* 2000). Plant architecture is dictated by the organization of metameric or phytomeric units, which consist of a node (point along a stem where a leaf attaches), an internode (stem segment between nodes), at least one associated leaf, and an axillary meristem (secondary shoot meristem or bud at an axil, or region adjoining the leaf-node attachment point) (Geber 1990; Marquis 1996; Domagalska and Leyser 2011; Gallavotti 2013), while sectoriality is the arrangement of those units into higher-level morphological units that are modular, share a common vascular bundle, and within which resource and hormone allocation is restricted (Marquis 1996; Stowe *et al.* 2000). Thus, plant architecture is impacted by the number of axillary meristems available for elongation after apical dominance is disrupted by damage or removal of the stem apex (Marquis 1996; Stowe *et al.* 2000; Domagalska and Leyser 2011). Sectoriality can dictate how certain types of herbivory, such as stem galling or boring and phloem or xylem feeding, impact the acquisition of and allocation of plant resources during attack (Marquis 1996; Stowe *et al.* 2000). Plant size may also affect a plant's ability to

compensate for damage, with larger plants having an advantage over smaller plants (Islam and Crawley 1983).

Timing of herbivory relative to plant phenology is perhaps the most important external factor dictating the type of compensation resulting from herbivory, with the availability of nutrients and the intensity of herbivory also playing crucial roles (Crawley 1983; Islam and Crawley 1983; Maschinski and Whitham 1989; Whitham *et al.* 1991; Paige 1992; Aarssen 1995; Marquis 1996; Fornoni *et al.* 2003). Plants that experience herbivory early in the growing season often have more time to recover and often are in the midst of nutrient flushes that may occur at that time, but semelparous species, or those that reproduce once, generally undercompensate or equally compensate when herbivory occurs late in the growing season, since little time remains for them to recover (Crawley 1983; Whitham *et al.* 1991; Paige 1992). Lennartsson *et al.* (1998) found that in Sweden, a region with a short growing season, the biennial *Gentianella campestris* overcompensated for simulated herbivory (clipping) in terms of fruits and seeds during only a narrow window in the growing season: too early, and affected plants were constrained by the availability of resources for regrowth; too late, and plants were unable to develop new flower-carrying nodes before frost. In this experiment, clipping during the optimal overcompensation window increased flower-carrying nodes on newly regrown branches by up to 4.3 times (Lennartsson *et al.* 1998). In a study involving transplanted *Thlaspi arvense* (Brassicaceae) seedlings and nutrient supplementation, the removal of the apical meristem, and the timing of these events in relation to plant phenology, Benner (1988) found that production of secondary branches increased with apex removal, and seed production increased with apex removal only when it occurred early (20 d. after seedling transplantation) and concurrently with nutrient supplementation. Additionally, nutrient supplementation resulted in more secondary branches, fruits, and seeds, with and without removal of the apical meristem, compared to

controls. Timing of herbivory later in plant development, when flowering occurred, was also cited by Inouye (1982) as a factor in the negative response of *Jurinea mollis* to the consumption of its terminal flower heads by small mammals. The Serengeti grasses *Sporobolus ioclados*, *Eustachys paspaloides*, and *Pennisetum mezianum* experienced poor compensatory regrowth and reduced total biomass when defoliated under extremely phosphorus-deficient conditions, suggesting that high levels of nutrients and efficient nutrient cycling typically observed in the Serengeti are necessary conditions for compensation (Chapin and McNaughton 1989).

Intraspecific and interspecific competition among plants for light, nutrients, and other resources may be another important factor influencing a plant's response to herbivory (Crawley 1983). In a three-year study on the semelparous species *Ipomopsis arizonica* involving natural and simulated herbivory, plant associations (interspecific competition), nutrient (nitrogen) and water supplementation, and attention to the timing of these events in relation to each other, Maschinski and Whitham (1989) determined that full compensation of fruits was the most common response under natural conditions, while undercompensation of fruits occurred in 16% of the plants when associated with neighboring grasses and subjected to herbivory late in the season (mid-July or later). Overcompensation of fruits occurred only when herbivory occurred early in the growing season or when late-season herbivory was accompanied by nutrient (nitrogen) supplementation, which was applied each year from about May or June through July or August, and neighbors were absent (Maschinski and Whitham 1989). The tolerance of rosinweed (*Silphium integrifolium*) to apical meristem galling by the cynipid wasp *Antistrophus silphii* was tested in field and garden experiments, where field conditions were natural (e.g. competitive and resource-limiting) and garden conditions were competition-free and subject to nutrient and water manipulation (Fay *et al.* 1996). Generally, galled *Silphium integrifolium* recovered better from galling in the garden than in the field (Fay *et al.* 1996). Although galled

plants experienced initial loss of height and leaf area in both the field and garden when galled, field subjects also experienced early stem and leaf senescence, no regrowth, and reduced reproductive output, although stem mass and gall mass increased (Fay *et al.* 1996). Axillary leaf buds failed to activate in galled field plants, even though galling occurred early in the growing season (late April and May) (Fay *et al.* 1996). After initial height and leaf area loss in the garden, galled *S. integrifolium* experienced vigorous regrowth from axillary leaf buds with 28% greater total leaf area averaged from May through August compared to ungalled plants in the garden and little or no loss in biomass or reproductive output compared to galled plants in the field (Fay *et al.* 1996). Growth was most vigorous under conditions combining galling and water- and nutrient-supplementation, but plants in this treatment also experienced the most rapid loss in axillary leaf area throughout the summer (Fay *et al.* 1996). Ungalled, unsupplemented plants in the garden also experienced more axillary growth than ungalled, unsupplemented plants in the field, suggesting that competition-induced light limitations maintained apical dominance (Fay *et al.* 1996). Fay *et al.* (1996) concluded that competition, resource limitation, and galling interacted to limit regrowth in the field, and that the meristem gall in this system may mimic the apical meristem in exerting apical dominance and preventing resource allocation away from the gall when competition is high and resources are limited.

A well-resourced plant may be more likely to respond positively to herbivory than a poorly resourced one, but clear predictions cannot be made easily on the general abundance of resources alone. The Limiting Resource Model (Wise and Abrahamson 2005, 2008) predicts that a plant's response to herbivory is based on more specific factors: (1) whether the resources that affect the plant's fitness are the same resources impacted by herbivores, (2) whether those resources limiting or not limiting when the herbivore is absent, and (3) whether herbivory, when it occurs, affects the acquisition of those resources or exacerbates existing limitations. The

acquisition of resources potentially impacted by herbivory includes the ability to take in soil nutrients or water through the roots or carbon dioxide through the leaves, as well as the ability to produce an adequate supply of photosynthates for cellular processes, growth, and reproduction. However, the degree of herbivory's impact on these resources depends on their relative abundance in relation to other resources. For example, as Wise and Abrahamson (2005) point out, in a high-nitrogen environment (e.g. where regular pulses of assimilable nutrients are introduced), carbon is limiting. Leaves are a carbon source (Crawley 1983), with carbon entering leaves in the form of carbon dioxide and being used during photosynthesis to produce various carbon-based molecules (e.g. glyceraldehyde-3-phosphate, sucrose, amylose) for energy. Since plants can use nitrogen and carbon for their biological processes only in relative proportions, a plant in a high-nitrogen environment can benefit from the abundant nitrogen only to the extent that carbon is also available (Wise and Abrahamson 2005). In this environment, herbivory that destroys or removes leaves, or the carbon-producing organs of the plant, exacerbates the limitation of carbon and impacts plant fitness to a greater extent than the same kind of herbivory occurring in an environment where nitrogen, not carbon, is the limiting resource (Crawley 1983; Wise and Abrahamson 2005).

According to Wise and Abrahamson (2005, 2008), the Limiting Resource Model also can be applied to the effect of herbivory on the number of meristems on an attacked plant. The number of axillary meristems can be a primary factor determining a plant's growth, development, and reproduction (Whitham *et al.* 1991; Aarssen 1995; Bonser and Aarssen 1996; Marquis 1996; Lehtilä 2000; Stowe *et al.* 2000; Wise and Abrahamson 2005, 2008; Gallavotti 2013). Meristem allocation dictates the fate of available meristems, which may either differentiate for vegetative growth by producing new shoots, differentiate for reproduction by sprouting a flowering stem (inflorescence), or remain dormant until later differentiation (Geber

1990; Bonser and Aarssen 1996). Differentiation for growth often produces additional meristems along the elongating shoot, while differentiation for flowering essentially terminates further activity and prevents any contributions of additional meristems to the plant's reserve pool (Geber 1990; Bonser and Aarssen 1996). Meristems removed by an herbivore or other damage also are essentially removed from the reserve pool, but this event also can increase the number of available meristems indirectly by disrupting apical dominance and releasing dormant lateral buds to produce new shoots bearing additional meristems (Whitham *et al.* 1991; Marquis 1996; Lehtilä 2000; Stowe *et al.* 2000; Wise and Abrahamson 2005, 2008). According to the Limiting Resource Model in relation to meristems, plants with weak apical dominance or indeterminate flowering may, in the absence of apical meristem damage, respond to higher nutrients with lateral bud break or additional flowers per stem, respectively (Wise and Abrahamson 2005, 2008). Indeterminate flowering occurs when only lateral stems produce flowers while the primary stem continues growing vegetatively. Determinate flowering occurs when the meristem of the primary stem differentiates from a vegetative growth function to a reproductive function; in this case, the meristem is removed from the pool of growing meristems and, if many flowers are produced, may cause a meristem limitation in the plant, and apical meristem damage can exacerbate the limitation. With indeterminate flowering, however, the availability of apical meristems is not expected to limit reproduction when damage is absent (Wise and Abrahamson 2005, 2008). Plants with very strong apical dominance that inhibits lateral bud break even under high resource conditions are likely to be severely limited by the number of activated meristems available for reproduction and may benefit from apical meristem damage (Wise and Abrahamson 2008). *Solidago altissima* (tall goldenrod) exhibits extremely strong apical dominance and did not break lateral buds in the absence of apical meristem damage, but did so after damage and, with nutrient supplementation, produced three

times more flowers compared to clipped and unfertilized ramets, although clipping generally resulted in fewer seeds per flower compared to unclipped ramets (58% reduction in seed count for clipped/unfertilized and 6% reduction for clipped/fertilized) (Wise and Abrahamson 2008).

Compensation as an Evolved Trait (Tolerance)

Much work and debate has ensued over the past few decades regarding whether overcompensation as a positive response to herbivory exists and, if it does, whether it is an evolved trait (McNaughton 1983, 1986; Belsky 1986; Whittam *et al.* 1991; Stowe *et al.* 2000; Fornoni *et al.* 2003). Whitham *et al.* (1991) reviewed several hypotheses for the evolutionary basis for overcompensation. The Nonadaptation Hypothesis proposes that overcompensation occurs in plant species having strong apical dominance but not having yet become highly adapted to an environment where strong apical dominance is no longer beneficial. Since apical dominance is genetically controlled, this hypothesis suggests that the genetic and/or developmental limitations under which the plant currently finds itself prevent it from breaking apical dominance without the assistance of an herbivore, making the plant-herbivore relationship mutualistic for as long as the plant is unable to disrupt apical dominance on its own (Whitham *et al.* 1991). The Extreme Adaptation Hypothesis proposes that plant species that overcompensate are extremely adapted to the high probability of being eaten, such that an absence of herbivory is an abnormal condition (Whitham *et al.* 1991). The Mutualism Hypothesis proposes that overcompensation resulting from herbivory is a mutualism that evolved out of antagonistic interactions that were inevitable and could not be avoided, with selection favoring any plant traits that might lessen herbivory's negative impact on themselves (Whitham *et al.* 1991). For example, grasses likely coevolved with their grazers and have adapted traits in response to being grazed that simultaneously limit and encourage grazing, such as chemical and

physical defenses and vegetative modes of reproduction (McNaughton 1979; Owen 1980; Owen and Wiegert 1981; McNaughton 1985, 1986). The Bet-Hedging Hypothesis proposes that plants with a high probability of being eaten, especially early in the growing season, can cut losses in the long-term by limiting the amount of plant tissue (e.g. stems) initially produced and, thus, lost to herbivory, and responding to herbivory with overcompensatory growth later (Whitham *et al.* 1991).

In commenting on the results of Paige and Whitham (1987) involving overcompensation in scarlet gilia (*Ipomopsis aggregata*), Van Der Meijden (1990) proposed that limited early season growth (e.g. an initial single inflorescence in *I. aggregata*) and overcompensation via activation of dormant buds or meristems following herbivory might become selective traits under certain conditions: (1) the timing of herbivory must be limited to a particular period, rather than occurring continuously, after which opportunity for regrowth may occur; (2) the probability of herbivory during that time period must be consistently high; and (3) the herbivory must be independent of other environmental cues in activating dormant meristems, such as changes in photoperiod that might trigger bud break in the plant species when herbivory is low or absent. Lennartsson *et al.* (1998) similarly expressed the importance of predictable damage as a necessary selective agent for overcompensation, namely, a consistently high risk of damage during a predictable, specific time period every year. Crawley (1983) suggested that compensation might evolve in plant species with specialist herbivores that are most active early in the growing season (e.g. migratory ungulates) and experience a period of decline when the plant may grow or flower with relatively fewer herbivore attacks. Compensation might also evolve when herbivores are regulated by natural enemies or disease, but not food-regulated, which would encourage undercompensation (Crawley 1983).

While compensation and overcompensation may occur in terms of increased growth or biomass, they do not always result in higher seed production or fitness. Herbivory-induced alterations to plant architecture often are detrimental by delaying and altering sexual maturity, expression, and reproduction (Inouye 1982; Crawley 1983; McNaughton 1983; Benner 1988). Energy and nutrients, such as carbohydrate or nitrogen, that might otherwise be used for reproduction become reallocated to regrowth following herbivory and other types of damage, often delaying or inhibiting flowering or seed maturation, producing smaller seeds, and negatively impacting seed set, germination, seedling success, and, therefore, fitness (Jameson 1963; Inouye 1982; Crawley 1983; Paige and Whitham 1987; Benner 1988; Lindroth 1989; Price 1991; Whitham *et al.* 1991; Fornoni *et al.* 2003).

Thus, in order for overcompensation for tissue loss to herbivory to benefit the plant, one would expect that the number of flowers, fruits, or seeds, and seed size (mass) would exceed that when herbivory is absent. This may be especially challenging to determine under leaf-focused herbivory because the carbon limitations that result from defoliation may significantly impact fruit and seed set and cause the plant to draw heavily from carbohydrate reserves (Crawley 1983). Additionally, decreases in seed set may not equate to decreased fitness, since other factors such as survival rates of mature plants after herbivory, seed size, and seedling survival are other factors important to fitness (Crawley 1983). Because seeds compete for available nutrients during development, seed number and seed size are inversely related, especially when nutrient or energy limitations exist (Harper 1977; Schaal 1980; McNaughton 1983). Further complicating matters are any fitness benefits that may occur from delaying reproduction: delayed seed maturation may be beneficial if late-maturing seeds escape seed predation (Islam and Crawley 1983). Thus, lifetime reproduction of both the plant and its seed-derived progeny (fitness) must be shown to have been enhanced by herbivory (Crawley 1983).

This is difficult to evaluate in species with multiple reproductive events during the plant's lifecycle (iteroparity), especially if some of the reproductive events are asexual (e.g. clonal growth from rhizomes, not genetically unique individuals from seed) (Paige and Whitham 1987; Whitham *et al.* 1991; Fagerström 1992; Wikberg 1995).

Another consideration is whether increased fitness from overcompensation equates to plant-herbivore mutualism. It is prudent to distinguish overcompensation from "tolerance," which involves evolutionarily adapted traits that buffer fitness losses caused by stress, whether the stress originates from damage by herbivores or storms, resource depletion, or other causes (Stowe *et al.* 2000; Fornoni *et al.* 2003). Compensation is one possible trait of tolerance, along with reallocation of resources, photosynthetic increase in remaining tissues, and increased growth rate (Stowe *et al.* 2000; Fornoni *et al.* 2003). In order for any plant response to be considered an adaptive trait of tolerance in the face of herbivory, it must be demonstrated that the response is a heritable variation in phenotype, that the herbivore is the primary selective agent acting on that phenotype, that the herbivory results in higher plant fitness for the plant and its progeny, and that other sources of stress or damage are not ultimately responsible for the response at the evolutionary level (Fornoni *et al.* 2003). As a result, one also would expect the frequency of tolerance-related genotypes in the population that encourage herbivory (e.g. ones that are more palatable, have fewer defenses, are more nutritious) to increase relative to resistance-related genotypes that discourage herbivory (e.g. those that are less palatable or nutritious, have more defenses) over many generations when herbivory is present (Belsky 1986; Stowe *et al.* 2000). Determining the agent evolutionarily responsible for the plant's response (ultimate cause), regardless of the agent eliciting the response in the present (proximal cause), is an important point, since what appears to be overcompensation or higher fitness in individual plants as a result of herbivory actually may be an unintended consequence of an evolved

response (e.g. disruption or weakening of apical dominance) to non-specific damage or some other agent (Aarssen and Irwin 1991; Aarssen 1995; Stowe *et al.* 2000; Fornoni *et al.* 2003).

Several hypotheses have been proposed to explain the evolutionary adaptation of apical dominance, most of which involve apical dominance in relation to competition for light, space, and other resources (Table 1). For instance, the Reserve Meristem Hypothesis is most related to overcompensation from apical meristem damage; it proposes that selection favors apical dominance because it holds dormant meristems in reserve for recovery from damage or stress (Salisbury and Ross 1991; Whitham *et al.* 1991; Aarssen 1995; Lehtilä 2000). This hypothesis proposes that the adaptive advantage associated with apical dominance is in having it disrupted, not in initiating or maintaining it, and preservation of apical dominance is costly (Van Der Meijden 1990; Aarssen and Irwin 1991; Aarssen 1995; Irwin and Aarssen 1996; Lehtilä 2000). However, since apical dominance could not have evolved for the sole purpose of being disrupted, it is likely that a reserve of meristems for recovery from damage is only a consequence of apical dominance and apical dominance is beneficial mostly under conditions in which the selective pressures typically associated with favoring apical dominance for the plant species are not present (balancing selection) (Aarssen 1995; Irwin and Aarssen 1996). In other words, for a plant species usually associated with dense, light-competitive conditions, apical dominance is costly when the plant is found occasionally in open, uncrowded habitats (Aarssen and Irwin 1991; Aarssen 1995; Irwin and Aarssen 1996). Removal of apical dominance in such a circumstance may benefit the plant by prompting it to grow lateral stems and increase its potential fitness (overcompensation), but loss of apical dominance under typical, crowded conditions would be costly (Aarssen and Irwin 1991; Aarssen 1995; Irwin and Aarssen 1996). Thus, given that natural selection favors traits that benefit fitness, competitive habitats should lead to strong apical dominance accompanied by overcompensation when the shoot apex is

removed, and non-competitive habitats should lead to weak apical dominance and no overcompensation (Irwin and Aarssen 1996). In a field test involving shoot apex removal of three herbaceous species (*Hypericum perforatum*, *Melilotus alba*, and *Ambrosia artemisiifolia*) growing naturally in open (non-competitive) habitats, Irwin and Aarssen (1996) found that all three species exhibited lengthening of remaining shoots (compensation) but no increase in branching (overcompensation) as a result of apex removal. This result supported the authors' predictions, and they proposed three possible explanations: these species have either weak apical dominance, apical dominance that did not incur any cost under non-competitive conditions, or apical dominance that was so strong that remaining lateral shoots quickly restored it (Irwin and Aarssen 1996).

Lehtilä (2000) developed a series of models to propose how a strategy of gradual bud activation (e.g. gradual weakening of apical dominance over time) may have evolved to protect most buds in the bud bank from damage early in the growing season, while avoiding the cost of activating them before herbivory has occurred, activating them and producing seeds too late in the season, or not activating them at all. Under intense, acute episodes of herbivory that results in overcompensation, selection favors the activation of a few buds early in the growing season, which are sacrificed to herbivory, and after which the remaining dormant buds are activated (Lehtilä 2000). Additionally, Lehtilä (2000) proposed that under low pressure from herbivory, selection should not favor bud dormancy, and when herbivory is moderate, gradual activation of dormant buds would be favored. Surprisingly, Lehtilä (2000) also suggests that damage occurring late in the growing season is more likely to result in overcompensation, provided late-growing shoots are able to successfully produce seeds, and that selection for overcompensation requires strong herbivore pressure. These latter hypotheses are surprising, considering that most cases of overcompensation in the published literature indicate that overcompensation is

most likely to occur under low to moderate herbivory, and that undercompensation will occur under intense herbivory (Crawley 1983; McNaughton 1983; Marquis 1984; Whitham *et al.* 1991; Paige 1992; Lennartsson *et al.* 1998; Fornoni *et al.* 2003).

Resource Regulation – Overcompensation and Opportunism

Since full or overcompensation may be a plant species' evolutionarily adapted attempt to regain tissue to unspecified damage, and many herbivores are attracted preferentially to young, tender, vigorously growing plants and plant parts, the opportunistic habit of resource regulation may be a consequence in many plant-herbivore systems. Resource regulation is the maintenance or increase in availability of high-quality food resources as a result of herbivore activities, with the resources being immediately available for subsequent generations of the same herbivore species in relation to the same individual plant or ramet (Craig *et al*. 1986; Price *et al.* 1987a, b; Roininen *et al.* 1988; Price 1991). For example, moose (*Alces alces*) (Danell *et al*. 1985), bud-galling sawfly (*Euura mucronata*) (Roininen *et al*. 1988), shoot-galling sawfly (*Euura lasiolepis*) (Craig *et al*. 1986) appear to engage in resource regulation by preferentially feeding on young, long, rapidly growing shoots, which are attacked further by members of the same herbivore population. Danell *et al*. (1985) found that the birch species *Betula pendula* responds to moderate browsing by moose (*A. alces*) with substantial regrowth of long shoots, which are preferred by moose during subsequent browsing. Moreover, moose in this study preferentially browsed the same birches in their migratory range over successive years and generally tended to prefer birches that had been browsed in the past. Since browsing reduces the capability of the birch to flower and produce seeds, only the moose appear to benefit from this relationship (Danell *et al*. 1985). The willow species *Salix cinerea* exhibits an ancient adaptation of fast, vigorous regrowth in response to general damage from snow, ice, and herbivores that may allow

it to compete favorably in early forest succession. This regrowth features shoots that are longer and bear more buds per shoot, which are favored by the bud-galling sawfly *Euura mucronata* due to optimal larval performance achieved on this type of plant tissue (Roininen *et al*. 1988). Craig *et al*. (1986) similarly found that the stem-galling sawfly *Euura lasiolepis* maintains high-quality food on its host plant *Salix lasiolepis* by inducing it to grow young, tender shoots that are targeted heavily by the sawfly and always available for subsequent generations. Craig *et al*. (1986) further noted that galling by *E. lasiolepis* keeps *S. lasiolepis* in a perpetual state of youth by progressively shifting the demographic of relative stem age classes from older to increasingly younger stems and prevents the plant for maturing beyond a point where it is no longer susceptible to the sawfly, a process the authors call "juvenilization." In such a state, *S. lasiolepis* is unable to achieve sexual maturity and cannot sexually reproduce (Craig *et al*. 1986). Additionally, vigorous regrowth of shoots do not adequately compensate for loss in photosynthetic area because repeated galling results in regular shoot stunting and senescence (Craig *et al*. 1986). Thus, Craig *et al*. (1986) propose that *S. lasiolepis* gains no benefit from this relationship with *E. lasiolepis*. Since herbivores benefit and plants do not, resource regulation is generally an antagonistic relationship.

Resource regulation cannot be assumed for all herbivore-plant systems where plants compensate for herbivory-induced damage. In order for resource regulation to be adaptive for the herbivore, the herbivore and its immediate offspring must demonstrate sufficiently high philopatry, or tendency to stay in or near birthplace, and herbivore generation times and plant recovery times must coincide so that subsequent herbivore generations can benefit reliably from the plant's response (Price 1991). Since plants do not benefit from resource regulation and would not have evolved specifically to produce tissues that are attractive for subsequent herbivory, resource regulation is not likely a product of coevolution, with reciprocal adaptations

in both plants and herbivores leading to this arrangement. Rather, resource regulation may be considered merely an opportunistic activity, where the herbivore takes advantage of the plant species' evolutionarily ancient regrowth response to general damage resulting from browsing, storms, frost, and other various biotic and abiotic events, which allows it to compete successfully in early succession for canopy openings and other newly available resources (Benner 1988; Roininen *et al*. 1988; Aarssen and Irwin 1991; Aarssen 1995).

Coevolution Between Plants and Herbivores

Strong coevolution between plants and herbivores is generally considered rare. Rather, most plant species are subjected to attack by a variety of different herbivores and abiotic events which cause damage and may impose conflicting selection pressures that result in "diffuse coevolution" and prevent reciprocal coevolution from occurring between a specialist herbivore and its associated plant species (Janzen 1980; Janzen 1983; Futuyma and Slatkin 1983; Strong *et al.* 1984; Lindroth 1989; Fleming 1991). Regarding the relationships of insect and plants, sequential evolution, rather than coevolution, may have occurred, meaning that a lag may have occurred in the evolution of modern insects in response to plant adaptations that evolved much earlier during the angiosperm diversification (Futuyma 1983). Coevolution in the strict sense (e.g. strong, specialized, direct, pairwise coevolution) between plants and herbivores is most likely to occur when few species are involved in each side of the relationship and they exert selective pressures on each other that result in reciprocal, species-level responses to each other's adaptations (Futuyma 1983; Futuyma and Slatkin 1983; Abrahamson 1989; Weis and Berenbaum 1989). Strong coevolution is not likely to occur in generalist insects and mammals that feed on a broad range of plant species or between organisms that exert weak or spatio-temporally incompatible selective pressures on each other or have widely different rates of

evolution (Janzen 1983; Lindroth 1989; Fleming 1991). Although many phytophagous insects exhibit some degree of host-specificity at the species (monophagy), genus, or family (oligophagy) level (Futuyma 1991) and may evolve responses to certain host species (Futuyma 1983), most vertebrate herbivores are polyphagous and generalists (Crawley 1983). Additionally, most plant species are associated with many herbivorous species, both generalist and specialist, and thus must respond to an array of selective pressures (Feinsinger 1983; Futuyma 1983; Janzen 1983). Finally, strong coevolution is difficult to identify, since perceived coevolved traits, such as defenses, actually may have developed in response to another species that is no longer a selective agent (Janzen 1980). Thus, most plant-animal interactions are, at best, likely examples of diffuse coevolution, rather than strong, direct, specialized coevolution (Feinsinger 1983; Futuyma 1983; Janzen 1983; Lindroth 1989; Fleming 1991).

Despite the tendency for herbivores and plants to coevolve in a diffuse manner, phytophagous insects include more specialists (e.g. monophagous) than any other herbivorous group (Futuyma 1983). Any of a number of agents, some behavioral, may be responsible for the general evolutionary trend toward specialization in herbivorous insects including sequestration of plant toxins in the bodies of some insects for defensive; aposematic purposes and selection of certain host plants as “enemy-free space” where parasitoids and predators may venture less frequently (Gilbert and Singer 1975; Futuyma 1983; Rossi *et al.* 1999; Stokes *et al.* 2012); changes in resource utilization to alleviate interspecific competition(Futuyma 1983); ovipositional mistakes where a female is attracted to and accidentally oviposits on an unsuitable host plant (Futuyma 1983; Rossi *et al.* 1999; Stokes *et al.* 2012); and tendencies to focus on the most abundant plant species and ignore less abundant ones (Optimal Foraging Theory) (Futuyma 1983). Additionally, their small size, short life spans, and ability to meet their individual nutritional needs with the tissues of a single plant make them more responsive

evolutionarily to the traits of a single or a few related plant species with which they associate (Feinsinger 1983; Futuyma 1983). Gall-inducing, a peculiar and complex form of specialist herbivory, often features monophagy and strong host-specificity (Crawley 1983) and may have resulted from strong, direct coevolution between gall-inducing organisms and their host plants (Abrahamson and Weis 1987; Dreger-Jauffret and Shorthouse 1992).

Compare, for example, the differences in ovipositional behavior between specialist and generalist insects. Gall-inducing females often exhibit extremely strong selective behavior for oviposition. Such selective behavior likely evolved because these larvae are endophytic and remain localized to the site of eclosion (sessile) and their performance depends on the mother's choice in oviposition location, which provides strong adaptive feedback for selective behavior that optimizes larval performance (Whitham 1978, 1980, 1992; Price *et al.* 1987 a, b; Price 1991; Fernandes and Price 1991). For example, the size of *Populus angustifolia* leaves chosen by females is strongly correlated with a number of fitness-related metrics in the leaf-galling aphid *Pemphigus betae*, including the number and development rate of offspring and the number of embryos produced by offspring (Whitham 1978). The ovipositional preferences of meristem-galling midge *Asphondylia borrichiae* females for well-resourced (high nitrogen) *Borrichia frutescens* translated to larger, less crowded galls, larger pupae, and hence higher potential fitness (Rossi and Stiling 1998; Rossi *et al.* 1999). Conversely, exophytic species such as leaf chewers (e.g. lepidopterans) exhibit little or no linkage between female selective behavior for oviposition and larval performance (Price 1991; Price 1992). In these species, oviposition often occurs several months before larvae hatch and may take place on a variety of substrates, some of which may not be edible. These larvae are mobile, can travel short distances to feeding sites, and may even exhibit territorial behavior in defending these sites against competitive cohorts. With essentially no linkage between female behavior in selecting optimal ovipositional sites and

larval performance, exophytic leaf-chewing species evolved to feed generally on any foliage available (Price 1991; Price 1992).

Gall-making and Apical Meristem Damage

Cecidogenesis, or gall-making, is a unique, complex form of herbivory that can be found in nearly all plant groups except for the algae, and is most commonly associated with dicotyledons (broad-leafed plants), particularly the perennial flowering plant families Asteraceae (e.g. asters, daisies, and sunflowers) and Rosaceae (e.g. roses,) and the woody plant order Fagaceae (e.g. oak) (Abrahamson and Weis 1987). Other dicots that may be galled include the orders Salicales (e.g. willow, poplar) and Fabales (e.g. legumes) (Dreger-Jauffret and Shorthouse 1992; Roskam 1992). Some species of monocotyledons, ferns, and conifers are also associated with gall-inducing organisms (Abrahamson and Weis 1987; Roskam 1992).

A gall is an overgrowth of tissue at the site of a gall-inducing organism that is initiated in response to and maintained by the presence and activities of the gall inducer (Mani 1992; Raman *et al.* 2005). The gall provides food, shelter, and protection from desiccation, predators and parasites (Enemy Hypothesis), and weather events, which may have been strong selection pressures that, over time, favored biochemical and physiological adaptations in the gall-inducer to exploit the ecological niche created by the plant's production of a gall (Abrahamson and Weis 1987; Price *et al.* 1987c). Gall-inducing also may allow the insect to compensate for low-quality plant tissue by manipulating host plants into improving the nutritional quality of tissues at the galling site (Rossi and Stiling 1998; Awmack and Leather 2002). Both gall-inducing dipterans *Lipara lucens* (De Bruyn 1995) and *Giraudiella inclusa* (Tscharntke 1989) performed best on thin, stressed shoots of *Phragmites australis* that contain lower levels of silica and may be more susceptible to attack, possibly by compensating for low-nutrient plant tissues by inducing the

production of high-quality gall tissues. During a water and fertilizer experiment, galls initiated by *Euura lasiolepis* on *Salix lasiolepis* contained significantly greater protein concentrations and lower phenol concentrations, regardless of protein or phenol levels in ungalled plant tissue, suggesting that *E. lasiolepis* alters the biochemistry of gall tissues to create conditions favorable for larval development (Waring and Price 1988).

Gall-inducing is found among many species of insects, mites, fungi, nematodes, bacteria, and viruses (Abrahamson and Weis 1987; Dreger-Jauffret and Shorthouse 1992; Mani 1992). Among insects, gall-inducers may be found in the orders Hymenoptera, Diptera, Hemiptera, Homoptera, Thysanoptera, Coleoptera, and Lepidoptera (Crawley 1983; Dreger-Jauffret and Shorthouse 1992; Raman *et al.* 2005). The gall has been viewed as the plant's attempt to encapsulate or isolate an invader from the rest of the plant, with the invader benefiting only incidentally from the plant's response (Mani 1992). However, many authors now consider the gall to be an extended phenotype resulting from both the genotype of the gall-inducer (e.g. its activities) and the genotype of the host plant (e.g. its response) (Weis and Abrahamson 1986; Abrahamson and Weis 1987; Weis *et al.* 1988; Weis and Berenbaum 1989; Rohfritsch 1992; Raman *et al.* 2005; Yukawa and Rohfritsch 2005). Indeed, galls on the same host plant species, even on the same plant organ, are morphologically and histologically distinctive among gall-inducers, suggesting that gall development is heavily influenced by the galling species (Abrahamson and Weis 1987; Dreger-Jauffret and Shorthouse 1992; Rohfritsch 1992; Yukawa and Rohfritsch 2005). Additionally, gall-inducers tend to be specialists and highly exclusive to their particular host species, genus, or family, and to the plant organ they attack, suggesting that the gall may be a product of strong coevolution between inducer and host in many systems (Abrahamson and Weis 1987; Weis *et al.* 1988; Dreger-Jauffret and Shorthouse 1992).

Gall characteristics influenced by the inducer include shape, size, and any tendency to increase gall size or number of cohabitating larvae through communal oviposition by female gall-makers (Abrahamson and Weis 1987; Dreger-Jauffret and Shorthouse 1992); texture, toughness, and rigidity (Abrahamson and Weis 1987); the production of exudations (sticky or sweet, ant-attracting substances), hairs, and other bizarre growths (Taper *et al.* 1986). Although gall tissue fed upon by the gall-inducer is often lower in phenolic compounds than other plant tissue (Price *et al.* 1987c), plant allelochemicals and stress metabolites like lignins and tannins are often sequestered in gall layers distal from the larval chamber (Abrahamson and Weis 1987; Weis *et al.* 1988; Rohfritsch 1992). Many of these gall characteristics may protect the gall-inducer from parasitoids and other predators (Janzen 1977; Abrahamson and Weis 1986, 1987). Lignins and tannins may protect the gall from other herbivores by increasing gall toughness (Strong *et al.* 1984; Price 1991). Larger gall size (diameter) increases the distance that a parasitoid (e.g. an insect whose larvae parasitize and kill their insect hosts) must penetrate with its ovipositor to reach the gall-inducing larva or pupa, which prevents attack by parasitoid species with short ovipositors (Gall-Diameter Hypothesis) (Weis and Abrahamson 1986; Rossi *et al.* 1992; Rossi and Stiling 1995; Stiling *et al.* 2003; Rossi *et al.* 2006). Galls induced by the gall midge *Asphondylia borrichiae* on sea oxeye daisy *Borrichia frutescens* increase in toughness as they mature and increase in size, which further deters oviposition by three of four parasitoid hymenopterans (Rossi *et al.* 1992; Rossi and Stiling 1995; Stiling *et al.* 2003; Rossi *et al.* 2006). Weis and Abrahamson (1986) genetically investigated variations in gall diameter in the *Solidago*-*Eurosta* system (goldenrod and goldenrod-galling fruit fly) and determined that gall size is influenced by competing selective forces acting on both genotypes. *Solidago* spp. favors smaller galls which redirect fewer nutrients and energy from plant reproduction and result from lower

reactivity to the gall-inducer stimulus, while *Eurosta* spp. favors larger galls that protect its larvae from parasitoids (balancing selection).

Natural selection also may act on behavioral phenotypes in gall-inducers, such as their ability to initiate a galling response in a host plant, identify plant genotypes that will produce a galling response, and coincide oviposition with optimal times of reactivity based on plant phenology (Abrahamson and Weis 1987; Weis *et al.* 1988; Raman *et al.* 2005). As noted earlier, larval performance is dictated by the mother's choice of host plant for oviposition, providing a powerful selective feedback loop (Whitham 1978, 1980, 1992; Abrahamson and Weis 1987; Fernandes and Price 1991). Gravid females are known to preferentially select locations for oviposition and time the event optimally to coincide with nutrient-rich flushes of new growth (Abrahamson and Weis 1987; Raman *et al.* 2005). In any given habitat, females also may be selective for particularly healthy, vigorous, and well-resourced plants (Price 1991).

With no common gall-making ancestor linking the diverse groups of insects, mites, fungi, nematodes, bacteria, and viruses that are members of this polyphyletic guild, gall-inducing is considered a homoplasy that evolved independently across several orders and even within families (Bissett and Borkent 1988; Roskam 1992; Raman *et al.* 2005). Ancestral feeding habits among arthropods that likely developed into gall-inducing include stem boring, leaf mining, leaf chewing, sap sucking, spore or pollen feeding, detritus feeding, and parasitism of these various feeders (Roskam 1992).

Gall types can range from simple, slight alterations in normal plant tissue (organoid galls) to more altered, complex growth patterns that feature more or less organized layers of abnormal tissue (histioid galls, such as prosoplasmic and kataplasmic galls) (Rohfritsch 1992). Cecidomyiids (gall midges) and cynipids (gall-inducing hymenopterans) are most often associated with highly organized prosoplasmic galls, while gall-inducing sawflies, lepidopterans

(moths), beetles, and homopterans (e.g. aphids) are most associated with the simpler kataplasmic gall type (Abrahamson and Weis 1987; Rohfritsch 1992). Galls contain more water than normal plant tissue and create a humid environment for inhabitants, which is important in xeric (dry) environments (Price *et al.* 1987c; Fernandes and Price 1991; Birch *et al.* 1992).

Generally, cecidogenesis, or gall initiation and development, involves the redirection in plant tissue development as a result of the presence and activity of the gall-inducing organism. In the case of gall-inducing insects, either oviposition by females, which may involve injecting a fluid with the eggs, and/or the feeding activities of newly hatched juveniles (e.g. tissue wounding by puncturing or scraping and sucking, extra-intestinal digestion via salivary secretions) generates and maintains an inflammatory response in the plant's tissues that inhibits normal tissue development (hypoplasy) (Abrahamson and Weis 1987; Dreger-Jauffret and Shorthouse 1992; Mani 1992; Rohfritsch 1992; Raman *et al.* 2005; Yukawa and Rohfritsch 2005; Rohfritsch 2008). The remaining stages include abnormal cellular division (hyperplasy) and enlargement (hypertrophy) and metaplasy, which either redirects the surrounding undifferentiated cells or de-differentiates normal cells to become nutritive cells that orient around the gall-inducing larva and connect to the plant's normal vascular tissues. Depending on the gall type, other plant cells, such as modified parenchyma, sclerenchyma, vascular, and epidermal cells, also may form in successive layers around the innermost nutritive cells and larval chamber, with the nutritive layers often constituting a cytological gradient and representing a nutrient sink (Weis and Berenbaum 1989; Bronner 1992; Rohfritsch 1992). The activities of gall-inducing arthropods are likely responsible for controlling and maintaining the nutritive cells (Abrahamson and Weis 1987; Bronner 1992; Dreger-Jauffret and Shorthouse 1992; Rohfritsch 1992; Raman *et al.* 2005), and organized layers of tissue in the gall acquire a

unique polarity and symmetry oriented around the larval chamber and its inhabitants (Rohfritsch 1992).

Galls can significantly alter and divert, or partially block and accumulate, the flow and allocation of nutrients and photosynthate from the host plant's organs and life functions to feed the gall-inducing organism, essentially becoming a nutrient sink (Abrahamson and Weis 1987; Weis *et al*. 1988; Bronner 1992; Dreger-Jauffret and Shorthouse 1992; Marquis 1996; Raman *et al.* 2005; Rohfritsch 2008). In most arthropod-induced galls, the larva either feeds directly on nutritive cells derived from plant tissue or induces the nutritive cells to become short-transport conduits for plant nutrients (Dreger-Jauffret and Shorthouse 1992; Rohfritsch 1992; Raman *et al.* 2005; Rohfritsch 2008). Not all galls have plant-derived nutritive tissue, however. "Ambrosia galls," typically associated with many gall-inducing midge species (Diptera: Cecidomyiidae), are lined by the mycelium, or mat of thread-like hyphae, of a fungal symbiont that is inoculated into plant tissue with the egg during oviposition, in which case plant-derived nutritive cells are absent and the larva feeds on the fungal mycelium, which are cytochemically similar to nutritive tissue (Borkent and Bissett 1985; Abrahamson and Weis 1987; Bissett and Borkent 1988; Gagné 1989; Bronner 1992; Dreger-Jauffret and Shorthouse 1992; Yukawa and Rohfritsch 2005; Rohfritsch 2008; Heath and Stireman 2010). Heath and Stireman (2010) demonstrated that the larvae of the cecidomyiid *Asteromyia carbonifera* placed on agar plates with the conidia of their fungal symbiont can thrive on a fungus-only diet. They also noted that the fungal mycelium quickly grows and envelops the larva after inoculation, allowing little time during which the larva is in direct contact with plant tissues. Rohfritsch (2008) reported that *Schizomyia galiorum* larvae do not graze on fungal hyphae, but pierce the fungal cells, which maintains the fungal mycelium as a thin, flat, translucent layer within the larval chamber that may be mistaken for plant nutritive tissue (pseudo-parenchyma). Rohfritsch (2008) also observed that larvae of

another cecidomyiid, *Lasioptera arundinis*, facilitate the spread of fungal hyphae into deeper plant tissues and feed on both fungal hyphae and plant tissues (activated host cells and vascular parenchyma).

