January 2013

Associational Resistance and Competition in the *Asphondylia - Borrichia - Iva* System

Keith Stokes
University of South Florida, khstokes@mail.usf.edu

Associational Resistance and Competition in the *Asphondylia – Borrichia – Iva* System

by

Keith Howard Stokes

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Biology
Department of Integrative Biology
College of Arts and Sciences
University of South Florida

Major Professor: Peter Stiling, Ph.D.
Earl McCoy, Ph.D.
Jason Rohr, Ph.D.
Anthony Rossi, Ph.D.

Date of Approval:
30 August 2013

Keywords: enemies hypothesis, parasitoid–mediated associational resistance,
Torymus umbilicatus, stemborers, gallformers, interguild competition

Table of Contents

List of Tables

List of Figures

Abstract

Indirect ecological effects such as associational resistance and resource competition have the potential to affect ecological interactions and influence the structure of ecological communities. Although resource competition is commonly studied, the effects of associational resistance are not as evident if studies are not designed to detect them. Additionally, the relative strengths of different ecological mechanisms ought to be measured in studies, rather than the strength of singular mechanisms. This permits proper attribution of causes and effects in community structure and detection of higher order interactions in a way that naïve reductionism will not.

In a series of experiments, I looked at the effects of large-scale addition and removal of *Borrichia frutescens* on associational resistance of *Iva frutescens* to the gallformer *Asphondylia borrichiae* in order to test the mechanism and strength of associational resistance in the system. Additionally, I measured the effects of relative host abundance and interpatch distance of hosts on associational resistance. Finally, I looked at the effect of the presence of stemborers competing with the gall former for host plant resources on parasitism rate and parasitoid guild composition.

I found evidence for a strong effect of associational resistance by natural enemies over short distances, although the phenomenon is likely of minor importance in comparison to other factors known to affect gall former population density, such as environmental effects and host plant genotype. Competitors also had a weak effect,

reducing mean gall diameter, but not significantly altering total parasitism rate. However, the presence of stemborer competitors did slightly alter the composition of the parasitoid guild.

In sum, both associational resistance and competition from stemborers have detectable effects on *A. borrichiae*, albeit weak ones. Environmental factors, such as soil nitrogen content, are likely much stronger determinants of gall former population size.

Chapter 1:

An Introduction to Associational Resistance and Competition in the *Asphondylia* – *Borrichia* – *Iva* system

Community composition may be influenced by biotic and abiotic factors. Biotic factors may be direct or indirect interactions between species. Direct interactions include predation, parasitism, interference competition and mutualisms. While indirect effects are more numerous in type as well as occurrence and include such interactions as exploitative competition, apparent competition and associational resistance, among others (Holt 1977, Strauss 1991, Wootton 1994, Raffel et al. 2008). Ohgushi (2005, 2008) has argued that the importance of plant-mediated indirect interactions in influencing the structure of arthropod communities has not been adequately appreciated. Species interactions are emergent properties of communities, with indirect interactions occurring only if a minimum of three species comprise the communities (Strauss 1991). Because many species directly interact with more than one other species, which then interact with still other species, a single direct interaction may result in multiple indirect interactions. The cumulative indirect effects of such interactions may potentially exceed the effects of direct interactions and ought to be quantified in community ecology studies. Associational resistance and exploitative competition are indirect species interactions known to affect phytophagous insects (Kaplan and Denno 2007, Barbosa et al. 2009).

Associational Resistance

Associational resistance (AR), an emergent property of plant communities, is a pattern of reduced herbivory on a plant species by the presence of other plant species, which may or may not be food items for the herbivore. Cases of the converse situation, increased vulnerability to herbivory resulting from the presence of nonfocal plant species, have also been documented and are referred to as associational susceptibility (Stiling et al. 2004, Barbosa et al. 2008) Several mechanisms have been proposed to account for this phenomenon, including the repellent plant hypothesis (Tahvanainen and Root 1973), the enemies hypothesis (Root 1973, Russell 1989), the attractant decoy hypothesis (Atsatt and O'Dowd 1976), and the semiochemical diversity hypothesis (Hambäck et al. 2000, Jactel et al. 2011), among others. The proposed mechanisms are not mutually exclusive nor are they all well-defined. In the repellent plant hypothesis, plant associates of the herbivore’s focal species produce noxious volatile chemicals to which the herbivore has a negatively chemotactic response, reducing foraging by the herbivore in the vicinity of the repellent plant and thus reducing herbivory rates on the focal species where it coincides with the repellent species. One alternate scenario is the attractant decoy hypothesis, wherein an associate of the target species has higher attractive qualities to the herbivore, but lower nutritional value, potentially reducing both the herbivore’s foraging rate on the target species and the herbivore’s population growth rate. Yet another mechanism is described by the semiochemical diversity hypothesis, which posits that AR may occur as a result of neutral masking of the presence of the target species from the herbivore.

