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Effects of abiotic factors on host plant quality and community structure of the parasitoid guild that attacks *Asphondylia borrichiae* midges on *Borrchia frutescens*

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EFFECTS OF ABIOTIC FACTORS ON HOST PLANT QUALITY AND COMMUNITY
STRUCTURE OF THE PARASITOID GUILD THAT ATTACKS *ASPHONDYLIA*
BORRICHIAE MIDGES ON *BORRICHIA FRUTESCENS*

by

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A thesis submitted to the Department of Biology
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This thesis titled Effects of Abiotic Factors on Host Plant Quality and Community Structure of the Parasitoid Guild that Attacks *Asphondylia borrichiae* Midges on *Borrchia frutescens* is approved:

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ABSTRACT

Asphondylia borrichiae is a gall-inducing insect that attacks its ancestral host plant *Borrichia frutescens*: through ovipositional mistakes it has acquired two additional hosts, *Iva frutescens* and *Iva imbricata*. Oviposition results in the formation of a gall, a tumor-like growth of plant tissue within which immature *A. borrichiae* develop. During development, immature *A. borrichiae*, are attacked by a suite of four generalist hymenopteran parasitoids. Of these, *Galeopsomyia haemon* and *Torymus umbilicatus* are facultative hyperparasitoids, more common and exhibit non-random attack patterns. In the present study, soil quality was manipulated through bi-weekly application of either ammonium nitrate fertilizer or sodium chloride rock salt: resulting in variation in host plant quality. Bottom-up manipulation cascaded upwards through trophic levels and indirectly shifted the composition of the parasitoid guild community. Decreases in host plant quality significantly decreased available leaves (64%), reduced stem growth (17%) and limited growth of the plant and galls when compared to vigorous, higher quality plants. Galls produced from salt-treated plots had significantly lower gall diameter (20%), fewer gall chambers (30%) and significantly increased attacks by *G. haemon* when compared to control galls (56%). Increasing plant quality significantly increased total leaf size (21%), retained more leaves, and increased growth of the host plant when contrasted with poorer quality plants. Fertilized plants produced galls with significantly greater gall diameter with increased number of *T. umbilicatus* when compared to controls (12%). Results support the gall-diameter hypothesis as present parasitoids were distributed across gall diameter. This study was successful in changing the natural enemies present within a system through indirect effects of soil quality, these bottom-up effects could potentially shape future top-down control by the parasitoids. Specifically, in instances of potential host-range expansion in which parasitoid

composition as mediated by lower trophic levels can either increase or decrease the availability of enemy free space.

INTRODUCTION

For many years ecologists have argued over top-down vs bottom-up control as the dominant mechanism in shaping populations as well as community structure. Proponents of bottom-up control such as Ohgushi and Sawada (1985), argued that local populations of lady beetles *Henosepilachna niponica* (Lewis), were density-dependent and stable during periods of abundance of their host plants. The implicit importance of bottom-up regulation led Schultz (1988) to argue in favor of increasing studies of plant chemistry and its impact on herbivore diets. Over time, disparities between the role of density dependent vs quality in shaping communities further widened the divide in proponents for bottom up control. Root (1973) concluded that density-dependent bottom-up factors were the driving force for the organization of plant-arthropod associations in simple and diverse habitats. Conversely, others supported the importance of quality as the driving force for regulation of herbivores in which selection of host plant quality in maximization of fitness was crucial in shaping communities (White, 1978; White, 1984; Price, 1991). Oppositely, proponents had gathered in support of regulation of herbivores in which natural enemies (i.e. top-down) control had a far-greater impact than any bottom up effects. Instances in which the removal of a keystone predator resulted in the unchecked growth of prey, and their potential and/or realized ramifications are well-known and well documented (see Hairston *et al.*, 1960; Paine, 1969; Ripple & Beschta, 2004). Yet, measures of top-down control do not need to reach the levels of a keystone species but may simply alter or shift the trophic levels beneath them. (Hairston *et al.*, 1960; Stiling *et al.*, 1992; Moon & Stiling, 2004). According to Lawton and Strong (1981) natural enemies in conjunction with autecological factors (species' response to the threat of natural enemies) are reasons behind shifts in community structures as opposed to the established notion of natural enemies culling the

spread of their prey. Thus, study of multi-trophic level interactions has resulted in a more holistic approach rivaling and revealing limitations of previously established dichotomous thinking regarding factors that regulate populations and shape communities. For instance, Hunter and Price (1992) proposed that populations and communities are influenced by a host of biotic and abiotic factors, and the classical models of bottom-up and top-down regulation must be utilized in an integrative framework to fully understand community structure. Multi-trophic level studies recognize the need for investigation of both direct and indirect effects that may occur within a food web and the importance of interactions between levels. Plants form the macrobiotic basis of most terrestrial food webs and plant heterogeneity acts as the foundation upon which the resulting trophic levels are built upon. Therefore, shifts in abiotic factors such as soil nutrients may have cascading effects that influence top-down regulation.

If abiotic factors do influence both bottom-up and top-down control, then an already stressed natural system that exhibits these multi-trophic interactions would be a viable source for further study. Modification of plant quality or heterogeneity caused by shifts in abiotic factors could therefore alter interactions between the plant, its herbivores, their pathogens and even natural enemies of the herbivores. Salt marshes have been important ecosystems for studying plant-herbivore (especially insects) interactions because of their heterogeneity in nitrogen and phosphorous compared to other terrestrial ecosystems (Pigott, 1969; Valiela & Teal, 1974; Kiehl *et al.*, 1997; see Van Wijnen & Bakker, 2001). Moreover, salt marshes, which typically exhibit a range of tidal variations, also experience a wide range of salinity, in turn, leading to further changes in heterogeneity. Plants, vary in their responses to salt stress and osmoregulation, potentially adding further abiotic influences on higher trophic levels (see Antlfinger, 1981; Munns, 2002; Richards *et al.*, 2010). Recent changes in rising sea levels in conjunction with

increases in practical salinity units(p.s.u.) in the Atlantic Ocean could likely present with another potential abiotic factor to help shape coastal salt marsh communities (Curry *et al.*, 2003; see Beckett *et al.*, 2016). Changes in tidal regimes or salinity could interact with plant quality or heterogeneity through complex interactions; for instance, availability and uptake of nitrogen may be reduced by plants under highly saline conditions in their effort to prevent or reduce salt uptake (Jefferies & Perkins, 1977; Osgood & Zieman, 1993; Bowdish & Stiling, 1998).

Measures of plant quality can be expressed in a myriad of ways; including growth rates, C:N ratios, leaf symmetry, etc. (Bagchi *et al.*, 1989; Møller & Swaddle, 1998; Møller & Elm, 1999; Cornelissen & Stiling, 2005). Plants of higher quality exhibit greater and faster growth maintain higher levels of nitrogen, lower C:N ratios, maintain more leaves and, interestingly, may overcompensate for growth lost by herbivores (Coley *et al.*, 1985; Price, 1991). For example, Maschinski and Whitham (1989) reported that *Ipomopsis arizonica* (Greene) Wherry that received nutrient supplementation and were subsequently attacked by herbivores (rodents and ungulates) overcompensated for the damage and produced greater overall biomass through increased fruit and seed production. Incidences of grazing by a snow goose *Anser caerulescens caerulescens* L. reportedly increased the net above-ground primary production of vegetation within a tundra salt marsh (Cargill & Jefferies, 1984). The beneficial effects of some limited types of herbivory on vigorously growing plants may also result in higher regrowth. For example, the biennial, scarlet gilia *Ipomopsis aggregata* (Pursh) V.E. Grant, reportedly increased their relative fitness 2.4-fold upon instances of mammalian herbivory over non-grazed scarlet gilia (Paige & Whitham, 1987). Furthermore, in the salt-marsh halophyte, *Borrchia frutescens* (L.) de Candolle (Asteraceae), exhibited faster growth of stems after attack on their apical meristems by the gall midge, *Asphondylia borrichiae* Rossi and Strong (Diptera:Cecidomyiidae)

(Spirko & Rossi, 2015). Alternatively, plants experiencing stress, tend to exhibit stunted growth, produce fewer leaves and exhibit greater rates of leaf senescence. Internally, stress in plants is often characterized by the breakdown and mobilization of soluble nitrogen away from impacted or damaged sites, and exhibit higher levels of leaf asymmetry (Jefferies & Perkins, 1977; White, 1984; Bagchi *et al.*, 1989; Osgood & Zieman, 1993; Bowdish & Stiling, 1998; Møller & Elm, 1999).

Plant quality can affect both attractiveness or suitability of the plant to herbivores and, as a result, bottom-up factors typically have a strong influence of herbivore community composition. Interestingly, phytophagous insects have been shown to favor both plants of poor quality (i.e. stressed or damaged), or conversely, those of high quality (i.e. nitrogen-rich, vigorously growing) (White, 1978; White, 1984; Price, 1991) Stressed plants may increase the abundance of soluble nitrogen, especially as it is transported away from stressed or senescing tissue (Beever, 1976; Hill, 1980; Stewart & Larher, 1980; see White, 1984) making more nitrogen available for herbivore growth (White, 1969). One such example, a scale insect *Icerya seychellarum* Maskell appeared to infest an evergreen bush *Scaevola taccada* (Gaertner) and preferentially fed on their weakened senescent leaves. As the infestation grew, coccids further reduced the growth of leaves and increased the likelihood of leaf senescence. Thus, initiating a negative feedback loop in which increasing coccid numbers further inhibited biomass production and decreased the quality of the plant (Newbery, 1980). Price (1991) provided evidence of insects that prefer to feed on high quality plants; for example forest insects appeared to preferentially attack younger more vigorous trees (Craighead, 1950), instances of heavy herbivory by invertebrates on vigorously growing plant species (Coley *et al.*, 1985), and preference by galling insects to attack larger than average plant modules (see Bernays, 1990). Due to the complex intimate association with their

host plants, galling insects provide ideal systems for studying multi-trophic level interactions (Craig *et al.*, 1986; Price & Clancy, 1986; Roininen *et al.*, 1988; Stiling & Rossi, 1997; Moon & Stiling, 2002). These plant-galler systems exhibit non-random attacks by the galling insect and/or their natural enemies. Two such examples, which both involve stem-galling sawflies, *Euura lasiolepis* Smith (Hymenoptera: Tenthredinidae) and *Euura mucronata* (Hartig), preferred to attack long rapidly growing shoots across both species of their respective plant hosts *Salix lasiolepis* Benth (Salicaceae) and *Salix cinerea* L. (Price & Clancy, 1986; Roininen *et al.*, 1988); this preference for long rapidly growing shoots has provided them with a temporary means of escaping their natural enemies (in this instance parasitoids).