The role of the galling organism in the initiation and maintenance of galls is complex and poorly understood. Further complicating matters in ambrosia galls is the role of the fungus and whether it or the insect is the causal agent. Generally, the feeding activities of insects and mites in gall formation has been correlated with increased levels of both IAA and cytokinins (Hale and Orcutt 1987; Raman *et al.* 2005), and an imbalance of IAA and cytokinins have also been associated with fungi- and bacteria-induced galls (Raman *et al.* 2005). Camp (1981) reported that *Sclerotium asteris*, the fungal symbiont of the gall midge *Asteromyia carbonifera*, may secrete compounds that are responsible for inducing the blister galls on the leaves and stems of two species of *Solidago*. Bissett and Borkent (1988), however, propose that secretions produced by the larva may be responsible for ambrosia galls because proliferation of fungal mycelium can be delayed for weeks while the gall develops, suggesting that the fungus cannot be the gall initiator. Yukawa and Rohfritsch (2005) similarly propose that the first instar larva initiates ambrosia galls in many cecidomyiid species. However, Lebel *et al.* (2012) found that the growth of fungal hyphae in the galls of all *Asphondylia* species studied began very early in the development of the gall and larval chamber, suggesting an important role for the fungal symbiont. Rohfritsch (2008) reported that larvae of *Lasioptera arundinis* initially wound the stem tissues of its host the common reed (*Phragmites australis*), after which the symbiotic fungal hyphae invade through the wound and spread deeply between the intercellular spaces toward the plant's vascular bundles; despite the initial wounding caused by the larvae, Rohfritsch (2008) considers the primary causative agent to be the fungus, which has direct access to the plant's vascular bundles and, therefore, is responsible for the growth and shape of

the gall. Hori (1992) reported in a review of published literature that the components of salivary secretions produced by gall-inducing hemipterans and homopterans, which may include amino acids, IAA or auxin-like compounds, phenol oxidases, and other compounds, may diffuse into the tissues surrounding feeding sites, but whether they are responsible for disrupting the balance of plant hormones is unknown. Hori (1992) and Raman *et al.* (2005) indicate that IAA originating from arthropod saliva is negligible in quantity compared to the amount of IAA produced by plants or found within galls. Thus, the causal agent of increased hormones in plants during infection or galling is poorly understood and may be the result of either synthesis and accumulation by the plant in response to the invader or synthesis by the invader itself (Cooke 1977; Abrahamson and Weis 1987; Hale and Orcutt 1987). Some researchers now conclude that it is unlikely that galling organisms secrete cecidotoxins that directly cause gall formation; rather, galls are likely the host plant's physiological response to either the mechanical wounding and feeding activities of the gall-inducer (Mani 1992) or chemicals present in the inducer's salivary secretions that sensitize or "condition" the plant's cells to its presence and stimulate gall formation (Hori 1992). Further complicating matters is evidence that some galls may be initiated by the female during oviposition and maintained through maturity by the larva (Hori 1992). Hori (1992) thus proposes that cecidogenesis be viewed as three distinct phases with three factors or agents, which may be mechanical or chemical and may be the same or different: (1) cell conditioning stimulated by a conditioner, (2) gall induction initiated by an inducer, and (3) gall maturation controlled and maintained by a maturator.

In ambrosia galls, the association between gall midge and fungus appears to be mutualistic, with the insect relying on the fungus as a food source and as an aid for invading and activating plant tissues, and the fungus relying on the insect for dispersal to and initial deposition inside the tissues of host plants (Bissett and Borkent 1988; Bronner 1992; Dreger-

Jauffret and Shorthouse 1992; Raman *et al.* 2005; Rohfritsch 2008; Heath and Stireman 2010). The developing larva may control excessive fungal growth in the larval chamber and/or inhibit the growth of unwanted, competing fungi (Bronner 1992; Rohfritsch 2008). Female gall-making cecidomyiids typically bear mycangial structures (e.g. pockets) either on one of the abdominal sternites or the ovipositor that collects and transports fungal conidia, suggesting that the association between midge and fungus has evolved over time and is not opportunistic or incidental, nor the fungus an inquiline (Borkent and Bissett 1985; Bissett and Borkent 1988; Rohfritsch 2008; Heath and Stireman 2010; Lebel *et al.* 2012). All gall-inducers display high levels of host fidelity, with most being associated with a single host-plant species (monophagous) or with a few closely related species (oligophagous), often within the same genus or family (Abrahamson and Weis 1987; Gagné 1989; Dreger-Jauffret and Shorthouse 1992; Raman *et al.* 2005; Yukawa and Rohfritsch 2005), but gall-inducing with a fungal symbiont that is collected and inoculated into plant tissues as the larva's food source may allow gall midges some flexibility in expanding its range to new host plants by removing the host's potential as a food source from the midge's host selection criteria (Bissett and Borkent 1988; Yukawa and Rohfritsch 2005; Uechi and Yukawa 2006). The fungal symbiont may also aid in protecting the gall-inducer, since the mycelium may contribute to the gall's rigidity and make it impregnable to parasitoids (Abrahamson and Weis 1987; Heath and Stireman 2010). Finally, since gall-associated fungi appear incapable of infecting living plants without the assistance of a vectoring insect, symbiotic fungi also may benefit through host plant expansion and bypass of plant defenses facilitated by the insect and improved nutrition obtained from access to living plant tissue (Bissett and Borkent 1988).

Gall-inducers may synchronize ovipositional and developmental stages to the growing, flowering, and fruiting seasons of their host plants, when these plant organs contain highly

assimilable nutrients for larvae (Abrahamson and Weis 1987; Rohfritsch 1992; Yukawa 2000; Raman *et al.* 2005; Yukawa and Rohfritsch 2005). Galls mature with the development of the larvae, and some species emerge from the gall as larvae and pupate in the ground, while others pupate inside the gall (Rohfritsch 1992; Yukawa and Rohfritsch 2005).

Gall-inducing arthropods (e.g. insects and mites) tend to induce galls either in young tissues that have recently undergone cell division and are newly differentiated, or in undifferentiated meristematic tissues (Abrahamson and Weis 1987; Hori 1992; Raman *et al.* 2005), which contain the greatest concentrations of assimilable nitrogen (McNeil and Southwood 1978) and other nutrients and can be readily inhibited and redirected (Crawley 1983; Abrahamson and Weis 1987; Price 1991; Rohfritsch 1992; Raman *et al.* 2005). Thus, the availability of undifferentiated tissue, such as that found in active meristems, is vital to most gall-inducing insects (Abrahamson and Weis 1987; Weis and Berenbaum 1989; Rohfritsch 1992). Espirito-Santo *et al.* (2007) found that the diversity of galling insects on 17 species of *Baccharis* was positively correlated with the number of available meristems, measured via the quantity of fourth-level stems present in the plant's architecture (Meristem Dynamics Hypothesis). While cecidomyiids are associated with young, undifferentiated plant tissues, cynipids may attack either undifferentiated or differentiated tissues (Rohfritsch 1992).

Generally, galls may be found on any plant organ, including leaves, stems and shoots, buds, seeds and flowers, and roots (Dreger-Jauffret and Shorthouse 1992; Mani 1992), and gall-inducers are specialized in their choice of organ (Weis and Berenbaum 1989) and may be selective in oviposition sites on the organ itself (Crawley 1983). For example, the gall-inducing aphid *Pemphigus betae* preferentially oviposits near the petiole on the surface of large *Populus angustifolia* leaves, with both variables (location relative to petiole and leaf size) being correlated with higher average fecundity of offspring (Whitham 1978, 1980).

The direct fitness effects of galls on host plants may be negligible when occurring on vegetative organs like leaves and stems at low or moderate endemic levels, but any plant tissue destined for plant growth or reproduction that is instead diverted in purpose to become a gall that grows and persists for many weeks may impact fitness indirectly (Abrahamson and Weis 1987). The most significant and direct negative impact on fitness is likely to occur among galls on reproductive organs like seeds and flowers (Abrahamson and Weis 1987; Bissett and Borkent 1988; Yukawa and Rohfritsch 2005). The gall-inducing wasp *Andricus quercus-calicis* attacks the flowers of *Quercus cerris* and then the fruits of *Q. robur* in the U.K. over two successive generations annually, but *Q. robur* suffers devastating acorn mortality, sometimes exceeding 90%, because it sheds the entire peduncle bearing both attacked and unattacked acorns (Crawley 1983). Galls found on leaves can reduce photosynthesis and shorten leaf longevity (Yukawa and Rohfritsch 2005).

Galls that disrupt apical dominance by directly invading meristematic tissue or simply killing the stem below the meristem can significantly alter architecture and growth patterns via removal of apical dominance (Yukawa and Rohfritsch 2005). *Asphondylia borrichiae* galls on the apical meristem of *Borrichia frutescens* were observed to disrupt apical dominance and cause branching (Rossi and Strong 1990). Both the bud-galling sawfly *Euura mucronata* (Roininen *et al.* 1988) and the stem-galling sawfly *E. lasiolepis* (Craig *et al.* 1986) disrupt apical dominance and induce lateral branching in their respective *Salix* species. While galls on meristems can destroy apical dominance, they may also reexert apical dominance by mimicking the apical meristem via its own auxin and cytokinin production, high metabolic rates, and nutrient sink characteristics (Fay *et al.* 1996). *Rhabdophaga strobiloides* galls on the terminal bud of *Salix cordata* appeared to assert apical dominance over lateral buds, thereby preventing their growth into new shoots which might compete with the gall-inducer for plant resources (Weis 1984).

Chapter 2: Study system

Compared to other forms of herbivory, systems involving gall-inducing organisms are ideal for studying the effects of herbivory and apical meristem damage because each gall is a discrete and easily identified and quantified event. This thesis project focuses on the gall midge *Asphondylia borrichiae* and its effects on the performance and fitness of its primary host plant, *Borrichia frutescens*.

The Host Plant

Borrichia frutescens (L.) DC and two related members of the Asteraceae family, *Iva frutescens* L. (marsh elder) and *I. imbricata* Walter (dune elder), are attacked by the gall midge *Asphondylia borrichiae* Rossi and Strong (Diptera: Cecidomyiidae) (Rossi and Strong 1990; Rossi and Stiling 1995; Rossi *et al*. 1999; Stokes *et al*. 2012). The current study will utilize only the gall midge's primary host, *B. frutescens*, because of the three hosts, it experiences the highest galling incidence (Rossi and Stiling 1995; Rossi *et al*. 1999) and previous studies have found high levels of galling for this host species at the proposed study site (Rossi *et al*. 2006; Stokes *et al*. 2012).

Borrichia frutescens, commonly known as sea oxeye daisy or bushy seaside tansy, is a small to medium-sized, herbaceous, perennial shrub measuring up to 1 m in height (Figure 2) (Carlton 1975). It is rhizomatous and forms distinct patches of clonal plants, or ramets, in brackish-to-saline back-marsh areas and salt flats (Cronquist 1980; Antlfinger 1981). It propagates primarily vegetatively through extensive rhizomes (Stalter and Batson 1973; Duncan

and Duncan 1987), but also produces small, monoecious flower heads ringed by at least a dozen yellow petal-like ray flowers and bearing numerous darker yellow disc florets that yield numerous seeds per flower head (Figure 3) (Carlton 1975; Cronquist 1980; Antlfinger 1981; Eleuterius 1990; Biber *et al.* 2013). The flower heads contain rigid receptacular bracts that each hold a disc flower and, later, an achene (dry fruit) housing a single seed; the bracts bear a single spine at the tip that makes the heads difficult to break open (Cronquist 1980; Duncan and Duncan 1987; Biber *et al.* 2013). The achenes are black or metallic-gray, approximately 3 to 4 mm long, tapered, glabrous, and with sides that meet at 3 or 4 angles (Figure 4) (Duncan and Duncan 1987; Eleuterius 1990; Biber *et al.* 2013). *Borrichia frutescens* flowers year-round, with peak flowering between May and July (Duncan and Duncan 1987). In mid-marsh habitat along the coast of west-central Florida, some populations of *B. frutescens* experience low flowering rates (3% or less) (Rossi *et al*. 1992; Rossi and Stiling 1995), but in low marsh elevations that experience regular tidal inundations of nutrients, flowering rates often exceed 20% (Rossi *et al*. 1992). It should be noted that previous studies covered relatively short periods and likely underestimated flowering rates. Finally, a study on salt marsh plant taxa in coastal South Carolina found that 97% of seeds produced by *B. frutescens* were viable (Stalter and Batson 1973).

Of the other two host plant species, *Iva frutescens* is often found with *B. frutescens* in salt marshes, but at higher elevations, while *I. imbricata* prefers growing among beach dunes above high tide line (Rossi and Stiling 1995). Both *Iva* species flower from August through October (Rossi *et al*. 1999).

The Gall-Inducer

Asphondylia borrichiae Rossi and Strong (Diptera: Cecidomyiidae) belongs to the tribe Asphondyliini, subfamily Cecidomyiinae, family Cecidomyiidae, or the gall midges ("cecido" = "gall"), although gall-inducing is not found in all members of this family (Strong *et al.* 1984; Gagné 1989; Dreger-Jauffret and Shorthouse 1992; Rohfritsch 2008). The Cecidomyiidae include over 3000 described species, of which only about half within a few lineages found in the subfamily Cecidomyiinae actually exhibit the gall-making habit (Bissett and Borkent 1988; Roskam 1992). Mycetophagy (fungus eating) is the plesiotypic (primitive) feeding habit of the subfamily Cecidomyiinae, with non-cecidogenetic phytophagy (plant feeding with no galling involved) and cecidogenetic endophytophagy and endomycetophagy (gall-inducing, internal plant feeding and fungus feeding) being derived trophic types (Bissett and Borkent 1988; Roskam 1992). Additionally, the larvae of some cecidomyiids are predatory or saprophagous (feeding on dead or decaying organic matter) (Strong *et al.* 1984). Endomycetophagous gall-inducing cecidomyiids like *A. borrichiae* are found in the tribes Asphondyliini, Alycaulini, Cecidomyiini, Lasiopterini, and Oligotrophini (Bissett and Borkent 1988; Roskam 1992; Yukawa and Rohfritsch 2005), and all species within the *Asphondylia* genus are associated with a fungal symbiont upon which they rely for food (Gagné 1989). All Cecidomyiinae adults tend to be small (<3 mm long) and fragile (Strong *et al.* 1984), weak flyers, mosquito-like in appearance, and adapted for passive, aeroplankton dispersal with weakly costalized (poorly enforced) wings and long fragile limbs and antennae (Gagné 1989; Roskam 1992).

Mature *Asphondylia borrichiae*, like all cecidomyiids (Gagné 1989; Yukawa 2000), live for only 2-3 days, during which their sole activity is breeding (Stiling *et al.* 1992). Female *A. borrichiae* oviposit and deposit a fungal symbiont inside the terminal meristematic tissue of the host plant, which induces the plant to produce a tumor-like overgrowth of tissue, or gall, within

which the immature midges develop (Figure 5) (Rossi and Strong 1990; Rossi *et al*. 1992; Stiling *et al*. 1992; Rossi *et al*. 2006). The galls are approximately 1 cm or greater in diameter (Rossi and Strong 1990; Rossi and Stiling 1995), polythalamous (many chambered), and roughly spherical (Stokes *et al*. 2012). Galls contain usually one to four chambers, but up to eight are common, and a gall containing greater than 20 chambers was found in the Florida Keys (Rossi and Strong 1990; Stiling *et al.* 1992; A. M. Rossi, pers. comm.). Each larva develops in its own chamber, surrounded by fungal mycelia (Rossi and Stiling 1995). With occasional exceptions, the midge appears to avoid flowering terminals, and the presence of a gall typically prevents the terminal from flowering (Rossi and Strong 1990; Rossi *et al*. 1992; Stiling *et al*. 1992; Rossi *et al*. 2006). One gall per apical meristem is typical, but occasionally, two or more galls may develop on a single meristem (Stiling *et al*. 1992).

Like all *Asphondylia* (Gagné 1989), *A. borrichiae* also deposits the conidia of at least one obligate fungal symbiont, or associated, interacting organism, along with its eggs (Rossi *et al*. 1999; TeStrake *et al.* 2006). Mature female endomycetophagous cecidomyiids typically bear a mycangial structure (e.g. setae or pouch) on the abdomen used to collect fungal conidia from environment (Borkent and Bissett 1985; Bissett and Borkent 1988; Gagné 1989; Rohfritsch 2008; Heath and Stireman 2010; Lebel *et al.* 2012;). In all Asphondyliini, the mycangial structure is a pouch located on the seventh abdominal sternite and is an identifying characteristic for this tribe (Borkent and Bissett 1985; Gagné 1989; Yukawa and Rohfritsch 2005; Rohfritsch 2008). Female *Asphondylia* spp. have a needle-like ovipositor with which it deposits both eggs and fungal conidia inside the plant tissues, but the exact mechanisms for identifying, collecting, and transporting the correct obligate fungal conidia in the environment and inoculating plant tissues with the conidia are poorly understood (Borkent and Bissett 1985; Gagné 1989; Yukawa and Rohfritsch 2005; TeStrake *et al.* 2006; Rohfritsch 2008). Borkent and Bissett (1985) and Bissett

and Borkent (1988) reported that most gall-making cecidomyiids they reared from galls in the laboratory did not carry fungal conidia, suggesting that mature females must collect conidia from unknown locations in the environment before ovipositing. Heath and Stireman (2010) reported that *Asteromyia carbonifera* failed to initiate galls within experimental mesh enclosures, possibly due either to the enclosures' effect on midge behavior in collecting conidia from the environment or the lack of the fungal source within them; at any rate, it was apparent that *A. carbonifera* females do not eclose from galls with conidia already present in their mycangia. Rohfritsch (2008) reported that females of the cecidomyiid species *Lasioptera arundinis* and *Schizomyia galiorum* emerged from galls with no fungal conidia. Attempts to develop galls from lab-reared *Asphondylia borrichiae* also have been unsuccessful (Rossi *et al.* 1999), and TeStrake *et al.* (2006) were able to identify no conidia in the mycangia of newly emerged adults in the field, although conidia were found on the surfaces of 15% of emerging adults. Borkent and Bissett (1985) suggest that Asphondylinii females may collect conidia in the environment by using the mycangial pouch as a shovel or scoop. *Lasioptera arundinis* were observed to collect into their mycangial pouches fungal conidia from the decaying leaf sheaths of their host *Phragmites australis*, which they then deposited into new shoots with their eggs (Rohfritsch 2008). In a study investigating the array of mycoflora associated with *Borrichia frutescens*, TeStrake *et al.* (2006) identified numerous endophytic fungi, that is, fungi residing within plant tissues, in both galled and non-galled *B. frutescens* and suggested that the obligatory fungi associated with *A. borrichiae* galls may either preexist in plant tissues and extend into the gall as it develops or may be inoculated by the gall midge and invade surrounding plant tissues as the gall matures. The diverse community of fungi in *A. borrichiae* galls suggests that the gall midge may not be solely responsible for introducing all of the possible fungal species associated with this plant species (TeStrake *et al.* 2006).

A broad variety of fungi have been found associated with *Asphondylia borrichiae* galls, particularly *Alternaria* sp. and *Bipolaris* sp., both of the family Pleosporaceae (TeStrake *et al.* 2006). *Botryosphaeria dothidea* aff. of the family Botryosphaeriaceae has also been isolated from *A. borrichiae* galls collected from the field during the current project (J. A. Smith, personal comm., unpubl. data). Fungi associated with and suspected in the formation of ambrosia galls of many other cecidomyiids belong to the Botryosphaeriaceae, which are endophytic, saprophytic, and sometimes opportunistic primary and secondary pathogens of woody plants (Bissett and Borkent 1988; Lebel *et al.* 2012), including many ornamental and horticultural crops (Heath and Stireman 2010).

Although a formal study of the larval digestive system in *A. borrichiae* has not been conducted, it can be expected to resemble that of other Cecidomyiinae: simplified and with reductions in the peritrophic membrane, cardial sac, Malpighian tubules, and/or other parts, and all of which accompany extra-intestinal digestion, or regurgitation of salivary secretions onto food substrate (Abrahamson and Weis 1987; Gagné 1989; Roskam 1992). Such digestive simplifications may suggest efficient digestion and assimilation of nutrients and/or high-quality food containing little or no indigestible components (Abrahamson and Weis 1987; Weis and Berenbaum 1989). Cecidomyiid larvae also tend to have rudimentary mouthparts that are adapted for scraping cells and sucking fluids that are apparently exuded through the cell walls and predigested with salivary secretions (Bronner 1992; Rohfritsch 1992; Rohfritsch 2008).

Asphondylia borrichiae larvae are essentially sessile and dependent on the mother's choice of host plant for oviposition. As with most cecidomyiids (Gagné 1989; Yukawa 2000; Yukawa and Rohfritsch 2005), adult *A. borrichiae* lack feeding mouthparts and, thus, do not feed and cannot use mouthparts to taste and identify suitable host plants (Rossi *et al.* 1999). The

mechanisms by which gravid females locate suitable hosts are unknown, but may involve olfactory and/or visual cues (e.g. plant size, texture) (Rossi *et al*. 1992).

Galling density on *B. frutescens* can vary widely, with none (0%) to over 100 galls per 200 terminals (50% galling rate of plant terminals), depending on the site and season (Stiling *et al.* 1992; Rossi and Stiling 1995). Galls can persist on the host plant during spring and summer for an average of about seven to ten weeks (Stiling *et al*. 1992). *Asphondylia* spp. have multiple larval stages and one pupal stage, all of which occur within the gall (Gagné 1989). After pupating, the pupa "drills" through the gall tissue using the antennal horns on its head, creating a ragged emergence hole to which the posterior end of the pupa attaches before the adult ecloses, leaving behind its puparium (Figure 6) (Gagné 1989; Rossi and Strong 1990).

Cecidomyiids exhibit a number of varying life history patterns, and in many *Asphondylia* species, larvae overwinter inside galls and emerge as reproductively mature adults in the spring, coinciding with the flush of fresh growth and rich flow of assimilable nutrients in their host plants (Gagné 1989; Raman *et al.* 2005; Yukawa 2000; Yukawa and Rohfritsch 2005; Uechi and Yukawa 2006); *A. borrichiae* follow this life history pattern as well (Stiling *et al.* 1992). *Asphondylia borrichiae* populations are also multivoltine, or tend to produce multiple generations per year, with overlapping generations (Stiling *et al.* 1992). With an adult stage lasting between one and seven days, all cecidomyiids tend to mate, oviposit, and develop galls in generational waves, synchronized so that population density ensures their progeny emerge en massé over a few days and encounter each other (Yukawa 2000; Yukawa and Rohfritsch 2005; Uechi and Yukawa 2006).

After emergence, the gall senesces and results in the death of the galled stem terminal (Rossi and Strong 1990; Stiling *et al*. 1992). Stem senescence does not typically result in the death of the entire stem, but rather to the uppermost nodes below the gall (Rossi and Strong

1990; Stiling *et al.* 1992). When galling occurs on the apical meristem of its host plant, such as *Borrichia frutescens*, the stem's death appears to release apical dominance and allows the dormant lateral buds at nodes below the gall to become active and elongate into axillary stems (Rossi and Strong 1990). Thus, in causing the original stem to bifurcate and produce additional stems, limited herbivory by *A. borrichiae* may increase the number of stems and, thus, the number of flower heads of the host plant. If flowering and seed maturation are not delayed and seed quality and viability are maintained or improved in this process, the plant may realize a benefit in fitness.

Borrichia frutescens is considered the ancestral host plant for the gall midge *Asphondylia borrichiae* and experiences highest galling rates from late winter through early autumn, with *I. frutescens* and *I. imbricata* being derived hosts that are galled primarily from early autumn to late winter (Stiling *et al*. 1992; Rossi and Stiling 1995; Rossi *et al.* 1999). Stokes *et al.* (2012) reported significant genetic differences between host-associated populations of *A. borrichiae* in two separate locations in Florida. Most Cecidomyiidae display remarkable levels of host fidelity, with most being associated with a particular host-plant species (monophagous) or with a few species within the same genus or family (oligophagous) (Gagné 1989; Yukawa and Rohfritsch 2005).

Four hymenopteran parasitoid species are associated with *A. borrichiae*: *Rileya cecidomyiae* Ashmead and *Tenuipetiolus teredon* Walker (Eurytomidae), *Torymus umbilicatus* Gahan (Torymidae), and *Galeopsomyia haemon* Walker (Eulophidae) (Rossi *et al.* 1992; Rossi and Stiling 1995; Rossi *et al.* 2006). These parasitoid species bear needle-like ovipositors with which they inject eggs through gall tissue into *A. borrichiae* larvae and/or pupae. Some of these parasitoid species have competitive advantages over the others. For example, although *G. haemon* has a short ovipositor that limits it to galls of small diameter, it is gregarious and

successful at hyperparasitizing the larvae of gall midges and other parasitoids (Stiling *et al. 1992*; Rossi and Stiling 1995; Rossi *et al.* 2006). *Torymus umbilicatus* is solitary but has the longest ovipositor of the four species, allowing it to attack the largest and oldest galls and hyperparasitize the other members of the parasitoid guild.

The responses of the host plant *B. frutescens* and its associated gall-making insect *A. borrichiae* to nitrogen supplementation have been investigated previously. Rossi *et al*. (1992) found that *A. borrichiae* is more likely to attack large, vigorously growing plants. In their study, nitrogen supplementation resulted in significantly larger leaves, higher nitrogen content in apical leaves, and higher flowering rates, but stem density was unaffected. Supplementation also resulted in slightly higher galling rates and significantly larger galls. These results support the Plant Vigor Hypothesis, which proposes that many herbivore species, particularly gall-inducing insect species that have a strong linkage between female preference for oviposition sites and larval performance, preferentially feed on vigorously growing plant or plant parts because they are apparently better resourced and, thus, higher in nutrients (Price 1991).

Study Objectives

The overarching question asked for this project was, does galling by *Asphondylia borrichiae* lead to a net benefit (overcompensation) for *Borrichia frutescens* in terms of growth or reproductive fitness? Specifically, three primary objectives were investigated: (1) to determine whether stem number is correlated with gall number; (2) to determine the relative strength of apical dominance in *Borrichia frutescens* in relation to stem condition; and (3) to investigate the responses of *B. frutescens* in terms of survival, growth, and potential fitness based on stem condition and nutritional status.

Specific questions explored during these experiments included: Is stem number correlated with gall number, and what is the relative strength of the correlation? What is the relative strength of apical dominance in *B. frutescens* (e.g. does it effectively suppress axillary bud break when the apical meristem is intact)? How does the manner of apical dominance disruption (e.g. galling or clipping) impact the timing and positioning of the first axillary bud break? Do stem condition (e.g. apical meristem intact vs. galling by *Asphondylia borrichiae* vs. simulated herbivory by clipping) and/or nutritional status affect survival and growth (e.g. height, stem count)? Do stem condition and/or nutritional status impact flower and seed production and quality (e.g. flowering time, number of seed heads produced, seed mass and count, and seed germination), as well as flower bud mortality? Do stem condition and nutritional status interact with each other in impacting these variables? Is there a particular height or range of heights at which *B. frutescens* undergoes flowering?

Chapter 3: Methods

Field Site

The field portion of this thesis project was conducted at an intertidal salt marsh in the Theodore Roosevelt area of Timucuan Ecological and Historic Preserve in Jacksonville, Florida (GPS coordinates N30°22'45", W81°28'49"; Figure 7). This site hosts a back-marsh community consisting primarily of *Borrichia frutescens* and *Iva frutescens*, with the adjoining marsh consisting of *Juncus roemerianus* Scheele (black needlerush) (Figure 8). At this site, *B. frutescens* and *I. frutescens* occur in patches and overlap very little and only at higher elevations, with *B. frutescens* being located primarily in an area with few competing species. The back marsh gives way to hammock along the north boundary of the site, with areas of sun and shade occurring in patches. Previous studies (Rossi *et al*. 2006; Stokes *et al*. 2012) have found that incidences of galling on *B. frutescens* at Timucuan Preserve are very high (approximately 30-50% of terminals).

Borrichia frutescens patches used in this study occur in both low, intertidal areas that are flooded during high tides and areas elevated a few feet above the high tide line. The site is a shell midden, with the ground consisting a mixture of soil, sand, and large quantities of oyster shell. Small mud crabs (family Panopeidae) inhabit the area and dig burrows, ranging in diameter up to about two or three centimeters, among the marsh plants. Considering that waterlogged marsh soils tend to be anoxic, nitrogen was expected to be present primarily in a form unusable to plants and, thus, this soil was generally assumed to be nutritionally poor, although soils may be oxygenated in isolated spots by mud crab burrows. Additionally, low areas

experiencing regular tidal inundations may have somewhat higher levels of nutrients than areas above the tide line. To account for all of these variables, treatments were randomized across the site.

Because the two experiments required approximately 400 single-stemmed ramets, or individual clonal plants, this project was conducted along a trail near the edge of the marsh where plants had been trimmed to the ground periodically (about every eight or nine months) and, thus, included many single-stemmed ramets, many with young stems that appeared to be recently sprouted from older rhizomes. This area was last trimmed in mid- to late October 2012, nearly three months before the beginning of the start of the project. Due to the amount of time that had passed since trimming, ramets were expected to have regrown stems and reestablished apical dominance, which was likely disrupted by trimming. Trimming did not occur again in the area until after all experiments concluded. Single-stemmed ramets were also identified for the experiments a few feet away from the trail near the edge of the salt marsh, where trimming did not occur, and treatments were randomized across all of these areas to account for varying conditions.

Gall Count vs. Stem Count Relationship Study

In a previous study at this site in 2005 involving 1,000 ramets, stem count and gall count were moderately correlated (A. M. Rossi, personal communication, unpubl. data). To confirm this relationship for the current project, 100 mature, undisturbed *Borrichia frutescens* not used for the other experiments were randomly identified in the field in July 2013 and the number of galls and the number of stems recorded. A stem was counted if it was alive (e.g. smooth and pliable), at least 1 cm long, and bore either an apical meristem, a live (e.g. smooth, green) gall, or at least one pair of unbroken buds or nodes (e.g. potential buds) along the terminal. All galls

present on the ramet were counted, including new galls and any old or senescent galls. Correlation analysis using Pearson's product moment was conducted to assess the relationship between gall number and stem number under natural conditions.

First Bud Break Experiment

Apical dominance can vary depending on the hormonal characteristics of the plant species, nutrition, light, and other resources (Phillips 1971; McIntyre 1977; Benner 1988; Cline 1991; Aarssen 1995; Smith and Whitelam 1997; Wise and Abrahamson 2005, 2008; Franklin 2008; Domagalska and Leyser 2011). Plants with weak apical dominance are more likely to sprout axillary stems with the apical meristem intact and are less likely to be limited by the number of available meristems than plants with strong apical dominance (Espirito-Santo *et al.* 2007; Wise and Abrahamson 2005, 2008). Additionally, apical dominance diminishes in strength with distance from the apical meristem (Jameson 1963; Cline 1991), making the node location in relation to the stem terminal at which lateral buds sprout new shoots informative in determining the relative strength of apical dominance. One aspect of apical dominance that appears to be underreported in the published literature is how the manner in which it is disrupted (e.g. suddenly, as from breaking, browsing, or clipping, or gradually, as from galling) impacts the rate at which lateral buds are activated and the proximity of those activated buds to the stem terminal. The purpose of this experiment was to investigate the relative effects of sudden vs. gradual vs. no removal of the apical meristem in order to discern whether *Borrichia frutescens* generally exhibits strong or weak apical dominance under undisturbed (undamaged) conditions.

This experiment began in May 2013 using three treatment groups, each containing 25 *Borrichia frutescens*: (1) clipped, (2) galled, and (3) stem intact (no removal). Each ramet began

the experiment with a single stem, which consisted of a primary stem bearing multiple pairs of dormant nodes (Figure 9). All ramets were similar in height, approximately 12 cm, and were haphazardly randomized across treatment groups. Ramets were tagged at the beginning of the experiment by gently placing a piece of tape bearing a unique identifier on the stem. All tape was placed along an internode, when spacing allowed, to ensure it would not hinder any bud breaks. Ramets in the clipped treatment group had their terminal meristem removed with scissors at the beginning of the experiment (Figure 10). Galled ramets began the experiment with a single, live gall (e.g. green, no emergence holes). All galls were assumed to be approximately the same age, having been initiated by the same generation of *A. borrichiae*. Intact (control) ramets were protected from galling by a mesh bag that was placed over each ramet's stem terminal and secured gently with a twist tie (Figure 11). Mesh bags were made of white 1 mm nylon tulle netting and initially measured approximately 10 cm wide by 14 cm long. They were replaced as necessary to accommodate plant growth and as they became soiled with mud and debris from rain and high tides. Mesh bags were used in both experiments during this project, and their possible effect on light penetration was an initial concern. However, preliminary data revealed no effect of the mesh bags on light penetration (Appendix A).

Every two weeks during an eight week period, experimental ramets were assessed for survival and the presence of bud break. At eight weeks, if at least one lateral stem was present, the node number bearing the bud to break first was recorded (0 = bud break at terminal, 1 = first node below the terminal, *etc.*). The distance from the terminal (apical meristem) to the first bud break also was measured to the nearest 0.1 cm using a standard tape measure. For clipped ramets, distance was measured from the clipped tip. For galled ramets, distance was measured from the bottom of the gall, which would have been synonymous with the clipped terminal in clipped ramets. Because all surviving galled and clipped ramets broke bud by eight weeks, they

were no longer monitored during the remainder of the experiment. Because no intact ramets broke bud by eight weeks, they were monitored weekly until at least 25% of them broke bud (seventeen weeks), at which time node number and distance to the first bud break from the apical meristem terminal was measured.

Survivorship at eight weeks was assessed by comparing the counts of alive and dead ramets using a chi-square (X^2) test. Additionally, the number of surviving ramets that broke bud at eight weeks were assessed by comparing counts of ramets that broke bud and those that did not using a X^2 test. All X^2 tests throughout were performed on counts; some data are presented as percentages for clarity.

The number of days to first bud break were compared among stem treatment groups using one-way ANOVA. Preliminary checks were conducted to ensure no violation of the assumptions of normality or homogeneity of variances. Variances were homogeneous, but due to very small sample sizes (n = 7 for clipped and n = 4 for intact), normality could not be achieved through transformation and was assumed; however, a probability plot of residuals was linear.

Distance to first bud break was also assessed among groups using one-way ANOVA. Preliminary checks were conducted to ensure no violation of the assumptions of normality or homogeneity of variances. Distance data were square-root transformed successfully before ANOVA to achieve normality and homogeneity of variances, but due to very small sample sizes, results should be viewed with caution.

The frequency of nodes bearing first bud break were assessed between treatment groups using a X^2 test. Due to low sample sizes, frequencies for nodes two and above were pooled and the X^2 test compared nodes 0, 1, and 2+ across stem treatments.

For all instances of one-way ANOVA, significant results led to a Tukey's honestly significant differences (HSD) test with Bonferroni correction ($\alpha = 0.017$) to examine pairwise comparisons between treatment groups.

Stem Condition and Nutritional Status Experiment

Experimental Protocols

To assess the effects of galling on seed set, quality, and viability, and any interaction with plant nutritional status (e.g. nitrogen levels), a 3X2 fully factorial experiment involving three categories of stem condition (galled, clipped, and intact) and two nutrient levels (fertilized and not fertilized) was conducted. Specifically, approximately 300 single-stemmed *Borrichia frutescens* were haphazardly assigned to one of six groups: (1) galled and fertilized; (2) galled and not fertilized; (3) clipped and fertilized; (4) clipped and not fertilized; (5) stem intact and fertilized; and (6) stem intact and not fertilized (control). Each of these groups began the experiment with approximately 50 ramets.

Because of a documented history of high galling rates on *B. frutescens* at Timucuan Preserve (up to 50% of terminals) (Rossi *et al*. 2006; Stokes *et al*. 2012), many ramets in the intact groups were expected to be galled as the experiment progressed. Any ramets in the intact groups that became galled were reclassified in the data records to the corresponding galled treatment group based on nutritional treatment and continued to be monitored, but ultimately were excluded from all post-experiment statistics. To mitigate the possibility that high galling rates may severely impact sample size in the control group, a second control group of 20 additional intact, unfertilized ramets was selected randomly and protected from galling by a mesh bag that was placed over each ramet's stem terminal and much of the primary stem, and secured gently with a twist tie. Mesh bags were identical to those used in the first bud break

experiment: white 1 mm nylon tulle netting with dimensions of approximately 10 cm x 14 cm. They were replaced with larger mesh bags as needed to accommodate plant growth and elongation of new meristems. Mesh bags were also replaced as they became soiled with mud and debris from rain and high tides. After the study ended, the effects of mesh bags on a number of factors including survivorship, growth, stem count, flowering, seed production, and seed germination were assessed by comparing bagged and unbagged control groups (see Appendix A). Because no significant differences were found between the two groups, the data from the two control groups were pooled into a single control group for all remaining statistical analyses. Unless specifically stated otherwise, the terms "control" and "intact/unfertilized" refer to this pooled control group consisting of both bagged and unbagged ramets.

The two galled treatment groups began the experiment with single-stemmed *B. frutescens* already bearing a single gall. Ramets in the clipped treatment groups initially had no galls and had their single, terminal meristem removed with scissors at the beginning of the experiment (Figure 12). No additional clipping (e.g. of lateral shoot meristems) occurred during the experiment.

The effect of plant nutritional status (i.e. nitrogen level) on plant survival, growth, and fitness and any interaction with stem condition in its effect were assessed by applying nitrogen to the three fertilized treatment groups (galled/fertilized, clipped/fertilized, stem intact/fertilized). Ramets in these treatment groups received a small amount (c. 2 g) of nitrogen fertilizer in the form of blood meal (12-0-0) every four weeks (press application). Blood meal is a natural, organic, slow-release granular fertilizer. The fertilizer was applied by carefully forming a hole approximately 3 to 4 cm deep, or as allowed by the soil compacted with oyster shells, near the base of the ramet using a screwdriver, pouring about 2 to 3 g of fertilizer into the hole, and covering it again. Applying the fertilizer in this manner, as opposed to broadcasting it on the

surface of the ground around the ramet, was intended to prevent it from being washed away to neighboring ramets or into the marsh during high tides or heavy rains. Any effects on the roots by the repeated formation of a hole was controlled in all non-fertilized treatment groups by forming a hole approximately 3 to 4 cm deep near the base of each ramet in those groups and covering it again.

The height of each ramet was recorded initially. Since the experiment began with single-stemmed ramets, initial stem count per ramet in all groups was 1. Initial gall count per ramet was either 1 or 0, depending on whether the group was a galled group or an ungalled group. The ramets were reassessed for survival, height, stem count, and gall count, as well as the presence of flower buds, every two weeks for 25 weeks, from January through June 2013.

For survival, a ramet was considered alive if it had any visible green or living tissue, including buds forming, turning green, or beginning to break. If a ramet was deemed dead based on these criteria, it was monitored bi-weekly for survival and, if green tissue appeared, data recording for that ramet resumed. For a few ramets that were completely destroyed or uprooted (e.g. by animals or humans or decomposition after death), the ramet was considered permanently lost for the remainder of the study (e.g. was not replaced) and the data recorded for it was not used in statistical analyses unless otherwise noted.

Height was measured to the tip of the tallest live stem (e.g. apical meristem, not leaf tips) to the closest 0.1 cm using a standard tape measure (Figure 13). Once axillary shoots began to grow, the tallest stem was often a lateral stem. In the event that a live gall was present on the tallest stem terminal, ramet height was measured to the top of the gall (e.g. the top of the swollen meristem). In the event a flower head or bud was present on the tallest stem, height was measured to the point where the sepals attach to the receptacle. For clipped ramets, height was measured initially to the tip of the clipped stem if it was alive or to the highest point

exhibiting live tissue, until axillary stems elongated and superseded this point, at which point the tallest meristem was used. Height for ramets in all stem treatment groups was expected to decrease periodically as stem and gall tissue senesced and abscised.

A stem was counted if it was alive (e.g. smooth and pliable) and bore either a meristem, a live (e.g. smooth, green) gall, or at least one node. The primary stem continued to be counted as a separate stem as long as it was alive and bore at least one unbroken node between its terminal and the next highest broken bud. The primary stem was no longer counted if the terminal had senesced or fallen away to the lateral stems or if the highest node had sprouted lateral stems. New stems were included in the stem count after they had elongated at least 1 cm from the bud break (Figure 14). In some cases, new stems were galled shortly after elongation, eventually transforming the little stem into a gall that appeared to be growing from the side of the main stem; these short galled stems were counted as separate stems as long as the gall was alive and green. In other cases, new lateral stems sprouted from directly below a gall, giving the appearance that they were part of the gall; these stems were counted separately from the gall above them (Figure 15). Stem counts were expected to decrease periodically as stems and galls senesced and abscised.