The AR phenomenon has important implications commercially and in experimental design and ecological inference with regard to the population dynamics of herbivores. From a commercial standpoint, knowledge of AR could be used to design improved crop layouts (intercropping) as a method of reducing the quantity of pesticides needed or to improve yields in organic farming, analogous to crop rotation's use to improve soil quality while reducing or eliminating the use of chemical fertilizers. The use of trap crops such as alfalfa (*Medicago sativa*) interspersed with a focal crop species, cotton (*Gossypium* sp.), to draw away the insect pest *Lygus* (Hemiptera: Myridae) is an example of a known mechanism of AR (attractant decoy) being applied to reduce crop damage without the expense and potentially adverse secondary effects of pesticides (Altieri 1991). Nor is this mechanism of AR the only one which can be enhanced to improve ecosystem services. The enemies hypothesis suggests a strategy for reducing herbivore damage by judicious increase of biodiversity adjacent to or within crop plots to enhance populations of beneficial insect species which are natural enemies of pest species. This enhancement can occur by addition of plant species which may be hosts of alternate seasonal prey or may provide other resources (e.g., nectar, pollen) to increase, or at least maintain stability of, populations of predators and parasitoids. For example, placement of blackberry bushes (*Rubus* sp.) adjacent to fields of grape (*Vitis*) allowed *Anagrus epos* (Hymenoptera: Mymaridae), a parasitoid of the significant crop pest the grape leafhopper (*Erythroneura elegans*, Hemiptera: Cicadellidae), to colonize an alternate host in which to survive the winter when grape leafhoppers are unavailable as a consequence of abscission of grape leaves and grape leafhopper hibernation (Altieri 1991).

In the field of parasitology, associational resistance can be broadly attributed to two mechanisms: zooprophylaxis, in which the frequency of encounters between humans (or other focal species) and disease vectors is reduced by an increase in abundance of the populations of nonfocal species, and the dilution effect, wherein vector abundance and vector/ focal species encounter rate remain the same, but the vector infection rate is reduced as non-competent hosts replace competent ones, which results in a lower incidence of infection acquisition by vectors (Randolph and Dobson 2012). Such outcomes are lauded as practical, utilitarian rationales for conservation of biodiversity as public policy because they reduce the risk of zoonotic disease. Although reviews have found a high frequency of AR-type outcomes in parasite-vector-host systems (i.e. either zooprophylaxis, dilution effect or a combination thereof), associational susceptibility and amplification of disease risk also occurs (Cardinale et al. 2012). Randolph and Dobson (2012) have criticized the dilution effect concept as a Panglossian strawman, lacking adequate empirical support for the claims of its proponents. Ostfeld (2013) has countered by asserting that Randolph and Dobson's critique is itself a strawman of the dilution effect argument and that evidence abounds for a negative correlation between biodiversity and disease risk, even though the contrasting effect of amplification of disease risk occurs as well.

As do all indirect effects, associational resistance exemplifies a potentially significant problem with reductionism in the practice of community ecology, particularly with regard to plant–herbivore interactions. As noted by Nurse (1997), although reductionism has been a successful strategy in the sciences, it can limit understanding if misapplied. Attempts to explain natural phenomena by reference to an inappropriate level of understanding might provide the illusion of explanation by providing an excess of detail. Further, failure to include relevant components of a system or to consider alternate mechanisms for the interaction of components and the resultant patterns that emerge will likely result in inaccurate descriptions of systems and unreliable predictions about those systems. To illustrate, if herbivore abundance on a particular host species is significantly influenced by indirect interactions mediated via the larger community of plants, then studies of plant–herbivore interactions which are limited to the herbivore and its target species and natural enemies of the herbivore will regularly result in incorrect conclusions regarding factors influencing herbivore population sizes and densities. Consequently, the magnitude of herbivore population regulation attributed to top-down and/or bottom-up forces will inevitably be incorrectly estimated and effects will be falsely attributed to incorrect causal mechanisms as a result of failing to account for the effect size of herbivore population regulation by associational resistance. Because AR is a pattern resulting from the outcome of species interactions by multiple possible mechanisms, and not a singular phenomenon, use of the term should always be modified to identify the causal mechanism at work when invoked, if possible.