Although galls are a product of the host plant, they represent an interaction between the insect and their host (i.e. interaction between an herbivore and the plant). Galls are exclusively plant tissue, and gall induction involves an interaction that results from a signal or stimulus produced by the insect, and subsequent response of the plant (Mani, 1964; Weis & Abrahamson, 1985; Weis & Abrahamson, 1986; Raman, 2011; see Miller & Raman, 2019). Stimuli for gall induction can vary amongst gall-inducing taxa, although, a common stimulus is an oviposition, eclosion of the egg, and damage caused by first-instar larval feeding on plant tissue, which results in a wounding response by the plant and eventual gall development (Rohfritsch, 1992; see Raman, 2011; Miller & Raman, 2019). Gall-inducing insects continue to develop and feed from within the gall, although, some members of the tribe Asphondyliini within the family Cecidomyiidae feed upon fungal conidia deposited during the initial ovipositional event (Rohfritsch, 2008; Tokuda, 2012). Gall shape or structure is partially directed by the gall-inducing insect, with the shape and form believed to represent an extended phenotype of the insect as expressed through the plant (Dawkins, 1982; Tokuda, 2012; Miller & Raman, 2019).

However, because the gall tissue is derived from the plant, expansion and growth of the gall (i.e. size) is entirely dependent on the quality of the plant as galls act as physiological sinks drawing in resources from the supporting tissues near the galled site. A study by Larson and Whitham followed the movement of ^{14}C radiocarbons and discovered that gall-inducing aphids *Pemphigus betae* Doane (Hemiptera: Aphididae) acted as non-mobilizing sinks and regularly intercepted carbons flowing from plant source tissue (Larson & Whitham, 1991). If galls act as a physiological sink and draw upon the resources of the host plant upon which they are developing, it would stand to reason that galls developing on higher quality plants will produce larger galls of higher quality.

Due to the highly specific nature of these plant-insect interactions it has been suggested that gall-inducing insects exhibit some manner of host-plant selection (see Miller & Raman, 2019). Variations in galling characteristics such as thickness, overall diameter, and within gall crowding have all been purported to increase or decrease the fitness of the developing gall-inducer. For instance, Rossi *et al.* (2001) found that larger, less crowded galls produced significantly larger *A. borrichiae* that had a higher potential fitness (i.e. more eggs of similar size at emergence) compared to midges from smaller galls. Weis and Abrahamson (1985) suggested that interactions between host plant, gall-maker and natural enemies (i.e. birds and parasitoids) have led to potential stabilizing selection. This is because the gall-inducer *Eurosta solidaginis* Cocquillet (Diptera: Tephritidae) appeared to suffer from higher avian predation in large galls while smaller galls were more likely to suffer from increased rates of attack by a parasitoid wasp *Eurytoma gigantea* Walsh (Hymenoptera: Eurytomidae). Gall size and shape may be a potentially heritable trait in gall-inducers as well as mitigating attacks by the natural enemies within the system. Gall-diameter may be a primary determinant in its suitability for oviposition.

For instance Price and Clancy (1986) reported that for another gall-inducing sawfly *E. lasiolepis*, the *Pteromalus* sp. (Hymenoptera: Pteromalidae) parasitoid that attacks it were actively searching for and attacking smaller galls, because they have relatively short ovipositors and have difficulty attacking larvae in large galls on the arroyo willow *S. lasiolepis* (Price & Clancy, 1986).

Asphondylia borrichiae is a small mosquito-sized gall-inducing insect of varying shades of brown with black eyes. Like other members of the supertribe *Asphondyliidi*, *A. borrichiae* is an ambrosia galler; that is, oviposition by the female results in a fungal-lined gall within which the immature stages of the midge feed and develop. Female *A. borrichiae* alight on the apical meristems of their host plants *B. frutescens* where they oviposit near the meristematic tissue, and deposit fungal conidia. Unlike other gall-inducers, it is believed that a combination of attacks by the developing gall midge, coupled with expansive growth of fungal hyphae cause the induction of the gall (Rohfritsch, 2008). These galls lack nutritive tissue, instead immature stages of *A. borrichiae* feed on the fungal mycelium that bear cytochemical features of typical nutritive tissue (Meyer, 1987; Bronner, 1992; Rohfritsch, 2008). *Asphondylia borrichiae* possess a membranous sac or mycangium located dorsally on the seventh sternite (Borkent & Bissett, 1985) used to house and transport the symbiotic fungal conidia. As previously mentioned, growth of the fungal hyphae causes the meristematic tissue to become hyperactive, resulting in the formation of a tumor-like gall. Most of *A. borrichiae*'s life cycle (approximately 95%) occurs within the gall, the midge undergoes several larval instars until developing into a pupa (Gagné, 1989; Rossi & Stiling, 1995). At the conclusion of the pupal stage, mature pupae use a pair of ventrally curved antennal horns to bore through the gall (Gagné, 1989). Spines present along the length of the puparium anchor it to the gall, allowing for emergence by the imago along the anterior of the

puparium. The spent puparium is oft found attached to the gall alongside a ragged emergence hole (Stiling & Rossi, 1997). The life cycle within the gall averages five to nine weeks (depending upon host plant and season), with the imago life cycle lasting 24-48 hours (Gagné, 1989; Stiling *et al.*, 1992). Should the shed puparium be dislodged from the surface of the gall, emergences leave behind a significantly ragged exit hole and can be used to determine the successful development and subsequent emergence of a midge. Galls senesce within one to two weeks (Stiling *et al.*, 1992) after successful insect emergence, which typically kills the apical meristem of *B. frutescens*.

Galls induced by *A. borrichiae* are largely spherical in structure (Stiling *et al.*, 1992; Craig, 1994; Stiling & Rossi, 1997; Rossi *et al.*, 2001; Rossi *et al.*, 2006) and may be found year-round because *A. borrichiae* is a multivoltine insect and can have several overlapping generations in a year (Stiling & Rossi, 1994), with peak galling events during the summer months of May through August (Rossi & Stiling, 1995). Galls normally contain on average one to four chambers, within which, a single midge larva develops (Rossi *et al.*, 2001). Natural enemies (e.g. the guild of parasitoids) are the primary source of mortality for juvenile midges with insignificant events by bird predation. During gall development, immature stages of *A. borrichiae* are attacked by a guild of four generalist hymenopteran parasitoids. The parasitoid guild or community includes *Rileya cecidomyiae* Ashmead (Hymenoptera: Eurytomidae), *Tenuipetiolus teredon* (Walker)(Hymenoptera: Eurytomidae), *Galeopsomyia haemon* (Walker)(Hymenoptera: Eulophidae), and *Torymus umbilicatus* (Gahan)(Hymenoptera: Torymidae). All four species of parasitoids use their ovipositors to penetrate the gall and parasitize the developing midge larvae and pupae. Rates of parasitism have been reported to

reach 100% causing temporary local extinction events of *A. borrichiae* (Stiling *et al.*, 1992; Stiling & Moon, 2005).

Two guild members *G. haemon* and *T. umbilicatus* are of note because in addition to attacking immature stages of *A. borrichiae* they are facultatively hyperparasitic, that is, they may also parasitize other members of the parasitoid guild. Studies conducted within this system (Stiling & Rossi, 1994; Rossi & Stiling, 1998; Rossi *et al.*, 2006) suggested non-random distributions of parasitoids across gall size classes and stem lengths. Incidentally, during a bagging study, *T. umbilicatus* the largest parasitoid, represented 75% parasitism events as galls matured and grew, with a mean gall diameter of 0.707cm (Stiling & Rossi, 1994). These parasitism events increased to 100% after 21 days of development with a mean diameter of 0.97cm. Due to its significantly longer ovipositor (a 2-fold increase), it appears as though *T. umbilicatus* has a selective advantage over other guild members in larger galls. This advantage is two-fold, larger and older galls have larger more developed hosts and second, *T. umbilicatus* can reach larval chambers that are inaccessible to the other parasitoid species (Rossi *et al.*, 2001; Rossi *et al.*, 2006). Conversely, the smallest member of the parasitoid guild, *G. haemon*, is gregarious, parasitism by *G. haemon* typically involves multiple larvae/pupae found within the same gall chamber; no other member of the parasitoid guild is gregarious (Stiling *et al.*, 1992). As a result, *G. haemon* can therefore overwhelm their host and/or competitors in small galls that it can reach with its relatively short ovipositor. In younger smaller gall cohorts, *G. haemon* has been shown to account for more than 50% of parasitism events (Rossi *et al.*, 2006).

Borrichia frutescens is a perennial wildflower with bright yellow flowers commonly referred to as sea oxeye daisy. This coastal halophyte typically grows in warm temperate subtropical coastal habitats extending from Maryland to Florida along the Atlantic Coast and westward

along the Gulf Coast towards Texas and areas in Mexico (Antlfinger, 1981; Stiling *et al.*, 1992; See review on *B. frutescens* Lonard *et al.*, 2015). This coastal halophyte can survive relatively high salinity gradients ranging from 20-130 ppt including growth in salt pans (Antlfinger, 1981; Richards *et al.*, 2004; Richards *et al.*, 2010). *Borrchia frutescens* exhibits both euhalophytic and pseudo-halophytic adaptations to minimize the effects of increasing salinity in the form of leaf succulence and salt ion exclusion from the roots to the shoots respectively (see Antlfinger, 1981; Antlfinger & Dunn, 1983; Munns, 2002; Meng *et al.*, 2018). Salt pans are normally bereft of nitrogen, potassium, phosphorus, and covered in wrack (Drawe *et al.*, 1981; Profitt *et al.*, 2005) and presence in coastal habitats leads to infrequent tidal action events (e.g. storm surges). Sea oxeye daisy is believed to be the host plant for *A. borrichiae* alongside two other members of the Asteraceae family marsh elder *Iva frutescens* (L.; Walter) and dune elder *Iva imbricata* (L.; Walter) (Stiling & Rossi, 1994; although see Clouse, 1995; Rossi & Stiling, 1998; Rossi *et al.*, 2001). Instances of galling events occurring within the two *Iva* species is considerably rarer than *B. frutescens*, with *B. frutescens* widely considered to be the original host due to increased natal host fidelity by emergent gall midges (although see Clouse, 1995; Rossi *et al.*, 2001), and movement unto the two *Iva* species is largely due to ovipositional mistakes. Galls produced on either *Iva* species are significantly smaller than galls formed on *B. frutescens* and confer negative fitness effects on reared midges. This decrease in gall size in conjunction with similar total number of chambers, increases the larval crowding present within both *Iva* species. However, the trade-off in reduced fitness allows for temporary refuge from predation (parasitism) during localized extinction events (Rossi *et al.*, 1994).