All galls present on the ramet were counted, including new galls and any old or senescent galls that formed earlier in the experiment but remained on the ramet. In the event that a single shoot terminal simultaneously bore more than one distinct gall or a single gall that was clearly a composite of multiple galls, a single gall was counted. In other words, “gall count” equates to the number of galled meristems. Gall count was expected to decrease periodically as senescent galls were abscised from the ramets.

Flower buds began appearing in late March (Figure 16). They were tagged at first appearance through the end of June with a piece of tape bearing a unique number identifying

the ramet and flower head that was gently placed around the flowering stem. The development of all flower heads tagged before the end of June was monitored biweekly until seed head harvesting, which concluded in early September. Tagged flower heads that had finished blooming (e.g. all florets senesced and/or abscised) had a small mesh bag placed over them and secured gently with a twist tie to capture any achenes (dry fruits bearing a single seed) that may be released before harvesting (Figure 17). For each seed head, dates for the following events were recorded: flower bud identified and tagged, flower head blooming (disc open), flower head completed blooming and was bagged, and seed head harvested. The date was recorded for any flower buds that aborted (died) before blooming. Although galling of flowers or flower buds is rare, several instances occurred in this study; in this case, they were noted and no longer monitored, since galling deforms flowers and prevents effective blooming and pollination (Figure 18). In the event that an intact ramet was galled during flowering, its flowers were monitored and seeds harvested, but the data was not used in any statistical analyses. Full maturity was reached when receptacular bracts were dry and brown and the flower stalk senesced (Figure 19). Then the bagged seed head was removed from the ramet by clipping the stalk and returned to UNF for further analysis.

Seed mass is indicative of seed quality and level of stored nutrients and is a better indicator of plant fitness than seed number alone (Harper 1977; Schaal 1980; Paige and Whitham 1987; Agrawal 1998). For this experiment, seed heads were stored in a dry location for one to two weeks, and then each seed head was carefully broken apart and each achene separated from its bract with forceps. Each achene contained a tiny seed; the term “seed” henceforth refers to both the achene and the seed within. For each seed head, the total seeds were counted and recorded. Abnormal seeds (e.g. shriveled, very small, or with bore holes) were counted separately and discarded. Normal seeds were counted and weighed collectively,

and the total mass was divided by the normal seed count to determine the mean mass per seed for the seed head. The general condition of the seed head was also recorded, including whether or not a substantial number of bracts were empty, indicating the associated floret was not pollinated. The presence of fungi or insects or evidence of insects (e.g. puparia) was also noted. Seeds for each seed head were then placed in a labeled paper coin envelope and stored at room temperature in a paper bag in the lab until the germination phase of the experiment.

Seeds were planted to determine germination rates in early September. Several recommended techniques were tested earlier in the year with varying success using sample seeds collected in the field from ramets not used in the study (Gann *et al*. 2012; Biber *et al.* 2013; Jenny Evans, Manager at Sanibel-Captiva Conservation Foundation Native Plant Nursery, personal comm.). Twenty-five seeds were randomly selected from each seed head. For seed heads producing fewer then twenty-five seeds, all seeds were selected. Seeds were soaked between 48 and 60 hours in tap water (Biber *et al.* 2013) in labeled plastic vials with caps loosely secured. Seeds were planted in two plastic horticultural flats each containing 18 cells (approx. 7.5 cm x 7.5 cm x 4.5 cm) and 11 individual plastic seedling pots (approx. 8 cm x 8 cm x 7.5 cm). All seeds for each seed head were planted together in a single cell or pot and the cell or pot was labeled with a piece of tape bearing the seed head identifier. Flats and pots were filled with approximately 3-4 cm of planting mixture, which was a 1:1 blend of Ace Horticultural Grade Vermiculite (A. H. Hoffman, Inc., Landisville, PA) and Organic Seed Starting Jiffy-Mix (Ferry-Morse Seed Company, Fulton, KY), the latter which contained 48-52% sphagnum peat moss, 48-52% vermiculite, lime, and an organic wetting agent. No fertilizers were included in the original manufactured products nor added to the final planting mixture. Seeds were very lightly covered with planting mixture, placed in light shade in the UNF greenhouse, and lightly watered under timed misters for four minutes every four hours for the duration of the germination phase

(Gann *et al*. 2012; Jenny Evans, Manager at Sanibel-Captiva Conservation Foundation Native Plant Nursery, personal comm.). Flats were placed side by side with individual pots placed along the back edges to "extend" the columns (Figure 20). Because the experimental treatments produced drastically unequal numbers of seed heads (replicates), a Latin square layout was not possible. Rather, it was mimicked as nearly as possible by arranging treatment replicates such that each row and column contained a variety of different treatments, and duplication of treatments was minimized where possible. Germination was expected to begin within about two to four weeks (Biber *et al.* 2013; Jenny Evans, personal comm.), but twelve weeks were allowed to ensure adequate time for germination, after which time the number of germinations for each seed head was recorded.

Statistical Analyses

Except where noted, all of the following statistical analyses were conducted on ramets surviving to the end of the experiment and all intact ramets used in these analyses were those that remained intact (ungalled) until the end of the experiment. Finally, because survivorship was not significantly different among stem treatment groups and among fertilization treatment groups, statistical analyses on stem counts, gall counts, flower counts, seed head counts, and other dependent variables could be performed with the assumption that surviving ramet counts across groups were similar.

Survivorship was assessed using a chi-square (X^2) test to compare counts of surviving and dead ramets at the end of the experiment across stem treatments and across fertilization treatments. Yates' correction was used in the X^2 test for fertilization treatment throughout all subsequent analyses because *df* = 1. Although all X^2 tests throughout were performed on counts, data are presented as percentages for clarity.

Because experiment set up took two weeks, the heights recorded at three weeks were considered initial heights. Preliminary analyses comparing the initial heights across stem treatment groups indicated that initial heights were not statistically equal (Kruskal-Wallis: X^2 = 23.794, *df* = 2, $p < 0.001$). Kruskal-Wallis was used because data in some treatment groups was not normal and could not be transformed without jeopardizing normality for other groups, and normality could not be assumed due to large sample size ($n \approx 50$). Mann-Whitney U tests for pairwise comparisons between stem treatments indicated that ramets in the galled group, in particular, were significantly taller compared to clipped and intact ramets. This situation can be explained by the likelihood that galled ramets are older and, therefore taller, than ungalled ones. Therefore, height was assessed in two alternate ways: overall percent change in height between the beginning and end of the experiment and average biweekly change in height. Initial height was used as a covariate when parametric testing (ANCOVA) could be performed on other dependent variables.

Due to the inability to successfully transform data to achieve normality and homogeneity of variances, overall percent change in height was assessed separately across stem treatments and fertilization treatments using two Kruskal-Wallis tests. Average biweekly change in height was assessed across stem and fertilization treatments by adding a value of 2 and square-root transforming the data to achieve normality and homogeneity of variances, and then using a two-way ANOVA.

Stem counts were assessed in two ways: the maximum number of stems at any one time during the experiment and the number of stems at the end of the experiment (week 25). Since all ramets began the experiment with one stem, a Poisson distribution was suspected. For each type of stem assessment, Poisson distribution analyses were performed in each treatment group using a chi-square (X^2) test to compare observed frequencies of each stem number

against expected frequencies in a Poisson distribution. Stem counts in all groups were found to adhere to a Poisson distribution (Table 3). Therefore, Kruskal-Wallis tests were performed to assess these maximum stem count and stem count at the end of the experiment across stem treatments and fertilization treatments. Only ramets surviving to the end of the experiment and intact ramets that were not galled during the experiment were used in these analyses.

To determine if a relationship exists between ramet height and stem count, Pearson's product moment correlation analysis was conducted on those data for intact/unfertilized ramets (control) at week 9. This date was chosen because it was in the middle of the growing season but prior to flowering, and had the broadest range of stem counts and heights, which were necessary for accurate analysis.

Galling was expected in unprotected ramets during the experiment. The number of starting ramets that were galled during the experiment was assessed across stem treatments and fertilization treatments using chi-square (X^2) tests to compare the number of galled ramets against the number of starting ramets. These analyses included ramets that did not survive to the end of the experiment and excluded intact ramets that were bagged. For the galled treatment group, ramets that were deemed to have been galled during the experiment were those that developed new galls after the initial gall.

Cumulative gall counts, or counts of unique galls that developed on each ramet surviving to the end of the experiment, were totaled and compared across stem treatments and across fertilization treatments using chi-square (X^2) tests. These analyses included ramets that began the experiment with intact stems and were unbagged but were later galled and excluded from most other analyses.

To determine if flowering was associated with ramet height or range of heights, all ramets that produced flower buds during the experiment regardless of treatment, except intact

ramets that were galled before flowering, were classified by height at first flower bud appearance. Then these height class frequencies were compared using a chi-square (X^2) test. The number of ramets that produced flower buds was assessed across stem treatments and across fertilization treatments using chi-square (X^2) tests. Because flower bud development occurred as early as week 11, this assessment includes all ramets that produced flower buds, including those with flower buds that later aborted and ramets that later died, as well as intact ramets that were galled after flower bud appearance.

Some flower buds were aborted, that is they died on the stem before blooming for unknown reasons. The number of flower buds that were aborted compared to total flower buds was assessed across stem treatments and across fertilization treatments using chi-square (X^2) tests. This assessment included intact ramets that developed flower buds and then were galled, but not intact ramets that were galled before flowering. Some flower buds or blooming flower heads were galled, which deformed the flower head and prevented or hindered proper blooming and pollination. The number of flowers or flower buds that were galled compared to total flower buds was assessed across stem treatments and across fertilization treatments using chi-square (X^2) tests. This assessment includes intact ramets that developed flower buds and then were galled, but not intact ramets that were galled before flowering.

Seed head production was assessed in two ways: the number of seeds produced by the end of the experiment and the number of flower buds that resulted in seed heads. Both analyses were conducted across stem treatments and across fertilization treatments using chi-square (X^2) tests. Intact ramets that were galled after flower buds appeared were excluded from these analyses.

Number of days to first flowering was assessed in two ways: flowering ramets only and all ramets. For flowering ramets, the number of days between the beginning of the experiment

and the day the first flower bud was discovered on the ramet were calculated and assessed across stem treatment and fertilization treatments using Kruskal-Wallis tests. For all ramets, non-flowering ramets were included and assigned the value of 182 days, or two weeks past the end of the experiment, and then assessed across stem treatment and fertilization treatments using Kruskal-Wallis tests. In both sets of analyses, data was normal and variances homogeneous only for some groups, and the data could not be transformed successfully without jeopardizing normality or homogeneity of variances elsewhere. Both of these analyses were conducted only on ramets that survived to the end of the experiment and included ramets with flower buds or flower heads that were later aborted or galled.

For the following analyses involving analysis of covariance (ANCOVA), preliminary checks were conducted for the assumptions of normality, linearity, homogeneity of variances, homogeneity of regression slopes, and reliable measurement of the covariate. Unless otherwise indicated, no assumptions were violated. These analyses include only ramets that survived to the end of the experiment and only flowers that developed normally from flower bud through seed head stage without being galled or aborting. Intact ramets that were galled before flowering and producing seed heads were excluded from the following analyses.

Two-way analysis of covariance (ANCOVA) was conducted with bootstrapping to assess the effects of stem condition and nutritional status on each of the following dependent variables, while controlling for initial height:

- Seed count (normal seeds)
- Seed mass
- Number of days in flower bud stage*
- Number of days blooming*
- Number of days ripening*

For the number of days in flower bud stage, blooming, and ripening (*), the data was not normal due to small sample sizes in the clipped group (n = 7) and could not be transformed without jeopardizing homogeneity of variances. Thus, normality was assumed and ANCOVA was performed, but results should be viewed with caution. For seed count and seed mass, data was normal and variances were homogeneous.

The number of abnormal seeds was compared to the number of normal seeds across stem treatments and across fertilization treatments by using chi-square (X^2) tests. Total seed germinations were compared to the total number of seeds that did not germinate and assessed across stem treatments and across fertilization treatments using chi-square (X^2) tests.

Lastly, significant ANCOVA results were further analyzed using contrasts with automatic Sidak correction. For all ANOVA analyses described previously where significance was found among stem treatments, Tukey's HSD tests were performed for pairwise comparisons of stem treatments with Bonferroni correction for significance adjusted to $\alpha = 0.017$ ($\alpha = 0.05/3 = 0.017$). Similarly, for all instances where the Kruskal-Wallis test was used and significance was found among stem treatments, Mann-Whitney U tests also were performed for pairwise comparisons between stem treatments with Bonferroni correction at $\alpha = 0.017$.

All parametric and non-parametric tests except for chi-square were performed using PASW Statistics (currently known as SPSS Statistics), version 18.0.0 (IBM, Armonk, NY). Chi-square (X^2) tests were conducted in Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA). Results charts in appendix B were created in SigmaPlot, version 11.0 (Systat Software Inc., San Jose, CA) and Microsoft Excel 2010.

Predicted Results

Gall Count vs. Stem Count Relationship Study

Previous, unpublished data investigating a possible association between gall count and stem count in *Borrichia frutescens* at Timucuan suggested that a moderate positive correlation exists between the two variables at this site (r = 0.415, n = 1000; A. M. Rossi, personal comm., unpubl. data). The gall-stem count study for this project was expected to further support this finding. However, the direction of this relationship could not be determined from this study alone. For instance, do higher stem counts result from higher gall counts via disruption of apical dominance and stem bifurcation, or do higher gall counts result from higher stem counts, which would mean more meristems and thus more galling sites for *Asphondylia borrichiae* females? Thus, manipulation experiments were necessary to investigate further.

First Bud Break Experiment

As previously described, the strength of apical dominance varies among species, and any differences in a plant species' propensity for sprouting lateral stems under various degrees of apical dominance (e.g. present, gradually disrupted, or suddenly removed), its timing, and the location of initial bud breaks may reveal much about its strength of apical dominance. Generally, strong apical dominance may be revealed by intense suppression of lateral buds when the apical meristem is present and rapid sprouting of lateral shoots and overcompensation for lost tissues after apical meristem removal (Aarssen 1995), as in the case of *Ipomopsis* spp. (Paige and Whitham 1987). In the first bud break experiment, intact ramets (control) were expected to exhibit evidence of stronger apical dominance compared to galled ramets, and clipped ramets were expected to exhibit evidence of the complete absence (sudden disruption) of apical

dominance. Galled ramets were expected to show evidence of a gradual decline in apical dominance compared to control and clipped ramets.

Since the sudden removal of apical dominance likely allows lateral buds to break faster than instances where apical dominance is still present to some degree, clipped ramets were expected to break bud faster than either galled or intact ramets, with intact ramets being the slowest to break bud, if at all. Clipped ramets were also expected to have the highest number of bud breaks among surviving ramets by the end of the experiment, followed by galled ramets, and then intact ramets.

Additionally, with apical dominance being completely absent in clipped ramets, lateral bud break was expected to occur closest to the stem terminal in that group, while some degree of apical dominance in galled ramets was expected to hinder bud break closest to the terminal but allow it at more distant nodes. Intact ramets were expected to have either no bud break at all (strong apical dominance) or bud break at nodes significantly distant from the apical meristem compared to the other groups (moderate apical dominance). This result was expected because apical dominance tends to weaken with increasing distance from the apical meristem (Jameson 1963; Cline 1991). Thus, in species where bud break may occur with the apical meristem intact, the probability of bud break can be expected to increase with increasing distance from the apical meristem to the point where hormonal suppression is weak enough to allow bud break; in other words more basally located lateral shoots breaking bud before buds closer to the terminal (basipetal bud break pattern) (Cline 1991).

Since gall-inducers are known to deplete nutrients and energy normally used by the plant for life functions (Abrahamson and Weis 1987; Weis *et al*. 1988; Bronner 1992; Dreger-Jauffret and Shorthouse 1992; Marquis 1996; Raman *et al.* 2005; Rohfritsch 2008), survivorship in the bud break experiment was expected to be lowest in galled ramets, although active galls

are generally unlikely to kill the host plant, since doing so would also kill the larvae within the gall (Crawley 1983). Since plant damage typically has detrimental effects on survival (Jameson 1963; McNaughton 1983; Strong *et al*. 1984; Lindroth 1989), survivorship was expected to also be somewhat lower in clipped ramets compared to intact ones.

Stem Condition and Nutrition Status Experiment

In the 25-week manipulation experiment involving stem condition and nutritional status, survivorship was expected to be similar to that in the first bud break experiment, at least initially: lowest in galled ramets, followed by clipped ramets, and highest in intact ramets. Survivorship was also expected to be higher in fertilized ramets than in non-fertilized ramets, particularly considering that the site was generally assumed to be nutrient-limited. However, other factors such as water availability, light, and weather conditions (e.g. storms) may impact survivorship (Crawley 1983; Strong *et al.* 1984), and the long-term survivorship of *Borrichia frutescens* at this site was generally unknown. Thus, it was expected that any differences in survivorship between the groups may decrease as time passed since initial damage (galling or clipping) occurred and other disturbances likely affected the plants.

Since gall-inducers tend to redirect nutrients and energy normally used for plant growth and reproduction, both measures of height (overall percent change in height and average biweekly change in height) were expected to be lowest in galled ramets. Both measures of height were expected to be somewhat higher in clipped ramets than galled ones due to the lack of gall-inducer and the vigorous regrowth expected after damage (Price *et al*. 1987a, b; Roininen *et al*. 1988), especially considering the timing of damage before the springtime growth spurt. However, clipped ramets were expected to have lower measures of height compared to intact ramets because removal of the actively growing apical meristem was expected to initially

disrupt growth in clipped ramets and any additional shoots that broke bud in clipped ramets would mean additional meristems (sinks) that would likely compete for resources. Conversely, intact ramets with a single apical meristem and uninterrupted apical dominance could be expected to direct all of their growth-allocated resources to that meristem and enable the single stem to grow tall quickly (Aarssen 1995). Nutrient supplementation was expected to result in larger plants, as in Rossi *et al*. 1992.

Since damage to the apical meristem may release *B. frutescens* from apical dominance and cause axillary buds to produce lateral shoots, it generally was expected that either galling or clipping would result in more stems compared to plants with intact stems, measured as maximum number of stems attained during the experiment and number of stems at the end of the experiment (week 25). Significant differences between ramets with apical meristem damage and those with no damage would suggest that *B. frutescens* responds to such damage with overcompensation in terms of growth. Because of the results in Rossi *et al*. (1992), fertilization was not expected to result in a significantly greater number of stems compared to the non-fertilized ramets.

This experiment included a correlation analysis of stem count vs. height. Because height is likely positively associated with age and stem count in plants, it can be expected to increase rather than decrease over time, it was generally expected that any correlation between these variables would be positive and not negative. However, if apical dominance is very strong in *B. frutescens*, or lateral stems never sprout in the absence of damage, a correlation between height and stem count may not exist, at least over relatively short time periods as in this study.

Previous studies have shown that *B. frutescens* have a mixture of genotypes susceptible and not susceptible to galling (Stiling and Rossi 1996; Rossi and Stiling 1998). Assuming that genotypes were distributed equally across all treatment groups, the number of ramets galled

during the experiment and cumulative gall counts were expected to be generally similar across stem treatments. Because *Asphondylia borrichiae* was found previously to prefer fertilized ramets (Rossi *et al*. 1992), nutrient supplementation was expected to result in somewhat higher galling rates in this experiment.

Because plants must reach sexual maturity during their development, first flowering was expected to occur at a certain height or range of heights, as opposed to occurring equally across all height classes. Because stem counts were expected to be higher in galled and clipped ramets, these groups were expected to produce more flowers and seed heads than intact ramets. First flowering was expected to occur earlier in intact ramets and later in galled and clipped ramets, due to the delay in reproduction that may occur as a result of regrowth after herbivory and similar damage (Jameson 1963; Inouye 1982; Bryant *et al.* 1983; Crawley 1983; McNaughton 1983; Paige and Whitham 1987; Benner 1988; Lindroth 1989; Price 1991; Whitham *et al.* 1991; Fornoni *et al.* 2003). First flowering was also expected to occur earlier in fertilized ramets compared to unfertilized ones, since nutrient supplementation, particularly under nutrient-poor conditions, may enable ramets to allocate more nutrients toward reproduction and allow damaged ramets to overcome nutrient limitations and delays from regrowth.

Since the gall-inducer may redirect a substantial proportion of resources that would be otherwise used for plant reproduction (Abrahamson and Weis 1987; Weis *et al*. 1988; Bronner 1992; Dreger-Jauffret and Shorthouse 1992; Marquis 1996; Raman *et al.* 2005; Rohfritsch 2008), galling may result in delayed flowering or fewer flowers, seed heads, or seeds. For this reason, the number of abnormal seeds and the number of aborted flowers were expected to be somewhat higher in galled ramets than intact ramets. Additionally, because developing seeds compete for available nutrients, especially when nutrient or energy limitations exist, and plants often initiate more seeds than needed and abort some seeds early in development to ensure

adequate nutrient and energy stores for remaining seeds (Harper 1977; Schaal 1980; Stephenson 1981; McNaughton 1983), some seeds were expected to not develop completely, particularly in the galled and clipped stem treatment groups and the unfertilized treatment group.

Seed mass typically varies little in most plant species (Harper 1977), with significant variations occurring instead in seed count; thus seed mass was expected to remain more or less constant across treatment groups. Nutrient supplementation was expected to increase seed count more than seed mass. Since seed mass is indicative of quality and energy reserves and larger and heavier seeds tend to experience higher germination success (Harper 1977; Schaal 1980; Paige and Whitham 1987), any variation in seed mass could be expected to result in higher germination success from heavier seeds. Damage from herbivory, actual or simulated, has been known to delay flowering (Jameson 1963; Inouye 1982; Crawley 1983; Paige and Whitham 1987; Benner 1988; Lindroth 1989; Price 1991; Whitham *et al.* 1991; Fornoni *et al.* 2003), so intact ramets generally were expected to flower sooner than galled or clipped ones. Nutrient supplementation was previously found to produce more flowers per ramet (Rossi *et al*. 1992), and this trend was expected in the experiment, as well as more seed heads per ramet and more seeds per seed head compared to unfertilized ramets.

Chapter 4: Results

Gall Count vs. Stem Count Relationship Study

The ramets assessed for this study had an average of 9.28 ± 1.12 (SEM) stems and 3.10 ± 0.38 galls. Pearson's product moment revealed a strong positive correlation between gall count and stem count (r = 0.852, n = 100) (Figure 21), with nearly 73% of the variation in one being explained by the other. However, the direction of influence in this relationship is not specified by this result. These variables are more strongly correlated than the same variables measured at the same site in 2005, when 17.2% of variation was explained by the relationship, which is probably due to yearly variation in both biotic and abiotic factors in the ecosystem (A. M. Rossi, personal comm., unpubl. data).

First Bud Break Experiment

Survivorship at Eight Weeks

Stem treatment had a highly significant effect on survivorship at 8 weeks (X^2 = 16.197, df = 2, p < 0.001). Presented as percent survivorship, intact (control) ramets had the highest survivorship (84%) and galled ramets the lowest (28%), while clipped ramets had a survivorship of 61% (Figure 22). Clipped ramets experienced an immediate decrease in survival during the first two weeks, while survival of galled ramets drastically decreased and surpassed clipped ramets after the second week. Intact ramets maintained a relatively moderate rate of mortality.

Days to First Bud Break

Stem treatment had a highly significant effect on the number of days to first bud break ($F_{2, 24}$ = 45.568, p < 0.001). Both galled and clipped surviving ramets broke bud within the first 40 days (note: values represent mean ± SEM throughout; 36.43 ± 6.25 and 33.64 ± 4.33, respectively) and were statistically similar, while intact surviving ramets broke bud about 80 days later (113.75 ± 1.75) and were significantly different from the other two groups (Figure 23).

Bud Break Among Surviving Ramets

When assessed for all surviving ramets at eight weeks, stem treatment had a highly significant effect on the number of surviving ramets that broke bud by that time (X^2 = 46.000, *df* = 2, p < 0.001) (Figure 24). Considered over time, slightly more clipped ramets than galled ramets broke bud in the first five weeks (Figure 25). By eight weeks, 100% of surviving galled and clipped ramets had broken bud, while no intact ramets had broken bud by this time. Intact ramets did not begin to break bud until sixteen and seventeen weeks, at which point the experiment ended, and then only 23.5% of surviving intact ramets had done so.

Distance to First Bud Break

Stem treatment also had a highly significant effect on the distance (cm) to first bud break ($F_{2, 24}$ = 12.474, p < 0.001), as measured from the stem terminal to the node bearing the first lateral meristem to break bud. Clipped ramets broke bud significantly closer to the terminal, with an average of 1.33 ± 0.23 (SEM) cm, compared to intact ramets, which broke bud an average 6.55 ± 2.03 cm from the terminal (Figure 26). Galled ramets broke bud an average of 2.97 ± 0.72 cm from the terminal and were statistically similar to both the clipped and intact groups. Note: Data were square-root transformed to meet normality and homogeneity of variance assumptions of ANOVA, but are presented untransformed for clarity.

Nodes Bearing First Bud Break

Stem treatment had a highly significant effect on the node bearing first bud break (X^2 = 14.534, *df* = 4, *p* = 0.006), as counted from the stem terminal (node 0) to the node bearing the first lateral meristem to break bud (Figure 27). Clipped ramets had an average node of 0.8 ± 0.2 and broke bud at nodes 1 and 0 (terminal). Approximately 25% of clipped ramets broke bud directly at the stem terminal, and was the only group to do so. Galled ramets had an average node of 1.9 ± 0.3 (SEM) and broke bud primarily at node 1, with the remaining bud breaks being split evenly between nodes 2 and 3. Intact ramets had an average node of 4.5 ± 1.2, and never broke bud closer to the terminal than node 3. Most intact ramets broke bud at nodes 3 and 4, but one ramet broke bud at node 8.

Stem Condition and Nutritional Status Experiment

Survivorship at 25 Weeks

Stem treatment had no effect on survivorship at week 25, or the end of the experiment (X^2 = 0.629, *df* = 2, *p* = 0.730) with intact ramets having the highest survivorship (79%), followed by galled ramets (77%), and clipped ramets (74%) (Figure 28a). Fertilization treatment also had no effect (X^2 = 0.357, *df* = 1, *p* = 0.550) with fertilized ramets having slightly higher survivorship (78%) compared to unfertilized ramets (75%) (Figure 28b). Considered over time (Figure 29), all treatment groups follow a similar trend in decreasing survivorship over time. Because survivorship was not significantly different across treatment groups, subsequent tests on height, gall count, and other variables did not need to take survivorship into account.

Initial Height

Heights of ramets at the beginning of the experiment varied significantly across stem treatments (X^2 = 23.794, *df* = 2, *p* < 0.001) (Figure 30a). Mann-Whitney U tests for pairwise comparisons with Bonferroni correction (α = 0.017) between stem treatments indicated that ramets in the galled group, in particular, were significantly taller (33.03 ± 1.95 cm) compared to clipped and intact ramets (23.39 ± 2.55 cm and 22.02 ± 1.73 cm, respectively). Clipped and intact ramets were not statistically different from each other. Initial height did not vary significantly across fertilization treatments (X^2 = 3.548, *df* = 1, *p* = 0.06) (Figure 30b), with an average height of 29.30 ± 2.03 (SEM) cm for fertilized ramets and 23.62 ± 1.54 cm for unfertilized ramets. Due to unequal initial heights across stem treatments, this variable was used as a covariate to control for its effect on other dependent variables when parametric testing (ANCOVA) could be performed.

Overall Percent Change in Height

Stem treatment had a highly significant effect on overall percent change in height between the beginning and end of the experiment (X^2 = 13.405, *df* = 2, *p* = 0.001) (Figure 31a). Mann-Whitney U tests for pairwise comparisons with Bonferroni correction (α = 0.017) between stem treatments indicated that galled ramets had a significantly lower percent change in height (111%) compared to clipped (227%) and intact (222%) ramets, which were statistically similar to each other. Fertilization treatment had no effect on overall percent change in height (X^2 = 0.875, *df* = 1, *p* = 0.349) (Figure 31b).

Average Biweekly Change in Height

Stem treatment had a significant effect on average biweekly change in height ($F_{2, 208}$ = 3.288, *p* = 0.039), but Tukey's HSD with Bonferroni correction (α = 0.017) revealed no significant

difference (Figure 32a). Without Bonferroni correction, a significant difference at the p = 0.05 level was found between the intact and galled groups. Average biweekly change in height for intact ramets was 2.69 ± 0.19 (SEM) cm, compared to 2.09 ± 0.14 cm for galled ramets and 2.25 ± 0.19 cm for clipped ramets. Fertilization treatment had no effect on average biweekly change in height ($F_{1, 208}$ = 0.261, p = 0.610) (Figure 32b). Average biweekly change in height for fertilized ramets was 2.36 ± 0.14 cm, compared to 2.30 ± 0.14 cm for unfertilized ramets. No significant interaction between stem and fertilization treatments was found ($F_{2, 208}$ = 1.708, p = 0.184).

Considered over time (Figure 33), average height differences between treatments groups are difficult to discern. It appears that intact ramets generally had steady gains in height initially compared to galled and clipped ramets, which did not appear to begin gaining height until several weeks into the experiment. Galled ramets may have had slightly larger gains than clipped ramets in the first seven weeks, although these differences are subtle. Average heights in all treatments appear to level off after 19 weeks.

Maximum Number of Stems

Stem treatment had no effect on maximum number of stems present on ramets during the experiment (X^2 = 1.798, df = 2, p = 0.407); galled ramets had an average maximum of 5.59 ± 0.52 (SEM) stems, compared to 5.32 ± 0.43 stems for clipped ramets and 4.97 ± 0.48 stems for intact ramets (Figure 34a). Fertilization treatment had a highly significant effect on the maximum number of stems per ramet (X^2 = 12.230, df = 1, p < 0.001), with fertilized ramets having an average maximum of 5.90 ± 0.33 stems, compared to 4.74 ± 0.43 stems for unfertilized ramets (Figure 34b).

Number of Stems at End of Experiment

Stem treatment had no effect on the number of stems at the end of the experiment (week 25) (X^2 = 0.986, *df* = 2, *p* = 0.611), with galled ramets having an average of 4.10 ± 0.57 (SEM) stems, compared to 4.08 ± 0.46 stems for clipped ramets and 4.59 ± 0.49 stems for intact ramets (Figure 35a). Fertilization treatment had a highly significant effect on the number of stems at the end of the experiment (X^2 = 11.973, *df* = 1, *p* = 0.001), with fertilized ramets having an average maximum of 4.90 ± 0.37 stems, compared to 3.60 ± 0.45 stems for unfertilized ramets (Figure 35b). Considered over time (Figure 36), the intact stem treatments appear to lag behind the galled and clipped treatments in terms of stem count until week 15, after which their stem counts are comparable to their galled and clipped counterparts. After week 17, fertilized groups have noticeably more stems than unfertilized groups.

Height vs. Stem Count

Pearson's product moment revealed a moderate positive correlation between ramet height and stem count (*r* = 0.369, n= 36) (Figure 37). Approximately 14% of the variation in stem count was explained by height.

Ramets Subsequently Galled

Stem treatment had a highly significant effect on the number of starting ramets that were galled during the experiment (X^2 = 10.146, *df* = 2, *p* = 0.006), with only 11% of clipped ramets being galled after the experiment began, compared to 31% of ramets in the galled group and 35% of unprotected (unbagged) intact ramets (Figure 38a). Fertilization treatment had no effect on the number of starting ramets that were galled during the experiment (X^2 = 0.622, *df* = 1, *p* = 0.430), with 28% fertilized ramets and 35% of unfertilized ramets (excluding bagged ramets) being galled after the experiment began (Figure 38b).

Cumulative Gall Count

Because starting ramet count and survivorship were homogeneous, cumulative gall counts could be assessed without regard to surviving ramet count. Stem treatment had a highly significant effect on cumulative gall count, or counts of unique galls that developed on each ramet surviving to the end of the experiment (X^2 = 91.876, *df* = 2, *p* < 0.001), with galled ramets having a total of 109 galls, clipped ramets having 13 galls, and intact ramets developing 39 galls (Figure 39a). Note that intact ramets used for this analysis are those that were galled during the experiment and excluded from all other statistical analyses. Fertilization treatment had no effect on cumulative gall count (X^2 = 0.000, *df* = 1, *p* = 0.992), with total gall counts in both treatment groups being essentially equal: 81 galls on fertilized ramets and 80 galls on unfertilized ramets (Figure 39b).

Height at First Flowering

Differences in first flowering events across height classes were highly significant (X^2 = 35.429, *df* = 8, *p* < 0.001), with 26% of first flowering occurring when ramets reached 50.1 to 60.0 cm, followed by 20% at 40.1 to 50.0 cm, 16% at 30.1 to 40.0 cm, and 13% at 60.1 to 70.0 cm (Figure 40). Smaller percentages of first flowering events occurred at height classes above 70 cm and below 30 cm. Due to small sample sizes, it was not possible to analyze this data at the treatment level without violating statistical assumptions, but first flowering events appear to be similar across treatments (Table 4).

Number of Days to Flowering

When assessed in flowering ramets only, stem treatment had a highly significant effect on the number of days before first flowering occurred (X^2 = 19.747, *df* = 2, *p* < 0.001), with clipped ramets flowering significantly later than either galled and intact ramets (Figure 41a).

Clipped ramets flowered at an average of 136.18 ± 4.66 (SEM) days after the beginning of the experiment, three weeks later than galled ramets (115.89 ± 3.16 days) and a full month later than intact ramets (106.16 ± 2.76 days). Only with Bonferroni correction ($\alpha = 0.017$) were galled and intact ramets not significantly different. Fertilization treatment had no effect on the number of days to first flowering ($X^2 = 1.323$, $df = 1$, $p = 0.250$), with fertilized ramets flowering an average of about five days earlier (111.64 ± 2.79 days) than unfertilized ramets (116.57 ± 3.98 days) (Figure 41b).

When non-flowering ramets were included in the analyses with 182 days assigned as the number of days before first flowering, or two weeks past the end of the experiment in late June, stem treatment continued to have a highly significant effect on flowering time ($X^2 = 38.166$, $df = 2$, $p < 0.001$), with intact ramets flowering significantly earlier than either clipped or galled ramets, which were statistically similar (Figure 42a). Clipped ramets developed flowers an average of 174.80 ± 2.13 (SEM) days, almost 10 days later than galled ramets (165.00 ± 3.57 days) and 36 days later than intact ramets (138.33 ± 4.91 days). Fertilization treatment had a marginal effect on the number of days to first flowering ($X^2 = 3.744$, $df = 1$, $p = 0.053$), with fertilized ramets flowering an average of about nine days earlier (155.10 ± 3.56 days) than unfertilized ramets (164.38 ± 3.05 days) (Figure 42b).

Number of Days in Flower Bud Stage

After controlling for the effect of initial height, stem treatment had no effect on the number of days in the flower bud stage ($F_{2, 46} = 0.298$, $p = 0.744$), with flowers on galled ramets spending an average of 24.50 ± 2.02 (SEM) days as flower buds, clipped ramets an average of 22.00 ± 2.83 days, and intact ramets an average of 23.48 ± 1.35 days (Figure 43a). Fertilization treatment also had no effect on the length of the flower bud stage after controlling for the

effect of initial height ($F_{1, 46}$ = 0.004, p = 0.950), with flowers on fertilized ramets spending an average of 23.57 ± 1.36 days as flower buds and on unfertilized ramets an average of 23.69 ± 1.65 days (Figure 43b). No significant interaction between stem and fertilization treatments was revealed ($F_{2, 46}$ = 0.057, p = 0.945). The covariate, initial height, also had no significant influence on flower bud stage ($F_{1, 46}$ = 0.004, p = 0.949).

Number of Days Blooming

After controlling for the effect of initial height, stem treatment had no effect on the number of days in the blooming stage ($F_{2, 46}$ = 1.859, p = 0.169), with flowers on galled ramets blooming for an average of 25.50 ± 1.39 (SEM) days, on clipped ramets for an average of 21.29 ± 2.65 days, and on intact ramets, an average of 26.52 ± 1.51 days (Figure 44a). Fertilization treatment also had no effect on the length of blooming stage after controlling for the effect of initial height ($F_{1, 46}$ = 0.848, p = 0.363), with flowers on fertilized ramets blooming an average of 25.80 ± 1.33 days and on unfertilized ramets, an average of 24.56 ± 1.45 days (Figure 44b). No significant interaction between stem and fertilization treatments was revealed ($F_{2, 46}$ = 0.267, p = 0.767). Again, the covariate, initial height, also had no significant influence on blooming time ($F_{1, 46}$ = 0.261, p = 0.612).

Number of Days Ripening

After controlling for the effect of initial height, stem treatment had a significant effect on the number of ripening days ($F_{2, 47}$ = 3.566, p = 0.038), with clipped ramets ripening significantly earlier than both galled and intact ramets, which were statistically similar to each other (Figure 45a; Figure 46). Planned contrasts indicated that clipping had a significantly negative effect on ripening time compared to the galled group (p = 0.006, 95% CI [1.800, 10.291]) and the intact group (p = 0.011, 95% CI [-12.616, -1.719]). The galled group was not

significantly different from the intact group (p = 0.419, 95% CI [-6.035, 2.563]). Seed heads on clipped ramets ripened for an average of 24.43 ± 2.75 (SEM) days, or about four days earlier than galled ramets (28.25 ± 1.61 days) and about five days earlier than intact ramets (29.38 ± 1.10 days). Fertilization treatment also had a highly significant effect on the length of the ripening stage after controlling for the effect of initial height ($F_{1, 47}$ = 8.281, p = 0.006), with seed heads on fertilized ramets ripening for an average of 29.63 ± 0.98 days, or nearly four days longer than unfertilized ramets (25.82 ± 1.65 days) (Figure 45b; Figure 46). No significant interaction between stem and fertilization treatments was revealed ($F_{2, 47}$ = 1.401, p = 0.258). The covariate, initial height, also had no significant influence on ripening time ($F_{1, 47}$ = 0.013, p = 0.911).

Number of Ramets that Produced Flower Buds

Stem treatment had a highly significant effect on the number of ramets producing flower buds (X^2 = 19.229, df = 2, p < 0.001), with intact ramets producing twice as many total flower buds (40) as galled ramets (19) and nearly four times as many flower buds as clipped ramets (11) (Figure 47a). Although consistent with previous studies, fertilization treatment had no effect on the number of ramets producing flower buds (X^2 = 0.700, df = 1, p = 0.403), with fertilized ramets producing 39 flower buds and unfertilized ramets producing 31 flower buds (Figure 47b).

Aborted Flower Buds

Stem treatment had no effect on the number of flower buds that were aborted (X^2 = 0.5581, df = 2, p = 0.757), with 32% of flower buds being aborted in the galled group, compared to 43% in the clipped group and 45% in the intact group (Figure 48a). Fertilization treatment also had no effect on the number of flower buds that were aborted (X^2 = 0.022, df = 1, p = 0.882),

with 38% of flower buds being aborted in the fertilized group, compared to 44% in the unfertilized group (Figure 48b).

Galled Flower Buds

Stem treatment had no effect on the number of flower buds that were galled (X^2 = 0.195, *df* = 2, *p* = 0.907), with 11% of flower buds being galled in the galled group, compared to 7% in the clipped group and 8% in the intact group (Figure 49a). Fertilization treatment also had no effect on the number of flower buds that were galled (X^2 = 1.723, *df* = 1, *p* = 0.189), with 4% of flower buds being aborted in the fertilized group, compared to 15% in the unfertilized group (Figure 49b).

Seed Heads Produced

In the analysis of seed heads produced, stem treatment had a highly significant effect on the number of seed heads produced (X^2 =9.234, *df* = 2, *p* = 0.010), with intact ramets producing a total of 24 seed heads compared to 16 for galled ramets and 7 for clipped ramets (Figure 50a). Fertilization treatment had no effect on the number of seed heads produced (X^2 = 3.064, *df* = 1, *p* = 0.080), with fertilized ramets producing 30 seed heads and unfertilized ramets producing 17 seed heads (Figure 50b).

When considered as the number of seed heads produced from total flower buds, stem treatment had no effect (X^2 =0.237, *df* = 2, *p* 0.883), with 57% of flower buds on galled ramets becoming seed heads, compared to 50% for clipped ramets and 47% for intact ramets (Figure 51a). Fertilization treatment also had no effect on the number of seed heads produced from flower buds (X^2 = 0.513, *df* = 1, *p* = 0.474), with 58% of flower buds on fertilized ramets become seed heads, compared to 41% for unfertilized ramets (Figure 51b).