Competition

Competition has long been considered an important ecological interaction in modern biology. Darwin (1859) invoked competition as a mechanism of natural selection, famously phrased as a “struggle for existence”. Competition can be of two general forms: interference competition or resource competition, sometimes called exploitation competition. The latter form of competition is a type of indirect interaction mediated by a resource, typically a prey species. Interspecific competition has been assumed by many researchers to be a major factor influencing community composition and structure, but this assumption has also been controversial. Hairston et al. (1960)

argued that competition between herbivores should be rare in habitats of high primary productivity (the “green Earth” hypothesis). The high productivity itself is evidence for minimal competition between herbivores, which are proposed to be reduced below their carrying capacity by natural enemies. As a consequence of herbivores being limited in a top-down manner, rather than limited by food resources, those same food resources, the plant community, are subjected to competition. Furthermore, natural enemies that keep herbivores in check are also limited by food availability and thus should experience competition. Oksanen and colleagues (1981) later developed a more generalized, mathematical model which permitted prediction regarding which trophic level(s) would be subjected to competition and which to top-down limitation, contingent on the amount of primary productivity in the system. They found that communities with low productivity would have herbivores limiting primary producers and would themselves be limited by competition. As productivity was increased in the model, higher trophic levels such as primary predators could be supported and become limiting to herbivores, resulting in a shift in competition to the trophic levels above (predators *sensu lato*) and below (plants and other primary producers). These predictions generally comported with published studies, with the exception of insect herbivores, which were suggested by Oksanen et al. (1981) to possibly be more strongly influenced by temperature than by other factors.

During the period from the mid-1970s to the mid-1980s, a vigorous debate ensued regarding the importance of competition to community structure. Diamond (1975) attempted to formulate assembly rules to explain the biogeography of bird communities and concluded the patterns observed were best explained by interspecific competition, with some allowance for variation in dispersal rates. That conclusion was challenged by Connor and Simberloff (1979), who showed that the patterns of species distributions observed could as easily be explained by random chance as by competition and proclaimed that studies should have to overcome a null hypothesis of random combinations of species. At around the same time, Connell (1980) published an argument that strong evidence of competition was lacking and was incapable of explaining species' distributions, despite its regular invocation as a mechanism for just that purpose and to explain coexistence of seeming competitors. Although past competition was purported to be the reason for the apparent divergence in niche occupation and for character displacement allowing coexistence by resource partitioning. Connell's argument included a prescription of six experimental treatments which would permit a researcher to conclude not only that competition was the causative mechanism for coevolution of proposed past competitors, but also that the resulting coevolutionary divergence was heritable, a necessary condition for evolved properties of organisms. The paper ended with the comment that in the absence of strong evidence for competition, the "Ghost of Competition Past" would no longer be a satisfactory explanation for observed patterns of species distributions and resource utilization. Similar conclusions were reached by Strong et al. (1979) in a review of character displacement studies against null hypotheses of randomly generated assemblages of avian species on islands: random co-occurrence of species could not be ruled out as an explanation in favor of character displacement, a predicted consequence of resource competition. This seeming consensus was broken by Roughgarden (1983), who objected to the conclusions above for philosophical and technical reasons. For example, Roughgarden rejected Connell's claim that heritability was a necessary condition of competition and contradicted the utility of randomized null communities as null models against which claims of competition discerned by biogeographic patterns could be tested. However, some of the disagreement is the result of exaggeration, as when Roughgarden wrote "This essay was provoked by the insistent claims of Connell (1980), Connor and Simberloff (1979), and Strong et al. (1979) that competition and coevolution of competitors are not real and important processes in nature, and hence, that theory for these processes is not worthy of testing." In actuality, those authors claimed simply that the available evidence was inadequate to support the predictions of competition theory.

Subsequently, independently conducted literature reviews of field competition studies by Schoener (1983) and Connell (1983) concluded that competition was frequent and important, although the studies did not compare the strength of the effect of competition nor its frequency against other ecological interactions such as predation. In fact, the studies were vote-counting exercises, lacking a composite of effect sizes. Gurevitch et al. (1992) conducted a meta-analysis, coming to largely the same conclusions: that competition was a frequent phenomenon and potentially a strong influence, although its strength was variable across studies. Also, *contra* Oksanen et al. (1981), the effect of competition on primary producers did not vary between habitats with low and high productivity. Similarly, the results contradicted Connell (1983), who found intraspecific competition was more frequently a stronger effect than interspecific competition among studies where both were evaluated. A more recent meta-analysis, restricted to competition among phytophagous insects (Kaplan and Denno 2007) also found competition to be a frequent phenomenon, against the predictions of the green

Earth hypothesis (Hairston et al. 1960), but consistent with the earlier observations of Oksanen et al. (1981), who noted that the predictions of the ecosystem exploitation hypothesis were not supported by the then-available studies on terrestrial arthropod herbivores. Additional studies have attempted to compare the effects of different ecological interactions with competition, including facilitation (Goldberg et al. 1999) and predation (Gurevitch et al. 2000). Competition and predation were found to have similarly-sized effects on population density, but otherwise acted by different mechanisms, with competition having greater effect on growth and mass and predation strongly affecting survivorship. An unexpected outcome was finding decreased competition with increased productivity, contrary to theoretical predictions (Oksanen et al. 1981). Further synthesis is needed to determine relative importance of competition to other mechanisms influencing community composition.