It is essential for the survival of *B. frutescens* to mitigate potential damage caused by increasing salinity. *Borrchia frutescens* achieves a modicum of salt tolerance through salt-

induced succulence within the leaves, salt sequestration within the vacuole, as well as the removal of salts within the root system (Antlfinger, 1981; Munns, 2002; Lonard *et al.*, 2015). These however, do not grant immunity to salt stress damage but allows for greater mitigation. A common response to salt compartmentalization within the vacuoles of the leaves, is the synthesis of N-rich compatible solutes such as proline and glycine-betaine (Cavaleiri & Huang, 1979; Antlfinger & Dunn, 1983; Moon & Stiling, 2000; Richards *et al.*, 2010). Although, the accumulation and synthesis of these compatible solutes incurs a heavy energy cost (Raven, 1985). A study by Richards *et al.* 2010 proposed that *B. frutescens* exhibits phenotypic plasticity regarding salinity among local conditions. Therefore, susceptibility to salt-stress among *B. frutescens* appears to be highly dependent upon already present abiotic conditions. Any shifts in abiotic heterogeneity to promote nitrogen uptake, or increase salinity, would likely increase or decrease host plant quality respectively.

This study proposes that changes in abiotic soil conditions within a salt marsh would trigger upwards and/or downwards cascading effects. These effects as exhibited by the tritrophic system of interactions of a host plant (*B. frutescens*), its herbivore (*A. borrichiae*), and the suite of parasitoids that attack them. Manipulation of soil quality (bottom-up influences) through treatment should result in variations in plant quality or heterogeneity. Interactions between quality and successful attacks by the herbivore within the system hereafter galling events, and the characteristics expressed by these produced galls would allow for further investigation of host plant quality on the development of herbivores. Morphometric changes of the galls as regulated by plant quality could in turn cause shifts in availability of attack by the natural enemies of the system as precluded by galling characteristics such as gall diameter. By establishing plant heterogeneity through assessing plant quality, this study monitored the upward

cascade of plant quality and the resulting interactions of herbivores and their natural enemies, specifically, the resulting composition of two hymenopteran hyperparasitoids and their potential top-down regulation.

METHODS

This experimental field study was conducted at Round Marsh within Timucuan Ecological and Historical Preserve (N30°22'42.6", W81°28'47.7"). Fifteen 1-m² plots were established along the edge of a transect at Round Marsh on 21 March, 2019. The transect was situated along the upper tidal region in a near monoculture of *B. frutescens* with sparse growth of *I. frutescens*. Plots were kept one meter apart to minimize any potential cross of treatments and were marked by four polyvinylchloride (pvc) stakes placed at the corners of each plot. Plots were randomly assigned to one of three treatments: 1) control; 2) salted or 3) fertilized and a corresponding colored/numbered survey flag was placed in the center of each plot. To ensure that plots had been effectively randomized, several plant characteristics were recorded at the time of plot establishment. Variables measured included mean number of galls per 100 stems, number of flowering terminals per 100 stems, mean stem length, and gall diameter. Gall and flower counts were assessed using multiple analysis of variance (MANOVA), while initial stem length and gall diameter were compared using analysis of variance (ANOVA). Additionally, due to the potential for flowering terminals to preclude galling events (Rossi *et al.*, 2001) a chi-square with Yates' correction was conducted on 300 haphazardly selected stems to determine observed frequency of the co-occurrence of galls on flowering terminals (see results for details).

Fertilized plots received 500g of a commercially available 6-6-6 ammonium nitrate fertilizer (Sunniland, Longwood, FL), while salted plots received an equivalent amount of sodium chloride rock salt (Morton Salt, Chicago, Illinois) biweekly as a press study. Due to the pressed

nature of the study, along with smaller plot sizes, treatments were kept low, to minimize any potential chemical damage to the plants. Tidal inundations in the current study were infrequent and diminished the likelihood of treatments washing away between applications. Treatments were broadcast evenly throughout the plots by hand to minimize any potential concentration of treatment.

*Determining treatment effects on *Borrchia frutescens**

Effects of treatments were assessed by host plant quality through comparisons of gall density, gall diameter, stem heights, leaf thickness, total leaf size, leaf asymmetry, and presence of leaf pairs. Each week the number of *A. borrichiae* galls per 100 *B. frutescens* terminals per plot were recorded. Similarly, number of flowering terminals per 100 stems were also recorded for each plot bi-weekly. To ascertain the extent of treatment effects on maximal gall diameters, five young incipient galls were identified during the onset of one of the larger *A. borrichiae* pulses in late May. Incipient galls were marked by wrapping numbered tape gently between the pair of apical leaves below the galled terminal. Maximal gall diameter per gall was measured weekly using digital dial calipers (nearest 0.1mm), lost galls through death or other means were not replaced and instead used for survivorship rates across treatments. Gallings and flowering rates of *B. frutescens* terminals were conducted until the conclusion of the study on 6 September 2019.

To determine the soil treatment effects on the growth and survival of nascent *B. frutescens* stems, five young ramets per plot were haphazardly selected in early May. All selected *B. frutescens* ramets exhibited green non-wooded stems, suggesting new growth (Moon *et al.*, 2001; Moon & Stiling, 2002). Each stem was banded using numbered tape marked with its respective treatment, plot number and stem number. Stem heights were measured from the tip of the highest apical meristem to the ground using a long tape measure (accurate to nearest 0.1cm). These same

marked stems were remeasured at the conclusion of the study, survivorship rates and increase in growth was calculated and expressed as a percentage for statistical comparison (see below).

Changes in leaf characteristics due to treatment effects as an indicator of host plant quality were determined by comparing leaf thickness, leaf size, crude leaf asymmetry, and number of terminal leaf pairs present upon conclusion of the study. Five similar-sized stems per plot per treatment were selected haphazardly and the number of leaf pairs present within 50cm of the apical meristem on each stem was recorded. Reduction of leaf pairs may suggest stress and reduction in the quality of the plant, a natural response to the accumulation of salt ions within leaves during transpiration is to eventually shed salt-stressed leaves (Munns, 2002). However, it has been argued that despite the halophytic nature of *B. frutescens* these shed leaves are not salt sinks (Antlfinger, 1981). To determine the effects of treatments on leaf characteristics, ten mature, fully expanded leaves per plot per treatment were haphazardly collected, placed in plastic bags, placed on ice, and returned to the lab. Leaf thickness was measured at the leaf's midpoint using digital calipers (nearest 0.1mm) to assess succulence. Leaf succulence has been purported to increase in high salinity (Longstreth & Nobel, 1979; Romero-Aranda *et al.*, 1998; Flowers & Colmer, 2008) with salt-stress indicative of diminished host plant quality. Individualized measurements of leaf size were taken using a leaf area meter (accurate to 0.01cm² CID Bio-Science CI-202 Camas, WA). Afterwards, each leaf was divided along the midrib using a scalpel and the resulting halves measured separately for leaf asymmetry. Total leaf size and leaf asymmetry (calculated as the absolute difference between the two halves) were compared as a measure of host plant quality.

Effects of host plant quality on the parasitoid guild community

Ten mature galls per plot per treatment were haphazardly collected on 6 September 2019, placed on ice and returned to the lab. For each gall, maximal diameter was measuring using digital dial calipers (nearest 0.1mm). Galls were then observed for emergences using a hand-held lupe and subsequently placed in small plastic dram vials. Midge pupae use ventral horns to bore their way out of the galls causing a noticeably ragged emergence hole along with a shed puparium. Conversely, the parasitoids chew their way out from within the gall chambers leaving clean exit wounds. Emergence holes were further subdivided by parasitoid, *G. haemon*. as the smallest member of the parasitoid guild community leaves pinhole sized emergence holes (personal observations) while the largest members of the community *T. umbilicatus* leave considerably larger emergence holes. If any parasitoid larvae or pupae could not be identified to species, they were scored as an unknown but used for comparison of overall parasitism rates between treatments. Galls were monitored for emergences daily with emergences recorded, any emerged insects were placed in 70% EtOH. After 2-3-weeks galls that had begun to senesce were dissected and any trapped parasitoids or midges left within the rotting galls were similarly and preserved in 70% EtOH. During this dissection process individual chambers present within each gall were also recorded for statistical analysis. Observations and dissections of the senescing galls were conducted daily until the exhaustion of collected gall samples. Resulting number of gall chambers per treatment and known parasitism events were used to determine the percentage and relative abundance of the parasitoids; but especially *T. umbilicatus*, *G. haemon* which are the primary parasitoids attacking *A. borrichiae* (Stiling *et al.*, 1992; Stiling & Rossi, 1994; Rossi and Stiling, 1995, Moon & Stiling, 2002; Rossi *et al.*, 2006). Further statistical analysis on the relationship between percent parasitism of both hyperparasitoids across gall diameter were used to assess the likelihood of parasitoid presence by gall diameter.

Except as noted, all statistical analyses performed passed normality and homogeneity of variances, however values are presented untransformed for clarity. Analyses on gall density, gall diameter, percent difference in stem heights and mean number of gall chambers were compiled using ANOVA. Following a significant main effect, treatment means were compared using a Tukey's post-hoc test. Repeated measures ANOVA of treatment effects on gall and flowering densities, however, did not pass Mauchly's Test of Sphericity, so resulting analyses were reported by Greenhouse-Geisser. Initial homogeneity of plots were established for gall density and flowering terminals as well as leaf characteristics: leaf thickness, total leaf size, leaf asymmetry (left and right leaf size) using MANOVA. Survivorship of incipient galls as well as stem tips were determined by G-tests of association.

RESULTS

*Initial treatment on *Borrchia frutescens* plots*

Plots used for the study were effectively randomized, the *a priori* analysis found no significant differences in initial gall ($F_{(2,12)} = 0.061$ $p = 0.941$) or flowering densities ($F_{(2,12)} = 2.600$ $p = 0.115$; Table 1). Initial gall densities averaged 5-6 galls per 100 *B. frutescens* terminals, while flowering rates were very low for all three treatments (Table 1). During the gall-flower co-occurrence comparison, although 21% (63/300) and 38% (114/300) of terminals possessed either a gall or flower respectively, only 3.3% (10/300) terminals had both. Thus, frequency of gall and flowers occurred 59% less often than expected ($X^2_{(1,300)} = 7.513$ $p < 0.01$).