Seed Count

After controlling for the effect of initial height, stem treatment had a significant effect on the count of normal seeds ($F_{2, 47}$ = 4.370, p = 0.019) (Figure 52a). Planned contrasts indicated that clipping had a significantly negative effect on seed count compared to the galled group (p = 0.020, 95% CI [6.039, 66.450]) and the intact group (p = 0.005, 95% CI [-95.368, -17.834]). The galled group was not significantly different from the intact group (p = 0.005, 95% CI [-95.368, -17.834]) but was lower, as expected. Average seed count per seed head was significantly lower for clipped ramets at 58.14 ± 14.02 (SEM) per seed head, compared to 83.94 ± 11.38 seeds per head for galled ramets and 100.67 ± 8.63 seeds per head for intact ramets. Fertilization treatment also had a significant effect on seed count after controlling for the effect of initial height ($F_{1, 47}$ = 5.881, p = 0.020), with average seed count per seed head being significantly higher in fertilized ramets (95.17 ± 8.45) compared to unfertilized ramets (77.12 ± 9.61) (Figure 52b). No significant interaction between stem and fertilization treatments was revealed ($F_{2, 47}$ = 1.223, p = 0.305). The covariate, initial height, also had no significant influence on seed count ($F_{1, 47}$ = 2.941, p = 0.094).

Abnormal vs. Normal Seeds

Stem treatment had a highly significant effect on seed viability, as assessed by comparing abnormal seed count against normal seed count (X^2 = 13.499, df = 2, p = 0.001), with 5% of clipped ramets and 4% of galled ramets producing abnormal seeds, compared to 2% for intact ramets (Figure 53a). Fertilization treatment had a highly significant effect on the seed development (X^2 = 60.314, df = 1, p < 0.001), with 11% of unfertilized ramets producing abnormal seeds, compared to only 3% for fertilized ramets (Figure 53b).

Seed Mass

After controlling for the effect of initial height, stem treatment had no effect on seed mass ($F_{2, 47}$ = 0.856, p = 0.433), with average mass per seed being 0.91 ± 0.06 (SEM) mg for galled ramets, compared to 0.78 ± 0.06 mg for clipped ramets and 0.78 ± 0.04 mg for intact ramets (Figure 54a). Although fertilized ramets produced slightly larger seeds, fertilization treatment also had no effect on seed mass after controlling for the effect of initial height ($F_{1, 47}$ = 0.362, p = 0.551), with average mass per seed being 0.85 ± 0.03 mg for fertilized ramets, compared to 0.78 ± 0.06 mg for unfertilized ramets (Figure 54b). No significant interaction between stem and fertilization treatments was found ($F_{2, 47}$ = 0.060, p = 0.942). The covariate, initial height, also had no significant influence on seed mass ($F_{1, 47}$ = 1.595, p = 0.214).

Seed Germination

Germinations were low across all treatments, but stem treatment had no effect on the ratio of seeds that germinated compared to seeds that did not germinate (X^2 = 1.582, df = 2, p = 0.453), with 9% of total seeds planted germinating in the galled group, compared to 7% in the clipped group and 10% in the intact group (Figure 55a). However, fertilization treatment had a highly significant effect on seed germinations compared to seeds that did not germinate (X^2 = 8.418, df = 1, p = 0.004), surprisingly, with 7% of total seeds planted germinating in the fertilized group, compared to 13% in the unfertilized group (Figure 55b).

Chapter 5: Discussion

Gall Count vs. Stem Count Relationship

The relationship study of gall count vs. stem count not only supported the 2005 study that these variables are positively correlated at this site, but revealed that they were more strongly correlated (r = 0.852) than in the 2005 study (r = 0.415) (A. M. Rossi, personal comm., unpubl. data). Possible explanations for this difference include variations in population genotypes, environmental conditions, and other biotic and abiotic differences between years that may influence either the population size of *Asphondylia borrichiae*, the susceptibility of *Borrichia frutescens* to galling, or both.

Survivorship

Ramet survivorship was expected to be relatively low in galled ramets due to competition between the plant and the gall-inducer for nutrients or secondary fungal pathogens (Abrahamson and Weis 1987; Weis *et al*. 1988; Bronner 1992; Dreger-Jauffret and Shorthouse 1992; Marquis 1996; Raman *et al.* 2005; Rohfritsch 2008), although galling by itself is unlikely to kill the host plant, since doing so would also kill the larvae within the gall (Crawley 1983). Ramet survivorship was also expected to be somewhat lower in clipped ramets compared to intact ramets, simply due to the propensity for some individuals to be unable to overcome damage (Jameson 1963; McNaughton 1983; Strong *et al*. 1984; Lindroth 1989). Survivorship in the stem-nutrition experiment was not significantly different among treatments, but the first bud break

experiment revealed significant survivorship differences between intact, clipped, and galled ramets. While survivorship in galled ramets in the bud break experiment dipped to 56% by week 4 and 28% by week 8, galled/unfertilized ramets never decreased to that level during the 25-week stem-nutrition experiment and dropped only to 71% survivorship by week 25. Clipped ramets also exhibited a similar, albeit less obvious, pattern in differential survivorship between experiments. This difference in survivorship between similar treatment groups in the two experiments likely resulted from a number of interrelated factors, including seasonal timing of the experiments and ramet height and condition. The stem-nutrition experiment began in January, while the first bud break experiment began in May, well after the springtime growth spurt had passed. Furthermore, because both experiments began with single-stemmed ramets, ramets meeting this requirement in May were also younger, shorter, and possibly in poorer condition compared to their larger neighbors. Although ramets in all three treatment groups had these characteristics, in conditions where competition for light is intense, which is likely at this field site, plants with apical meristem damage caused by galling or clipping may be unable to grow vertically quickly (Benner 1988; Aarssen 1995), and thus, this damage may have had a particularly detrimental effect compared to ramets galled or clipped before the growing season for the other experiment. Furthermore, mortality in plants is often related to size, with the highest mortality occurring in the smallest size classes (Crawley 1983). Additionally, although ramets that began the stem-nutrition experiment in January had a broader range of heights than those in the bud break experiment, the density of foliage in *B. frutescens* patches at that time was noticeably less compared to foliage density in May, which may have alleviated light competition early in the growing season (Figure 56). Additionally, several galled ramets in the first bud break experiment were not only shorter than their neighbors, but also notably devoid of leaves, which may have caused carbon limitation and reduced their survival. Generally, it was

observed in the field that short, single-stemmed ramets bearing a single gall and no foliage often died instead of breaking bud, especially if they were shaded by taller neighbors. Resource competition can exacerbate the negative effects of herbivory and other damage, as well as have a stronger negative effect than the damage itself (Whitham *et al.* 1991). Stress in these plants, especially the damaged ones, may also have allowed secondary pathogenic infection to occur. Surprisingly, survivorship was not influenced significantly by either nitrogen supplementation or the lack of it, suggesting that if a nitrogen limitation exists, it does not manifest itself in increased mortality. The amount of nitrogen supplemented in this experiment was relatively small and may have been insignificant compared to the amount of nitrogen delivered by daily tidal fluctuations.

Strength of Apical Dominance, Bud Break Incidence, and Stem Count

Hormonal suppression of lateral buds is strongest closest to the terminal where it originates and diminishes with distance when apical dominance is present (Jameson 1963; Cline 1991). Also, clipping of plants with moderate or strong apical dominance may result in lateral stem elongation at the highest nodes on the clipped stem (Cline 1991). Thus, *Borrichia frutescens* was expected to exhibit certain responses related to sudden and complete disruption of apical dominance (stem clipped), gradual disruption (galled), and no disruption (intact): Clipped ramets were expected to break bud fastest and very close to or at the clipped stem terminal, galled ramets less fast and less close to the galled stem terminal, and intact ramets breaking bud, if at all, significantly late and at nodes distant from the stem terminal.

All of the results in the first bud break experiment supported these expectations. Although both clipped and galled ramets broke bud almost immediately and slightly more clipped ramets than galled ones broke bud at week 2 and week 4, galled ramets surpassed

clipped ramets in bud breaks by week 6 (Figure 25), suggesting that clipped ramets responded more readily to the complete removal of the apical meristem removal and that a small amount of hormonal suppression of lateral buds was still present in galled ramets. Additionally, clipped ramets broke bud at or very close to the clipped terminal, while galled ramets broke bud most commonly at nodes 2 and 3, again suggesting that low levels of hormonal suppression were present in galled ramets. By contrast, intact ramets experienced a large temporal delay until bud break, doing so about four months after the beginning of the experiment, and fewer than a quarter of surviving intact ramets doing so. Additionally, bud break in intact ramets occurred no closer to the terminal than node 3 and as distant as node 8, suggesting strong hormonal suppression that may have varied among individual ramets. Conversely, sudden disruption of apical dominance via clipping activated dormant buds closest to the terminal, with some adventitious shoots sprouting directly at the terminal, while galling represents a gradual weakening of apical dominance in the nodes closest to the terminal and bud break occurring once hormonal suppression is weak enough to allow it. Although no galled ramets in this experiment broke bud at the terminal (e.g. from directly under the gall), several galled ramets in the stem-nutrition experiment were observed to do so, suggesting that galling may disrupt apical dominance nearly as completely as with clipping in some instances such as high soil nitrogen.

The apparent dramatic overcompensatory response in the galled and clipped groups compared to the control group in the first bud break experiment suggests that apical dominance appears to have a strong inhibitory effect on the ability of the plant to produce lateral stems. However, the results of the stem-nutrition experiment do not suggest strong apical dominance and, in fact, suggest quite the opposite, at least under certain conditions. Approximately 27% of intact/unfertilized (control) ramets broke bud and sprouted lateral shoots by week 3 of the

stem-nutrition experiment. By week 11, when the first flower buds began to appear, 44% of intact/unfertilized ramets had broken bud. As with survivorship, timing and related conditions like height and age may have been a factor. Apical dominance may weaken over time as a plant ages, and plant height typically is correlated with age in upright plants (Cline 1991; Aarssen 1995). Single-stemmed ramets began the stem-nutrition experiment before the springtime growing season, while single-stemmed ramets in the first bud break experiment were shorter and presumably younger than their taller neighbors and likely began their growth later in the season. Additionally, one must consider that both of these experiments were conducted over a relatively short period time, compared to the overall life span of *Borrichia frutescens*, which is a long-lived clonal perennial.

Another factor potentially influencing apical dominance in the stem-nutrition experiment may have been initial height. All bud break ramets began the experiment at a uniform height of about 12 cm, while initial heights across treatments in the stem-nutrition experiment covered a substantially broader range. The intact/unfertilized ramets began the experiment with height ranging from 5.8 cm to 59.5 cm. However, the shortest control ramet in the stem-nutrition experiment to break bud at week 3 did so at 13.8 cm height. Another control ramet broke bud at week 9 when it was only 6.5 cm tall. Plant size may affect a plant's ability to compensate for damage, with larger plants having an advantage over smaller plants (Islam and Crawley 1983). Additionally, apical dominance may be costly in taller plants, when shading by neighbors is not an issue, and thus is more likely to weaken with increasing height (Aarssen 1995; Bonser and Aarssen 1996). However, stem count and height in control ramets measured at week 9 in the stem-nutrition experiment had only a moderate positive correlation (r = 0.369), with only 13.6% of the variation in stem count being explained by height; clearly, other factors are at work. Stem treatment had no effect on stem count. Plant age may have had some effect

on the ability of ramets to compensate: Young plants or plant tissues that have not yet matured are not only more susceptible to herbivory (Price 1991), but are less likely to respond positively to herbivory with regrowth (Whitham *et al.* 1991).

Reproduction is also known to play a role in weakening apical dominance, with the onset of flowering being followed by lateral bud activation in many species (Cline 1991; Aarssen 1995). It was observed during this experiment that flowering destroys the apical meristem by transforming the meristem into a bud that flowers and produces a seed head that later senesces and causes the stem to die back to a lower node. Even aborted flower buds killed the stem and often prompted dormant lateral buds below it to sprout. However, some ramets in all treatments broke bud as early as week 3, or at least eight weeks before the first flower buds appeared, suggesting that flowering is not the sole reason for bud break in this experiment.

Changes in photoperiod, such as increased light in the spring, may trigger branching in some species (Van Der Meijden 1990; Franklin 2008; Domagalska and Leyser 2011), while shading or light competition has been known to strengthen apical dominance and reduce branching (Crawley 1983; Whitham *et al.* 1991; Aarssen 1995; Bonser and Aarssen 1996; Smith and Whitelam 1997; Franklin 2008). As noted in relation to survivorship differences, the timing of the two experiments in relation to growing season, relative height compared to neighbors, and the amount of foliage and sunlight may all have affected photoperiod, light availability, and their relationship to the strength of apical dominance in these experiments.

Nutrient availability near the dormant lateral bud is thought to be a primary requirement for its elongation (Cline 1991), and improved nutrition via the roots has been known to override apical dominance in plants with weak apical dominance and should result in higher stem count (Cline 1991; Aarssen 1995; Wise and Abrahamson 2008; Domagalska and Leyser 2011). This response to nutrient supplementation was demonstrated in the stem-

nutrition experiment, where fertilized ramets had an average of 1.3, or over 35%, more stems than unfertilized ramets, a highly significant difference, especially given the relatively short duration of the experiment. Thus, a combination of factors such as height (or ramet age), seasonal timing in relation to flowering, shading and proximity to larger neighbors, and nutrients (Phillips 1971; McIntyre 1977; Benner 1988; Cline 1991; Aarssen 1995) may all have played a role regarding strength of apical dominance in *B. frutescens* during these experiments.

Initial Height and Stem Condition

Before the results of the stem-nutrition experiment can be discussed further, the differences in initial ramet heights (week 3) between stem treatments must be examined. Clipped and intact ramets were similar to each other but significantly shorter than their galled counterparts at the beginning of the experiment. Galled ramets may have been significantly taller than ungalled ramets simply due to age, since older ramets are taller and are more likely to be galled than younger, shorter ones. The apical meristems on taller ramets are probably more visible to female *Asphondylia borrichiae* searching for oviposition sites. Additionally, statistically shorter ramets in intertidal zones may have been undesirable targets for oviposition due to regular inundation.

Despite removal of the apical meristem in clipped ramets, the process of clipping did not automatically result in initially shorter heights for clipped ramets and, in fact, intact ramets were slightly shorter than clipped ramets on average. Although ramets were randomized (except for galled ramets) according to treatment group, ramets in the clipped/unfertilized treatment group appear to have been dramatically shorter than the other groups, including the clipped/fertilized ramets (Figure 33). This difference appears to have been compensated statistically by taller clipped/fertilized ramets when all clipped ramets were pooled for analysis.

Responses in Height to Stem Condition and Nutritional Status

In the stem-nutrition experiment, average height appeared to follow a similar trend (Figure 33) for galled and clipped treatment groups: very little or no discernable change, and even a negative change for clipped/fertilized ramets, in average height during the first seven weeks, followed by steady increase in height until approximately week 19 or 21 and then gradual leveling off. By contrast, intact ramets had no lag in height gains during the first seven weeks, but the leveling off did occur around week 21. Slight decrease in average height in clipped, particularly clipped/fertilized, ramets in the first several weeks was probably due to die-back of the primary stem to a lower set of nodes, from which bud break eventually occurred. Generally, fertilized ramets appeared to have slightly stronger gains in height over time compared to unfertilized ramets.

Average biweekly change in height (growth rate) was higher in intact ramets, followed by clipped ramets, and then galled ramets, although these differences were not statistically different when analyzed after conservative Bonferroni correction, indicating a weak but detectable effect. Galled ramets had a significantly lower overall change in height between the beginning and end of the experiment compared to either clipped or intact ramets, while overall percent change in height was highest in the clipped group but not significantly higher than the intact group. Since clipping disrupted apical dominance immediately, it is likely that lateral branches in that group were able to sprout and grow sooner compared to stems in intact ramets, where hormonal suppression of lateral buds may have persisted longer. This suggests that galled treatment groups may have had significant periodic setbacks in height through stem senescence compared to clipped and intact groups. This explanation is reasonable: Galling kills the stem terminal (Rossi and Strong 1990; Stiling *et al*. 1992), and ramets in the galled treatment group were more likely to be subsequently galled than the other groups, leading to senescence

of more stem terminals in the galled group. Under such conditions, height in galled ramets can be shortened periodically if the tallest stems are galled and die back, and overall growth rate decreases as new stems are initiated and eventually compensate for height lost. Thus, in this experiment, average biweekly change in height is probably a better measure of compensation than overall change in height.

In this study, fertilization had no effect on either measure of height, while earlier research indicated fertilization resulted in larger plant size in *Borrichia frutescens* (Rossi *et al*. 1992). The likely explanation for this difference lies with the type and amount of fertilizer used. The current experiment involved relatively small amounts of slow-release blood meal, while Rossi *et al*. (1992) used relatively large amounts of fast-release ammonium nitrate. Since nitrogen in the current experiment led to a partial override of apical dominance and significantly higher stem counts but not greater heights or changes in height, small amounts of supplemented nitrogen may be allocated first to producing lateral shoots, rather than growing taller. This explanation is supported by the principle of allocation, which states that limited resources will be allocated among various life functions, and diversion of resources to any one activity results in less of the other activities (Abrahamson 1989).Thus, plants may be expected to allocate resources to either bushier growth or taller growth, but not both (Aarssen 1995).

Incidence of Galling

Not unexpectedly, some intact ramets not protected from galling by mesh bags were galled during the stem-nutrition experiment and, thus, excluded from most statistical analyses except for those assessing the number of starting ramets that were galled during the experiment and cumulative gall count in surviving ramets by week 25. A relatively low percentage (11%) of clipped ramets were galled during the experiment and cumulative gall

counts in surviving ramets in this group were also low (13). Comparatively, galled ramets had a relatively high percentage of subsequent galling (31%) and a very high cumulative gall count in surviving ramets (109), while intact ramets had a high percentage of galling (35%) but a moderate cumulative gall count (39). Thus, a similar percentage of galled ramets and intact ramets were galled, but more galls were found on ramets in the galled group than in the intact group. Indeed, 27.6% of subsequently galled ramets in the galled group developed more than one subsequent gall, compared to 17.1% of galled ramets in the intact group developed more than one gall.

Since stem count has already been shown to be generally the same across stem treatments, differential availability of galling sites (more meristems vs. fewer meristems) is not a likely cause for increased galling in galled ramets. The most likely explanation is that some genotypes of *Borrichia frutescens* have been found to be more susceptible to galling than others (Stiling and Rossi 1996; Rossi and Stiling 1998). Thus, most or perhaps all of the ramets that began the experiment as galled and were assigned to the galled treatment group may have been of a genotype susceptible to galling, while fewer ungalled ramets chosen for the clipped or intact treatments may have been susceptible to galling. In other words, the genotype susceptible to galling may not have been found equally in all stem treatment groups.

Although the galled group may have had a higher proportion of genotypes susceptible to galling compared to the clipped and intact groups, susceptible genotypes were probably equally represented between the fertilization treatment groups, since the experimental design was fully factorial and the experiment began with roughly equal numbers of galled, clipped, and intact ramets in each nutrition group. Thus, any effect of nutrient supplementation should have been revealed. A previous study (Rossi *et al.* 1992) found that nitrogen supplementation led to higher galling rates by female *A. borrichiae*, which may preferentially select healthy well-

resourced plants for oviposition. However, nitrogen supplementation in this experiment had no effect on the cumulative number of galls or the number of starting ramets that were galled during this experiment, despite the finding that stem (galling site) count increased with nutrient supplementation which should have resulted in a higher number of galls. In a study of creosote bush (*Larrea tridentata*) and eight associated *Asphondylia* species that induce galls on leaves, stems, and flowers, Waring and Price (1990) found that gall density was positively associated with bushier architectural forms (e.g. more stems) than plants with a less bushy form (e.g. fewer stems), suggesting that bushier forms provide more galling sites, resulting in more galls. Again, the relatively small quantities of slow-release blood meal compared to the large quantities of fast-release ammonium nitrate used in Rossi *et al.* (1992) is the likely explanation for this result. The amount of nitrogen used in the current study may have been insignificant compared to tidal inundations of nutrients and may have unable to produce the dramatic results observed in Rossi *et al.* (1992).

Stiling *et al*. (1992) indicated that stem terminals typically develop a single gall, although two galls may occur. In this experiment, only two terminals were recorded as having two distinct galls; they were recorded as a single gall. Several galls (9.3% of total galls across all treatments) appeared to be composite galls, or galls that had the appearance of two, three, or four galls coalesced together into a single gall. These galls may have been the result of multiple ovipositional events that occurred close to each other in time and space, possibly by different females, or the result of abnormal gall development.

First Flowering

Flowering is a function of plant size (Crawley 1983), and growing tall is thought to benefit plants in light- and pollinator-competitive environments and where height-aided seed

dispersal is important (Table 1; Salisbury and Ross 1991; Aarssen and Irwin 1991; Aarssen 1995; Irwin and Aarssen 1996). Thus, height was expected to play some role in flowering in this experiment. Height had a highly significant effect on first flowering when assessed using height class. Nearly half of first flowering events occurred between the heights of 40 and 60 cm, with the remaining height classes having progressively fewer first flowering events. This suggests that flowering and height may be associated, although seasonality and photoperiod also play a role. Flowering synchronization is caused in response to seasonal changes in photoperiod and ensures a large enough pool of flowers in the population for pollination (Crawley 1983). Field observations suggest that *Borrichia frutescens* appears to have determinate flowering, or flowering that occurs by redifferentiating a vegetative meristem for reproductive functions, which essentially prevents the meristem from further growth and, after flowering and seed head ripening, causes the stem to die back. Thus, one would expect that a balance between flowering on time and attaining an appropriate height that maximizes visibility and accessibility to pollinators (Aarssen 1995) would be important. The first flower bud was recorded on March 21, and some new flower buds continued to be recorded on the last day of data recording, June 25 (Table 5). For the first few weeks of flowering, flower buds first appeared exclusively on intact ramets, with galled ramets beginning to flower in early April and clipped ramets beginning to flower at the end of April. Peak flowering generally occurred at the end of April, with 27% of flower buds in all treatment groups being discovered on April 30, and 50% of flower buds having appeared by that date. Over 65% of new flower buds were identified between April 16 and May 14. Thus, timing, possibly photoperiod length, probably also plays a crucial role in triggering flowering besides height. Furthermore, timing and height may interact in inducing flowering, since very short plants were not likely to flower between April and May, and few ramets, even

those between 40 and 60 cm, would be expected to flower in winter, when pollinators would be less common and any pool of flowering ramets with which to outcross would be small.

Clipped ramets flowered about a month later than intact ramets, while galled ramets flowered about 9 to 21 days later than intact ramets. For the first few weeks of the experiment, field observations noted that many clipped ramets were essentially "sticks," or live stems lacking in leaves. This occurred because short ramets often had only a few leaves, most of which were at or near the terminal and would have been removed inadvertently during clipping. Thus, clipping may have caused carbon limitation by drastically reducing the number of leaves. Additionally, any amount of compensatory regrowth that eventually occurred, as evidenced by the greater overall percent change in height in clipped ramets, may have resulted in the redirection of nutrients away from reproduction and delayed flowering (Jameson 1963; Stephenson 1981; Inouye 1982; Bryant *et al.* 1983; Crawley 1983; McNaughton 1983; Paige and Whitham 1987; Benner 1988; Lindroth 1989; Price 1991; Whitham *et al.* 1991; Fornoni *et al.* 2003). Although galled ramets did not exhibit significant regrowth in terms of stem count or height, gall-inducers are also known to redirect resources away from plant functions like reproduction, which also can delay flowering (Abrahamson and Weis 1987; Weis *et al*. 1988; Bronner 1992; Dreger-Jauffret and Shorthouse 1992; Marquis 1996; Raman *et al.* 2005; Rohfritsch 2008). Nutrient supplementation appears to have somewhat ameliorated the effects of stem damage, with fertilized ramets flowering about five to nine days earlier than unfertilized ramets, depending on whether ramets that did not flower by June 25 were included in the analyses; the benefit provided by nutrient supplementation, however, was not statistically significant.

Flower Bud Galling and Abortion

It was generally observed that the flowering stem in *Borrichia frutescens*, a determinate flowering species, essentially undergoes a form of apical meristem damage as the seed head ripens and turns brown, presumably gradually releasing apical dominance. It was further observed in the field that flower buds that were aborted before blooming accelerated this process by killing the stem as the flower bud senesced and abscised. Flower buds on galled and clipped ramets were no more or less likely to become aborted than those on intact ramets, suggesting that flower buds between these groups were essentially similar in their likelihood of reaching blooming stage.

Previous studies of *Borrichia frutescens* indicated that flowering terminals are rarely galled and galled terminals rarely flower (Rossi and Strong 1990; Rossi *et al*. 1992; Stiling *et al*. 1992; Rossi *et al*. 2006). Although no galled terminals flowered during this experiment, a moderate percentage (up to 11%) of flower buds or flowers in the early stages of blooming developed galls. The number of flowers that became galled before or shortly after blooming was similar across stem treatments and across fertilization treatments. This suggests that emergence of *Asphondylia borrichiae* adults from older galls may have coincided with flowering to cause an increased incidence of flower heads being exposed to gall midges. Field observations noted that galling of flower buds sometimes appeared as a noticeable gall on the receptacle of the flower bud, but flower heads that had begun to bloom often revealed their galled state by severe deformation and blooming of only a few florets that were relatively unaffected by this deformation (Figure 18). The negative impact of galls on flowers is consistent with earlier studies (Rossi and Stiling 1998; Rossi *et al*. 1999).

Blooming and Seed Production

Both galling and clipping negatively impacted both flower head and seed head production compared to intact controls, with clipping having a particularly severe effect. Twice as many intact ramets flowered compared to galled ramets and four times as many compared to clipped ramets. Intact ramets also produced 50% more seed heads than galled ramets and over 240% more than clipped ramets. If seed count and seed mass were equal, this result alone would probably suggest a severe fitness disadvantage for galled ramets and, especially, clipped ramets. However, naturally occurring apical meristem damage similar to clipping, such as grazing by grasshoppers, is seen rarely in the field (A. M. Rossi, pers. comm.), suggesting that this type of damage is probably not a strong selective pressure.

This result was somewhat unexpected, since flower and seed head production was expected to be lowest in galled plants due to the removal of the stem from the pool of potential flower-producing meristems (Rossi and Strong 1990; Rossi *et al*. 1992; Stiling *et al*. 1992), as well as nutrient sequestration by the gall-inducer (Abrahamson and Weis 1987). However as already noted, any compensatory growth spurt seen in damaged ramets, whether the damage was caused by clipping or galling, may be expected to draw resources away from reproduction (Jameson 1963; Stephenson 1981; Inouye 1982; Bryant *et al.* 1983; Crawley 1983; McNaughton 1983; Paige and Whitham 1987; Benner 1988; Lindroth 1989; Price 1991; Whitham *et al.* 1991; Fornoni *et al.* 2003). In birch (*Betula pendula*), browsing of twigs and their buds by moose (*Alces alces*) reduces the capability of *B. pendula* to flower and produce seeds (Danell *et al*. 1985). Mechanisms cited for this reduction include the loss of twigs bearing flower buds, reduction in height (for seedlings or saplings), reduction in the number of meristems and decreased probability of flower initiation, and diversion of nutrients from reproduction to compensatory growth (Danell *et al*. 1985).

The number of days between flower bud appearance and blooming was not statistically different across either stem treatments or fertilization treatments, with the groups differing by only a couple of days. The number of days spent blooming also was not statistically different across either stem treatments or fertilization treatments, although intact ramets bloomed the longest (26.52 days) and clipped ramets bloomed the shortest (21.29 days), a difference of about five days or 20%. Galled ramets had an average blooming time of about a day less than intact ramets (25.50 days).

Seed head ripening time was significantly affected by stem treatment, with seed heads in the clipped group ripening over significantly fewer days compared to either galled or intact ramets, which were similar. Galled and intact ramets spent on average 4 or 5 days, or up to 20%, longer ripening than clipped ramets. Clipping also had a significant effect on seed count and, as already noted, a distinct albeit statistically insignificant effect on blooming time, trends that may be important biologically. Although individually these phenological stages did not differ in length, four or five extra days of blooming and another four or five days ripening can be expected to have additive effects that result in significant differences in other measures, particularly those related to seed production. More time blooming may provide a greater chance for additional florets to bloom, florets to be pollinated, and, thus, more seeds may be produced. More time ripening can also be expected to result in larger seeds or more seeds, since ripening involves the development of seeds and the building of the seeds' energy and nutrient reserves.

This explanation holds for clipped ramets: 4 or 5 fewer days for blooming and another 4 of 5 fewer days for ripening appears to have resulted in a significantly low count of abnormal seeds, with 31% fewer abnormal seeds compared to galled ramets and 42% fewer compared to intact ramets. Clipping, therefore, appeared to have a greater effect on normal seed set and

potential reproductive fitness. By contrast, galling did not have a statistically significant effect on blooming, ripening, or seed count, compared to intact ramets, although the trend clearly suggests that both clipping and galling divert resources from seeds. Also, the percentage of abnormal seeds for galled ramets was only slightly lower than clipped ramets, with the fewest abnormal seeds from intact ramets. The differences between groups range from only 2% to 5%, which may be biologically irrelevant. However, the trend supports the explanation that plant damage may divert resources from seeds. Seeds may develop incompletely or abort if resources are limited and multiple metabolic sinks are competing for those resources (Stephenson 1981); such sinks may include vigorously growing stems and galls.

While stem treatment ultimately had no significant impact on seed germination, the trend does suggest that clipping and galling do have a slight impact on germination compared to intact ramets, with the lowest percentage of seeds germinated being in the clipped group. Considering that clipping also had a significantly detrimental effect on the number of ramets that flowered and the number of seed heads and normal seeds produced, clipping or similar damage that completely removes the apical meristem (e.g. breaking) appears to have serious consequences for seed development and production.

Why would clipping have a detrimental effect on these variables while galling affected only some of them and not to the extent of clipping? The first consideration may be nutrients redirected to other functions and away from reproduction. Compensatory regrowth as measured by maximum stem count was not significantly different among stem treatments but suggested a trend that may have accounted partially for these differences, since galled ramets had the highest maximum stem count, followed by clipped and then intact ramets. When the average number of stems is viewed across treatments at biweekly intervals over the experiment (Figure 57), it is apparent that galled ramets broke bud very quickly and experienced a dramatic

increase in stem count within the first few weeks. By comparison, clipped ramets were slower to break bud and experienced dramatic increases in stem count between weeks 5 and 10. Cline (1991) noted that if clipping of plants with moderate or strong apical dominance is conducted just below the stem terminal where lateral buds are small, bud elongation may be delayed. Clipped ramets also had a slight decrease in average height during the first several weeks of the experiment. It should be noted that galled ramets also had no significant gains in height during that time. This decrease was probably due to stem senescence to lower nodes following clipping; this senescence was noted in the field. Considering that flowering generally began in the population around week 11, delayed regrowth in clipped ramets is probably the primary cause of their delayed flowering, which likely had a cumulative effect on other measurements of reproduction that resulted ultimately in fewer seeds. The importance of the timing of damage and regrowth relative to reproduction has been cited in numerous papers as being among the most important external factors influencing a plant's ability to fully or overcompensate for apical meristem damage (Inouye 1982; Crawley 1983; Islam and Crawley 1983; Benner 1988; Maschinski and Whitham 1989; Whitham *et al.* 1991; Paige 1992; Aarssen 1995; Marquis 1996; Lennartsson *et al.* 1998; Fornoni *et al.* 2003). Damage that occurs later in the season can result in a narrow window of recovery and regrowth that coincides with flowering time in the general population, resulting in delayed reproduction in damaged individuals (Crawley 1983; Whitham *et al.* 1991; Paige 1992).

One may question why apical meristem damage in the form of galling and clipping would have such different results in this experiment. Single-stemmed ramets in the galled group were already galled at experiment setup and may have been galled for days or weeks in advance, meaning that they already would have been undergoing the biochemical changes as a result of disrupted apical dominance by galling, while clipping occurred at experiment setup.

However, the first bud break experiment was set up the same way, and yet those galled and clipped groups broke bud at similar times and rates; indeed, clipped ramets broke bud faster than galled ones. Thus, the difference of days or weeks between the galled and clipped groups does not appear to be a factor in the stem-nutrition experiment.

The likely factor causing delayed growth in clipped ramets may have been carbon limitation. Many single-stemmed ramets chosen for this experiment were not only short (young), but also sparse in foliage, with experiment setup occurring in January. Many ramets bore only a few leaves, with all of them at or near the stem apex. Thus, in many cases, clipping the apical meristem had the unfortunate side effect of also removing all or most of the leaves on the ramet. Although some of these denuded ramets did not survive, most of them eventually broke bud. Survivorship in these ramets may have been much lower if foliage density in the population had been high, as occurred with the first bud break experiment when setup in May.

Seed Mass and Stem Condition

While the count of normal seeds was significantly different among stem treatments, normal seed mass was not. This result supports the general observation that many plant species produce seeds that tend to vary more in seed count, while keeping seed mass relatively constant within the constraints of seed size polymorphism for the species, even under stressed conditions (e.g. intraspecific competition, poor nutrients) (Harper 1977). The principle of allocation suggests trade-offs between seed size and seed quantity, with seeds of higher mass having higher energy reserves and increasing the survival of the seedling, while seed quantity increases the probability that at least some seeds will survive to germinate (Harper 1977; Crawley 1983). Since developing seeds compete for available nutrients, seed number and seed size (mass) are typically inversely related, especially when nutrient or energy limitations exist

(Harper 1977; Schaal 1980; McNaughton 1983). Additionally, although mean seed mass across a seed head is typically constant, actual seed size among individual seeds may be polymorphic, based on factors like position (Cavers and Harper 1966). This type of seed polymorphism is common in Asteraceae (Compositae), with ray florets and disk florets bearing varying sized achenes (Cavers and Harper 1966; Harper 1977). It was noted during the current experiment that seeds produced by ray florets around the edge of the receptacle were fatter compared to disk florets that bloomed within the disk.

Nitrogen as a Limited Resource

Nutrient supplementation was delivered in the form of granular, slow-release nitrogen. Increased survival, growth, and/or fitness of galled and/or clipped plants under nitrogen supplementation compared to normal levels may suggest that nitrogen is a limiting factor in the system (Wise and Abrahamson 2005). However, in this experiment, nitrogen supplementation lead to significant differences in only a handful of dependent variables, namely stem count, the number of days for seed head ripening, seed count, and ratio of abnormal to normal seeds. However, it should be noted that nitrogen supplementation in the current study utilized low levels of slow-release fertilizer, unlike previous studies (Rossi *et al.* 1992) that used relatively high concentrations of highly soluble ammonium nitrate. Additionally, some characteristics may be more phenotypically plastic, or susceptible, to nitrogen supplementation than other characteristics.

The addition of nutrients can be utilized by the plant for growth and reproduction and can alleviate any limitations that may occur in these plant functions as a result of nutrient deficiency from stressful environmental conditions, herbivory, or other types of damage (Stephenson 1981). Improved growth in the form of increased stem count resulted from

nutrient supplementation, with fertilized ramets having an average of 1.3, or over 35%, more stems than unfertilized ramets, regardless of stem treatment. This result may have occurred because supplementation via the roots may override apical dominance when it is weak (Cline 1991; Aarssen 1995; Wise and Abrahamson 2008; Domagalska and Leyser 2011).

Nitrogen supplementation also significantly increased the number of days for seed head ripening and seed count, and very significantly lowered the incidence of abnormal seed development compared to unfertilized ramets. Stephenson (1981) noted that nutrient supplementation, particularly after flowering, may alleviate resource limitations and increase production of normally developed seeds; in the current experiment, nutrients were supplied in monthly press applications, so fertilized ramets would have received nutrients before, during, and after flowering. The addition of nutrients may have enabled plants to allocate more nutrients to seed development and reserves, which is suggested by the increase in the number of days for seed head ripening. During these additional days, all fertilized ova would have had a better chance of acquiring adequate nutrient and energy stores and developing fully, and less chance of developing abnormally or being aborted. Increased seed count and fewer abnormal seeds are potentially important effects, as higher production of normally developed seeds is likely correlated with higher fitness if seedling survival and reproduction is at least equivalent to or better than those for unfertilized ramets. Seed mass, by comparison, was not significantly impacted by nutrient supplementation, although seed mass was slightly higher in fertilized ramets. Thus, the nutrient supplementation appears to have been allocated primarily to increasing seed yield.

Unexpectedly, a significantly lower percentage of seeds from fertilized ramets germinated (7%) compared to those from unfertilized ramets (13%). The reason for this result is unclear, but perhaps *Borrichia frutescens* seeds are resistant to additional nitrogen if the

environment is not nitrogen limited. Nutrient supplementation was expected to alleviate any nutrient limitations and ensure adequate reserves for seeds, which was expected to increase germination success (Harper 1977; Schaal 1980; Crawley 1983; Paige and Whitham 1987). Germination success was generally much lower than expected, averaging 9.2% across all treatments. Stalter and Batson (1973) reported 97% germination in *Borrichia frutescens* seeds collected in Georgetown, SC. Biber *et al.* (2013) indicated that germination success may vary annually but generally tend to be high. The generally low germination success in this experiment may be due to a number of factors. Seeds were planted in September and allowed to germinate through early December, which may have meant exposing them to photoperiods too short to germinate effectively. Additionally, recommended germination temperatures were 25 to 35^{o}C (Biber *et al.* 2013), which may not have been maintained in the greenhouse on nights with below-freezing temperatures. Damping off by pathogenic fungi is also more prevalent under these conditions. Finally, germination rates reported in the literature may have been based on highly artificial conditions, such as with the use of grow lights and temperature control, while conditions used in this experiment may have been more similar to those found in the natural environment. If accurate, this would suggest that seed set and sexual reproduction are of secondary importance to clonal reproduction in *Borrichia frutescens*.

Most of the remaining variables, although not significant, nevertheless revealed consistent trends. Fertilized ramets exhibited slightly more beneficial outcomes than unfertilized ramets, including slightly higher survivorship, and average biweekly change in height, as well as slightly more ramets that flowered and seed heads that were produced. Fertilized ramets also began flowering slightly earlier than unfertilized ramets and produced flower buds that began blooming slightly sooner and spent a few more days blooming and, thus, more days being exposed to pollinators. These trends, coupled with the significant results, suggest that nitrogen

is at least a somewhat limited resource in this system, although the degree of limitation probably is not severe. Finally, gall count was only slightly higher in fertilized ramets.

An earlier fertilization experiment on *Borrichia frutescens* (Rossi *et al.* 1992) found slightly different results, including no effect of nutrient supplementation on stem count and significantly increased gall density. The reasons for these differences may include the type and concentration of fertilizer used, which was fast-release and used in higher concentrations in the previous study, as well as possible location-specific environmental effects and timing of application. Galling differences may be an artifact of location-specific frequencies of genotypes susceptible to galling.

Does Overcompensation Result from Galling?

The overarching question asked for this project was, does galling by *Asphondylia borrichiae* lead to a net benefit (overcompensation) for *Borrichia frutescens* in terms of regrowth or reproductive fitness? The answer is probably not. Plants with very strong apical dominance are likely to be severely limited by the number of meristems available for flowering and may benefit from apical meristem damage (Wise and Abrahamson 2008), but *Borrichia frutescens* does not seem to exhibit strong apical dominance, particularly in the weeks leading up to flowering. Although bud break may occur faster in ramets that have apical meristem damage from galling or clipping (e.g. breakage), which may suggest short-term adjustments to mitigate the costs of damage, in the long term, this type of damage does not appear to have a significantly positive or negative effect on the number of stems compared to intact ramets, and any overcompensation in stem count that might occur initially appears to be matched eventually by bud break in intact ramets and probably does not have dramatic, long-term benefits for *B. frutescens*. *Borrichia frutescens* often grows in somewhat dense, light-

competitive patches, where apical dominance and growing tall may be most beneficial (Aarssen and Irwin 1991; Aarssen 1995; Irwin and Aarssen 1996). In this case, the timing of fluctuations in the strength of apical dominance may be important as related to growth or reproduction. Disruption of apical dominance by galling or other damage at inopportune times (e.g. when ramets are short and shaded by neighbors) probably has detrimental effects on survival and is not likely to result in beneficial overcompensation. Additionally, most reproductive measures of flowering and seed set were also generally lower in galled ramets than in intact ramets, although not as low as in clipped ramets. Thus, at best, *Borrichia frutescens* appears to respond to galling by *Asphondylia borrichiae* with moderate or mild undercompensation. Another possibility to consider is that *A. borrichiae* may be regulating galling sites as a resource in ramets susceptible to galling by attacking apical meristems before or as they differentiate into flower buds, hindering flowering, and maintaining a steady supply of apical meristems for future generations of offspring.

Future Research

Additional questions may be explored through further research, including:

- How do stem condition and nutritional status affect reproductive fitness in *Borrichia frutescens* when measured in seedling survival and reproductive performance?
- Is a patch of *B. frutescens* in an area like Timucuan Preserve composed of genetically identical clones of a single individual, or are there clones of multiple individuals present?

- Is the genotypic susceptibility to galling among some *B. frutescens* ramets a factor more important to female *Asphondylia borrichiae* than either the availability of galling sites or the health and vigor of the potential host?
- What are the factors that make such a genotype susceptible to galling?
- Is the cecidogenesis process in *B. frutescens* initiated by *A. borrichiae* or a fungal symbiont such as *Botryosphaeria dothidea* aff.? Is the initiation and maintenance of the gall performed by the same organism?
- What is the role, if any, of the fungal symbiont in potentially infecting and contributing to stem senescence after galling (e.g. as a systemic pathogen)?
- Does apical dominance fluctuate in this system regularly based on season (photoperiod) or physiological cues, and how does its timing relate to the overall phenology, particularly flowering, of the plant?
- What is the pattern of bud activation in *B. frutescens* in relation to factors like age, shading, and seasonality?
- Because clonal reproduction appears to be the primary mode of reproduction in *B. frutescens*, how does apical dominance relate to clonal reproduction (e.g. vegetative spread from rhizomes)?
- Are there measures of reproductive fitness in relation to clonal reproduction in this system, such as the number of potentially available (inactive) meristems on rhizomes?