Study System

Asphondylia borrichiae (Diptera: Cecidomyiidae) is a gall–forming midge which utilizes three species of salt marsh and coastal dune plants in the southeastern United States by oviposition near the shoot apical and lateral meristems. The three host plants have overlapping geographic ranges and are sea oxeye daisy, *Borrichia frutescens* (L.) DC., marsh elder, *Iva frutescens* L. and dune elder, *Iva imbricata* Walter (Rossi et al. 1999), which are all members of the family Asteraceae. Oviposition results in the formation of complex polythalamous galls, which are nearly spherical over–growths of plant tissue. *Asphondylia* produces ambrosia, or fungus–lined, galls surrounding the eggs and subsequent immature stages of the developing midge, each in its own chamber. Galls are significantly larger on *B. frutescens* in the spring and summer than on the two other host species (Rossi & Stiling 1995). Four hymenopteran species comprise a guild of parasitoids attacking *A. borrichiae*: *Galeopsomyia haemon* (Walker) (Eulophidae), *Torymus umbilicatus* (Gahan) (Torymidae), *Tenuipetiolus teredon* (Walker) (Eurytomidae), and *Rileya cecidomyiae* Ashmead (Eurytomidae) (Stiling et al. 1992). Emergence holes on the galls permit determination of parasitism rates since emerging *A. borrichiae* produce relatively large diameter holes with ragged margins; in contrast, the various parasitoid species produce holes with smooth margins and of various sizes, but relatively smaller diameter than the midge (Stiling and Rossi 1997). A comparison of parasitism rates inferred from emergence holes with rates taken from galls collected and cultured in vials found no significant difference between the two measures. As with many plant–herbivore interactions, much of the research into the midge has focused on the relative roles of host quality versus proportional composition of the guild of natural enemies and population density of natural enemies on directly regulating the midge population. However, more recently conducted research has concluded that composition of the plant community has a significant indirect effect on midge abundance on one of the host–associated populations (Stiling et al. 2003), likely via associational resistance mediated by natural enemies.

The presence of *Borrichia frutescens* has been found to reduce gall densities of *Asphondylia borrichiae* on *Iva frutescens*, an example of the indirect effect associational resistance (Stiling et al. 2003). Coincident with the decrease in gall density, gall parasitism rates on *I. frutescens* increased when *B. frutescens* was added to islands where it was not previously established. In addition to an increase in total gall parasitism rates, the species–specific parasitism rate of one particular member of the parasitoid guild, *Torymus umbilicatus*, increased significantly with *B. frutescens* addition, suggesting that the enemies hypothesis is operating in this system. The AR effect is asymmetrical in this system: the presence of *I. frutescens* altered neither parasitism rates nor gall densities on *B. frutescens*, either in the natural or manipulative experiments. Stiling and others concluded that the AR in the *Asphondylia – Borrichia – Iva* system was likely mediated via the parasitoid guild which attacks *A. borrichiae*, especially *T. umbilicatus*, the largest member of the guild. *Torymus umbilicatus* has an ovipositor twice the length of the ovipositors of the other species in the guild and also engages in hyperparasitism of other guild members. This hypothesis for AR is supported by the observation that the component of parasitism which is attributed to *T. umbilicatus* in *I. frutescens* galls in the presence of *B. frutescens* is nearly double that when *I. frutescens* occurs without *B. frutescens* (63.2% vs. 33.3%, Stiling and Rossi 1994). An underlying reason for this phenomenon may be the fact that *Torymus umbilicatus* emerging from *B. frutescens* galls have significantly longer ovipositors than those emerging from *I. frutescens* (Brown and Rossi 2013). Galls on *I. frutescens* are significantly smaller than those occurring on *B. frutescens*. Consequently, *T. umbilicatus* from *Borrichia* galls may be able to more readily parasitize *Iva*-occurring *Asphondylia* and engage in hyperparasitism in *Iva* galls than the converse situation. Also, the smaller galls may be susceptible to parasitism by *Borrichia* – origin *T. umbilicatus* for a longer period, whereas in contrast, *Borrichia* galls may potentially grow too large for smaller *T. umbilicatus* with shorter ovipositors emerging from *I. frutescens* to effectively parasitize. Further investigation of this phenomenon is warranted to confirm the findings of the first study and to determine the strength and relative importance of associational resistance in the *Asphondylia* – *Borrichia* – *Iva* system.