*Effects of treatments on *Borrchia frutescens**

Monthly gall densities per 100 stem terminals varied significantly by time, treatment and produced a significant interaction effect (Time $F_{(1.7, 20.353)} = 64.151$ $p < 0.001$; Treatment $F_{(2,12)} =$

21.792 $p < 0.001$; Interaction $F_{(3.392,20.353)} = 5.599$ $p = 0.005$; Figure 1). However, flowering densities only varied significantly by time (Time $F_{(2.447,29.366)} = 35.263$ $p < 0.001$; Treatment $F_{(2,12)} = 1.133$ $p = 0.354$; Interaction $F_{(4.894,29.366)} = 0.634$ $p = 0.672$). Moreover, maximal gall-diameter showed significant differences across treatments ($F_{(2,57)} = 5.333$ $p < 0.01$); specifically, maximal gall diameter was significantly larger for fertilized plants compared to salted ones (Figure 2). Survivorship on incipient galls over the field study showed no significant association with treatments and gall presence ($G_{\text{calc}} = 2.68$ $df = 2$ $0.1 < p < 0.5$; Table 2). Although, initial heights of *B. frutescens* stems showed no significant differences ($F_{(2,72)} = 0.765$ $p = 0.469$; Table 1). Treatment did significantly effect percent stem growth ($F_{(2,63)} = 7.017$ $p = 0.002$; $\log_{10}(10+x)$ -transformed data to meet homogeneity of variance; Figure 3). Stems growing in fertilized plots grew 8.75% more than controls and 16.87% more than salted plots relative to pre-treatment levels. *Borrchia frutescens* stem survival was high across all treatments and showed no significant association ($G_{\text{calc}} = 0.771$ $df = 2$ $0.5 < p < 0.9$; Table 2). However, effects of treatment on leaf thickness ($F_{(2,144)} = 0.865$ $p = 0.423$), total leaf size ($F_{(2,144)} = 7.807$ $p = 0.001$; Figure 4), leaf symmetry (left and right halves cut along midrib respectively) ($F_{(2,144)} = 8.159$ $p < 0.001$; $F_{(2,144)} = 6.665$ $p = 0.002$) and absolute difference of left and right halves ($F_{(2,144)} = 0.192$ $p = 0.825$) only found significant effects of total leaf size and leaf symmetry (Table 3). Although not significant, *B. frutescens* leaves in salted plots were found to be thicker than both control (3%) and fertilized leaves (6%), suggesting potential salt-induced succulence of the leaves. Fertilized plots exhibited the greatest total leaf size(cm^2) ($F_{(2,144)} = 7.807$ $p = 0.001$; $S\bar{x} = 3.8$ $C\bar{x} = 3.7$ $F\bar{x} = 4.5$; Figure 4) generating leaves that were 18% and 21% larger than salt and control leaves respectively. Interestingly, these leaves were also found to be lower in absolute differences (i.e. more symmetrical) than salted leaves, although these differences were not significant.

Significance in total leaf size as expressed by fertilized leaves persisted upon dividing the leaves along the midrib, with 23% and 18% increases in left half leaf size across salt and control leaves respectively and 19% increase in right halves. Number of leaf pairs was significantly affected by treatment, with a 64% decrease in number of leaf pairs between salt and control plots (Figure 5).

Effects of host plant quality on gall size and parasitoid guild

Gall diameter differed among treatments with smaller mean gall diameter on salt-treated *B. frutescens* based on *post hoc* analysis ($F_{2,132} = 18.288$ $p < 0.001$; ln-transformed data to maintain homogeneity of variance) compared to fertilized plots. Not surprisingly, differences in gall diameter led to differences in mean gall chambers ($F_{(2,132)} = 18.563$ $p < 0.001$; Figure 6). Mean number of gall chambers shows significant differences between all three treatments. In untreated control, an almost equal distribution between *G. haemon*, *T. umbilicatus* and unidentifiable unknowns coupled with *R. cecidomyiae* and *T. teredon* across known parasitism events is exhibited. Highly significant differences are observed in the salt-treatment with a 56% increase in known parasitism events being attributed to *G. haemon* and a 61% decrease in *T. umbilicatus* when compared to controls. However, this trend was almost reverted in the fertilized groups when compared to controls, with an increase in *T. umbilicatus* parasitism (12%) and a significant drop-off of *G. haemon* (30%) (Figure 7) from salt-treated galls to fertilized galls. Analysis to determine a correlation if any, between gall diameter and percent parasitism by both *G. haemon* and *T. umbilicatus* on known parasitism events found a moderately positive correlation between known *T. umbilicatus* parasitism events on maximal gall diameter and found non-significance between *G. haemon* parasitism events and gall diameter. ($r(133) = 0.33$ $p < 0.001$; $r(133) = -0.084$ $p = 0.335$).

DISCUSSION

For the gall midge, *A. borrichiae*, changes in plant quality altered gall characteristics resulting in a strong interaction with the parasitoid guild community exerting top-down regulation of the midge population. Gall characteristics such as diameter, symmetry and number of chambers are a combination of plant genotype, insect genotype, and their interactions with the environment (Price and Clancy, 1986; Weis and Abrahamson, 1986). Gall characteristics, as an extension of host-plant quality, provide partial bottom-up regulation of the midge population. Gall size has been shown to have effects on the size and potential fitness of *A. borrichiae* (Rossi *et al.*, 2001). However, gall diameter can also affect the suitability of galls to attacking parasitoids, thereby potentially shifting the composition of the guild of natural enemies (suite of parasitoids). Previous research within the system has suggested non-random attacks by two parasitoids and successfully shifted parasitoid guild composition through bagging studies (Stiling & Rossi, 1994; Rossi *et al.*, 2006). However, the present study achieved shifts in the parasitoid guild through the application of salt and fertilizer treatments. These treatment effects cascaded upwards through the lower trophic levels and indirectly shaped the parasitoid guild community. It is imperative in fostering greater understanding of population and community dynamics to identify and explore how biotic and abiotic factors interact amongst all trophic levels to influence a system.

As expected, over the course of this seven-month press experiment, gall densities varied seasonally. Gall densities were shown to significantly vary by treatment and time. Gall densities within salt-treated plots were consistently lower, compared to significantly greater density found within fertilized plots. Gall densities varied significantly by time due to the multivoltine nature of the galling insect (Stiling & Rossi, 1994), coupled with their five-to-nine week developmental

period (Stiling *et al.*, 1992). It is expected for galling events to rise and fall over the course of this study as pulses of the gall midges peter out or begin anew. Therefore, it is unsurprising that gall densities were decreasing steadily into the beginning of June and summarily sharply increased by the months end across all three treatments. This seasonal effect was seen across all three treatments equally. Over time, (approximately ten weeks) changes in plant quality began to affect gall densities between treatments, a distinct bottom-up effect.

Although flowering of *B. frutescens* occurs year-round, flowering rates vary seasonally and peak from May to July (Duncan and Duncan, 1987; Lonard and Judd, 1989; Rossi *et al.*, 2001). In the present study, treatment had no effect on flowering rates of *B. frutescens* however, salt and fertilizer treatment has been shown to increase and decrease rates of flowering respectively in similar studies (Stiling *et al.* 1992; Moon and Stiling 2002). Reduced rates of flowering may be caused by prolonged apical dominance caused by induction of galls which maintains the apical meristem (and suppresses lower stem buds) until gall senescence. Spirko and Rossi (2015) found galled ramets of *B. frutescens* produced 50% fewer flower buds compared to fully intact or clipped ones. Moreover, galling may partially regulate stem morphology, thereby preventing flowering and maintaining the terminal as a potential ovipositional site for the midge; a similar phenomenon has been reported in stem galling sawflies (Craig *et al.*, 1986; Roininen *et al.*, 1988). Co-occurrence of galls and flowers occurred on 10 of the 300 haphazardly selected stem tips (3%), this was 59% less than expected, indicating that flowering may prevent the terminal from galling events (Rossi and Strong, 1990; Stiling *et al.*, 1992).

Not surprisingly, percent growth of *B. frutescens* was nearly 50% higher in fertilized plots when compared to salt treated plants. Nitrogen is considered the limiting element for

growth within salt-marsh ecosystems (Valiela and Teal, 1974; Profitt *et al.*, 2005). Limiting nitrogen has been shown to decrease biomass production within coastal barrier salt-marsh ecosystems (Van Wijnen & Bakker, 2001) and the positive effects of nitrogen on plant growth is well established (Jefferies & Perkins, 1977; Kiehl *et al.*, 1997, Opik *et al.*, 2005). Nitrogen is an important macromolecule for plant growth, and is also an essential component of necessary biological molecules (e.g. proteins, nucleic acids, etc), and supplemental nitrogen may also increase cell metabolism through greater production of vitamins, cofactors for enzymatic processes, hormones, photosynthetic pigments (e.g. chlorophylls, phycobillins), and phytochrome photoreceptors (Opik *et al.*, 2005) Nitrogen-rich soluble compounds may play an important role in plant salt tolerance and osmoregulation (see below).

Halophytes, such as *B. frutescens* express a variety of adaptations for surviving in increasingly saline conditions. One of the most important adaptive features is regulating internal salt concentrations (i.e. minimizing entry of salt ions). Salinity effects in plants are magnified due to the accumulation of ions throughout the water column, plants transpire 30-70 times more water than is used for growth (Munns, 2002). It is believed that *B. frutescens* may selectively exclude salts at the roots (see Antlfinger, 1981; Antlfinger and Dunn, 1983) and therefore minimizes many of the deleterious effects of drawing water in a salt-marsh ecosystem (Munns, 2002). These effects will nevertheless accumulate over time, of which the common response is the abscission of the leaf. Leaf loss is particularly detrimental in perennial halophytes as leaves must be maintained over extended periods of time, and any loss will reduce biomass production (Munns, 2002). Although the implication of salt-stress on leaf loss is apparent, this physiological mechanism has yet to be determined (see Antlfinger, 1981; Heinsch *et al.*, 2004). Another adaptive feature in regulating salt concentrations is minimizing the concentration of salt ions

within the cytoplasm. Despite the propensity for halophytes to grow in areas of increasing salinity, their enzymes are equally as vulnerable as glycophytic-enzymes to salt damage (Flowers *et al.*, 1977; Zhu, 2001). Sodium and chlorine ions are normally sequestered within the vacuole of leaves, and as a means of balancing this irregular osmotic pressure, leaves either accumulate or synthesize organic solutes (Munns, 2002). Organic solutes such as proline and glycine-betaine have reportedly been produced by *B. frutescens* (see Cavalieri & Huang, 1979; Moon & Stiling, 2000) although the salinity thresholds necessary for production as reported by Cavalieri and Huang appear to be well outside the ranges observed in our study, and their synthesis incur a heavy energy cost (Raven, 1985). Both salt exclusion and compartmentalization act to prevent the negative effects of salt stress that leads to; inhibited plant growth, production of reactive oxygen species, decreases in chloroplast structures and lowered rates of photosynthesis due to stomatal closures, reduced growth, less leaves, woody stems and decreased flowering

The constant application (pressed) of salt to these halophytes appeared to have successfully stressed *B. frutescens*. Richards *et al.* 2010 purported that *B. frutescens* expresses high phenotypic plasticity in salt-stress responses, this in turn allows for growth in highly saline conditions such as salt pans. However, if surrounding abiotic conditions are kept relatively low, it is likely, that consistent application of treatments met and surpassed the average salinity in the local environment. Furthermore, it has been suggested that stem morphology of *B. frutescens* may be indicative of the plant's local abiotic conditions because the transition from non-woody to woody stems appears affected by nitrogen availability (Moon *et al.*, 2001). At the onset of this study 75 haphazardly selected ramets, exhibited green non-woody morphology, however, at the conclusion of the study all surviving stems had become woodier regardless of treatment. The overwhelming extent of woody stems across treatments suggests that transition of *B. frutescens*

ramets to woody stems may be partially associated with age, and/or in part due to the already present poor abiotic conditions (see Moon *et al.*, 2001).