Appendix A: Analysis of Mesh Bags and Light Penetration

Mesh bags were used in both experiments during this project, and their possible effect on light penetration was an initial concern. However, preliminary data before experimentation began and analysis of data collected for both bagged and unbagged control plants during the stem-nutrition experiment revealed no effect of the mesh bags on light penetration or plant survival, growth, or flowering.

Based on measurements captured in January 2013 using a light meter (Apogee Instruments Inc., Basic Quantum Meter, model BQM-SUN, Logan, UT), square-root transformed, and analyzed using two-way ANOVA with sunny/shady conditions and mesh/no mesh as factors. The differences in light readings taken inside and outside the mesh bags were not statistically significant ($F_{1, 39} = 0.004$, $p = 0.949$) and no significant interactions between mesh and light conditions were found ($F_{1, 39} = 0.022$, $p = 0.882$). As expected, a significant difference was found between sunny and shady conditions, regardless of the presence or absence of mesh ($F_{1, 39} = 3277.478$, $p < 0.001$). In sunny conditions, the mesh bags blocked an average of 0.5% of light ($\bar{X}$ ± SEM = 1253.40 ± 41.07 under mesh vs. $\bar{X}$ ± SEM = 1259.70 ± 35.29 not under mesh). Unexpectedly, average light readings under shady conditions were over 2% higher under mesh ($\bar{X}$ ± SEM = 76.90 ± 7.89 under mesh vs. $\bar{X}$ ± SEM = 75.40 ± 5.17 not under mesh).

The effect of mesh bags on a variety of morphologic and reproductive factors for bagged controls (intact/unfertilized ramets) in the stem-nutrition experiment also was analyzed

compared to unbagged controls using several statistical tests. No significant differences were discovered. See Table 2 in Appendix C for details about statistical analyses and results.

Appendix B: Figures

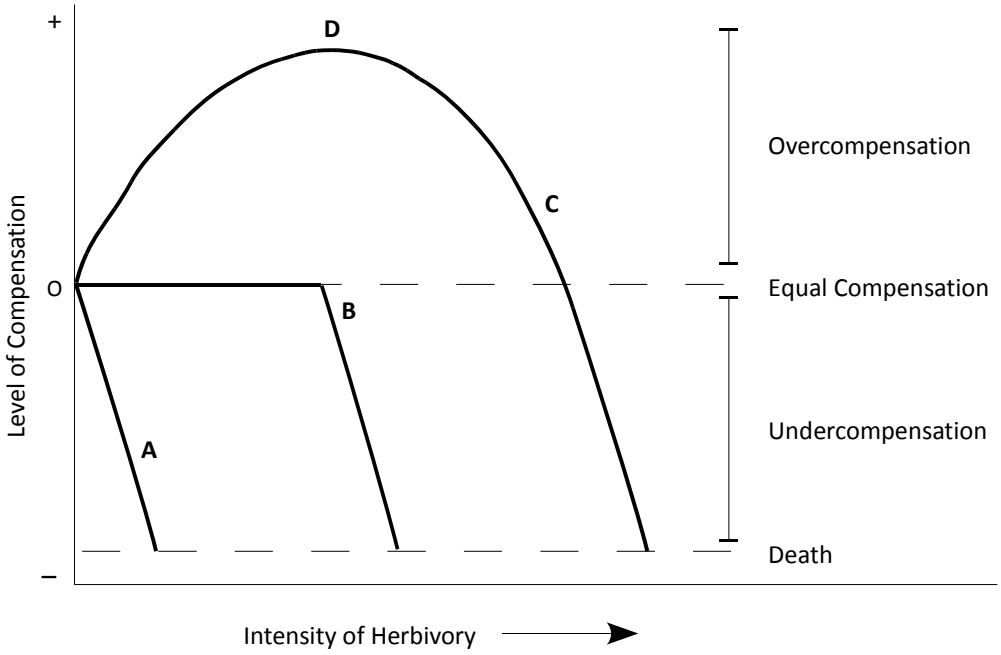


Figure 1. Plant responses to increasing intensity of herbivory may include (A) undercompensation, which may steadily worsen with increasing intensity, (B) equal compensation, which may become undercompensation at a certain intensity, or (C) overcompensation, which increases to an optimal level of herbivory (D), after which it steadily declines. Redrawn after Dyer 1975, McNaughton 1979, Dyer *et al*. 1982, McNaughton 1983, and Belsky 1986.

Figure 2. *Borrichia frutescens*, or sea oxeye daisy. Courtesy: Gary Wood

Figure 3. *Borrichia frutescens*, or sea oxeye daisy, flower head bearing disc florets and ray florets. Courtesy: Gary Wood

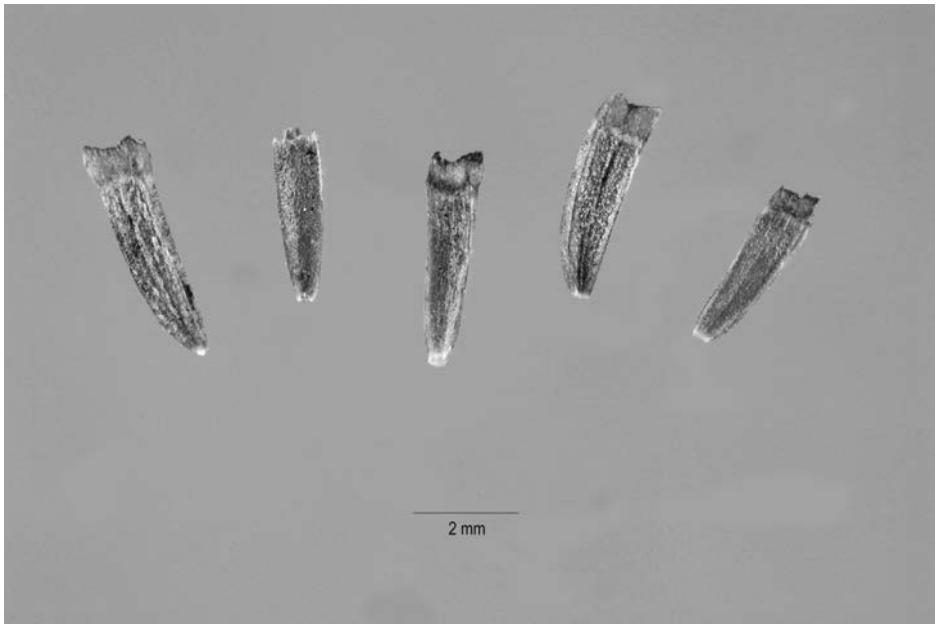


Figure 4. *Borrichia frutescens* achenes (seed-bearing fruits). Source: Steve Hurst at USDA-NRCS Plants Database, public domain/non-copyrighted

Figure 5. An *Asphondylia borrichiae* gall on *Borrichia frutescens*. Courtesy: Gary Wood

Figure 6. *Asphondylia borrichiae* gall with emergence hole and attached puparium at right. Courtesy: Gary Wood

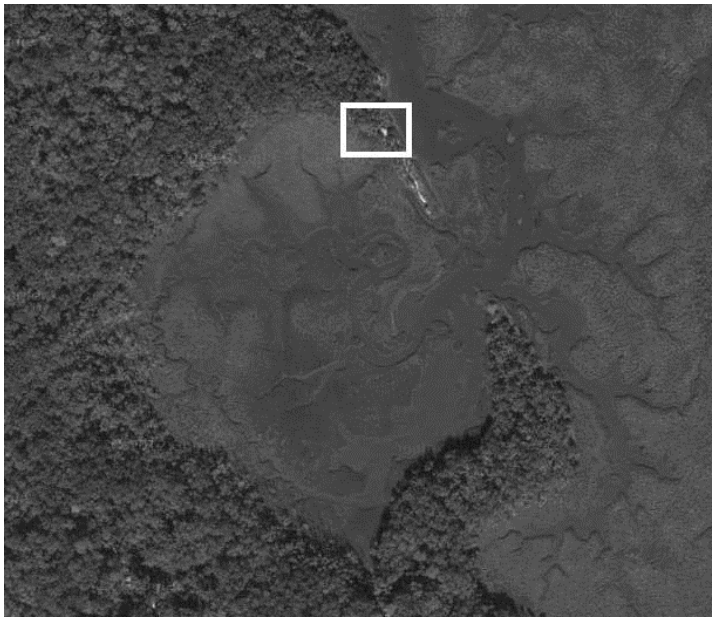


Figure 7. Satellite image of Round Marsh in the Theodore Roosevelt area of the Timucuan Ecological and Historic Preserve in Jacksonville, Florida, with field site indicated. Source: Google Maps.

Figure 8. Field site with *Borrichia frutescens* in foreground and *Juncus roemerianus* in background. Courtesy: Gary Wood

Figure 9. A single-stemmed ramet of *Borrichia frutescens* with apical meristem (stem terminal) intact. Courtesy: Gary Wood

Figure 10. A clipped ramet for the first bud break experiment. Courtesy: Gary Wood

Figure 11. A bagged ramet for the intact (control) group in the first bud break experiment. Courtesy: Gary Wood

Figure 12. Clipping method used for all clipped treatment groups. The apical meristem was removed by clipping just below the next lowest node. Courtesy: Melanie Masdea

Figure 13. Ramet height being measured to tallest apical meristem. Courtesy: Melanie Masdea

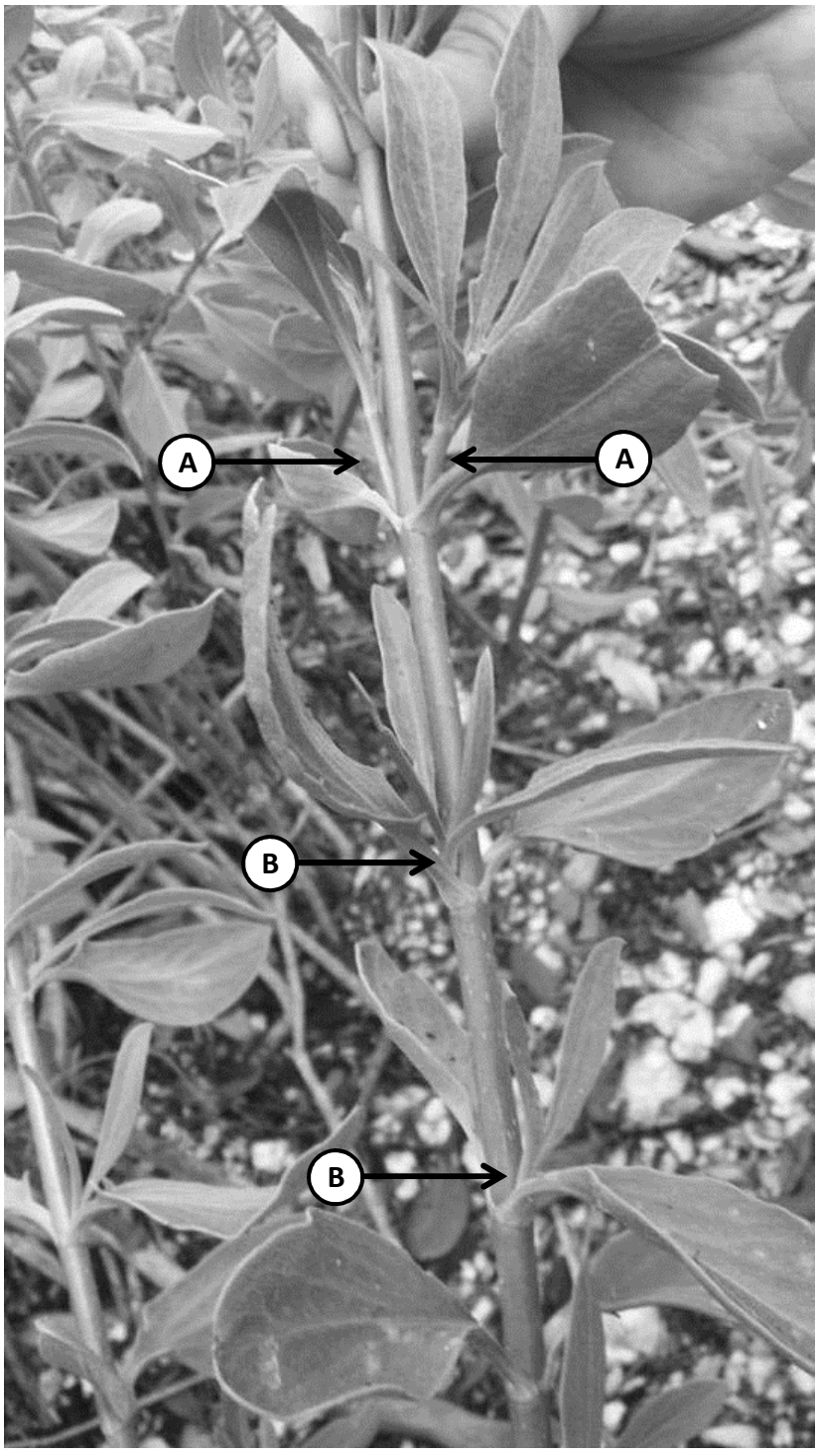


Figure 14. Axillary stems long enough to count (A) and not long enough to count (B). Courtesy: Melanie Masdea

Figure 15. A galled treatment ramet with (A) two new shoots breaking bud immediately under the gall and (B) another at the first node below the gall. Courtesy: Gary Wood

Figure 16. A flower bud of *Borrichia frutescens*. Courtesy: Gary Wood

Figure 17. A bagged experimental seed head in ripening phase. Courtesy: Melanie Masdea

Figure 18. A galled flower head, which deformed the receptacle and florets and prevented them from blooming normally. Courtesy: Melanie Masdea

Figure 19. Ripened seed heads of *Borrichia frutescens*. Courtesy: Gary Wood

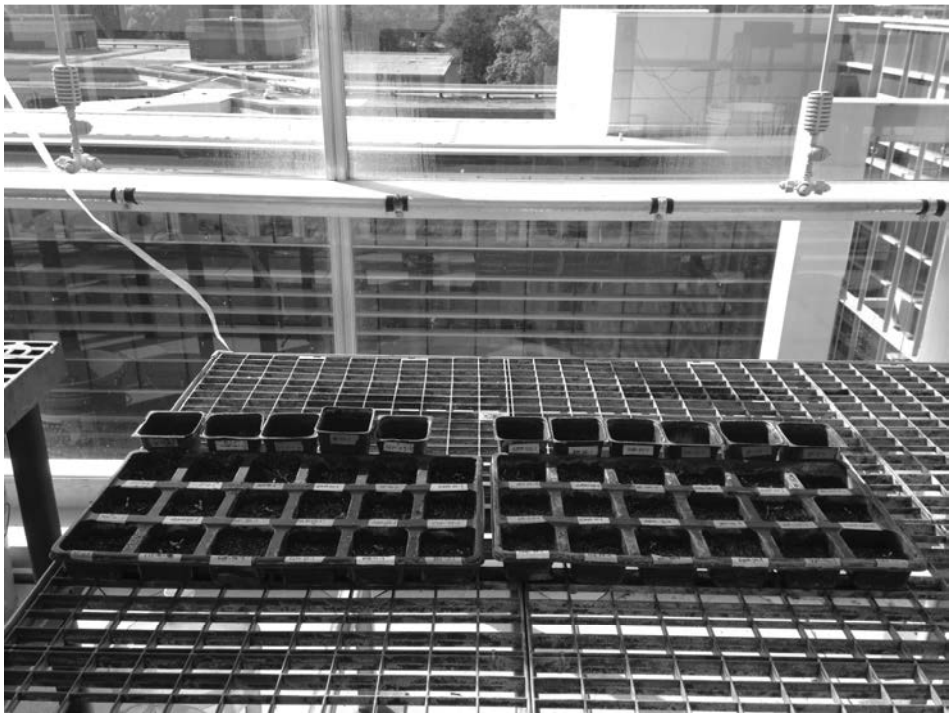


Figure 20. Layout of flats and pots for seed germination. Courtesy: Lisa Rowan

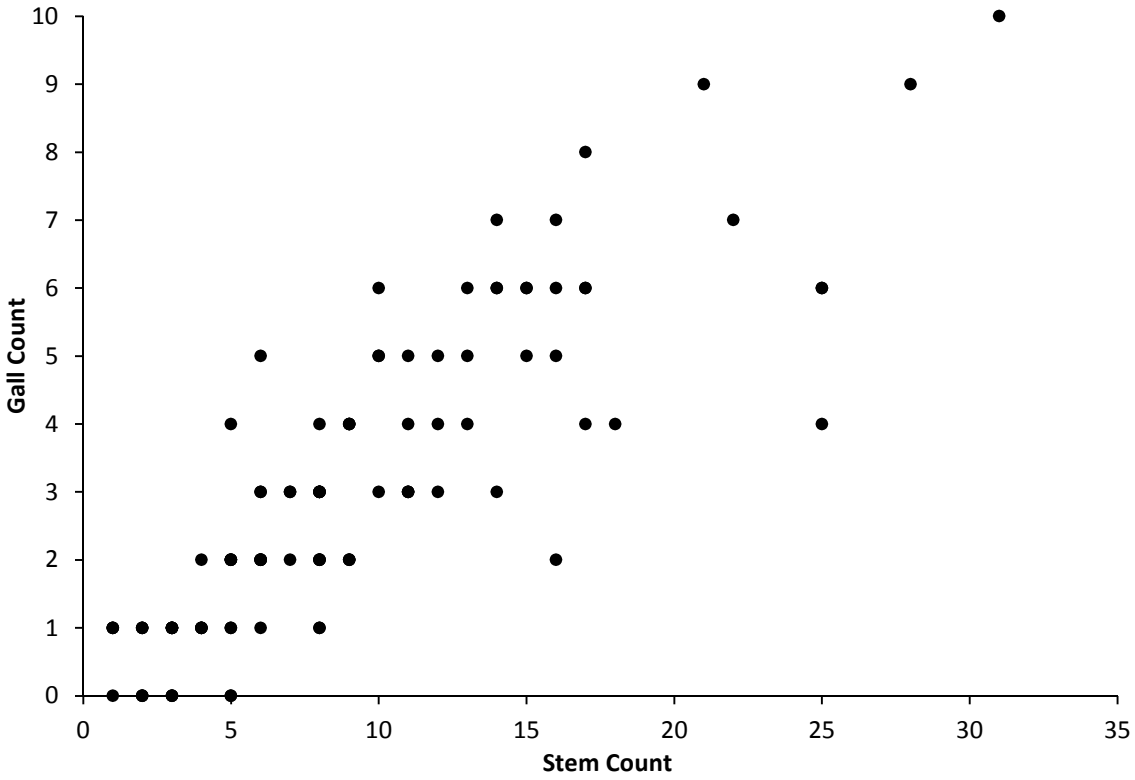


Figure 21. Correlation between gall count and stem count (*r* = 0.852, n = 100).

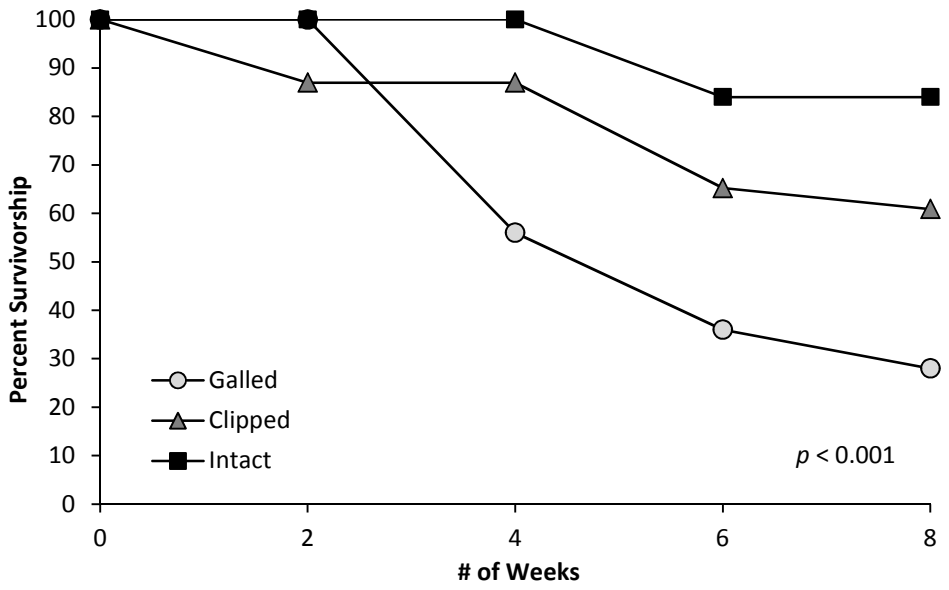


Figure 22. Percent survivorship depicted over the first eight weeks in the first bud break experiment. Statistical analysis was conducted on plant counts at week 8 (X^2 = 16.197, *df* = 2, *p* < 0.001). Although all X^2 tests throughout were performed on counts, data are often presented as percentages for clarity.

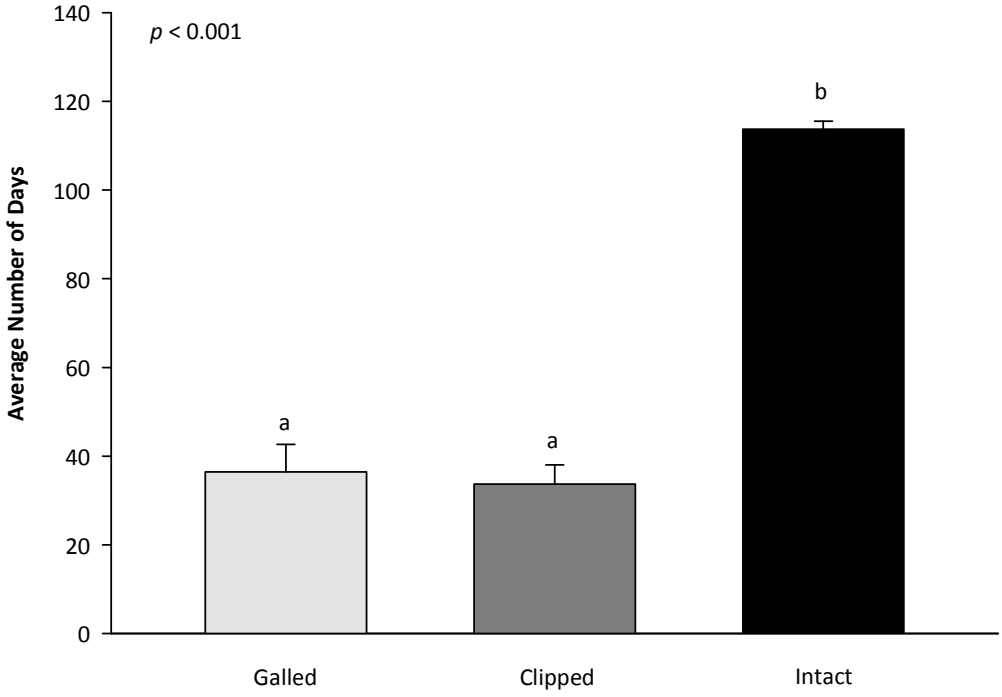


Figure 23. Average number of days to first bud break with standard error of the mean (SEM) error bars across stem treatments ($F_{2, 24}$ = 45.568, *p* < 0.001) in the first bud break experiment.

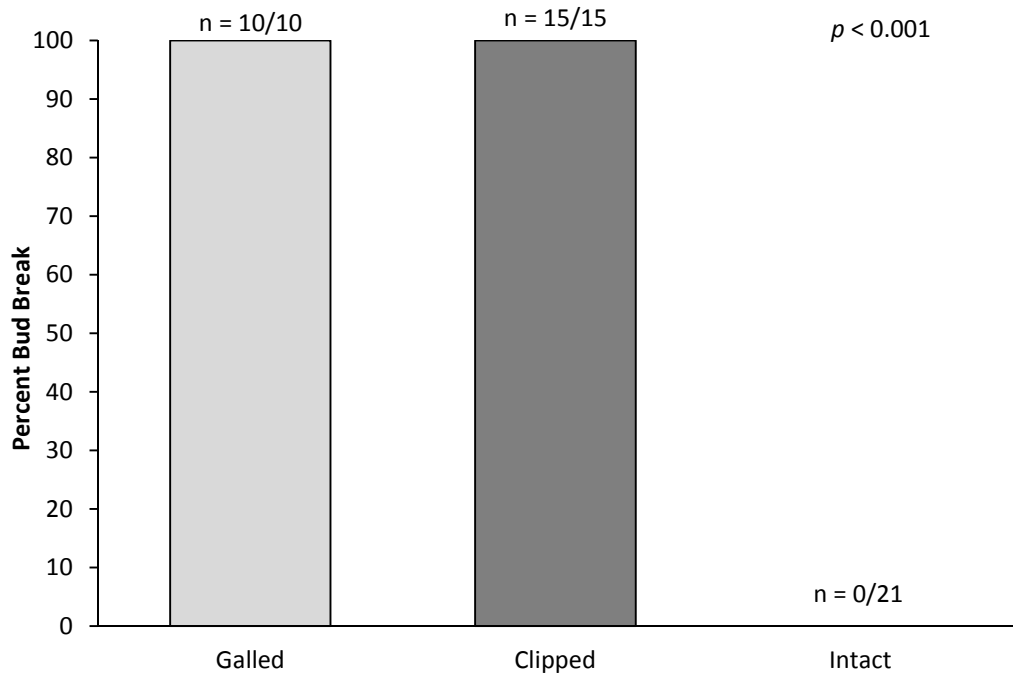


Figure 24. Percent bud break occurring by eight weeks across stem treatments (X^2 = 46.000, *df* = 2, *p* < 0.001) in the first bud break experiment.

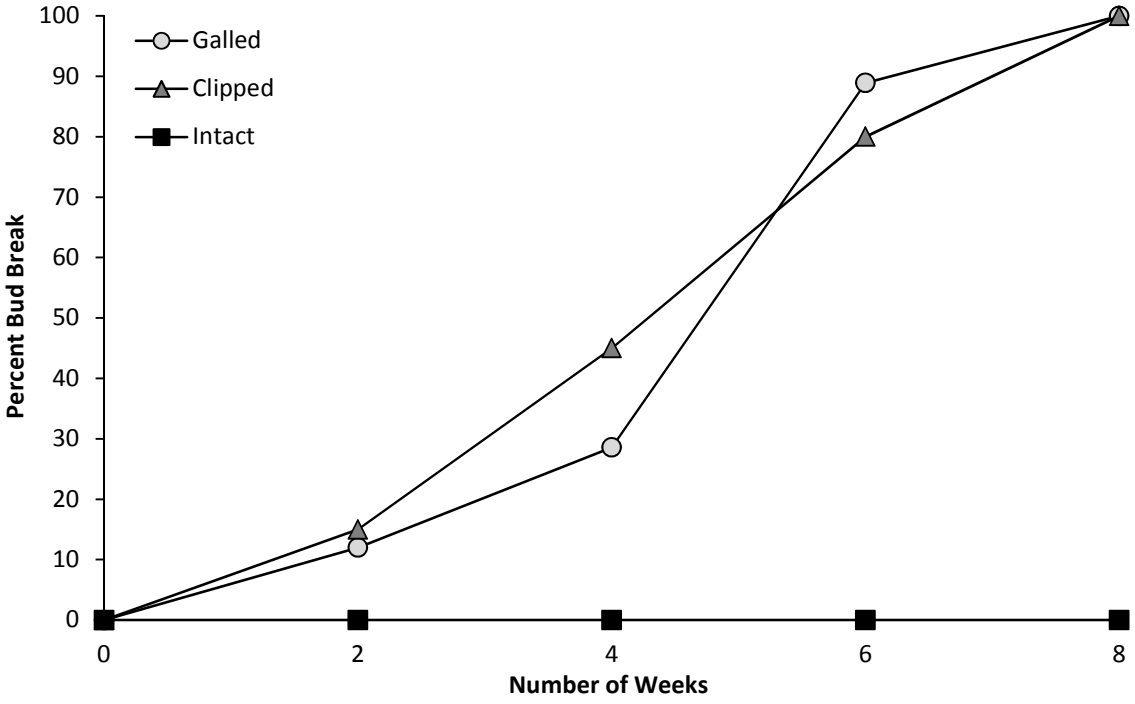


Figure 25. Percent bud break depicted over the first eight weeks in the first bud break experiment.

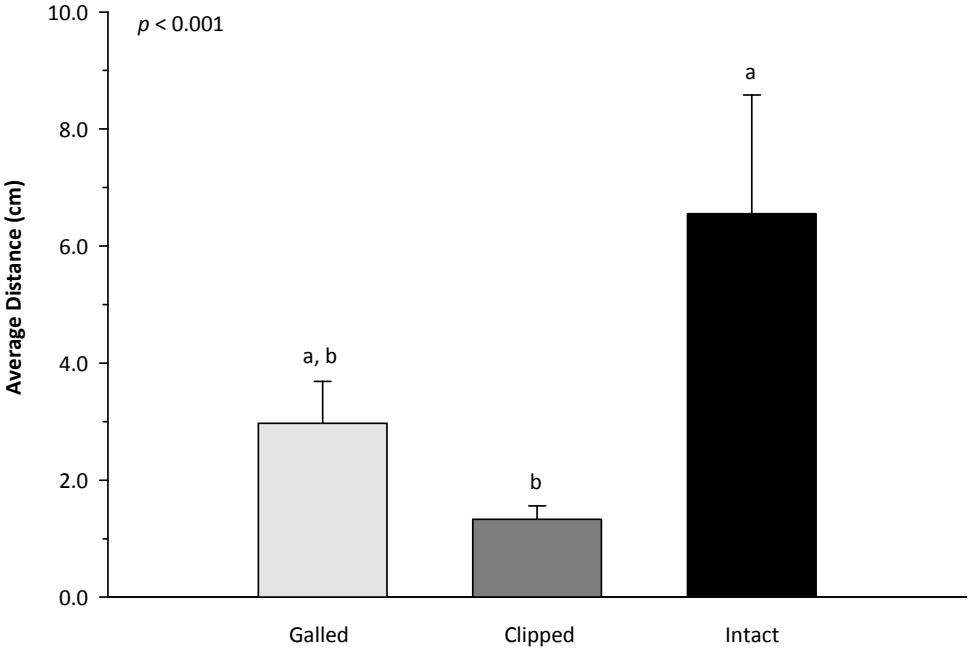


Figure 26. Average distance to first bud break in centimeters, as measured from the stem terminal to the node bearing the first lateral meristem to break bud, with SEM error bars ($F_{2, 24}$ = 12.474, p < 0.001).

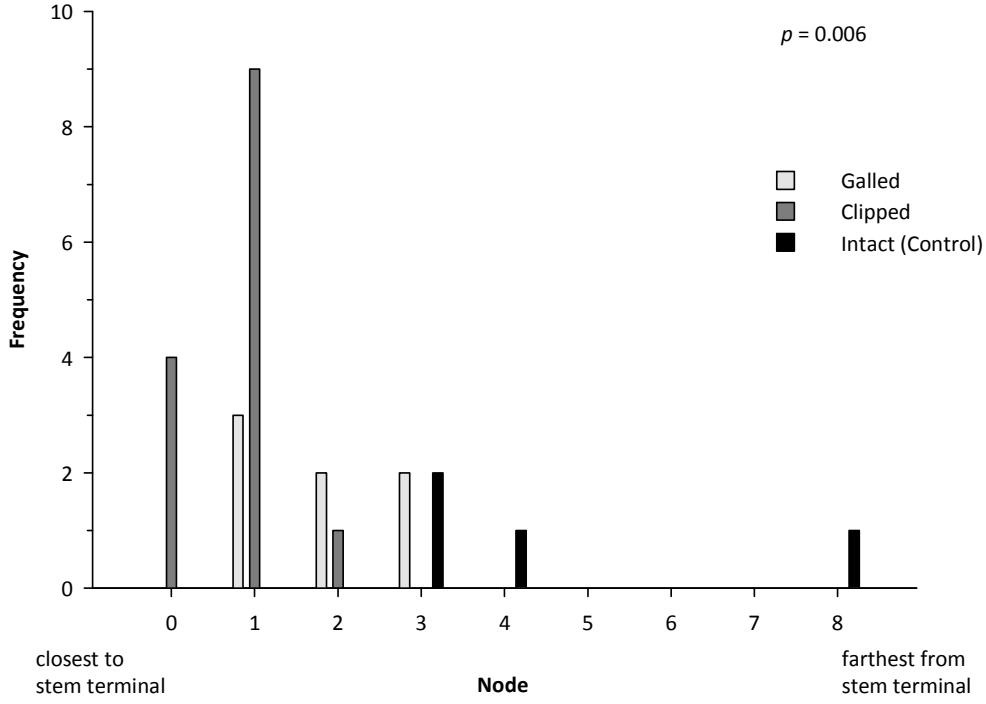


Figure 27. Frequencies of first bud breaks occurring at different nodes (0 = stem terminal, 1 = first node below terminal, *etc.*) (X^2 = 14.534, *df* = 4, p = 0.006).

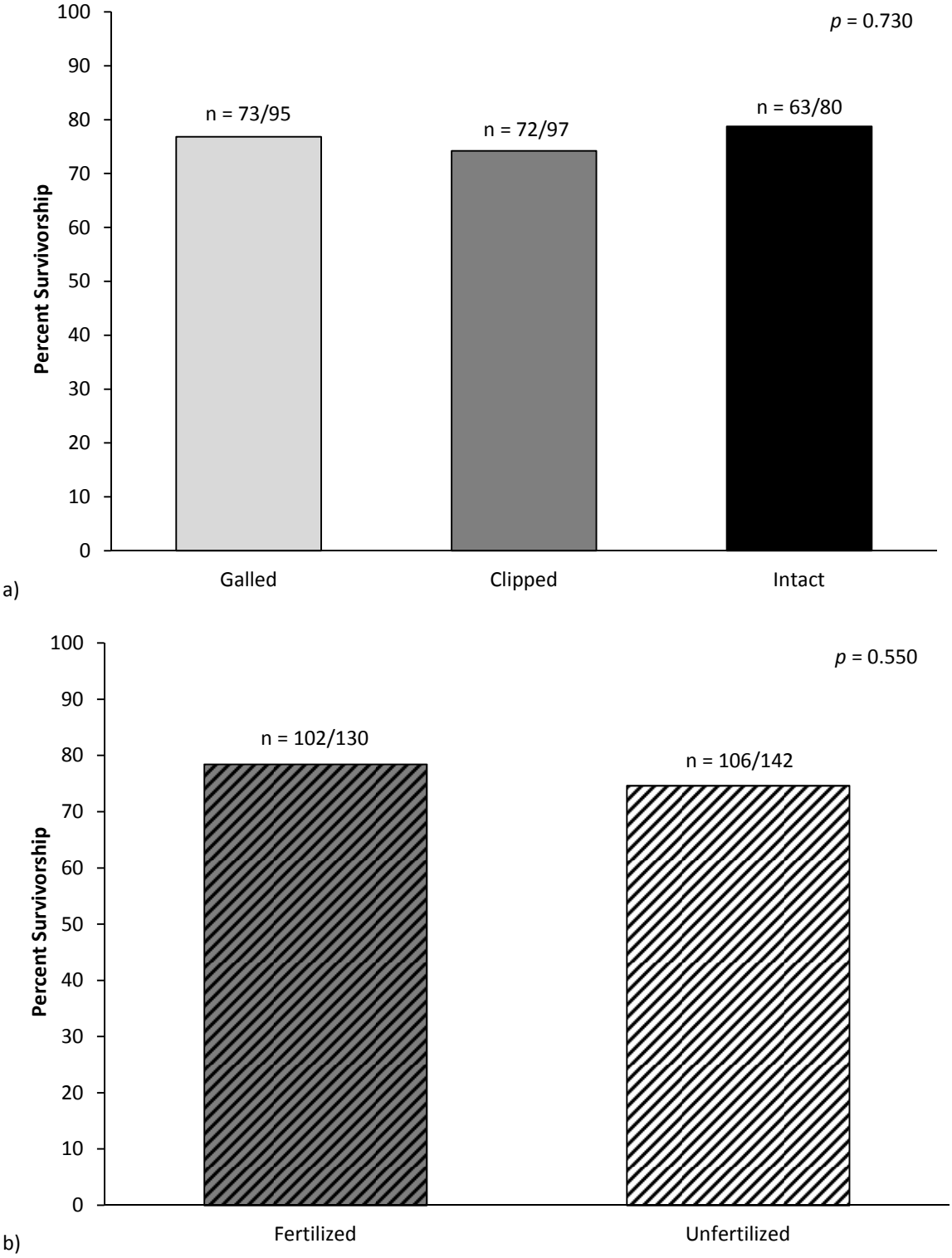


Figure 28. Percent survivorship at 25 weeks across (a) stem treatments (X^2 = 0.629, *df* = 2, *p* = 0.730) and (b) fertilization treatments (X^2 = 0.357, *df* = 1, *p* = 0.550) in the stem-nutrition experiment.

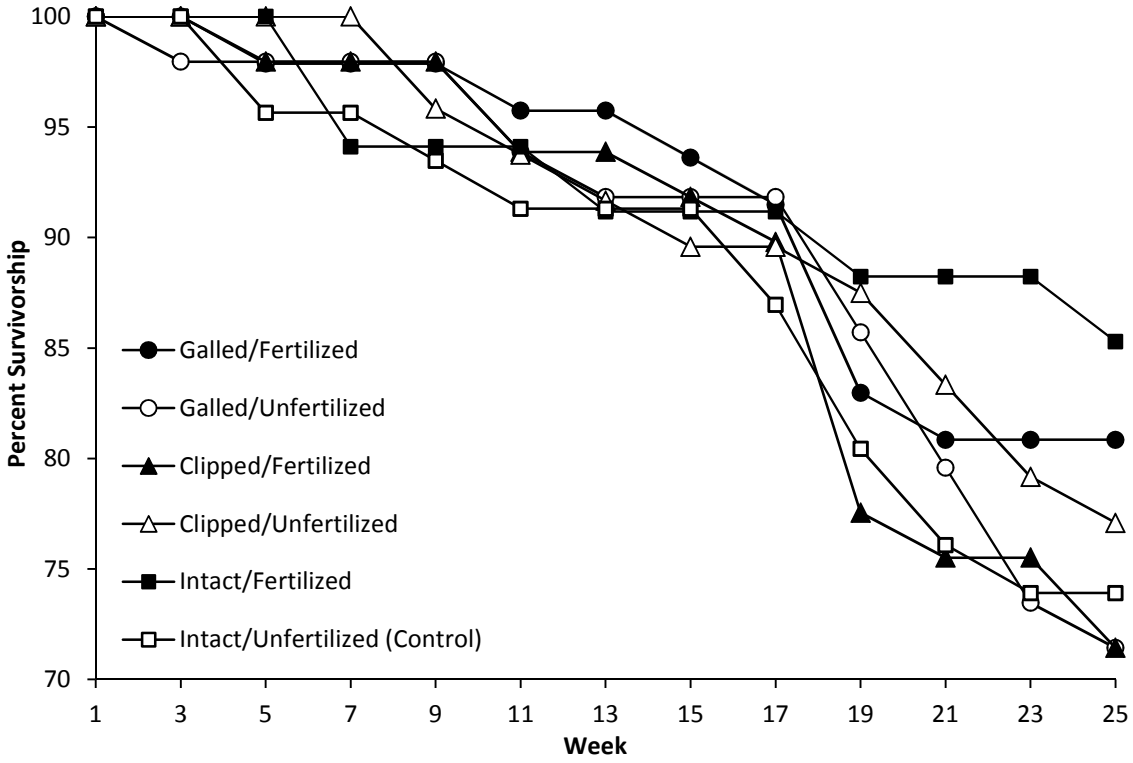
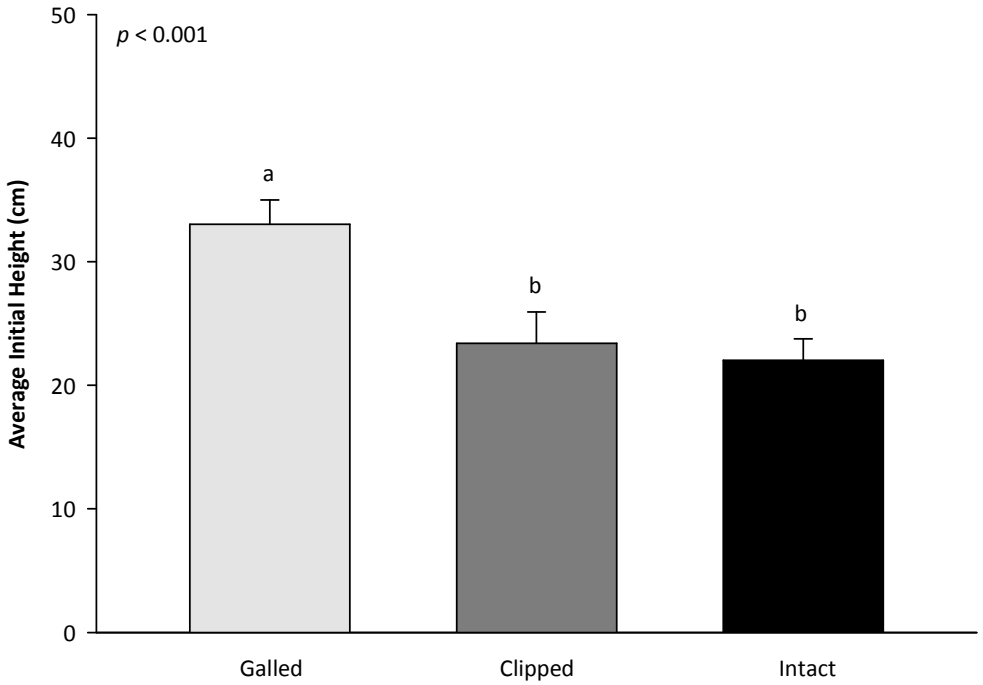
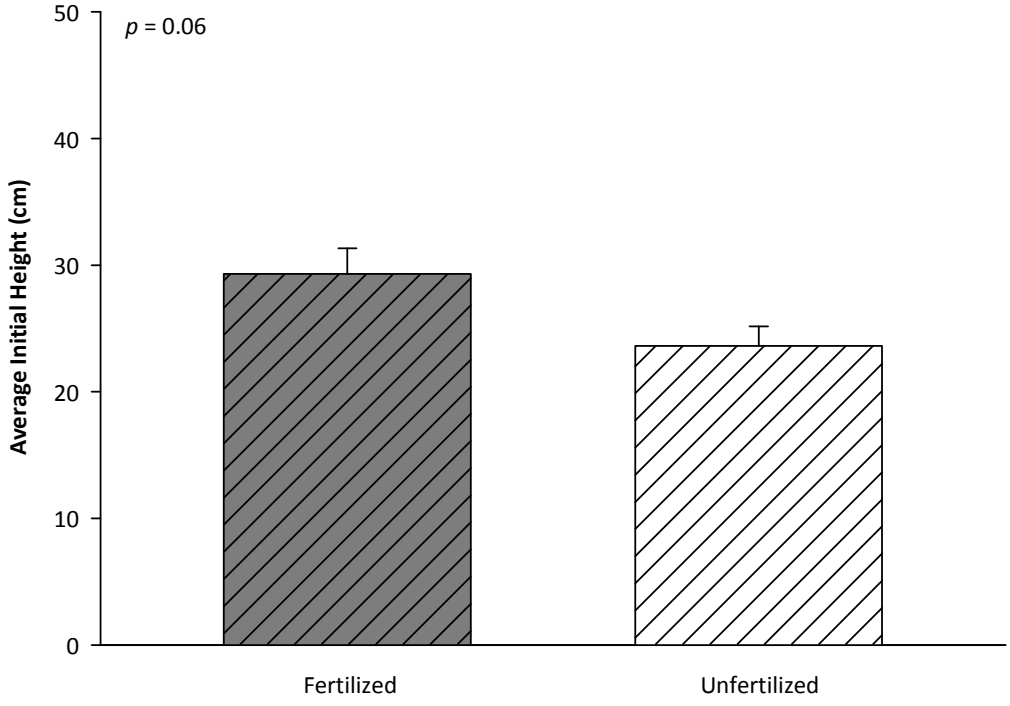


Figure 29. Percent survivorship depicted over 25 weeks in the stem-nutrition experiment. Note: The percent scale ranges from 70 to 100%.