In summary, I used the *Asphondylia–Borrichia–Iva* system to investigate the strength of parasitoid–mediated associational resistance and the competitive effects of stemborers on galls of *Asphondylia* and its parasitoids. By using observational data and manipulative field experiments described, I explored the effects of variation in relative host abundance, patch distance and ovipositional site areal density on associational resistance in the *Asphondylia–Borrichia–Iva* system, including the results of a large-scale manipulation of several sites. Stiling et al. (2003) documented the existence of AR in this ecological interaction by a small scale presence/ absence manipulation of the system, but did not attempt to quantify the magnitude and limits of the functional relationship between each of the variables of relative abundance, distance between host patches and host density with the AR effect. Additionally, I followed up a study by Stiling et al. (1999), who found a negative association between the stemborer *Neolasioptera* sp. and *A. borrichiae*, which was taken to be an indication of competition. The gall former oviposits in the leaf buds of *B. frutescens* and *I. frutescens* and was negatively associated with the stemborer on both host species. Stemborers interrupt the flow of nutrients to the shoot apices, potentially causing gall mortality, and the resultant negative association between the herbivores. Although the two sessile feeders co-occur less frequently than expected by chance, they do co-occur. If host–plant–mediated exploitative competition is a consequence of the co-occurrence, surviving galls should show some effect of competition. The probable result of co-occurrence in the absence of gall mortality is a reduction in gall diameter, gallformer size and fecundity and increased parasitism rate. I therefore collected stems bearing galls for comparison of gall diameter, gall midge wing length (which is correlated with body size and fecundity, see Rossi et al. 1999), parasitism rate and parasitoid guild composition in galls on stems with and without stemborers.

Chapter 2:

Effects of large–scale host plant addition and removal on parasitoid–mediated associational resistance in the gall midge *Asphondylia borrichiae*

Abstract

Associational resistance (AR) occurs when a plant species experiences less herbivory when growing in the presence of other plant species than when growing in monoculture. Densities of the gall midge *Asphondylia borrichiae* on the coastal plant *Iva frutescens* are depressed in the presence of a second coastal plant species, *Borrichia frutescens*. Previous studies suggested that hymenopteran parasitoids from *B. frutescens* galls spill over onto *I. frutescens* galls and reduce gall densities through increased parasitoid mortality of the immature stages of *A. borrichiae*, thereby effecting AR.

This study employs large–scale addition or near–complete removal of *B. frutescens* from a series of spoil islands near the west central Florida coast. Densities of galls on *I. frutescens* decreased where *B. frutescens* was added, increased where *B. frutescens* was removed and remained unchanged on islands where *B. frutescens* abundance was not manipulated, consistent with the hypothesis of associational resistance.

Relative to unmanipulated islands, total parasitism rate on *Iva* galls declined greatly on islands where *B. frutescens* was removed and increased on islands where *B. frutescens* was added. The species–specific parasitism rate on *Iva* galls of *Torymus*

umbilicatus, the parasitoid hypothesized to be primarily responsible for AR in the system, declined on islands where *B. frutescens* was removed and increased on islands where *B. frutescens* was added, supporting the hypothesis of parasitoid–mediated AR in this coastal plant–gall maker system.

Introduction

Associational resistance (AR) is a phenomenon wherein a species gains protection from attack by natural enemies by dint of its community affiliation (Tahvanainen and Root 1972). AR has been attributed to a number of different mechanisms, including the repellent plant hypothesis (Tahvanainen and Root 1972), the attractant decoy hypothesis (Atsatt and Dowd 1976) and the enemies hypothesis (Root 1973), among others. In the first case, a focal plant receives some protection from herbivory because of production of a noxious volatile chemical by a neighboring species, which tends to drive off herbivores from the area. In the second case, a neighboring species appears to herbivores to have greater desirability as food and draws herbivores away from the focal species, reducing herbivore populations. The enemies hypothesis proposes that neighboring species of the focal plant harbor and increase populations of natural enemies of herbivores, which spill over to the focal species and reduce herbivory. Although a recent review and meta–analysis by Barbosa et al. (2009) found that associational susceptibility, the converse phenomenon of AR, was more common among insect herbivores, whereas AR occurred more frequently among mammalian herbivores, I present experimental evidence that AR mediated by natural enemies affects herbivore abundance and is the likely mechanism of AR in this system.

Asphondylia borrichiae Rossi & Strong (Diptera: Cecidomyiidae) is a gall–forming midge which develops on sea oxeye daisy, *Borrichia frutescens* (L.) DC., marsh elder, *Iva frutescens* L. and dune elder, *Iva imbricata* Walter (Asteraceae) (Rossi et al. 1999). The first two species co–occur in salt marsh habitat, while the last is found primarily on beach dunes and is usually isolated from the others. Galls are significantly larger on *B. frutescens* than on the two other host species (Rossi and Stiling 1995). *Asphondylia* is attacked on each of its host plant species by a guild of four hymenopteran parasitoids: *Torymus umbilicatus* (Gahan) (Torymidae), *Galeopsomyia haemon* (Walker) (Eulophidae), *Tenuipetiolus teredon* (Walker) (Eurytomidae), and *Rileya cecidomyiae* Ashmead (Eurytomidae) (Stiling et al. 1992). In studies conducted during 1991–1993 on a series of spoil islands near the west coast of Florida, *A. borrichiae* gall densities on *I. frutescens* were significantly lower on islands with immediately adjacent patches of *B. frutescens* than on *I. frutescens* populations occurring on islands without *B. frutescens*. Percent parasitism overall on *I. frutescens* was significantly higher on islands with *B. frutescens* than on *I. frutescens* populations occurring on islands without *B. frutescens*. The species–specific parasitism rate of *T. umbilicatus* on *I. frutescens* was higher in the presence of *B. frutescens*, though not significantly so. The AR effect is asymmetrical in this system: the presence of *I. frutescens* altered neither parasitism rates nor gall densities on *B. frutescens*.