While leaves from plants in salted plots tended to be thicker than those from the other treatments this difference was not significant. Leaves from salt-treated plots were 3% and 6% thicker than control and fertilized plots respectively. Moreover, the lack of significant salt induced succulence of the leaves may be attributed to less effective accumulation of leaf water content in *B. frutescens*. For instance, other halophytes found growing within these marshes such as *Salicornia virginica* L., and *Batis maritima* L. expressed 93-98% water-content retention as compared to only ~73% for *B. frutescens* (Antlfinger, 1981). Our results suggest that retention of water within the leaves as a means of diluting salt ions accumulated during transpiration in salt-treated *B. frutescens* leaves may be lower than these other halophytes or it was not greatly skewed by addition of salt. A common adaptation by halophytes to mitigate salt stress is the accumulation of salt ions within the vacuole, which acts as a sink for the ions, until the leaf is abscised (Munns, 2002). In the present study *B. frutescens* leaf pairs were significantly reduced by 60% for *B. frutescens* within salt-treated plots. Increased leaf drop was reported in *B. frutescens* during periods of high salinity caused by drought and/or tidal inundations (Heinsch *et al.*, 2004). However, it is still unclear whether leaf drop occurs due to the accumulation of salt and subsequent abscission of the leaves, due to aging, or both (Antlfinger, 1981).

Increases in average leaf size showed that fertilized plots grew significantly broader leaves when compared to leaves from salted and control plots. Unsurprisingly, these significant effects were maintained upon measuring the individualized leaf halves (i.e. measurements of the left and right portions cut along the midrib). Not only did salted plots produce smaller leaves, they also exhibited the greatest absolute difference between leaf halves (i.e. they were the most

asymmetrical) which suggests the plant was stressed, although values for absolute differences were not significant. It has been suggested that reducing asymmetry has been shown to increase metabolism, fecundity, and susceptibility to parasitism (Møller & Swaddle, 1998; Møller & Thornhill, 1997).

Bottom-up effects of plant quality significantly affected gall diameter. Fertilized plots produced galls with larger gall diameter, compared to galls reared from salted plots (Stiling *et al.*, 1992; Rossi *et al.*, 2001; Rossi & Stiling, 1995; Rossi & Stiling, 1998; Stiling & Rossi, 1994; Stiling & Rossi, 1998; Moon & Stiling, 2004). Gallings enacts a toll on the plant, due to acting as a physiological sink that draws nutrients from nearby sources as a means of sustaining and increasing its abnormal growth (McCrea *et al.*, 1985; Larson & Whitham, 1991). Thus, changes in host plant quality, altered gall size and structure, which in turn cascaded through the gall midge and, ultimately, the parasitoid community. Increases in plant quality, via the application of fertilizer provided additional resources resulting in larger galls with more chambers (Stiling *et al.*, 1992; Clouse, 1995; Rossi and Stiling, 1995). Previous studies determined that minimizing larval crowding, through increased gall diameter, produced larger female gall midges with greater potential fecundity by means of increased egg production without a reduction in egg size (Rossi *et al.*, 2001). Increased gall development rates and/or gall diameter may also alter the “window of vulnerability” of *A. borrichiae* to attack to the guild of parasitoids (Stiling & Rossi, 1994). Previous research has shown that of the parasitoids that attack developing midges, only one, *T. umbilicatus* has an ovipositor that is significantly longer than other members of the parasitoid guild (Stiling & Rossi 1994). This longer ovipositor allows for *T. umbilicatus* to take advantage of its hyperparasitic nature to target and attack larger galls later in development that are too thick for the other members to effectively penetrate.

Salt-treated plots produced significantly smaller galls when compared to galls from either controlled or fertilized plots in both lab-returned and marked field-study galls. This significant difference in size can most likely be attributed to decreases in available nitrogen due to salt stress, as gall size appears to be mediated by the quality of the plant. It is likely that *B. frutescens* within salt-treated plots were not only exhibiting signs of salt stress such as inhibited plant growth, production of reactive oxygen species (ROS) (leading to damage to DNA, proteins, and lipids), potential damage to chloroplasts, and diminished rates of photosynthesis (Meng *et al.*, 2018; Zhu, 2001), but also interference, as increasing soil salinities likely inhibited *B. frutescens*' ability to absorb nitrogen (Jefferies & Perkins, 1977; Osgood & Zieman, 1993; Bowdish & Stiling, 1998). While this study did not actively measure the production of proline and glycine-betaine, two compatible solutes oft synthesized as a means of combatting increasing salinity levels and potentially used for ROS scavenging (Shen *et al.*, 1997). Previous research as conducted by Moon and Stiling reported increased frequency of *Pissonotus quadripustulatus* (Homoptera: Delphacidae) a sap-sucking herbivorous insect on salt-stressed *B. frutesces* and were believed to be a good indicator for the accumulation of organic solutes (Moon & Stiling, 2000). Further suggesting that this study was successful in pushing the boundaries of salt tolerance past the phenotypic plasticity normally expressed within the system. This lowered host-plant quality appeared to negatively affect all plant characteristics leading to smaller more asymmetrical leaves, lowered gall densities, significantly reduced stem heights, and significantly fewer leaves.

Mean number of gall chambers was determined to significantly vary across both treatments and unmanipulated controls. Fertilizer and control groups had significantly larger

galls, with significantly more chambers, as seen in previous studies of this *Asphondylia-Borrchia* system (Stiling *et al.*, 1992; Clouse, 1995; Rossi & Stiling, 1995).

Other studies have found a correlation between gall diameter and frequency of attacks by parasitoids. For instance, *Pteromalus sp.* parasitoids preferentially attack the sawfly *Euura lasiolepis*, in small galls on an arroyo willow *Salix lasiolepis* (Price & Clancy, 1986). It is possible that the *Borrchia-Asphondylia* system may also follow a similar non-random attack hypothesis dependent upon the diameter of the galls. The current study is consistent with previous ones that suggest that *G. haemon*, which is gregarious, can overwhelm the midge or other parasitoids in small galls (0.7-0.9mm); after this, its short ovipositor prevents it from reaching hosts in large galls (Stiling & Rossi, 1994; Stiling & Rossi, 1996).

In the current study, presence of *T. umbilicatus* increased (significantly) in fertilized plots. In addition, *T. umbilicatus* exhibited a moderately significant positive correlation with gall diameter across treatments. Conversely, while the smallest parasitoid, *G. haemon* increased in salted plots that produced smaller galls, it exhibited a very weak non-significant negative correlation to gall diameter. This difference in response of the largest and smallest parasitoids may have resulted because a previous study using artificial galls, found that *G. haemon* was significantly affected by stem length: *G. haemon* was significantly more common at the top of the *B. frutescens* canopy and less common on short stems (Rossi *et al.*, 2006). In the current study, galls were chosen regardless of stem length.

Over 60% of the known parasitism events within galls reared from salted plots can be attributed to the gregarious nature of *G. haemon*. A striking contrast when compared to the almost equal distribution of known events within controlled plots, as well as the marked decrease in *G. haemon* within fertilizer-reared galls, a roughly 55% decrease in their presence. These data

are indicative of bottom-up effects indirectly altering the parasitoid guild community. As gall characteristics change dependent upon abiotic factors, so too does the suite of natural enemies within this tritrophic system.

Gall diameter and thickness can have a large impact on the “window of vulnerability” and suitability for members of the parasitoid guild (Price & Clancy, 1986; Stiling & Rossi, 1994). Thus, this “window of vulnerability” of the parasitoids may shift under variable abiotic conditions, which may in turn shift dominance of the parasitoid guild between the two hyperparasitoids. Small galls (like those produced in salted plots) may not be large enough to support development of the largest parasitoid, *T. umbilicatus* while large galls (characteristic of fertilized plots) may be too thick for successful penetration by *G. haemon* (Stiling & Rossi, 1994; Brown & Rossi, 2013). Thus, the prevalence of *T. umbilicatus* and absence of *G. haemon* in large galls is likely due to the significantly longer ovipositor of *T. umbilicatus*. An identification of a breakpoint across known parasitism events will allow for greater use of gall diameters within field studies as a means of correctly attributing parasitism events to their respective parasitoid. The prevalence of *T. umbilicatus* is shown to be positively correlated with gall diameter, and although a breakpoint was not able to be determined with the generated data, percent parasitism on galls reared from salted plots unequivocally showed the increased presence of *G. haemon* in the significantly smaller salted galls.

The results of this study support the “upward cascading” effects as mentioned in Hunter and Price of abiotic factors on plant quality. Shifts in plant quality have been shown to cause significant differences in gall characteristics and ultimately influences composition of the parasitoid guild attacking *A. borrichiae*. Within this and similar study systems, environmental heterogeneity can be easily manipulated by natural means. Instances of growth within salt pans

cause drastic shifts in salinity levels over a distance of a few meters (Antlfinger, 1981), frequency of tidal inundations can accumulate or drive away wrack (Drawe *et al.*, 1981; Profitt *et al.*, 2005), topography can mitigate or prevent the effects of tidal inundations, these and many other factors can all interact in shaping the plant heterogeneity and in turn the herbivores and their natural enemies. It is likely that this and similar coastal systems may be further influenced by rising tidal regimes in conjunction with increases in salinity. Specifically, salinity within the Florida Current has increased by 3% and coastal levels have seen a steady increase over recent years (see Beckett, 2016; Szuts & Meinen, 2017). Tidal action in the current system albeit infrequent, did inundate most plots. If these trends continue, it is possible that increasing tidal events could lead to further salt-stress across the entirety of the system. Except for those *B. frutescens* located upon higher elevation leading to discrete areas with potentially lower salinity due to minimized tidal events. These changes could potentially present with a new avenue for abiotic heterogeneity in the *Borrchia-Asphondylia* system. Normally found in high salt marsh habitats, *B. frutescens* exposed to increasingly saline tidal actions may see greater shifts towards salt-stressed stunted growth and further increase the prevalence of attack by *G. haemon*.