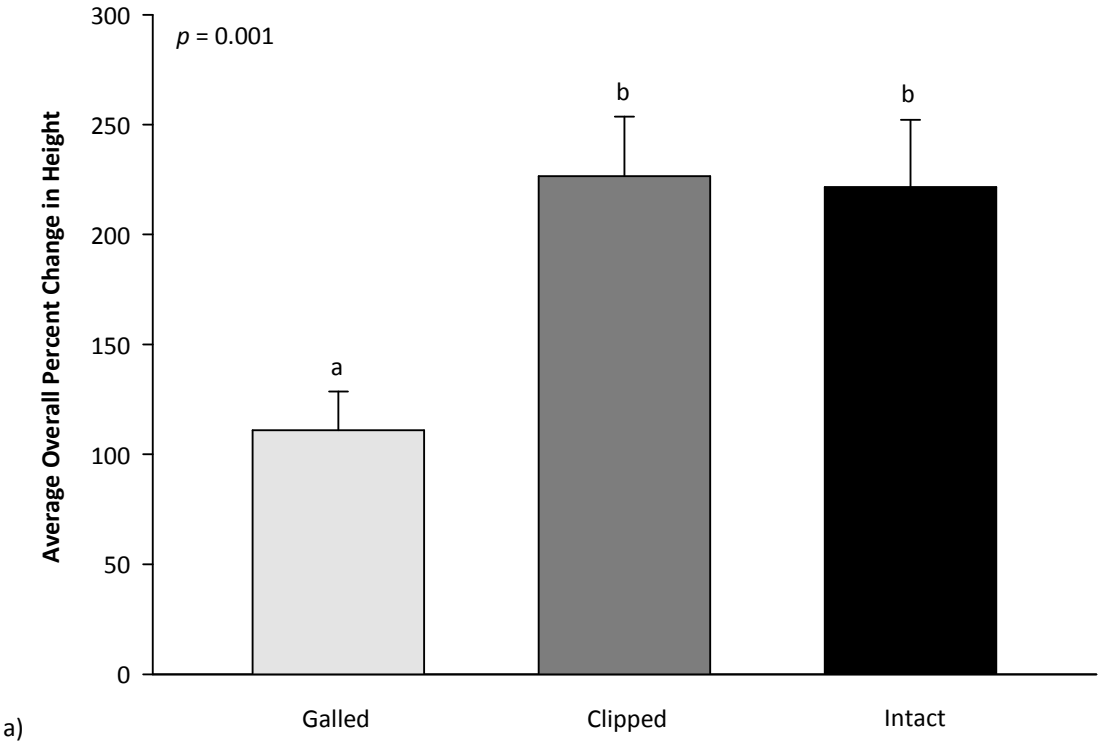


a)

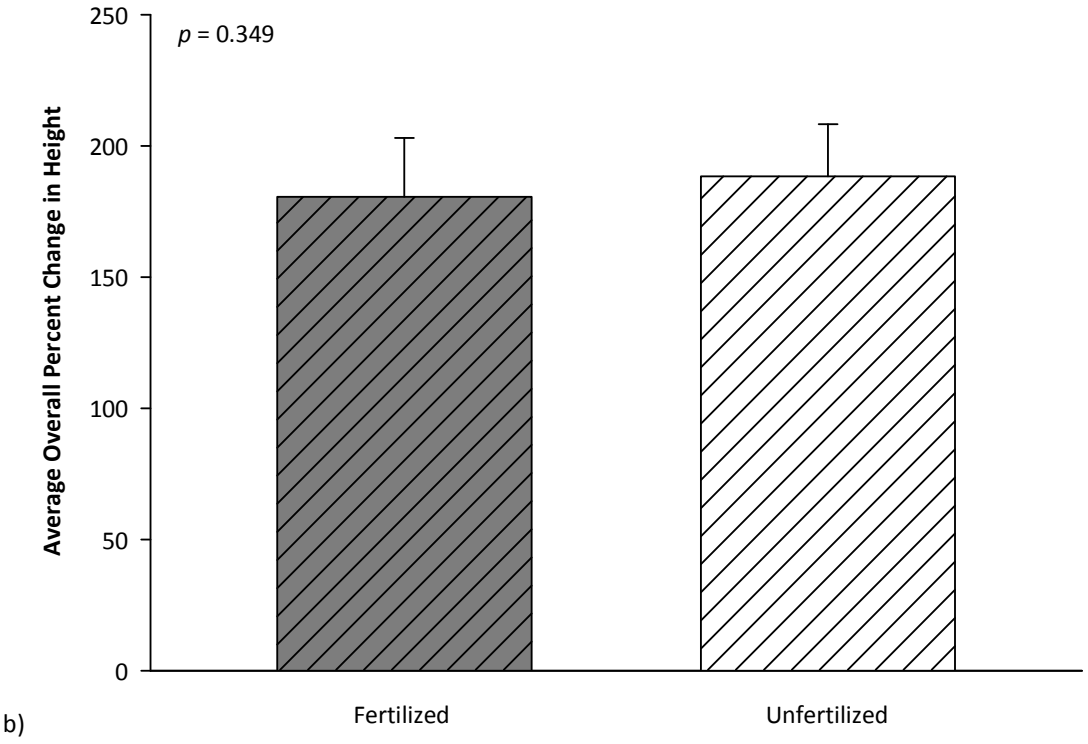


b)

Figure 30. Average initial heights (cm) of ramets with standard error of the mean (SEM) error bars across (a) stem treatments (X^2 = 23.794, *df* = 2, *p* < 0.001) and (b) fertilization treatments (X^2 = 3.548, *df* = 1, *p* = 0.06) in the stem-nutrition experiment.

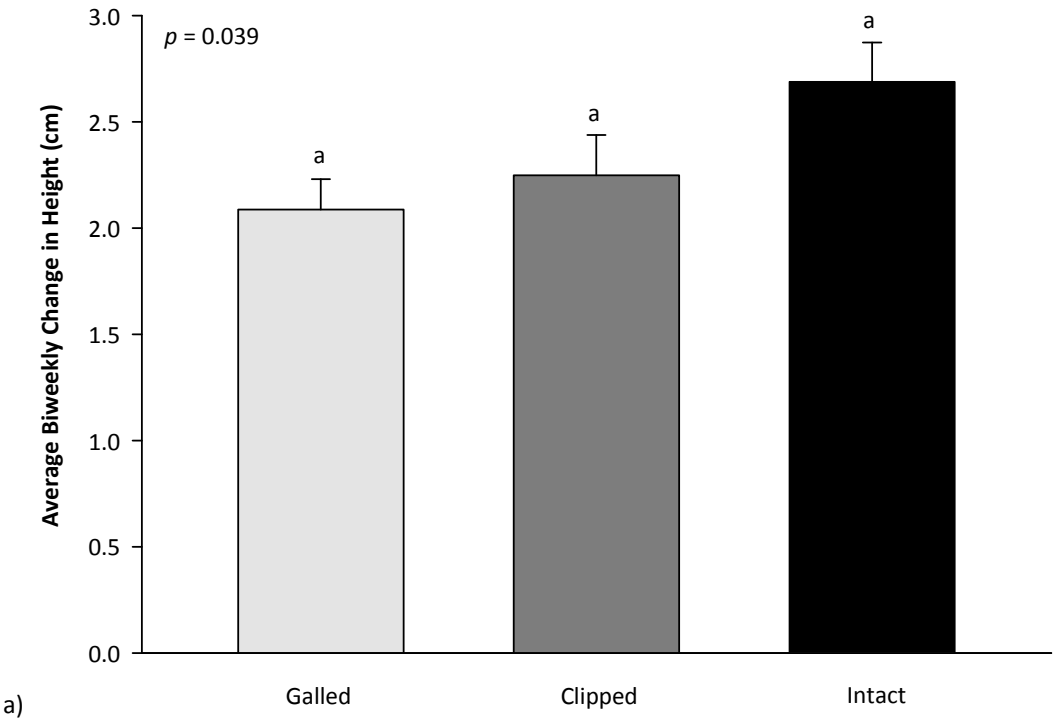


a)

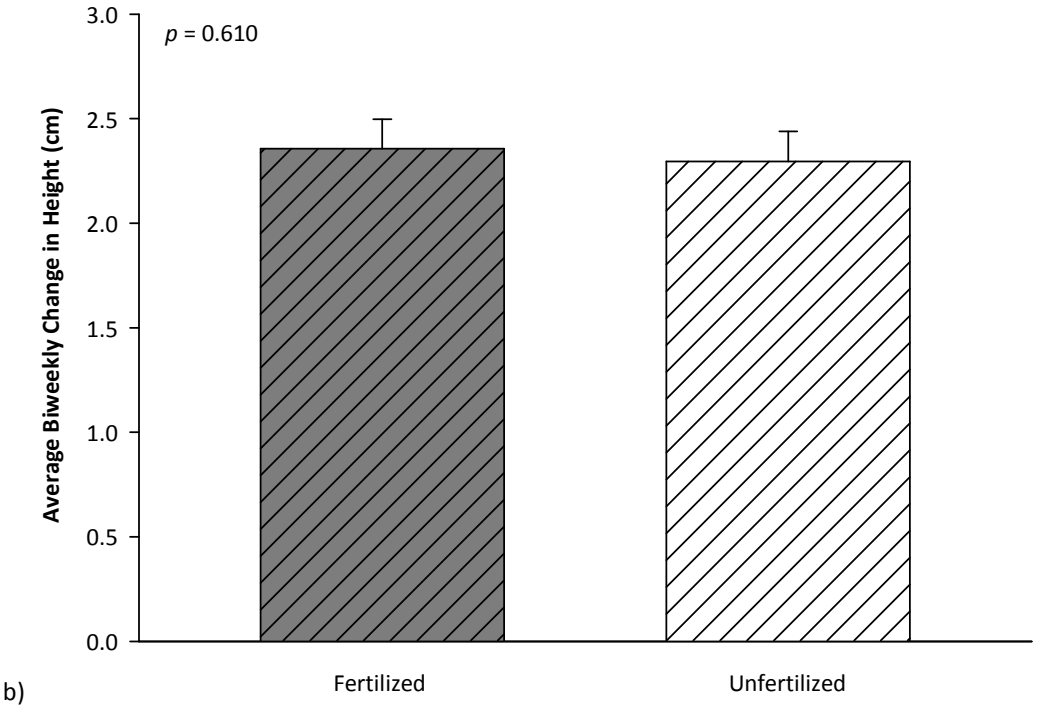


b)

Figure 31. Average overall percent change in height with SEM error bars across (a) stem treatments (X^2 = 13.405, *df* = 2, *p* = 0.001) and (b) fertilization treatments (X^2 = 0.875, *df* = 1, *p* = 0.349) in the stem-nutrition experiment.



a)



b)

Figure 32. Average biweekly change in height with SEM error bars across (a) stem treatments and (b) fertilization treatments in the stem-nutrition experiment (stem treatment: $F_{2, 208} = 3.288$, $p = 0.039$; fertilization treatment: $F_{1, 208} = 0.261$, $p = 0.610$; stem*nutrition: $F_{2, 208} = 1.708$, $p = 0.184$). Note: Stem treatment bordered on significance, which was lost in pairwise comparisons. Data is presented untransformed for clarity.

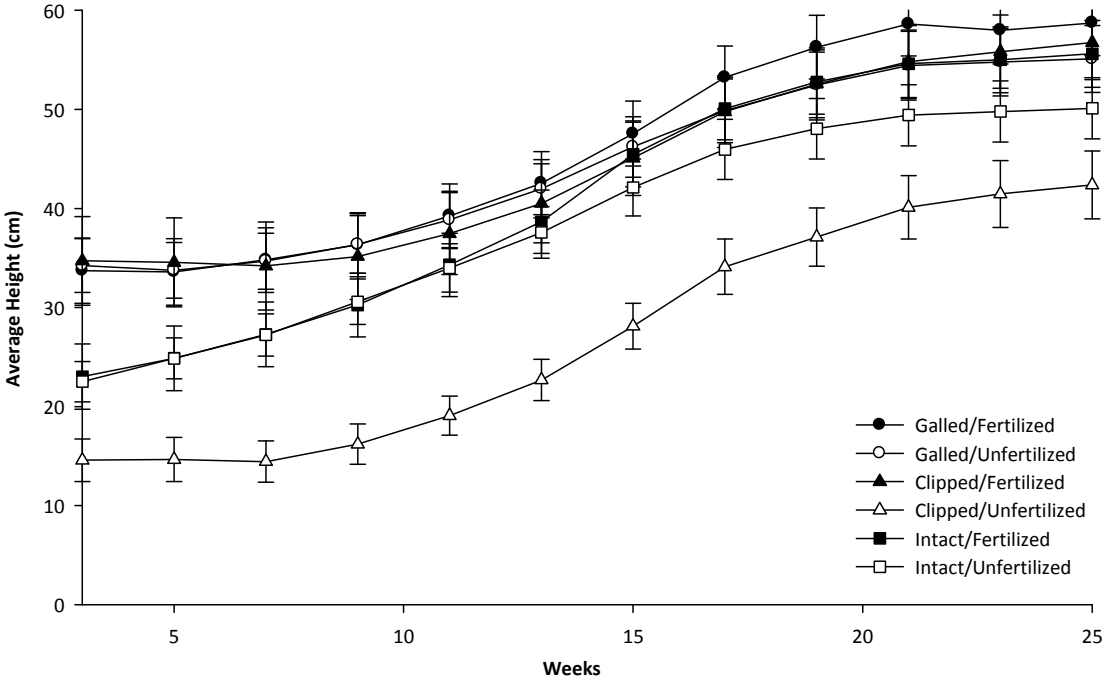
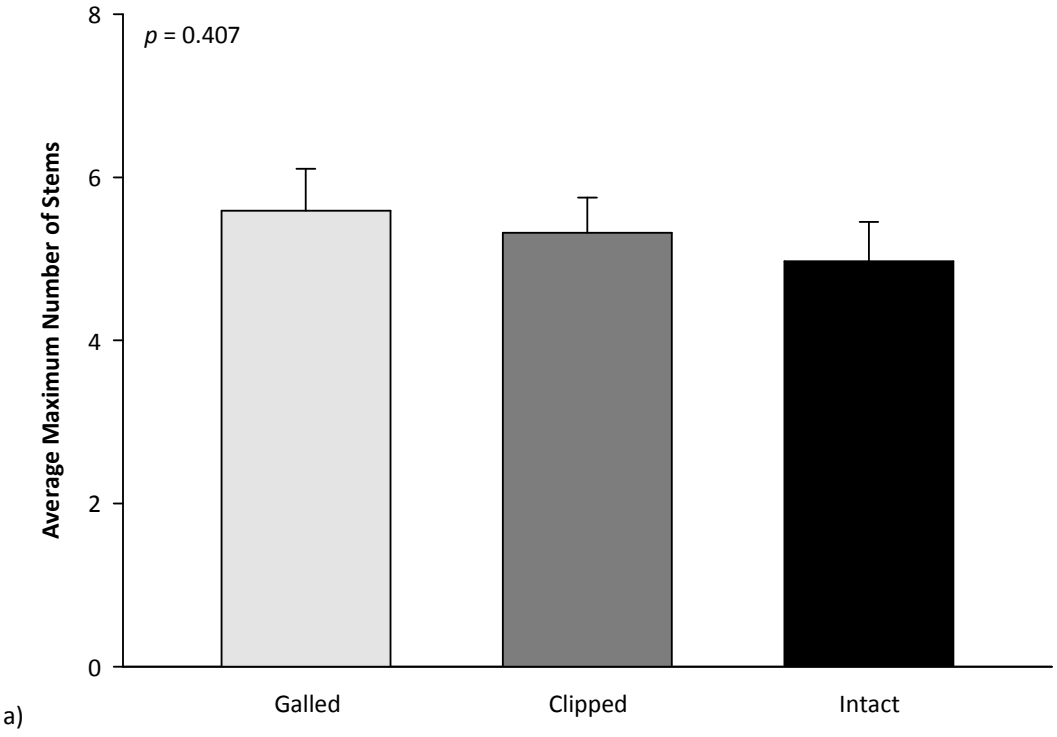
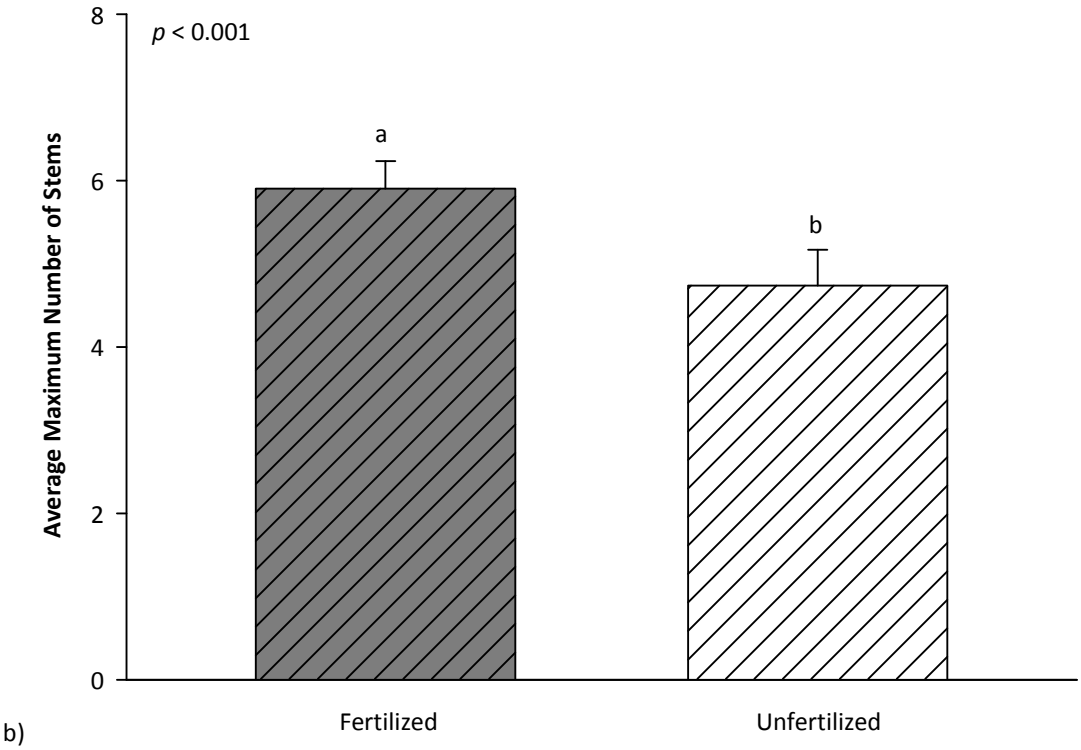


Figure 33. Average height (cm) depicted over 25 weeks in the stem-nutrition experiment with SEM error bars.

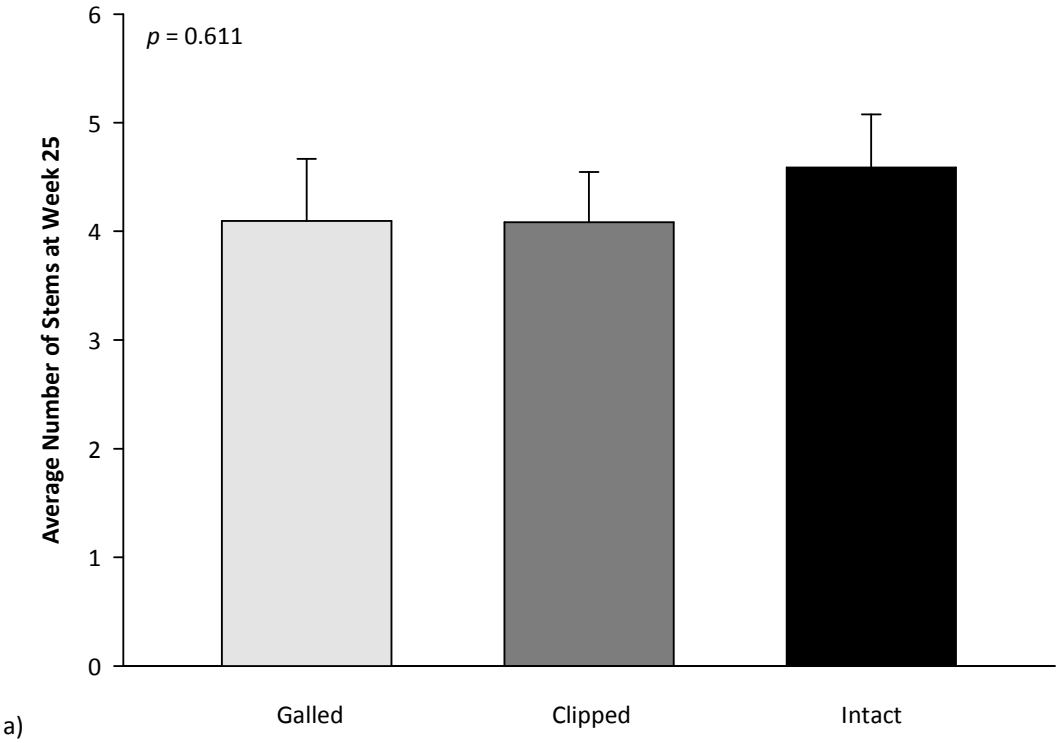


a)

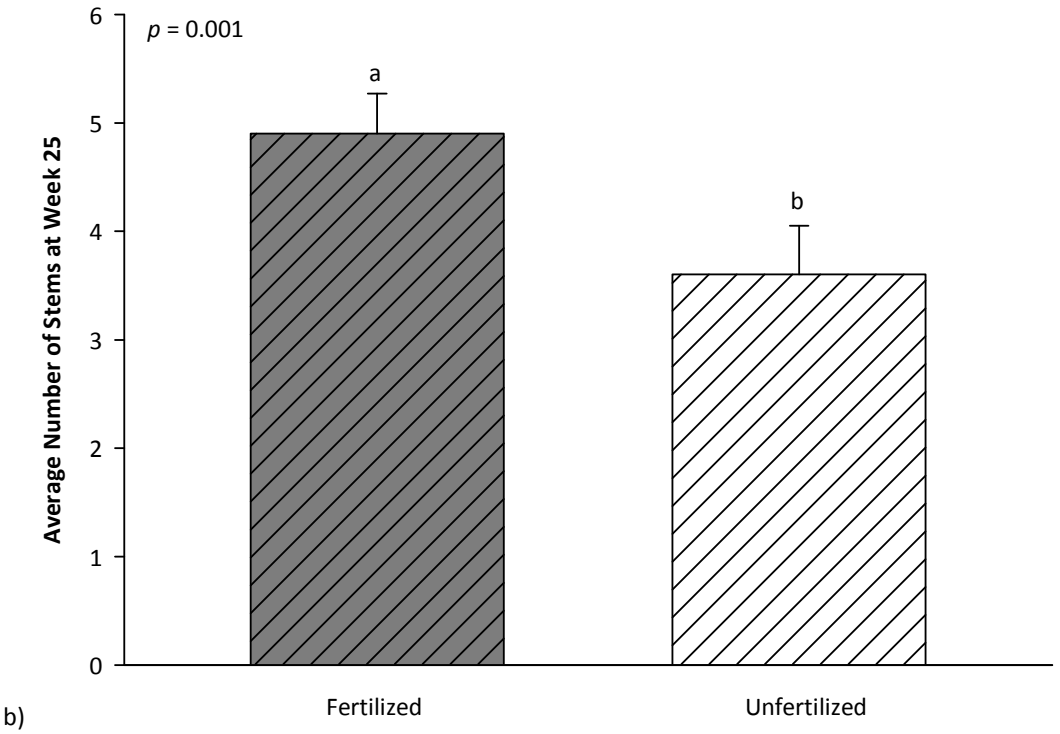


b)

Figure 34. Average maximum number of stems with SEM error bars across (a) stem treatments (X^2 = 1.798, *df* = 2, *p* = 0.407) and (b) fertilization treatments (X^2 = 12.230, *df* = 1, *p* < 0.001) in the stem-nutrition experiment.



a)



b)

Figure 35. Average number of stems at week 25 with SEM error bars across (a) stem treatments (X^2 = 0.986, *df* = 2, *p* = 0.611) and (b) fertilization treatments (X^2 = 11.973, *df* = 1, *p* = 0.001) in the stem-nutrition experiment.

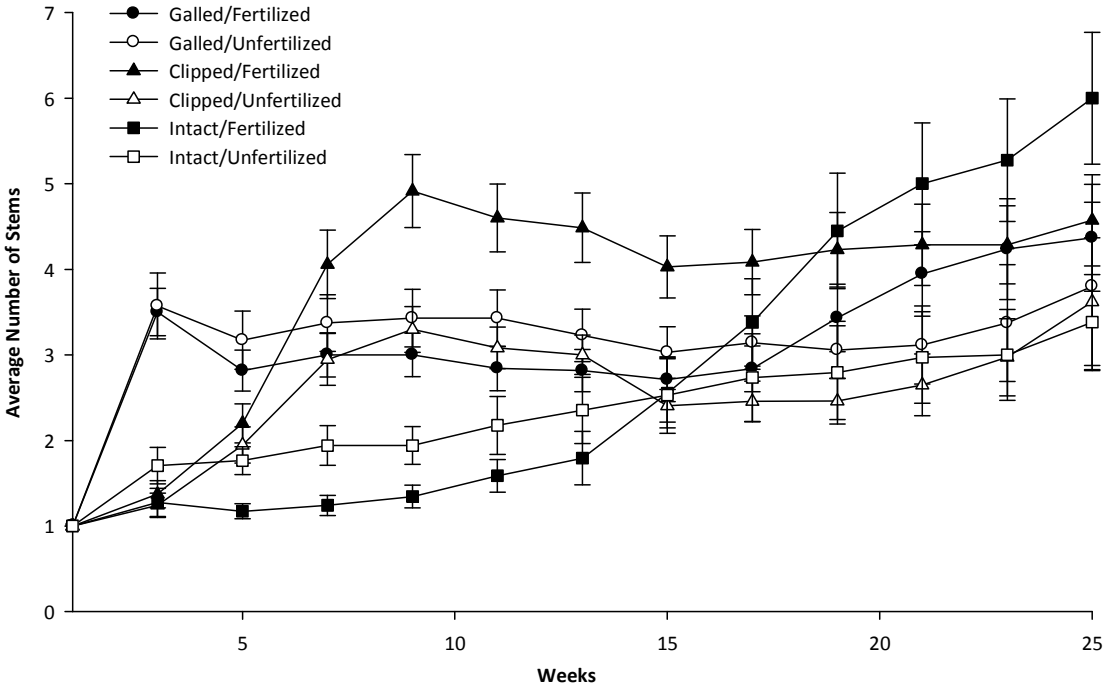


Figure 36. Average number of stems depicted over 25 weeks in the stem-nutrition experiment with SEM error bars.

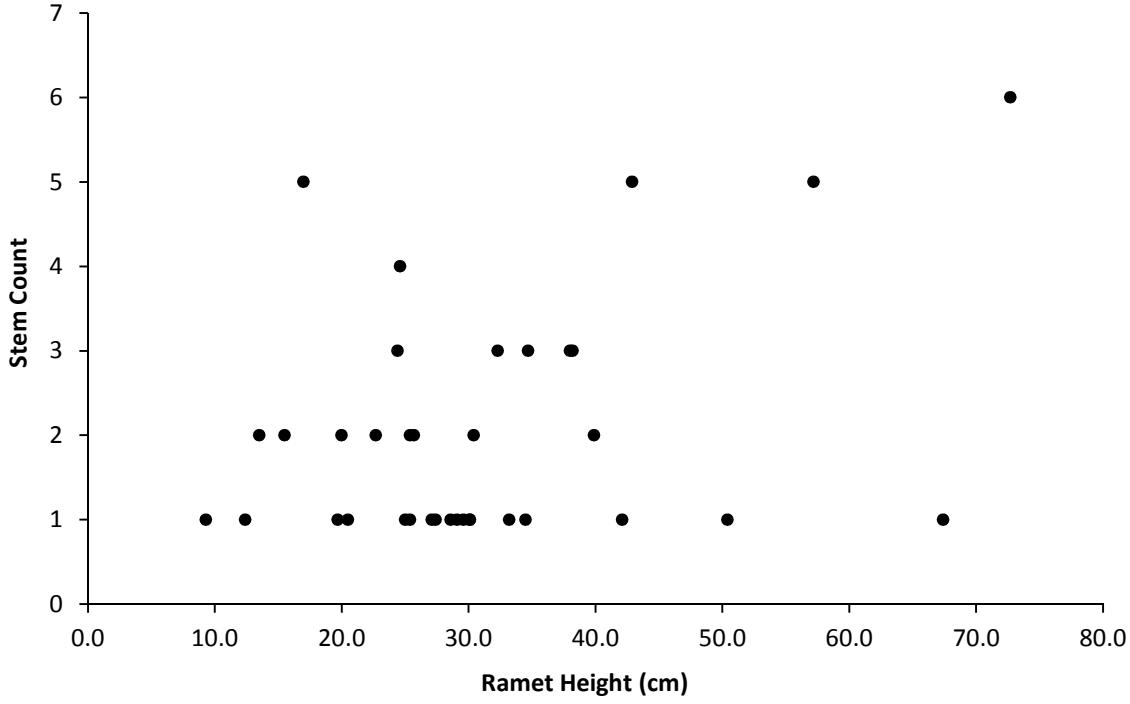


Figure 37. Correlation between stem count and ramet height at week 9 (r = 0.369, n = 36).

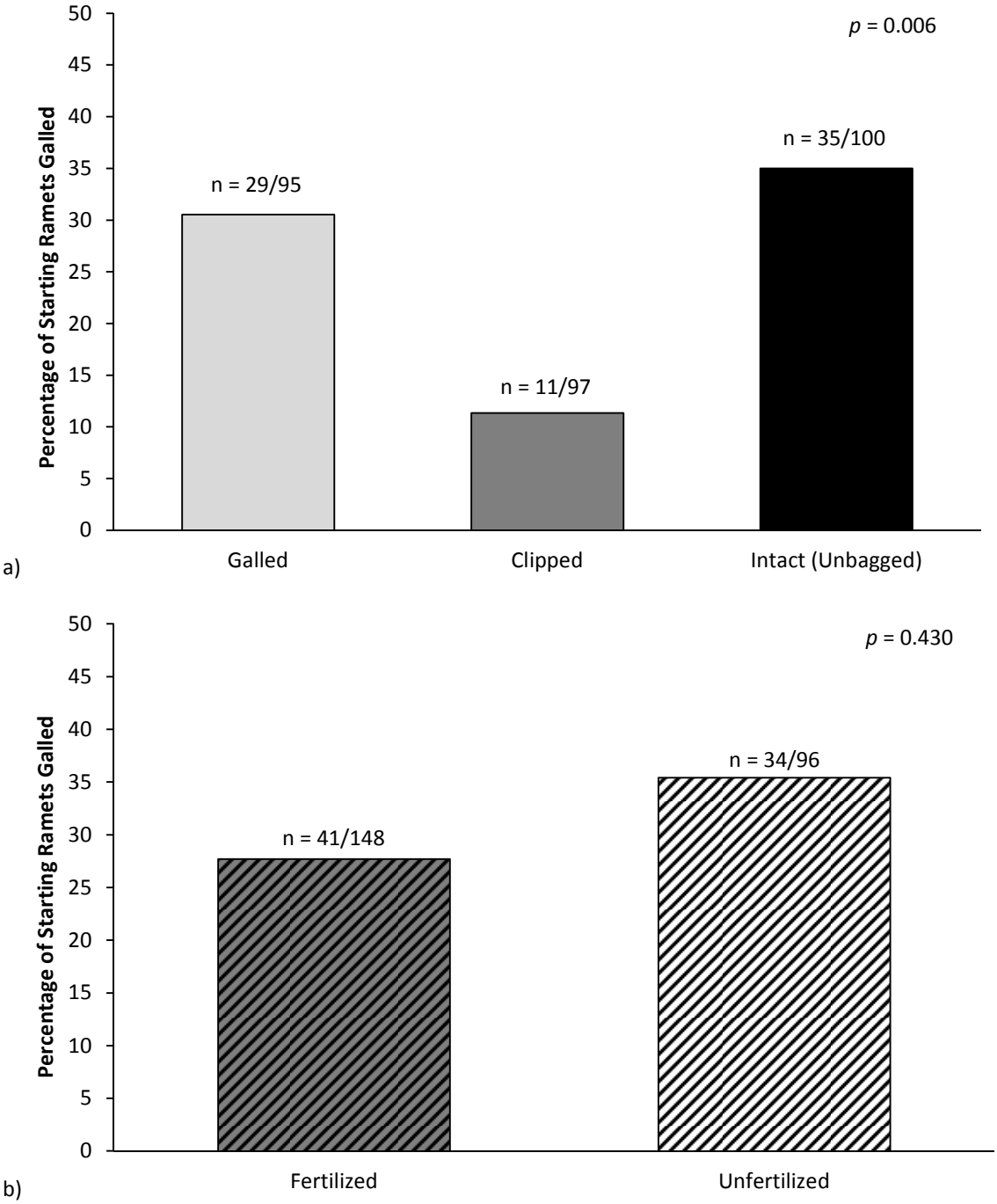


Figure 38. Percentage of starting ramets that were galled during the stem-nutrition experiment, assessed across (a) stem treatments (X^2 = 10.146, *df* = 2, *p* = 0.006) and (b) fertilization treatments (X^2 = 0.622, *df* = 1, *p* = 0.430). Includes ramets that died or were missing during the experiment and excludes bagged intact ramets (bagged controls).

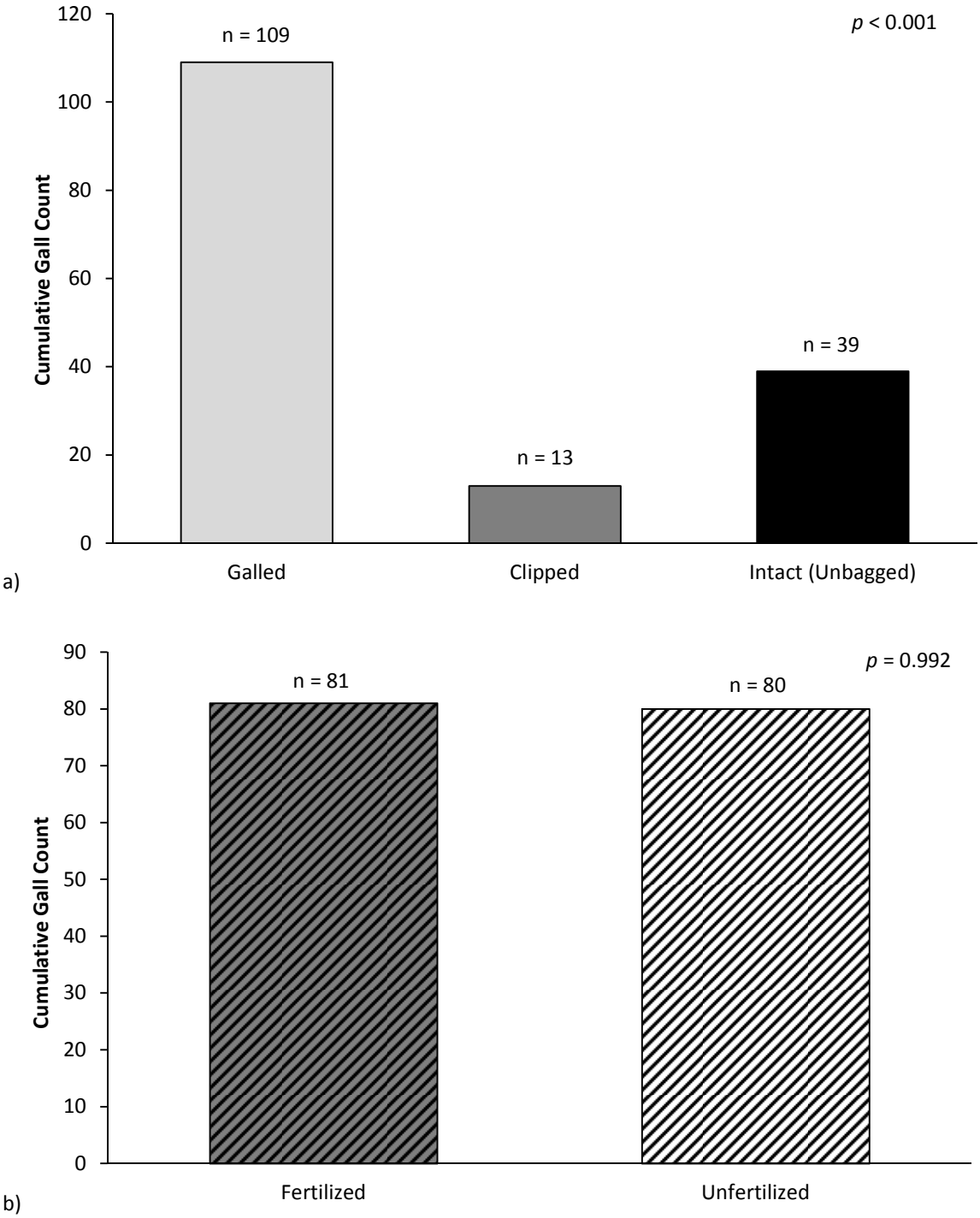


Figure 39. Cumulative gall counts across (a) stem treatments (X^2 = 91.876, *df* = 2, *p* < 0.001) and (b) fertilization treatments (X^2 = 0.000, *df* = 1, *p* = 0.992) in the stem-nutrition experiment, including ramets that began the experiment with stems intact but were later galled.