Stiling and others (2003) concluded that the AR in the *Asphondylia – Borrichia – Iva* system was likely mediated by *T. umbilicatus*, the largest member of the parasitoid guild, which possesses an ovipositor more than twice the length of the ovipositors of the other guild members, allowing it to parasitize *A. borrichiae* late in development, when the gall may be too large for the other species to successfully reach the midge for oviposition. *Torymus umbilicatus* also engages in hyperparasitism of other guild members and its long ovipositor gives it an advantage in a “last in wins” scenario. In addition, *T. umbilicatus* emerging from *B. frutescens* galls have significantly longer ovipositors than those emerging from *I. frutescens* (Brown and Rossi 2013). The greater ovipositor length of *T. umbilicatus* gives this species an advantage by permitting it to parasitize a large fraction of *A. borrichiae* on *I. frutescens*, which has smaller galls than on *B. frutescens*. Consequently, *T. umbilicatus* from *Borrichia* galls co–occurring with *Iva* galls were thought to be able to spill over from *Borrichia* and readily parasitize *Iva*-occurring *Asphondylia*, depressing gall densities on that host species. The presence of *I. frutescens* altered neither parasitism rates nor gall densities on *B. frutescens*.

The proposed explanation for the mechanism of AR in this system, that the phenomenon is mediated by its parasitoids generally and *T. umbilicatus* particularly, is a hypothesis derived from the data resulting from the initial study (Stiling et al. 2003). As such, using those data to test the hypothesis would constitute begging the question. Thus, an additional study was necessary as a minimal condition to test that hypothesis. Here I take advantage of large scale manipulations of whole islands where *B. frutescens* was added or nearly eliminated in the late 1990s and compare current gall densities and parasitism rates to those recorded in earlier studies. The use of islands as treatment replicates permits inference about community effects of AR resulting from changes in community composition.

Methods

The study was conducted on nine patches of *I. frutescens* on the same chain of spoil islands used by Stiling and coworkers (2003) for counting gall densities in the 1991–1993 study. The islands occur in the Gulf of Mexico near west central Florida, in the vicinity of Clearwater and Tarpon Springs. In 1999, a team from Tampa Bay Aquatic Preserves, an agency of the state of Florida, involving many workers, completed large–scale plantings of *B. frutescens* on some islands where this species was previously absent, thereby effecting a manipulation of island flora which would otherwise have been logistically impossible by researchers. These planted patches were on the order of at least 100 m^2 in area (P. Stiling, *pers. comm.*). At similar locales, *B. frutescens* has a mean stem density of 220/m^2 (K. Stokes, unpublished data). Assuming similar density values for the planted patches of *B. frutescens*, approximately 22 000 stems were added to each island, representing a substantial alteration of the community. Three islands also experienced near extirpations of their *B. frutescens* populations, consisting of reductions greater than 90% of their extent (P. Stiling, *pers. comm.*) as a consequence of removal of exotics, especially Brazilian pepper (*Schinus terebinthifolius*), by the Tampa Bay Aquatic Preserves team. Said exotic species removal effort incidentally altered abiotic conditions and destroyed most *Borrichia* on these three islands, effectively resulting in a treatment which was previously determined to be undesirable to perform intentionally because of the potential for adverse impacts on local habitat, as well as logistically difficult. Three islands had patches of *B. frutescens* which had neither addition of *B. frutescens* nor removal of exotics and therefore did not experience meaningful change in extent of *Borrichia* coverage from that observed in the 1991–1993 study.

Monthly gall counts conducted from April 2009 through January 2010 were used to estimate the proportional frequency of galling in all nine patches of *I. frutescens* on control or manipulated islands. At each sampling date, replicate gall counts were taken of 100 haphazardly selected *I. frutescens* stem tips on each of five different *Iva* bushes. Galling rates were averaged across sampling dates. Patches of *I. frutescens* bushes on each island were grouped into treatments according to changes in abundance of immediately adjacent patches of *B. frutescens* and galling rates on *Iva* were statistically tested for differences among these treatments.

Mature galls were collected from *I. frutescens* bushes on all islands and returned to the lab for culturing of gall inhabitants to determine total and species–specific parasitism rates of galls by members of the parasitoid guild. Maturity of galls was judged primarily by presence of emergence holes and secondarily by gall shape. Gall width was measured across the longest axis perpendicular to the stem to the nearest 0.01 mm using digital calipers. Insects which emerged from cultured galls were stored in 99% isopropanol for identification using a stereomicroscope. Cultured specimens were pooled across sampling dates to calculate an aggregate parasitism rate for each site. I used mean total parasitism rates and *T. umbilicatus*–specific parasitism rates from *I. frutescens* patches adjacent to those *B. frutescens* patches which did not change appreciably in extent as a baseline for comparison for the other treatment groups. Deviations from these baselines were calculated for each of the other *Iva* patches and Student's two–sample *t*–tests used to test for differences among sites grouped into treatments based on changes in abundance of neighboring *B. frutescens* patches.