Asphondylia borrichiae is believed to have formed host-associated races that have diverged at the level of their host plant genus (i.e. *Borrchia*- associated and *Iva*-associated populations)(Stiling & Rossi, 1998; Rossi *et al.*, 2001; Stokes *et al.*, 2012). One (temporary) benefit the midge acquired when expanding its host range (i.e. from *Borrchia* to either *Iva* species) was the acquisition of enemy free space on these novel hosts. Trade-offs on the novel hosts include lowered available galling terminals due to increased flowering rates (Stiling & Rossi, 1994; Rossi & Stiling, 1995; Rossi *et al.*, 2001), significantly smaller galls producing gall midges with reduced fitness (Rossi *et al.*, 2001), as well as seasonal variations in development

time (Rossi *et al.*, 2001). It is possible that galls grown on novel hosts that are of differing size than produced by *B. frutescens* may potentially prolong the refuge afforded by these novel hosts due to an inadequate search image as it appears parasitoids avoid smaller galls further away from the established canopy (Rossi & Stiling, 1998; Rossi *et al.*, 2006; Ishii & Shimada, 2010). It is also likely, that variations in galling size and growth due to plant quality as seen in this study may lead to a similar outcome. These results further emphasize the importance of investigating multi-trophic level interactions to understand the ecology and evolution of *Asphondylia borrichiae* and its host plants. Nevertheless, the significance in understanding the extent and complexity of abiotic-biotic interactions within systems and their role in shaping communities cannot be understated.

Treatment	Galls/100 stems ^{ns}	Flowers/100 stems ^{ns}	Initial stem height (cm) ^{ns}	Initial gall diameter (mm) ^{ns}
Control	5.00 \pm 1.1	0.8 \pm 0.4	41.6 \pm 2.5	9.7 \pm 0.6
Salted	5.40 \pm 1.5	0.4 \pm 0.4	44.4 \pm 1.8	9.4 \pm 0.5
Fertilized	5.60 \pm 1.0	2.0 \pm 0.7	40.4 \pm 2.6	9.9 \pm 0.6

Table 1. Initial effects of gall/flower density, stem heights and gall diameter. Gall and flower densities per 100 haphazardly selected stems stem height (cm) and gall diameter (mm) of *B. frutescens* recorded March 2019 when plots were established. Gall and flower densities were measured using a MANOVA, while mean stem height and gall diameter were assessed using ANOVA; ns indicates non-significant differences. No initial differences were found for any characteristic (see text for details) indicated values are mean \pm sem.

Treatment	Gall Survivorship ^{ns}	Stem Survivorship ^{ns}
Control	40% (10 of 25)	92% (23 of 25)
Salted	60% (15 of 25)	84% (21 of 25)
Fertilized	40% (10 of 25)	88% (22 of 25)

Table 2. Survivorship effects of galls and stems. Effects of treatment on survivorship of incipient galls from late May 2020 until the conclusion of the study in early September 2020. Many of the galls had naturally senesced due to the successful emergence of either *A. borrichiae* or parasitoids; ns indicates non-significant differences. Both gall and stem survivorship was compared using a G-test of association (see text for details).

Treatment	Thickness (mm) ^{ns}	Total Size (cm) ²	Symmetry Left Half (cm) ²	Symmetry Right Half (cm) ²	Absolute Difference ^{ns}	Percent Asymmetry ^{ns}
Control	1.18 ± 0.04	3.72 ± 0.14 a	2.02 ± 0.08 a	1.96 ± 0.07 a	0.145 ± 0.02	4.14 ± 0.51
Salted	1.22 ± 0.04	3.79 ± 0.18 a	1.92 ± 0.1 a	1.96 ± 0.1 a	0.16 ± 0.02	4.51 ± 0.55
Fertilized	1.14 ± 0.02	4.5 ± 0.14 b	2.37 ± 0.08 b	2.34 ± 0.07 b	0.158 ± 0.02	3.56 ± 0.41

Table 3. Effects of treatments on leaf characteristics of *B. frutescens*. Leaf thickness measured along thickest point at midrib, trends suggest possible salt-induced succulence of the leaves as a means of mitigating salt stress. Fertilized plots produced greater leaf size, with less asymmetry when compared to salted leaves (see text for details); ns signifies no significant differences, cells with different letters were found to vary significantly during post-hoc analysis.

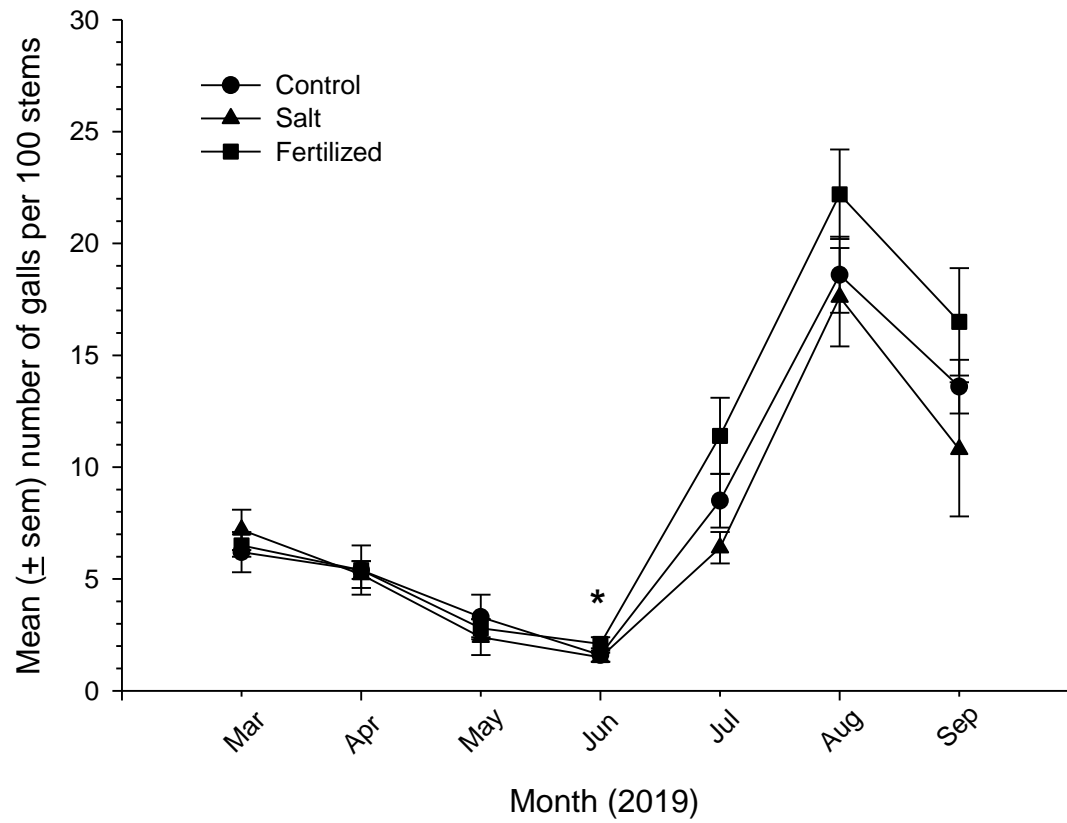


Figure 1. Gall densities over time. Mean gall densities per 100 stems per treatment of *Asphondylia borrichiae* galls on *Borrichia frutescens* host plant. Onset of the study occurred near the conclusion of one of the multivoltine generational pulses of the gall-inducer. Significant effect of time on gall densities as indicated by the asterisk denotes the start of one of the larger gall pulses, with significant effects of treatment showing trends.

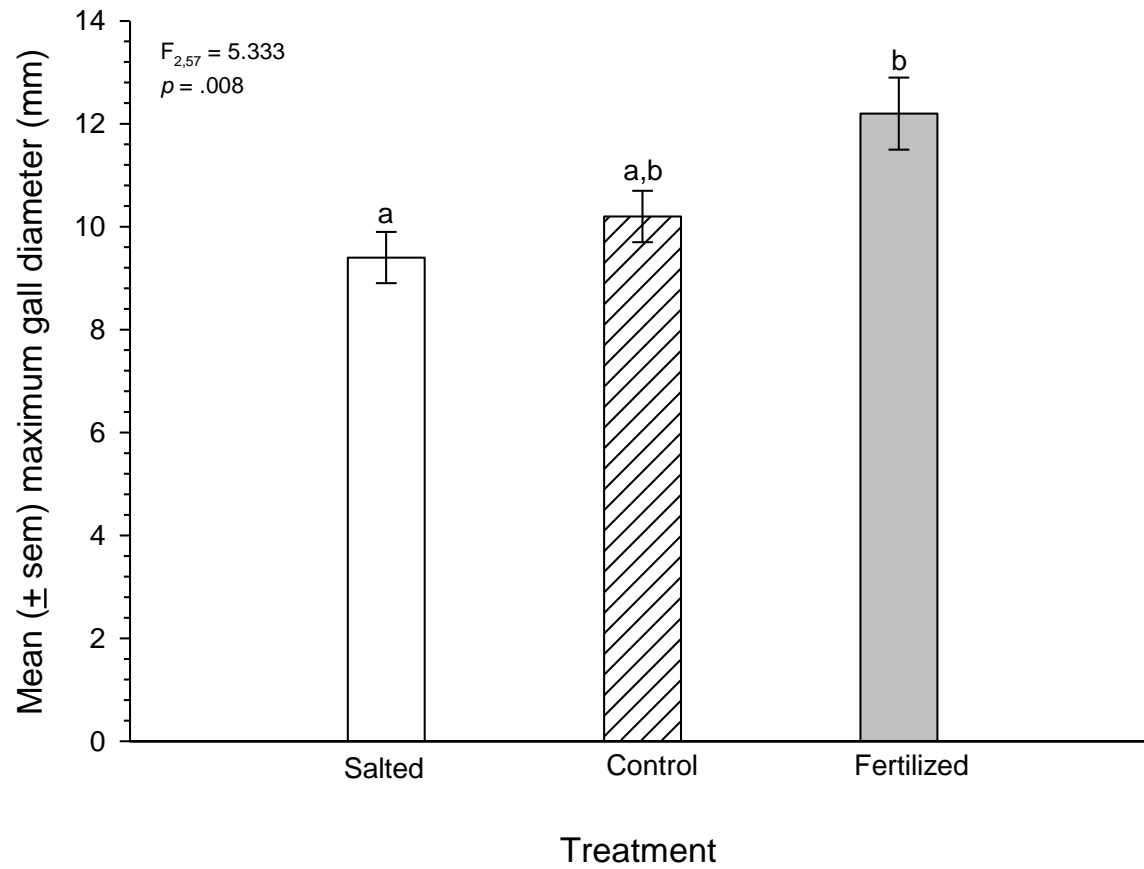


Figure 2. Mean maximal gall diameter of *A. borrichiae* galls as grown on host plant *B. frutescens* across treatments. Measurements taken along the greatest axis of each individual gall over the course of the study. Increasing gall diameter is likely to increase the fitness of developing herbivores and can help shape the window of vulnerability to attack by parasitoids.