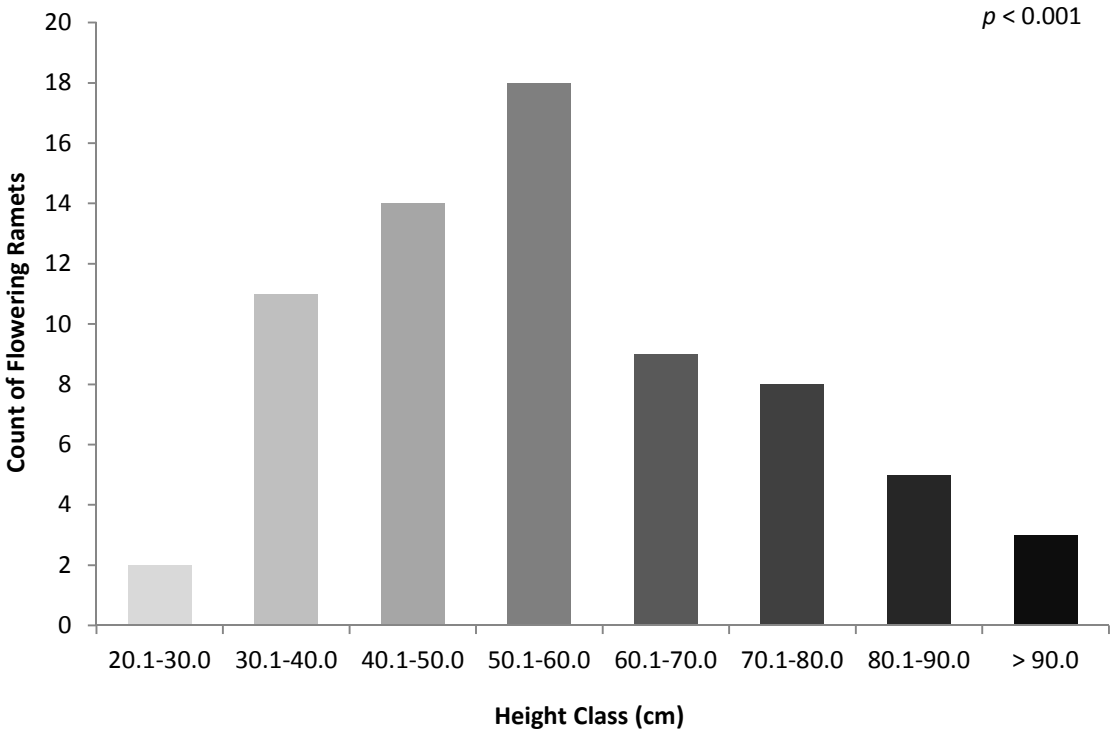
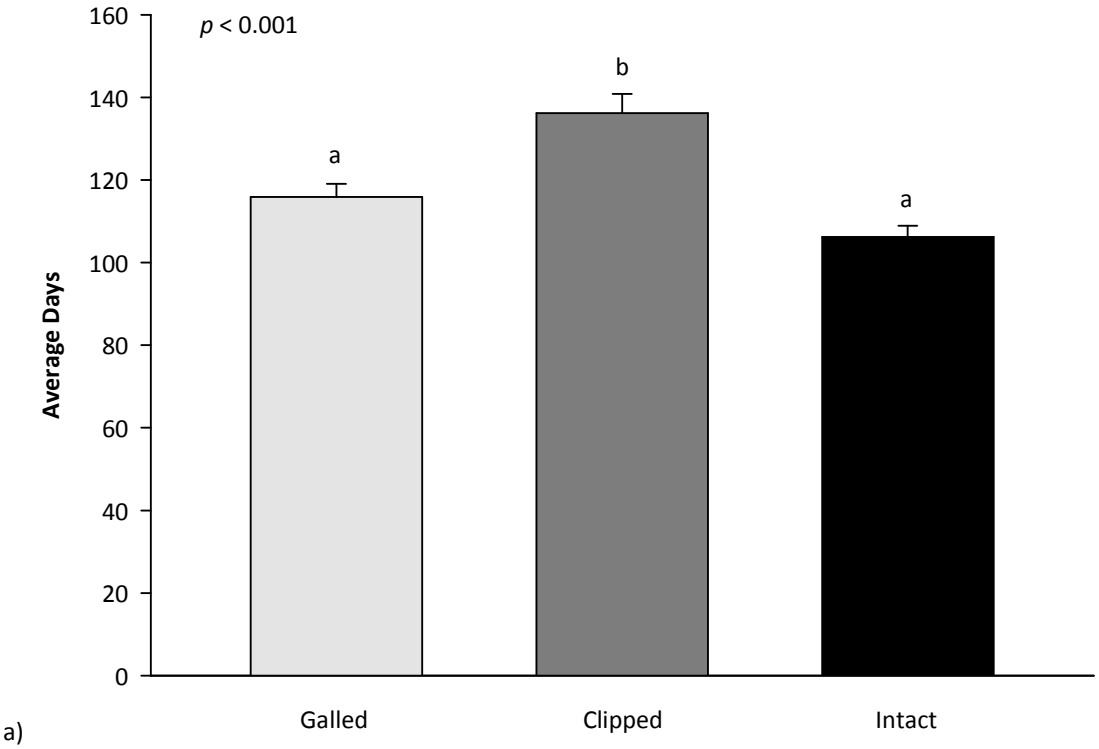
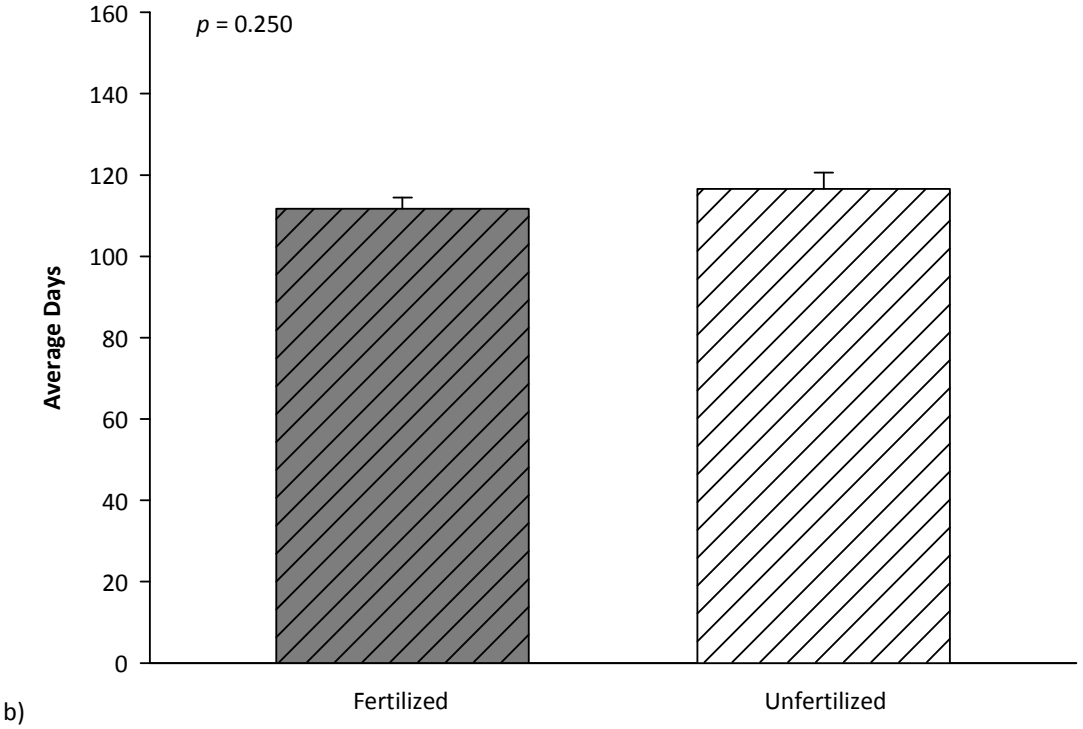


Figure 40. Counts of ramets in various height classes (cm) at first flowering, regardless of treatment in stem-nutrition experiment (X^2 = 35.429, *df* = 8, *p* < 0.001).

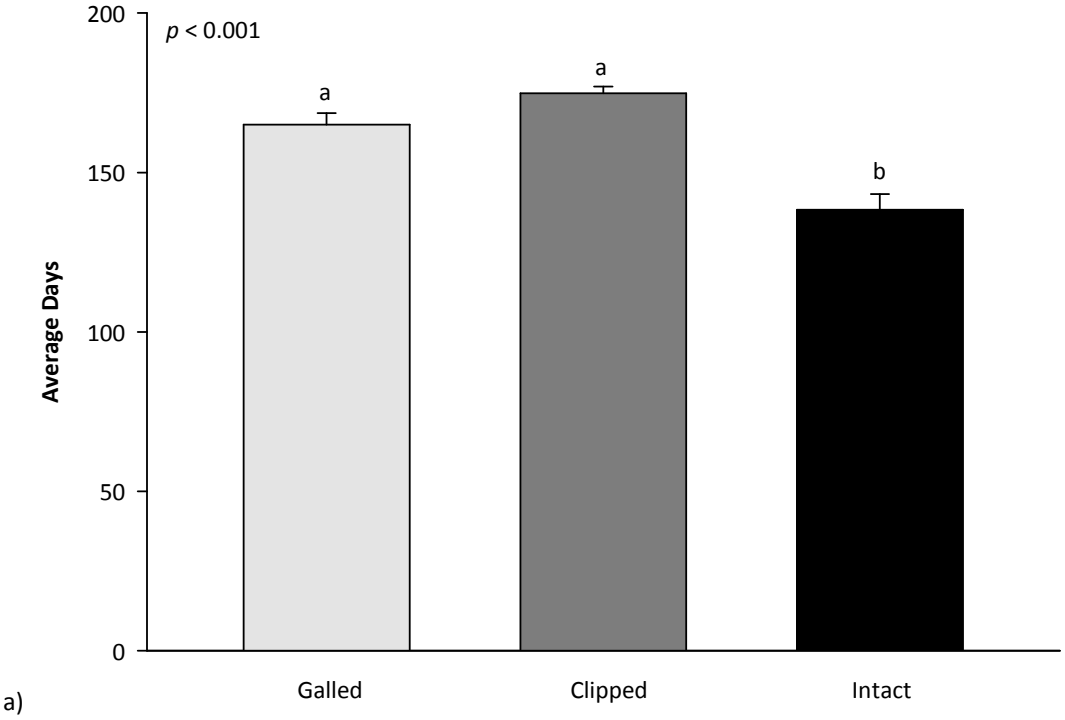


a)

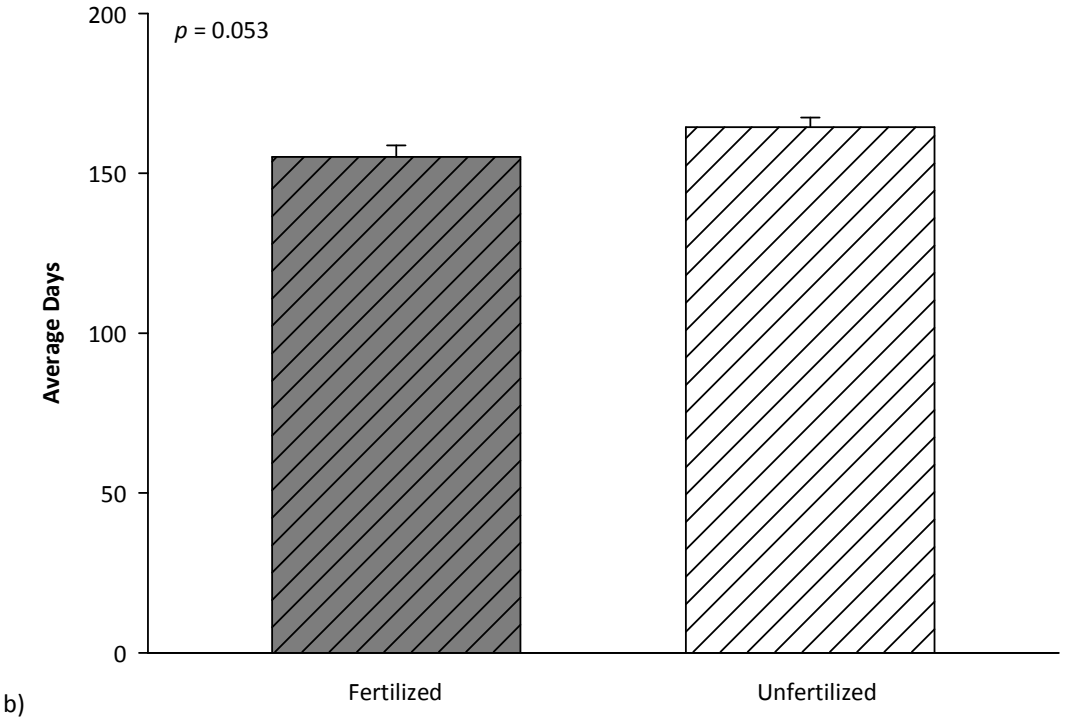


b)

Figure 41. Average number of days until appearance of first flower bud in flowering ramets only with SEM error bars across (a) stem treatments (X^2 = 19.747, *df* = 2, *p* < 0.001) and (b) fertilization treatments (X^2 = 1.323, *df* = 1, *p* = 0.250) in the stem-nutrition experiment.

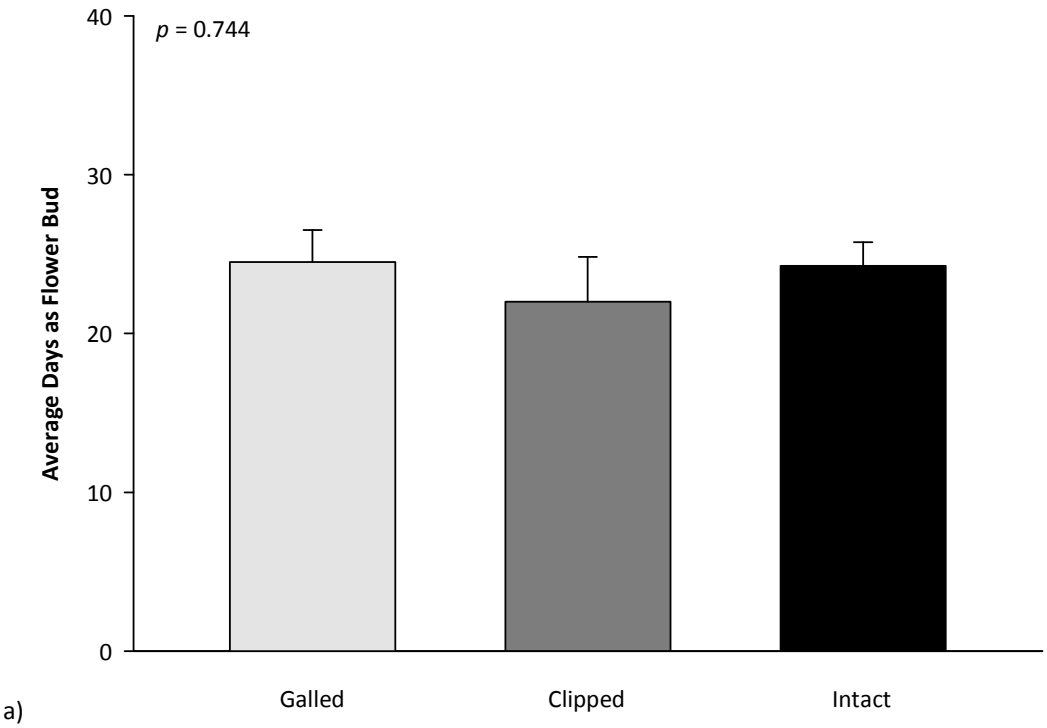


a)

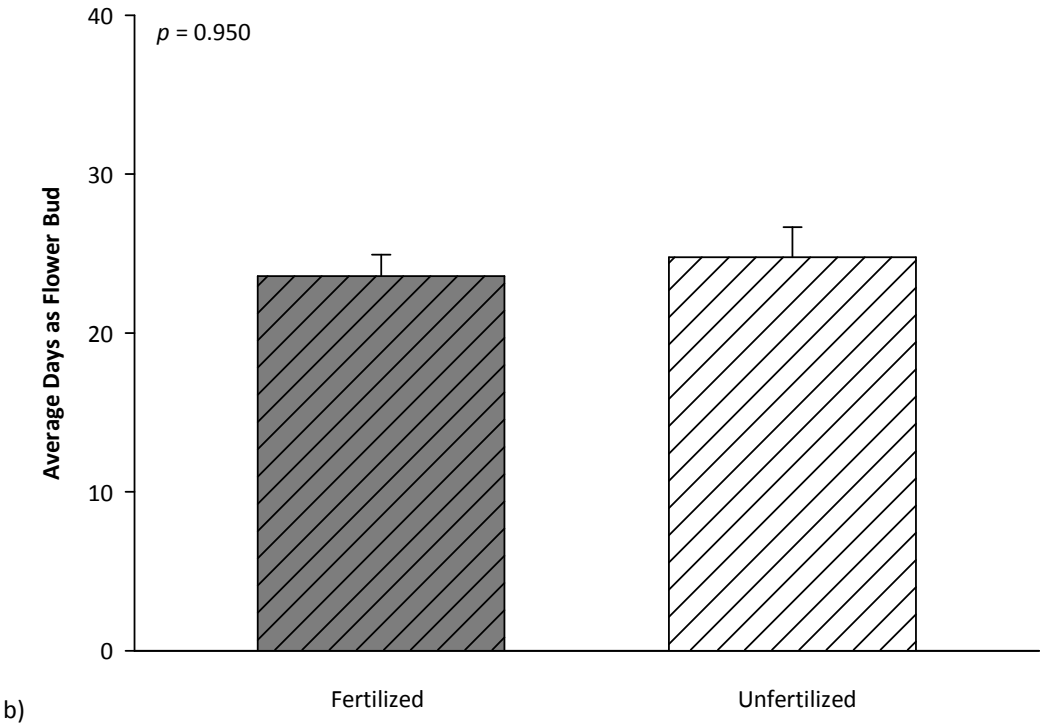


b)

Figure 42. Average number of days until appearance of first flower bud in all ramets with SEM error bars across (a) stem treatments (X^2 = 38.166, *df* = 2, *p* < 0.001) and (b) fertilization treatments (X^2 = 3.744, *df* = 1, *p* = 0.053) in the stem-nutrition experiment. Non-flowering ramets were assigned a value of 182 days, or two weeks past the end of the experiment.

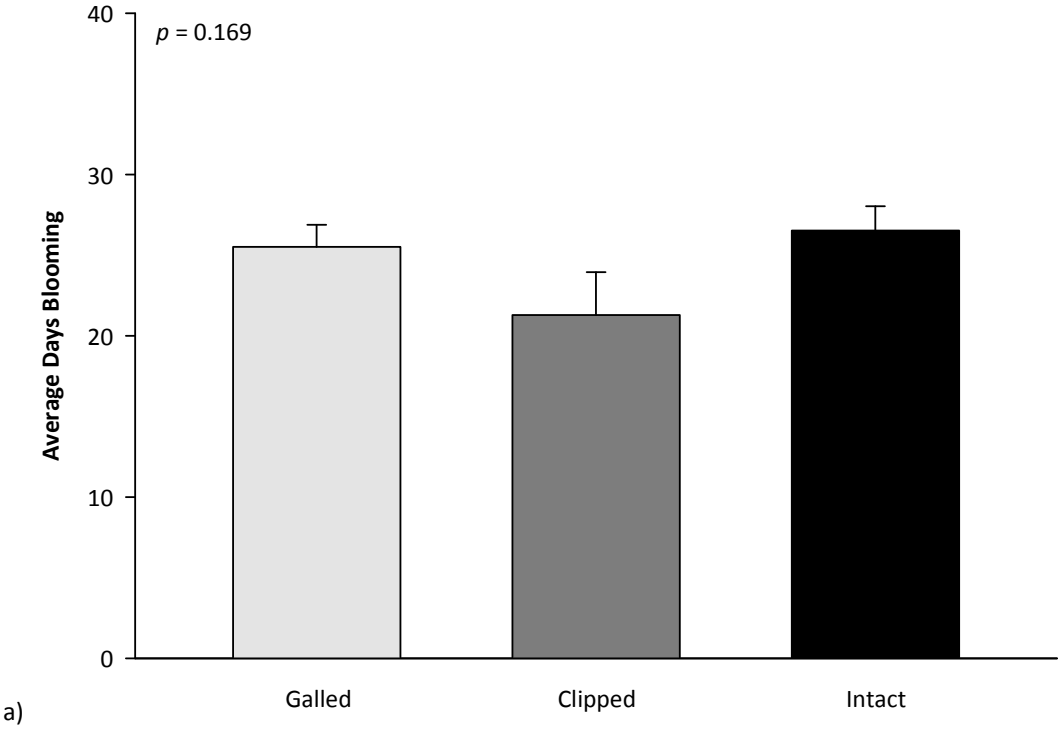


a)

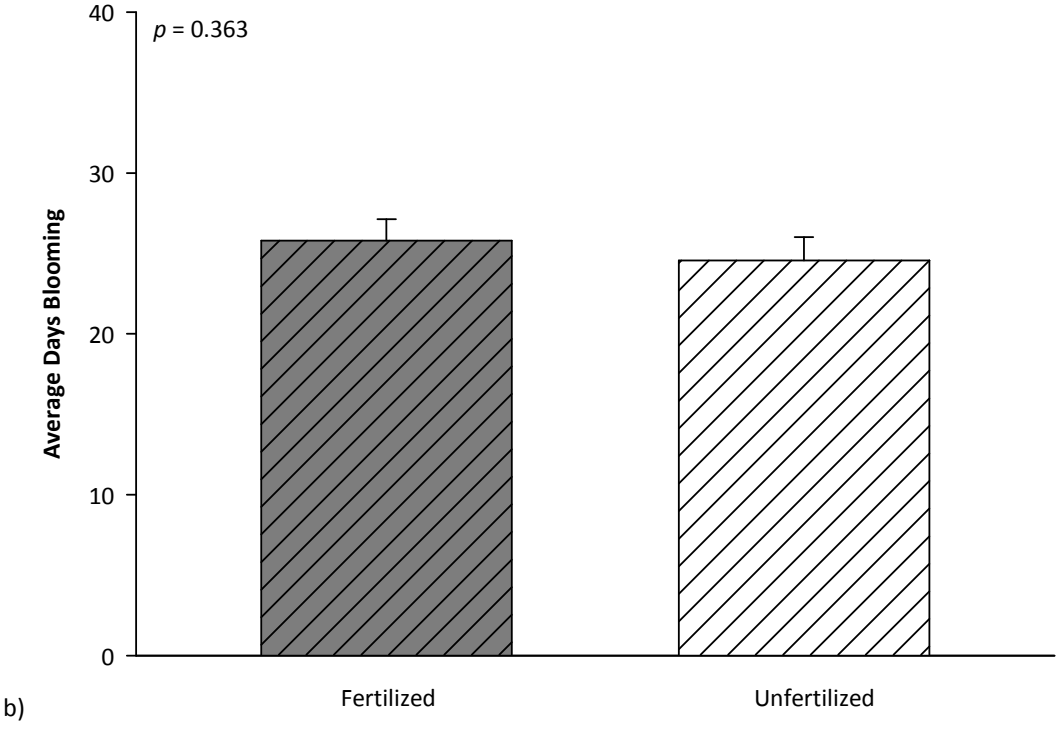


b)

Figure 43. Average number of days flowers spent as flower buds with SEM error bars across (a) stem treatments and (b) fertilization treatments in the stem-nutrition experiment (stem treatment: $F_{2, 46}$ = 0.298, p = 0.744; fertilization treatment: $F_{1, 46}$ = 0.004, p = 0.950; stem*nutrition: $F_{2, 46}$ = 0.057, p = 0.945; covariate: $F_{1, 46}$ = 0.004, p = 0.949).

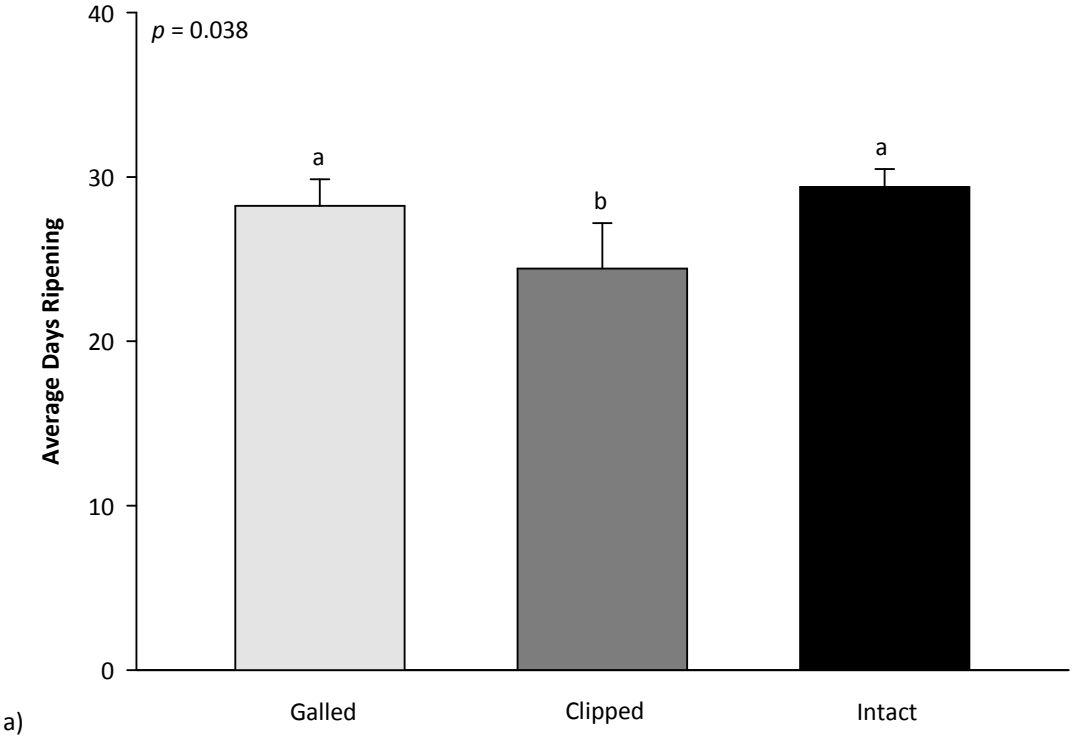


a)

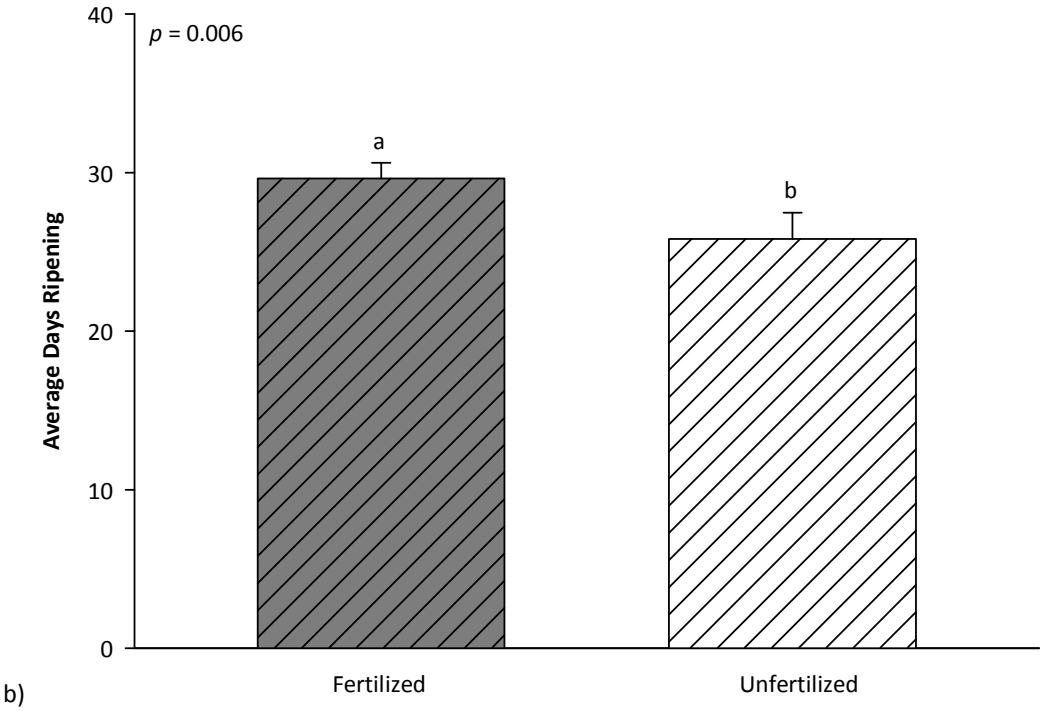


b)

Figure 44. Average number of days flowers bloomed with SEM error bars across (a) stem treatments and (b) fertilization treatments in the stem-nutrition experiment (stem treatment: $F_{2, 46} = 1.859$, $p = 0.169$; fertilization treatment: $F_{1, 46} = 0.848$, $p = 0.363$; stem*nutrition: $F_{2, 46} = 0.267$, $p = 0.767$; covariate: $F_{1, 46} = 0.261$, $p = 0.612$).



a)



b)

Figure 45. Average number of days seed heads ripened with SEM error bars across (a) stem treatments and (b) fertilization treatments in the stem-nutrition experiment (stem treatment: $F_{2, 47} = 3.566$, $p = 0.038$; fertilization treatment: $F_{1, 47} = 8.281$, $p = 0.006$; stem*nutrition: $F_{2, 47} = 1.401$, $p = 0.258$; covariate: $F_{1, 47} = 0.013$, $p = 0.911$).

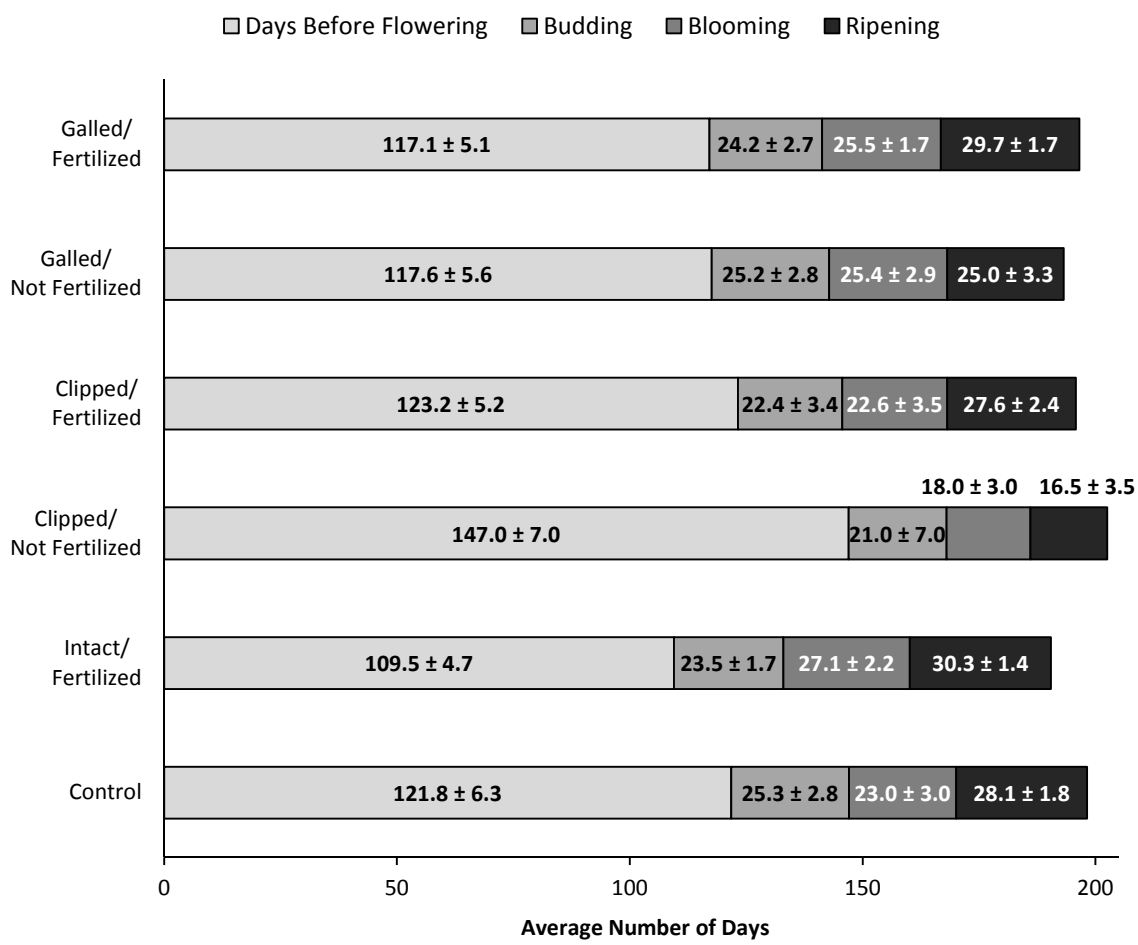


Figure 46. Phenology of sea oxeye daisy across stem-fertilization treatments (seed head-producing flowers only).

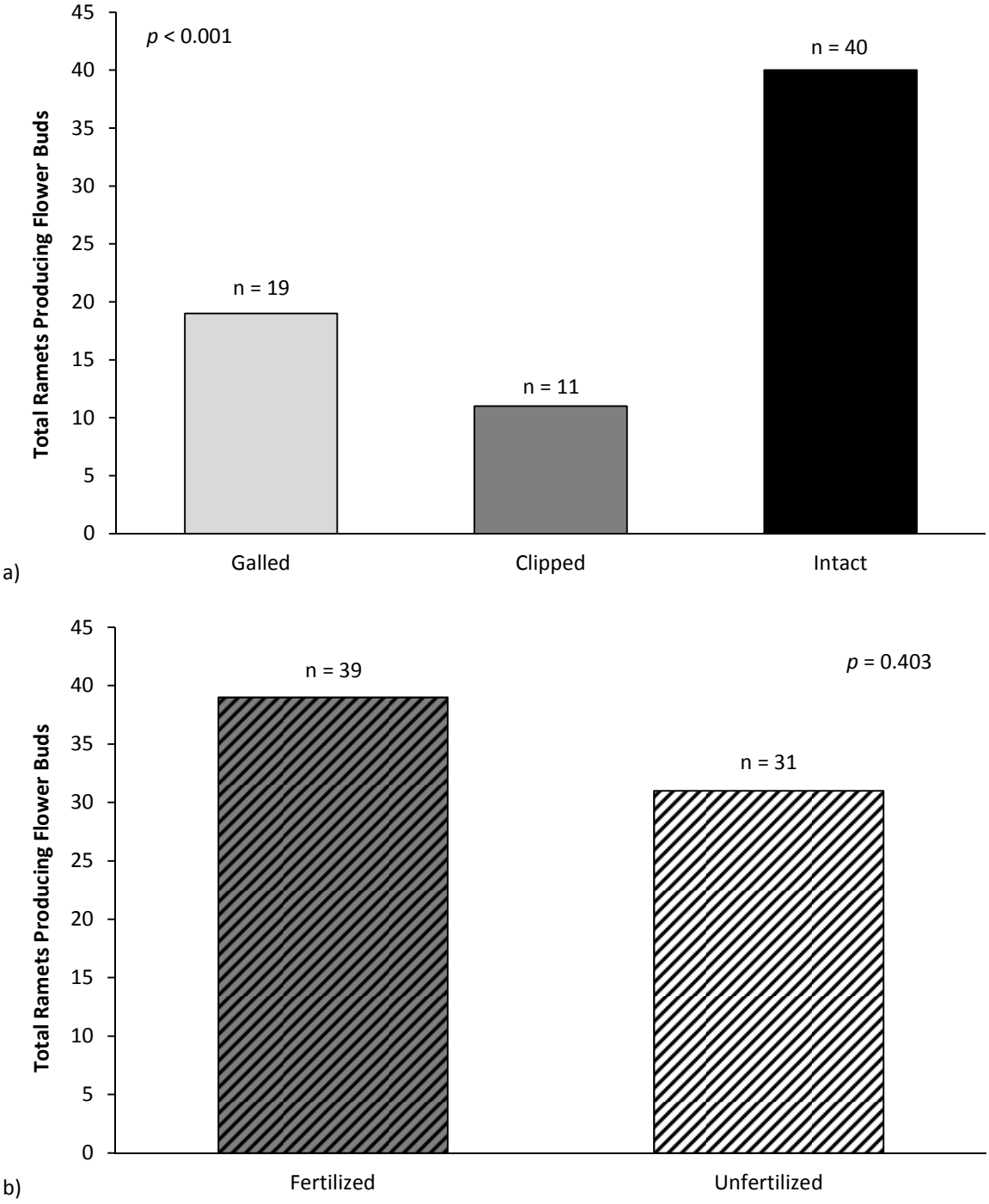


Figure 47. Total number of ramets producing flower buds across (a) stem treatments (X^2 = 19.229, *df* = 2, *p* < 0.001) and (b) fertilization treatments (X^2 = 0.700, *df* = 1, *p* = 0.403) in the stem-nutrition experiment. Includes ramets that later died and intact ramets that were galled after flower bud appearance.

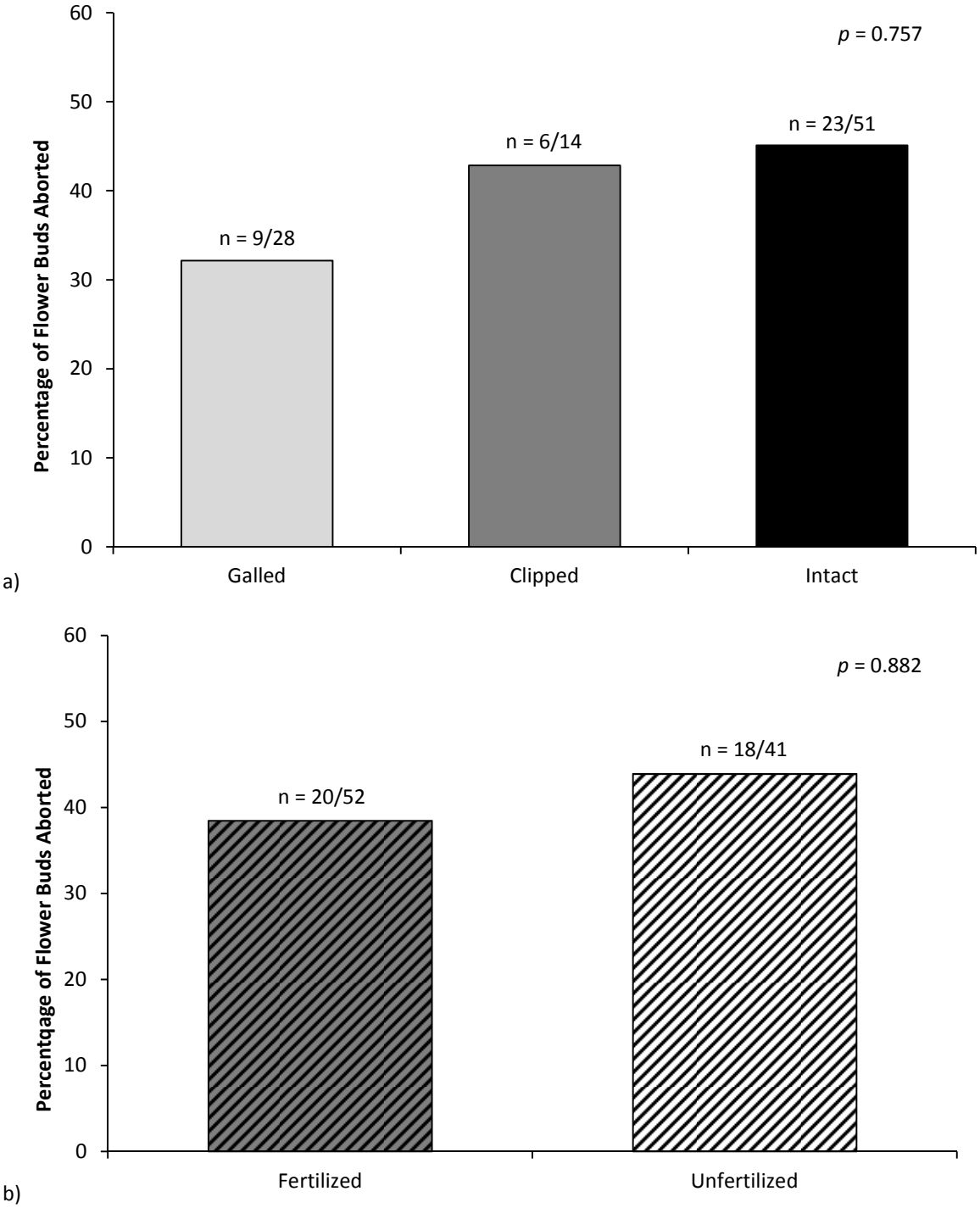


Figure 48. Percentage of flower buds aborted across (a) stem treatments (X^2 = 0.558, *df* = 2, *p* = 0.757) and (b) fertilization treatments (X^2 = 0.022, *df* = 1, *p* = 0.882) in the stem-nutrition experiment. Note: Percent scales range from 0 to 60%.