Results

Treatment variances of changes of *I. frutescens* galling rates between past and present studies were found to be heteroscedastic by a Levene's test for equality of variance ($F_{2,6} = 8.370$, $P < 0.02$), so differences among treatment groups were tested using the nonparametric Kruskal–Wallis test (Fig. 1, $P < 0.03$). Galling rates increased on patches of *I. frutescens* where the neighboring *B. frutescens* patch experienced a severe decline in abundance, while the converse was observed on *I. frutescens* patches on islands where *B. frutescens* was added. *Iva frutescens* near unmanipulated *B. frutescens* patches experienced little change in galling rates.

Significant differences were found in total parasitism rates ($t_4 = 3.13$, $P < 0.04$) and marginally significant differences detected in *T. umbilicatus*–specific parasitism rates ($t_4 = 2.35$, $P < 0.08$) between islands with additions or extirpations of *Borrichia*. *Iva frutescens* galls experienced an increase in total parasitism and parasitism by *T. umbilicatus* when neighboring *B. frutescens* patches were added and a decrease in total parasitism and *T. umbilicatus*–specific parasitism when neighboring *B. frutescens* patches decreased in area (Fig. 2).

Discussion

The change in *I. frutescens* galling rates between the 1991–1993 and 2009–2010 studies showed an inverse correspondence with the change in *B. frutescens* abundance (Fig. 1). In addition, *Iva* galls on islands which had *B. frutescens* added experienced a relative increase in total parasitism rates compared to unmanipulated islands while the *Iva* galls on islands where *B. frutescens* abundance decreased experienced a relative decrease in parasitism rates (Fig. 2a). Despite the lack of significant difference in *T. umbilicatus*

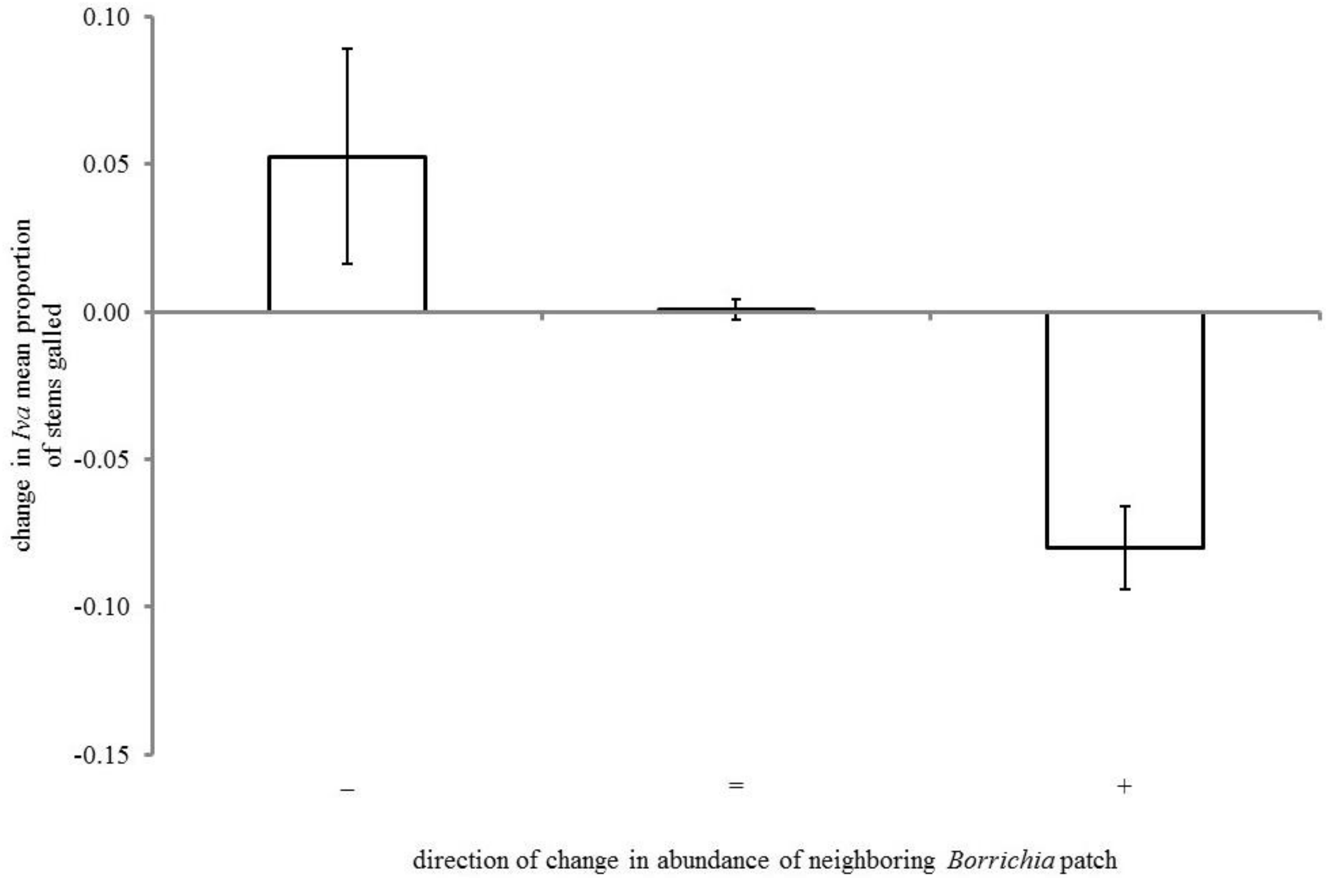
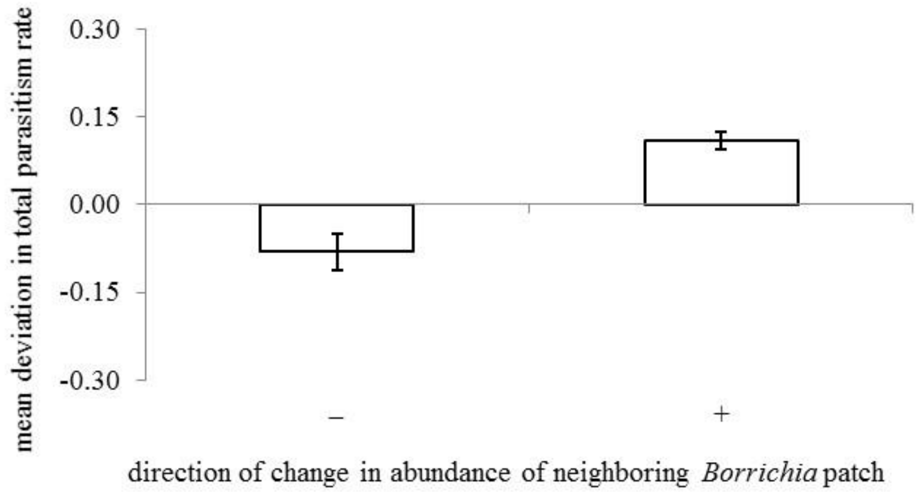