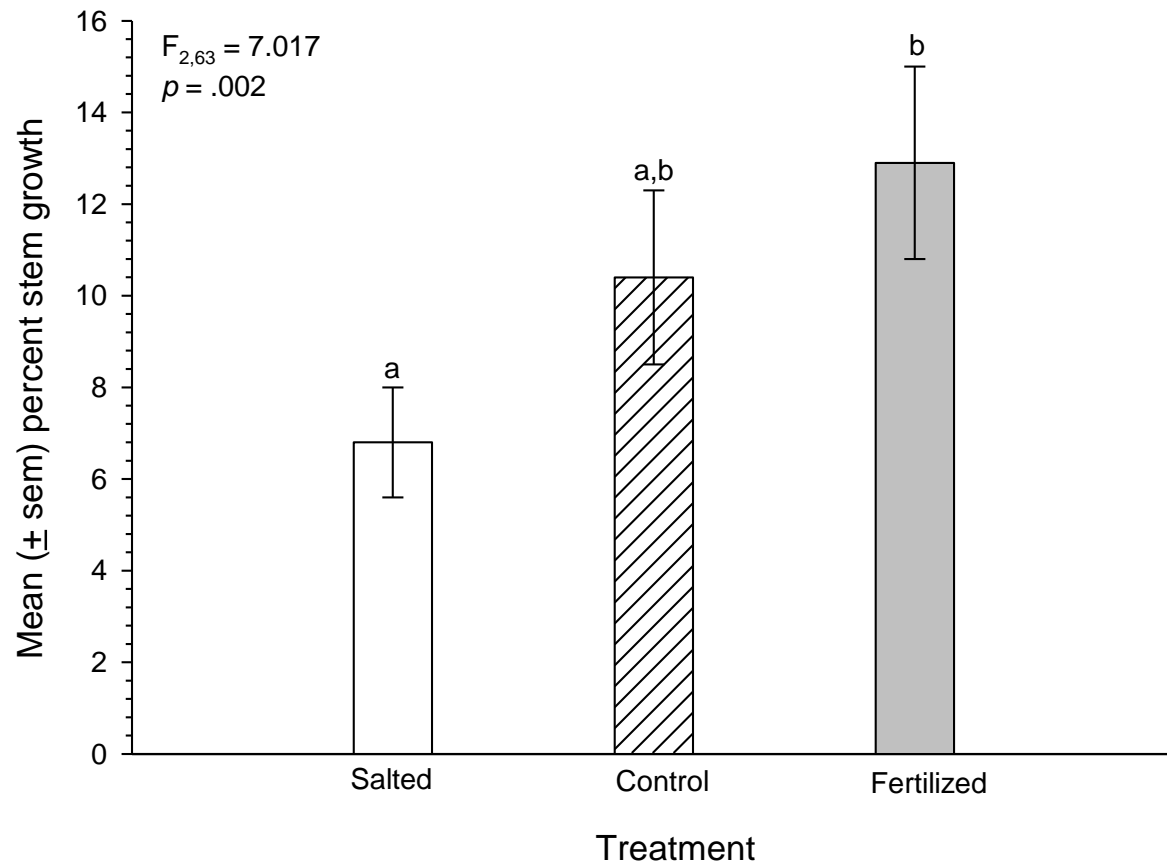


Figure 3. Percent stem growth of nascent *B. frutescens* relative to pre-treatment levels. Measurements taken from the surface of the soil to the highest apical meristem indicative of plant quality as mediated through treatments. Increases in available nitrogen as afforded by fertilizer treatments over the length of the study improved growth rates while further salt stress diminished growth *B. frutescens* stems.

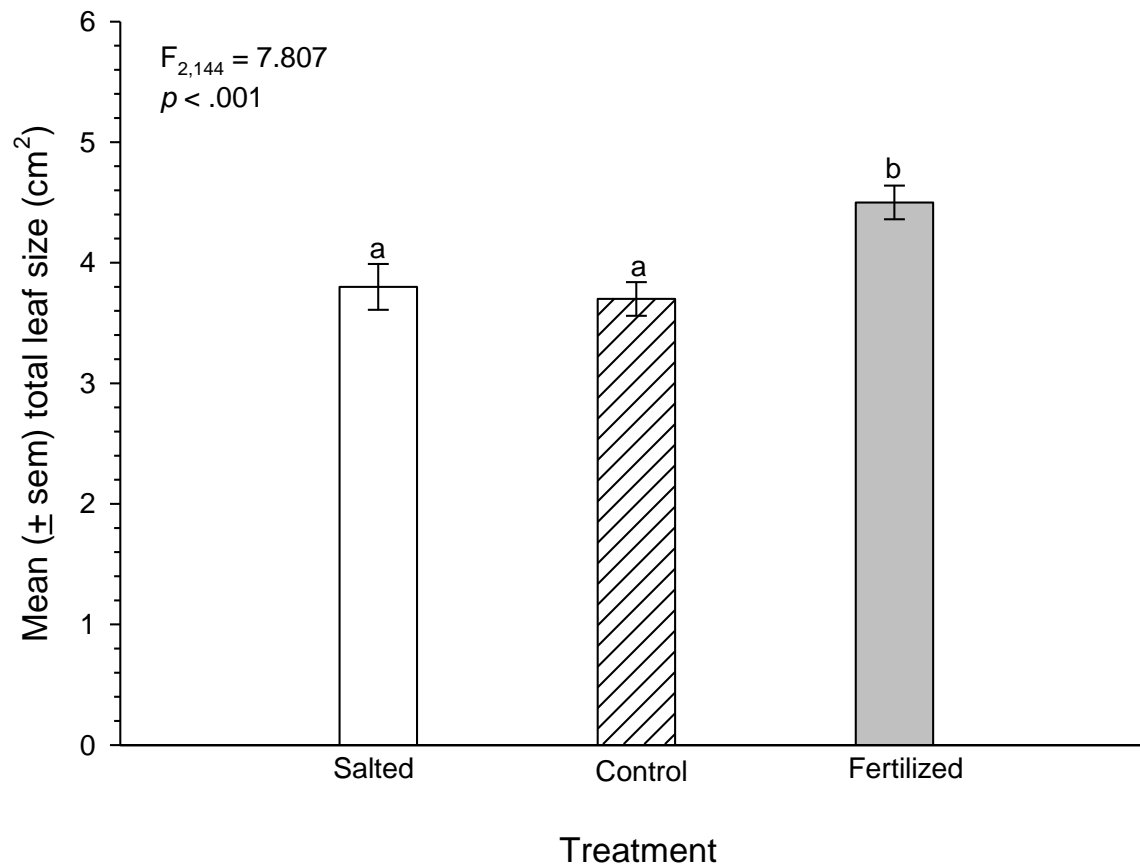


Figure 4. Mean total leaf size of *B. frutescens* leaves across treatments. Salted leaves may be exhibiting signs of shading effects on leaves prompting expansion of leaf size as previous data shows diminished growth of salt-stressed *B. frutescens* stems, corroborated by increased leaf thickness suggesting potential salt-induced succulence of the leaves (not-significant).

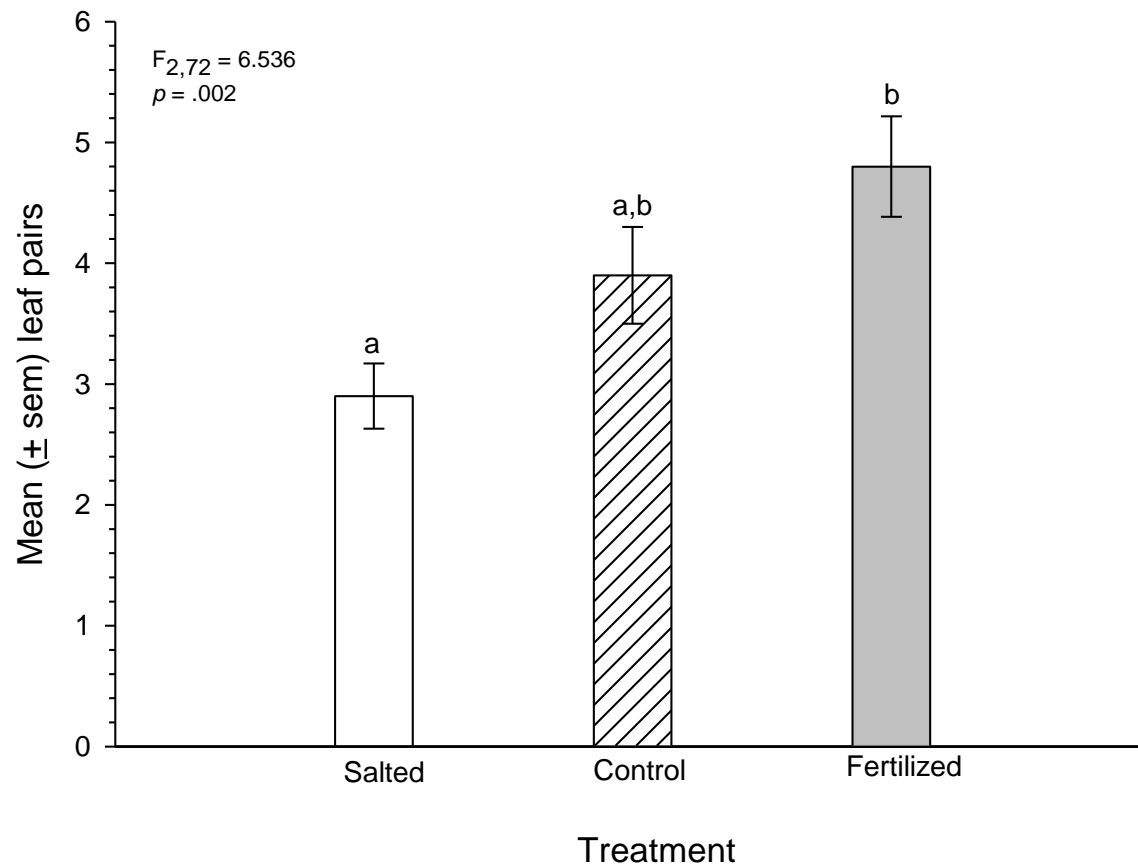


Figure 5. Presence of leaf pairs within 50cm of the highest apical meristem on haphazardly selected *B. frutescens*. A common response to increased salt-stress in halophytes is the sequestration of salts within the vacuole, production of compatible osmotica and the implementation of salt-stressed leaves as a salt-sink for abscission. These data show significantly fewer remaining leaves on salt-treated *B. frutescens* although this can be potentially attributed to less overall leaf production due to stunted growth as caused by increasing salt stress.