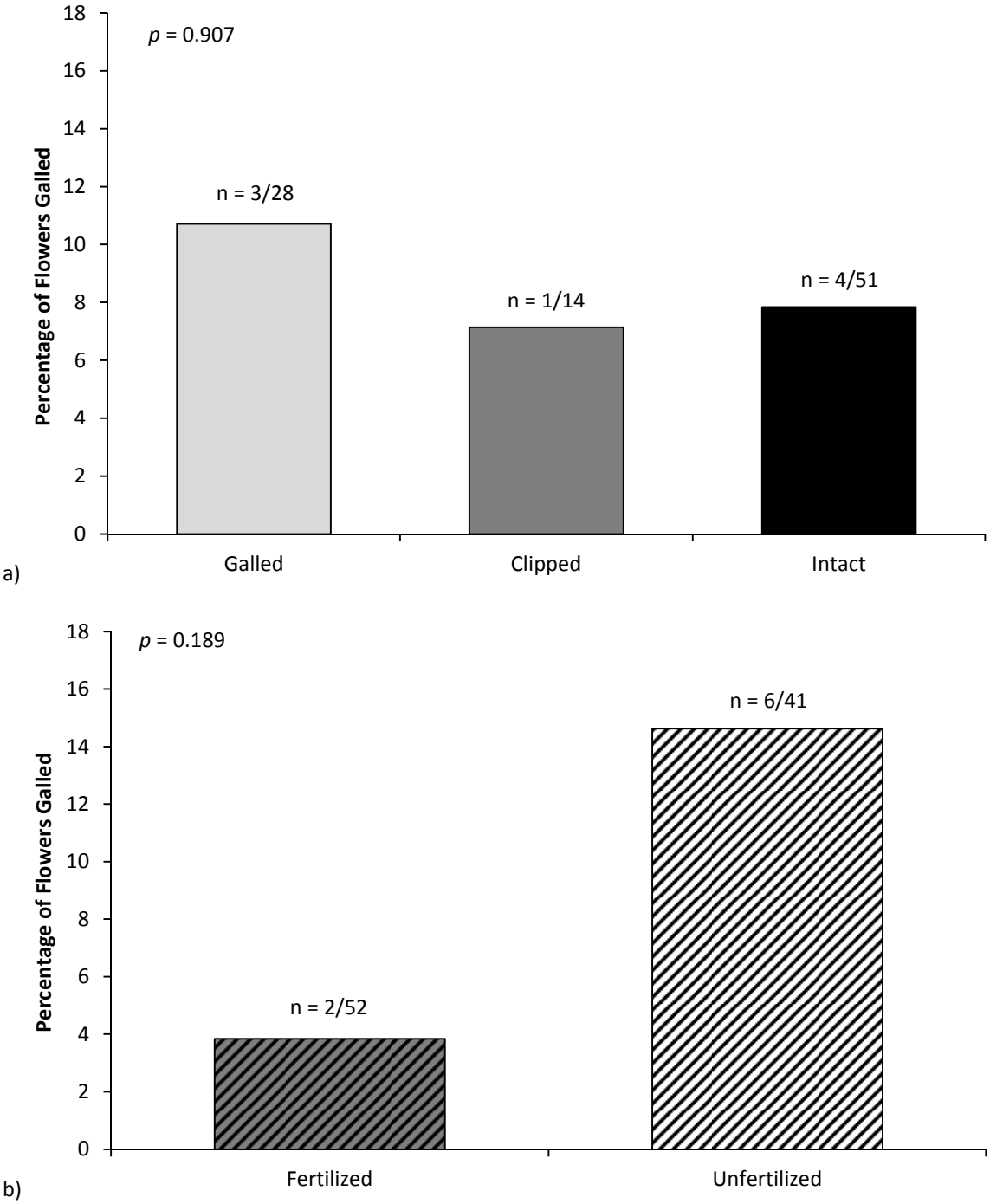
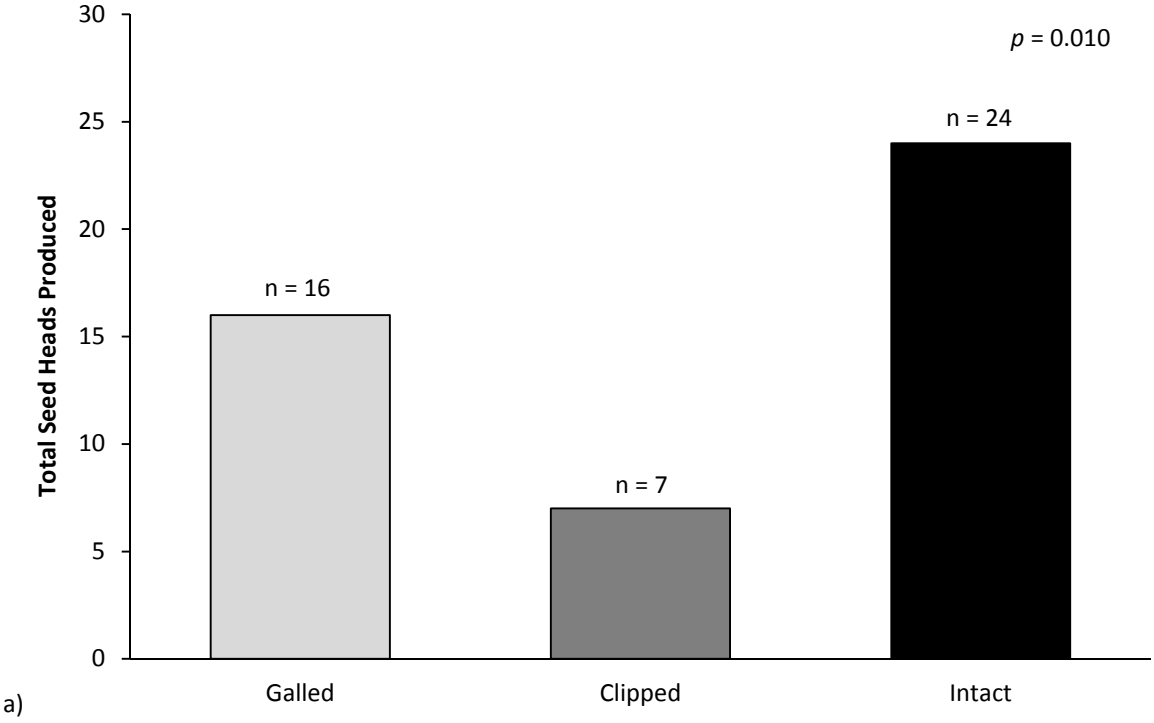
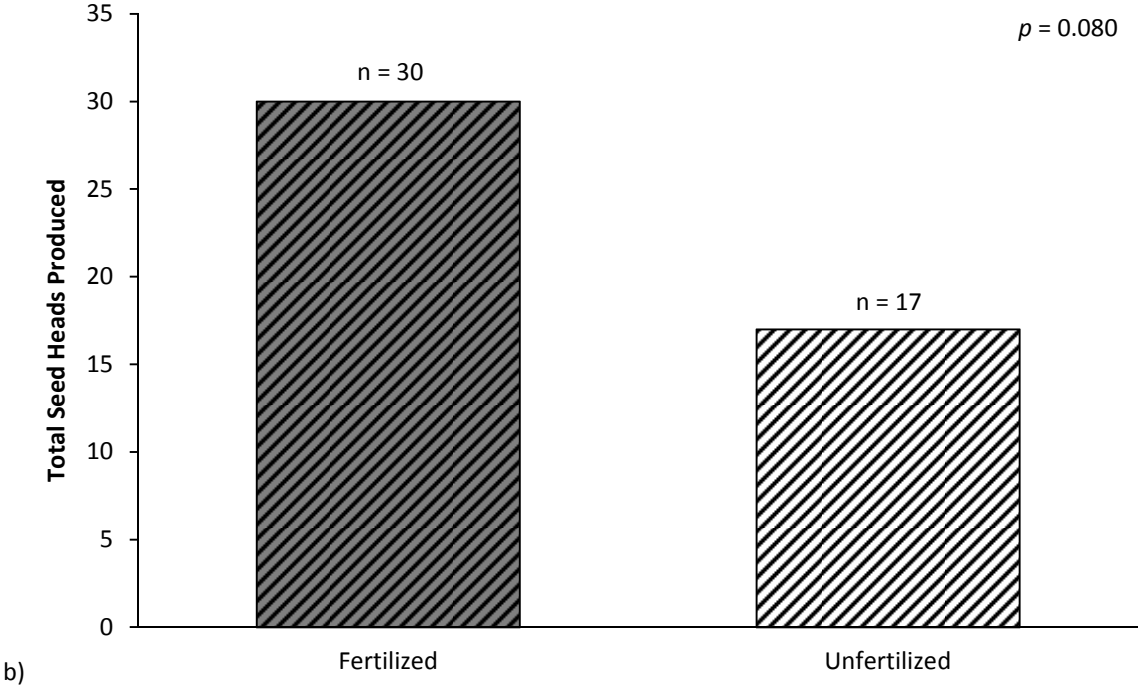


Figure 49. Percentage of flowers or flower buds galled across (a) stem treatments (X^2 = 0.195, *df* = 2, *p* = 0.907) and (b) fertilization treatments (X^2 = 1.723, *df* = 1, *p* = 0.189) in the stem-nutrition experiment. Includes intact ramets that developed flower buds and then were galled, but not intact ramets that were galled before flowering. Note: Percent scales range from 0 to 18%.



a)



b)

Figure 50. Total number of seed heads produced across (a) stem treatments (X^2 = 9.234, *df* = 2, *p* = 0.010) and (b) fertilization treatments (X^2 = 3.064, *df* = 1, *p* = 0.080) in the stem-nutrition experiment.

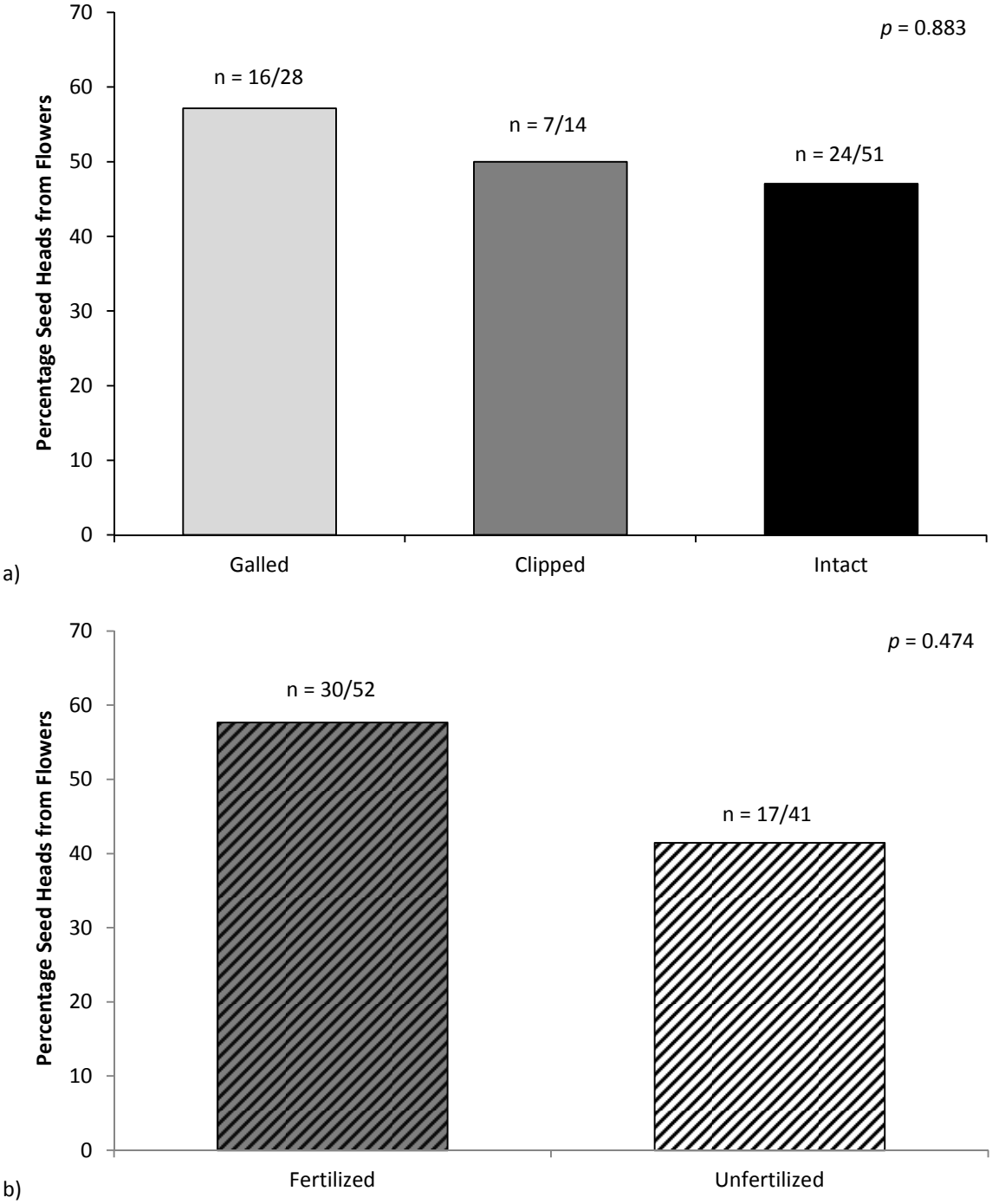
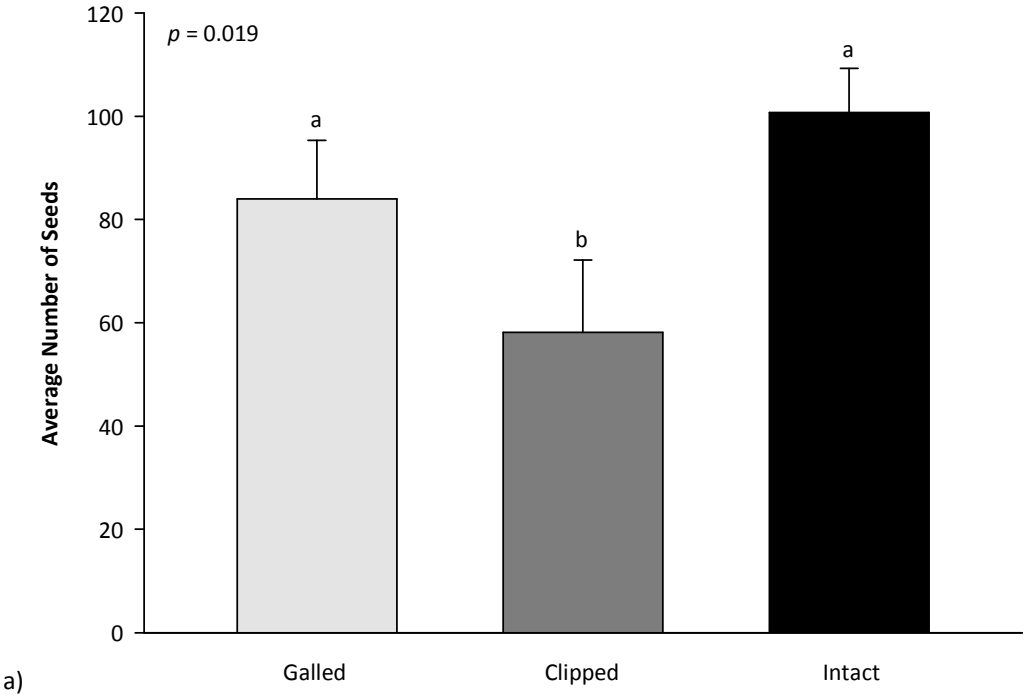
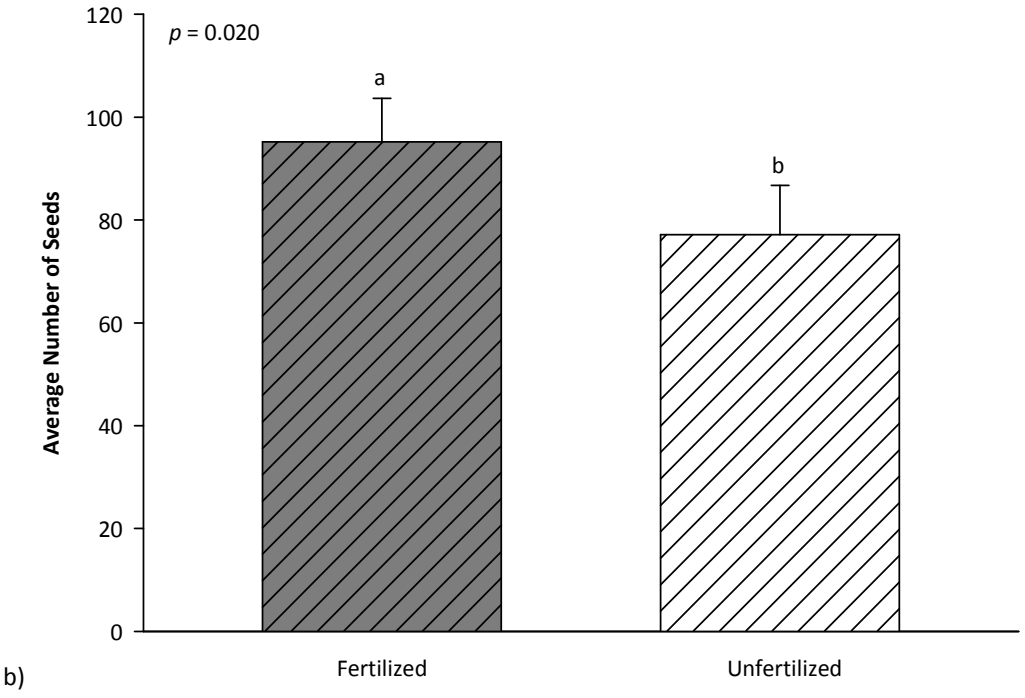


Figure 51. Percentage of seed heads produced from flowers across (a) stem treatments (X^2 = 0.237, *df* = 2, *p* = 0.883) and (b) fertilization treatments (X^2 = 0.513, *df* = 1, *p* = 0.474) in the stem-nutrition experiment. Note: Percent scales range from 0 to 70%.



a)



b)

Figure 52. Average seed count with SEM error bars across (a) stem treatments and (b) fertilization treatments in the stem-nutrition experiment (stem treatment: $F_{2, 47} = 4.370$, $p = 0.019$; fertilization treatment: $F_{1, 47} = 5.881$, $p = 0.020$; stem*nutrition: $F_{2, 47} = 1.223$, $p = 0.305$; covariate: $F_{1, 47} = 2.941$, $p = 0.094$).

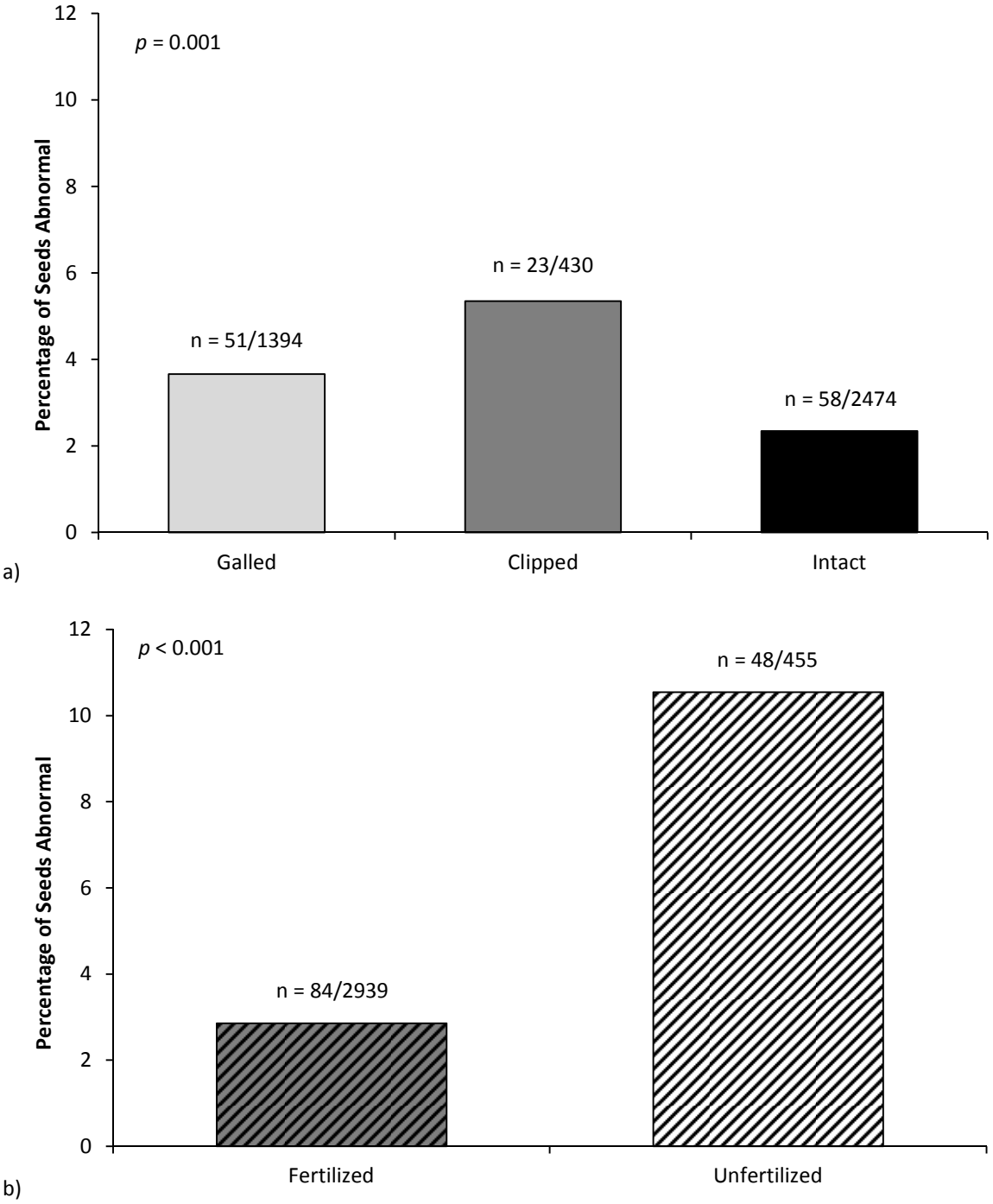
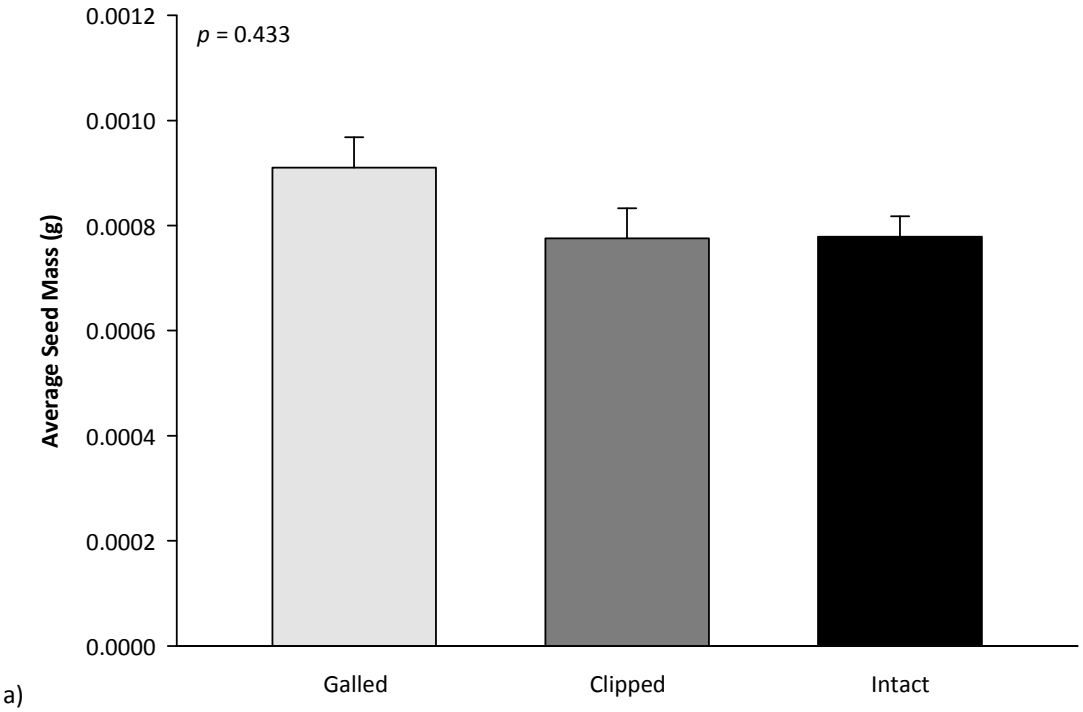
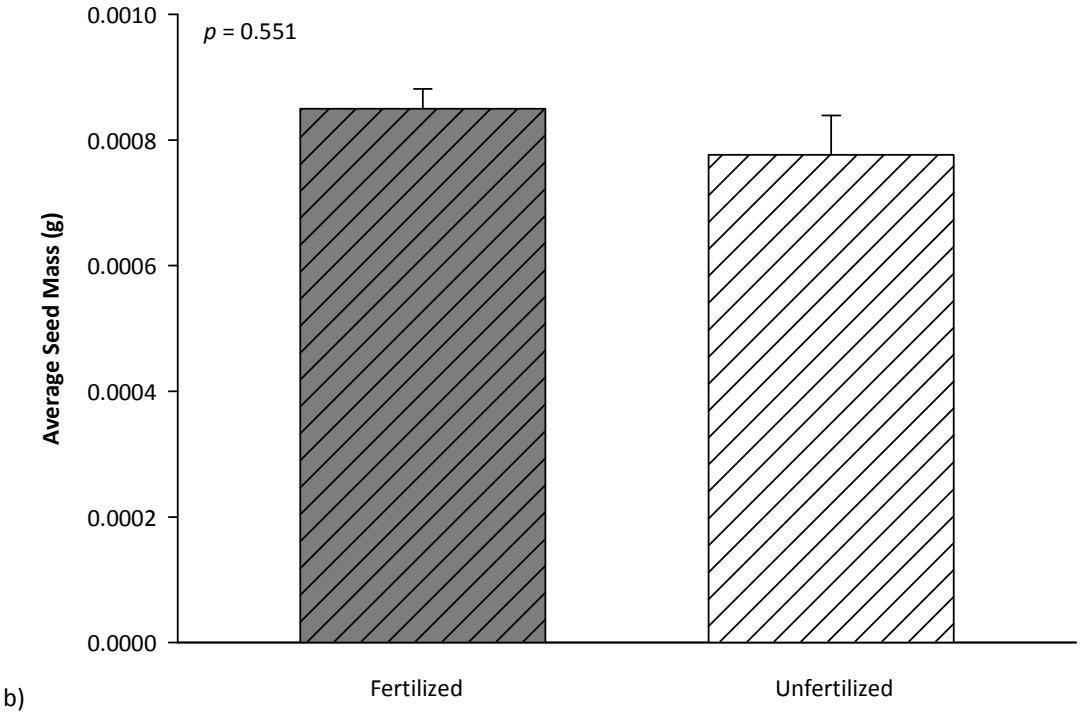


Figure 53. Percentage of seeds that developed abnormally across (a) stem treatments (X^2 = 13.499, *df* = 2, *p* = 0.001) and (b) fertilization treatments (X^2 = 60.314, *df* = 1, *p* < 0.001) in the stem-nutrition experiment. Chi-square was performed on abnormal vs. normal seeds, but percentage of total seeds that were abnormal is shown for clarity. Note: Percent scales range from 0 to 12%.



a)



b)

Figure 54. Average seed mass (g) with SEM error bars across (a) stem treatments and (b) fertilization treatments in the stem-nutrition experiment (stem treatment: $F_{2, 47} = 0.856$, $p = 0.433$; fertilization treatment: $F_{1, 47} = 0.362$, $p = 0.551$; stem*nutrition: $F_{2, 47} = 0.060$, $p = 0.942$; covariate: $F_{1, 47} = 1.595$, $p = 0.214$).

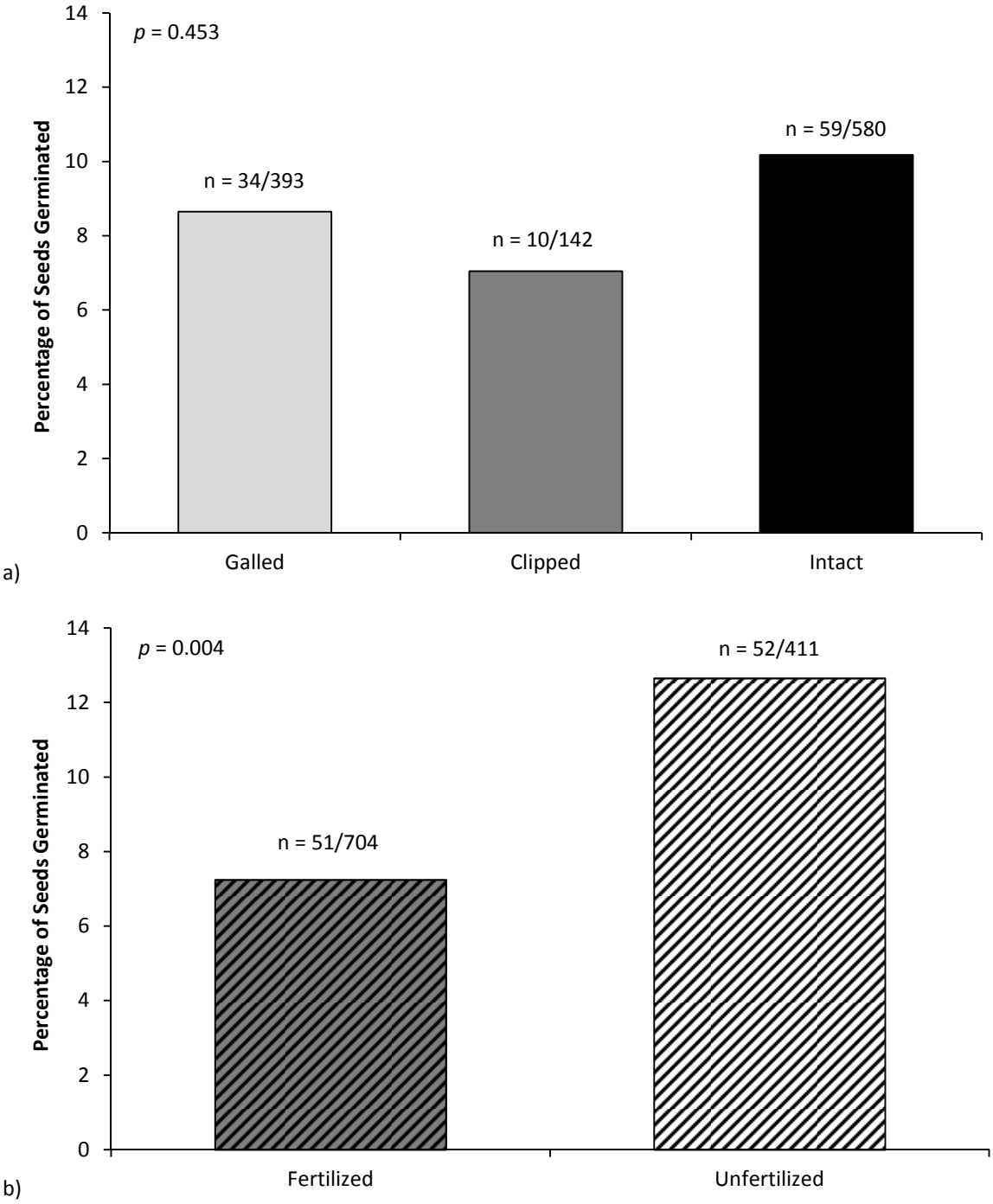


Figure 55. Percentage of seeds germinated across (a) stem treatments (X^2 = 1.582, *df* = 2, *p* = 0.453) and (b) fertilization treatments (X^2 = 8.418, *df* = 1, *p* = 0.004) in the stem-nutrition experiment. Chi-square was performed on total germinations vs. non-germinations, but percentage of planted seeds that germinated is shown for clarity. Note: Percent scales range from 0 to 14%.

a)

b)

Figure 56. *Borrichia frutescens* in (a) early January and (b) late May at the study site. Note that foliage is sparse in January and denser in May, possibly resulting in less intense light competition for short ramets early in the growing season and more competition for short ramets later. Courtesy: Gary Wood

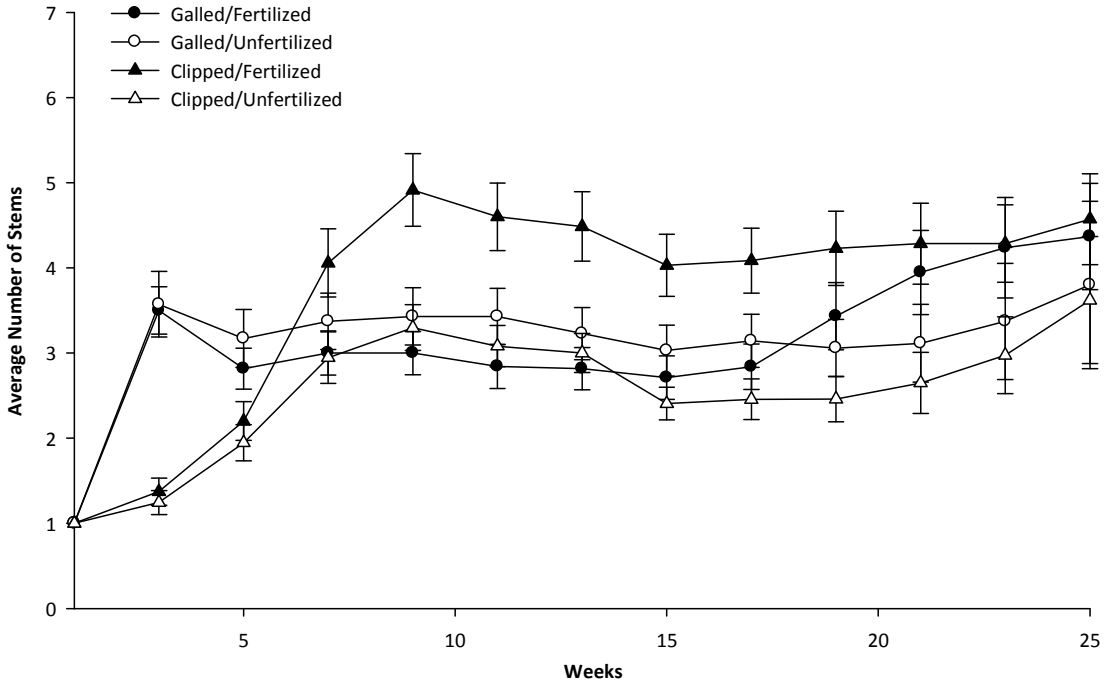


Figure 57. Average number of stems in galled and clipped stem treatments only, depicted over 25 weeks in the stem-nutrition experiment. Note that both galled treatment groups experienced an immediate increase in stems, while the clipped groups were slow to break bud.

Appendix C: Tables

Table 1. Hypotheses for apical dominance, which results in tall or long, spindly growth forms.

Hypothesis	**Explanation**	**Reference**
Light Competition	Plants near or above canopy capture more light, shade shorter neighbors	Salisbury and Ross 1991; Aarssen and Irwin 1991; Aarssen 1995; Irwin and Aarssen 1996
Light Interception	Vertical forms maximize capture of angled light rays at higher latitudes	Aarssen 1995
Effective Pollination	Taller plants more accessible to pollinators or wind	Aarssen 1995
Effective Dispersal	Seeds dispersed over greater distances from tall plants	Aarssen 1995
Phalanx Efficiency	In clumped/tufted forms, minimizes competition between plant's segments	Aarssen 1995
Guerilla Efficiency	In prostrate forms, minimizes competition between plant's horizontal shoots or runners	Aarssen 1995; Bonser and Aarssen 1996
Reserve Meristem	Holds meristems in reserve for recovery from damage	Aarssen 1995

Table 2. Statistical tests and results related to effects of mesh bags on bagged controls vs. unbagged controls in the stem-nutrition experiment.

Factor Tested	Methods	Results
Survivorship at 25 weeks	X^2 test w/ Yates' correction	$X^2 = 3.434$, $df = 1$, $p > 0.05$
Overall percent change in height	Added 10, square-root transformed, then one-way ANOVA	$F_{1, 33} = 0.006$, $p = 0.936$
Average weekly change in height (cm)	One-way ANOVA	$F_{1, 34} = 1.692$, $p = 0.203$
Total stem count at week 25	X^2 test w/ Yates' correction	$X^2 = 3.430$, $df = 1$, $p > 0.05$
Max stem count	X^2 test w/ Yates' correction	$X^2 = 3.425$, $df = 1$, $p > 0.05$
Seed head count	X^2 test w/ Yates' correction	$X^2 = 0.000$, $df = 1$, $p > 0.05$
Plants with flower heads	X^2 test w/ Yates' correction	$X^2 = 0.121$, $df = 1$, $p > 0.05$
Seed count	Kruskal-Wallis test*	$X^2 = 0.117$, $df = 1$, $p = 0.732$
Seed mass	Kruskal-Wallis test*	$X^2 = 2.922$, $df = 1$, $p = 0.087$
Seed germinations	X^2 test w/ Yates' correction	$X^2 = 0.089$, $df = 1$, $p > 0.05$
Number of days to first flowering (all ramets)	Kruskal-Wallis test*	$X^2 = 0.086$, $df = 1$, $p = 0.769$
Number of days to first flowering (flowering ramets only)	Kruskal-Wallis test*	$X^2 = 3.521$, $df = 1$, $p = 0.061$
Number of days as flower bud	Kruskal-Wallis test*	$X^2 = 1.102$, $df = 1$, $p = 0.294$
Number of days blooming	Kruskal-Wallis test*	$X^2 = 1.482$, $df = 1$, $p = 0.223$
Number of days ripening	Kruskal-Wallis test*	$X^2 = 0.223$, $df = 1$, $p = 0.637$

*Kruskal-Wallis was used because of difficulties achieving normality and/or homogeneity of variances due to very small sample sizes for bagged control ramets (n = 2 to n = 8). Results should be viewed with caution.

Table 3. Results of Poisson distribution analyses of maximum stem count and stem count at the end of the experiment (week 25) performed on all stem and fertilization treatment groups.

Treatment	Results of Poisson Distribution Analysis (X^2 Tests)	
	Maximum Stem Count	**Stem Count at End of Experiment**
Galled	X^2 = 1.015, *df* = 13, *p* > 0.05	X^2 = 1.24, *df* = 9, *p* > 0.05
Clipped	X^2 = 0.441, *df* = 12, *p* > 0.05	X^2 = 2.20, *df* = 10, *p* > 0.05
Intact	X^2 = 2.317, *df* = 12, *p* > 0.05	X^2 = 2.14, *df* = 11, *p* > 0.05
Fertilized	X^2 = 0.451, *df* = 14, *p* > 0.05	X^2 = 2.34, *df* = 12, *p* > 0.05
Unfertilized	X^2 = 0.161, *df* = 11, *p* > 0.05	X^2 = 0.800, *df* = 8, *p* > 0.05

Table 4. Percentages of flowering ramets in each stem-fertilization treatment group and their height class (cm) distribution at first flowering. Sample sizes were too small (n < 6) to make reliable statistical inferences.

Height Classes (cm)	Galled/ Fertilized	Galled/ Unfertilized	Clipped/ Fertilized	Clipped/ Unfertilized	Intact/ Fertilized	Intact/ Unfertilized (Control)
20.1-30.0	0%	0%	0%	0%	0%	6%
30.1-40.0	0%	0%	0%	17%	0%	22%
40.1-50.0	17%	14%	0%	50%	17%	17%
50.1-60.0	42%	29%	40%	17%	42%	22%
60.1-70.0	8%	0%	20%	17%	8%	17%
70.1-80.0	25%	29%	20%	0%	25%	11%
80.1-90.0	8%	14%	0%	0%	8%	0%
90.1-100.0	0%	14%	0%	0%	0%	6%
100.1-110.0	0%	0%	20%	0%	0%	0%
Total	100%	100%	100%	100%	100%	100%

Table 5. The number of flower buds identified at certain dates between 3/21 and 6/25/2013. Note that peak flowering occurred between mid-April and mid-May.

Date	**Number of New Flower Buds Identified**
3/21/2013	1
4/2/2013	6
4/16/2013	16
4/30/2013	27
5/14/2013	18
5/28/2013	11
6/11/2013	7
6/25/2013	7

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- **Documentation Specialist, Instructional Designer**
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- **Technical Writer**
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- **Technical Writer**
  Inet Technologies, Inc. – Richardson, TX — October 1998 to April 2001
- **Technical Writer**
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    - Timucuan Science and History Symposium, Jacksonville, FL, January 2014

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