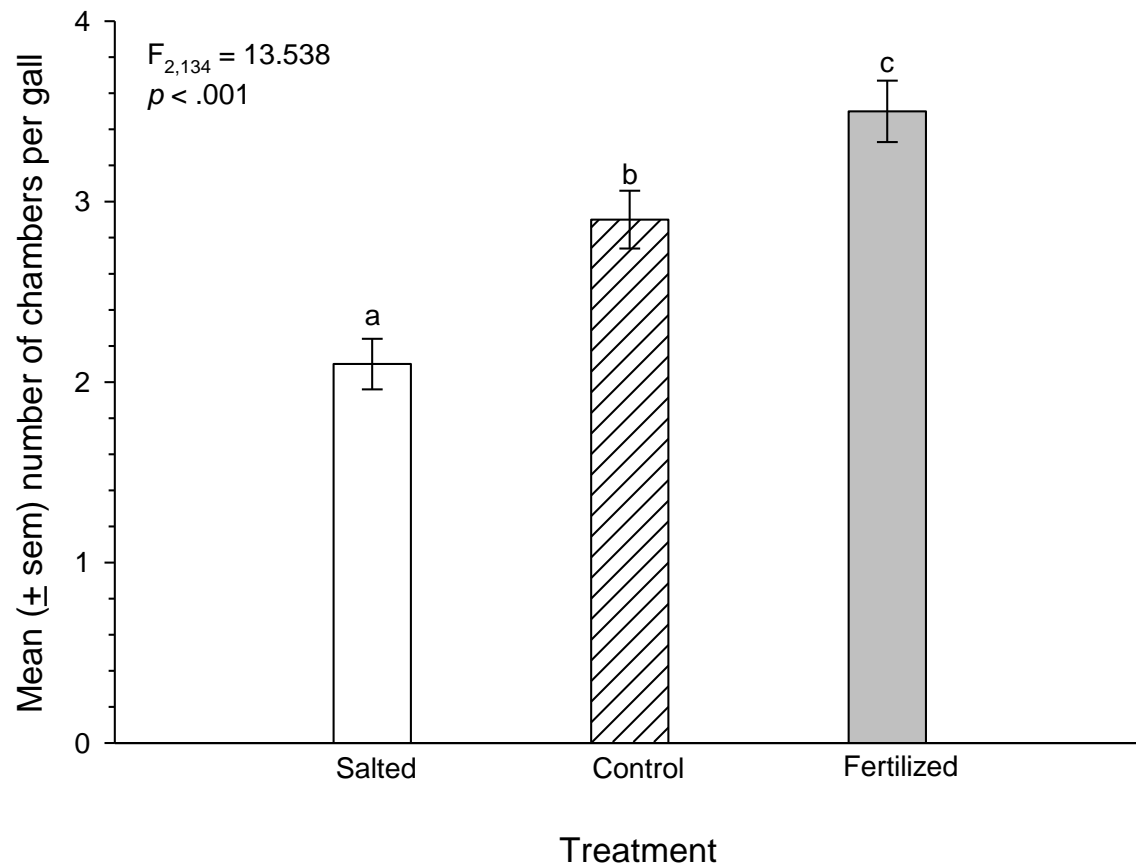


Figure 6. Mean number of chambers per *A. borrichiae* induced gall. Increases in available chambers coupled with increases in size likely increased the potential fecundity of *A. borrichiae* developing within the robust galls produced on fertilized *B. frutescens*. Decreased number of chambers in salted plots are likely indicative of aborted chambers early in development of the gall, and potentially increased salinity of gall tissue. Decreasing available chambers in conjunction with diminished growth of galls induced on salt-stressed *B. frutescens* will likely decrease the fitness of *A. borrichiae* imago.

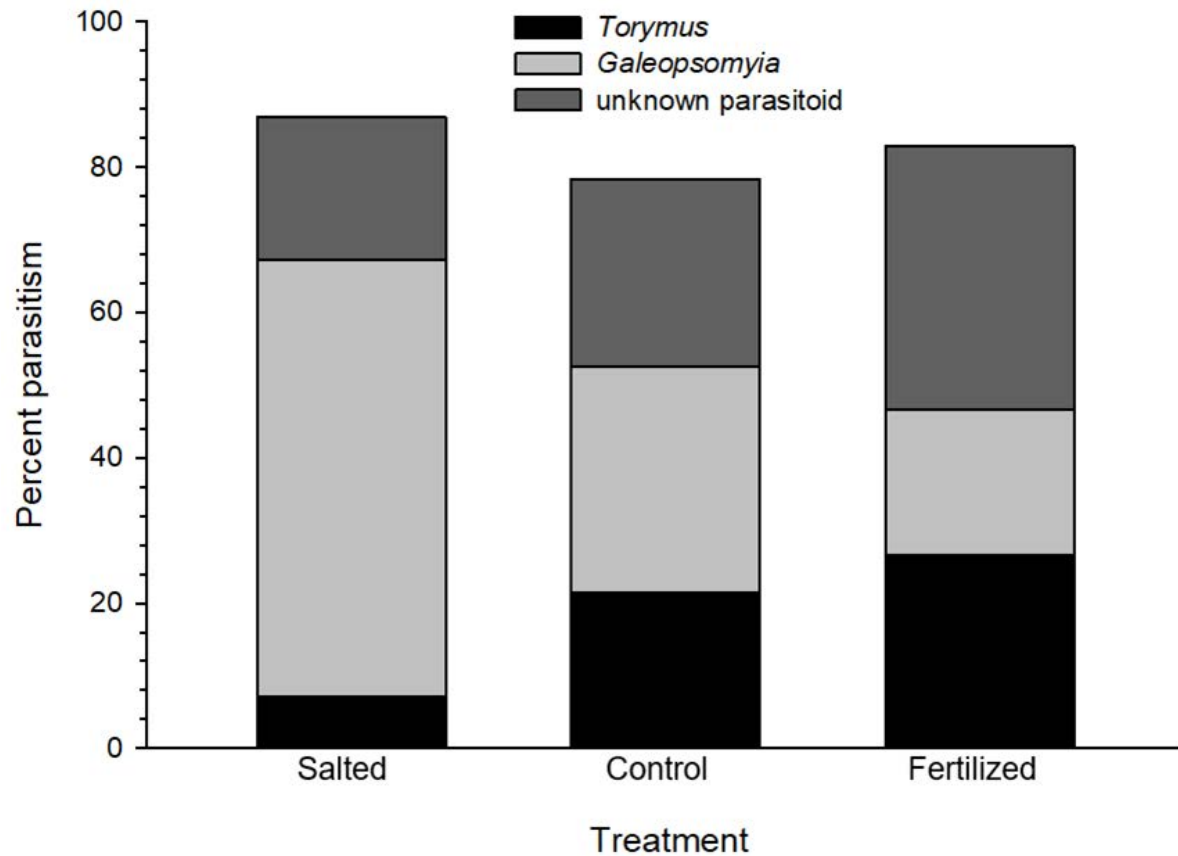


Figure 7. Parasitoid community composition across all known parasitism events. Unidentifiable stages in development of parasitoids as well as parasitism events attributed to *T. teredon* and *R. cecidomyiae* were kept as unknowns. Of the known parasitism events of the haphazardly collected galls, galls reared from salted plots were dominated by *G. haemon* due to numerically overwhelming other parasitoids. These advantages decreased as gall diameter increased across treatments with shifts towards dominance by *T. umbilicatus*. As the window of vulnerability decreases for other members of the parasitoid guild due to their significantly shorter ovipositor.

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VITA

EDUCATION

University of North Florida, Department of Biology Jacksonville, FL
M.S. in Biology Expected May 2020

Florida International University, Department of Biological Sciences Miami, FL
B.S. in Biological Sciences December 2016

RESEARCH EXPERIENCE

University of North Florida, Department of Biology Jacksonville, FL
Graduate Student with Dr. Anthony Rossi Spring 2019-Present

Ecological Entomology and Tritrophic Plant Interactions

Observed tritrophic interactions between an herbivore gall-midge, *Asphondylia borrichiae*, its host plant, *Borrichia frutescens*, and a suite of natural enemies in the form of a parasitoid guild community. Experimental manipulation of the abiotic factors surrounding this system allowed for observations in potential shifts within the parasitoid community in response to changes in gall characteristics as exhibited by the host plants.

University of North Florida, Department of Biology Jacksonville, FL
Graduate Student with Dr. Doria Bowers Fall 2017-Fall 2018

Mosquito Morphology and Vector Studies

Attempted to identify differential viral infections of *Sindbis virus* (SINV), an alphavirus within the *Togaviridae* family, in salivary glands of mosquitoes.

TEACHING EXPERIENCE

University of North Florida Jacksonville, FL
Graduate Teaching Assistant, General Biology II Laboratory Spring 2020, Spring 2019
Graduate Teaching Assistant, General Biology I Laboratory Fall 2017-Fall 2018, Fall 2019

PRESENTATIONS

Orta, K. Indirect effects of plant quality on the parasitoid composition of *Asphondylia borrichiae*. National Park Service. Presentation delivered at the Timucuan Science and History Symposium, Jacksonville, FL, January 2020.

Orta, K. and Rossi, A.M. Indirect effects of plant quality on the parasitoid composition of *Asphondylia borrichiae* (Diptera: Cecidomyiidae) galls. Entomological Society of America. Poster presentation delivered at the Entomological Society of America Annual Meeting, St. Louis, MO, November 2019.