Figure 1 Variation in change of mean *Iva* galling rate (±1 SE) between the 1991–1993 and the 2009–2010 studies among *Borrichia* abundance treatment groups.

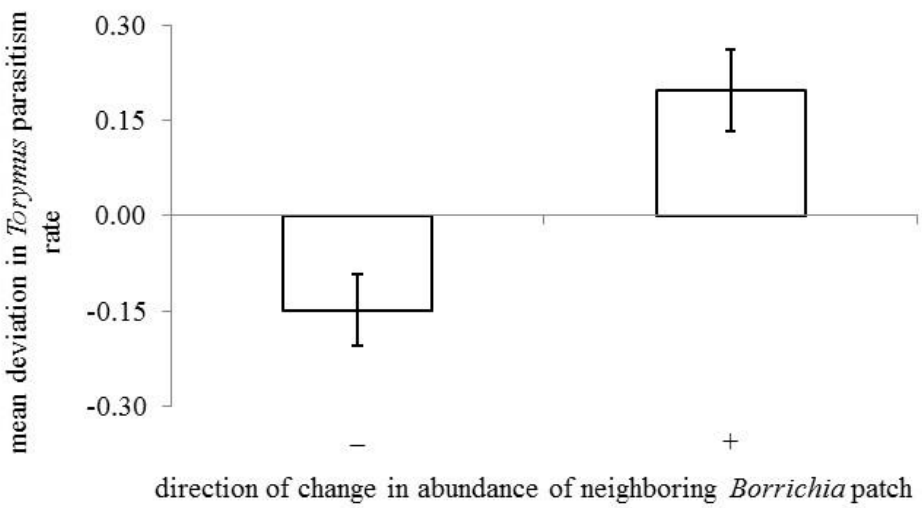
parasitism rates between increasing and decreasing *Borrichia* abundance treatment groups, a clear trend can be observed of increasing frequency of *T. umbilicatus* where *B. frutescens* was added and decreasing *T. umbilicatus* frequency where *B. frutescens* abundance decreased markedly (Fig. 2b). Treatment groups deviated from the baseline in opposite directions and consistent with the predictions of natural enemies–mediated AR for both total *Iva* gall parasitism rate and species–specific parasitism rate response variables.

Torymus umbilicatus is the member of the parasitoid guild thought to be most likely responsible for AR by Stiling et al. (2003). The increase in *T. umbilicatus* frequency in the current study is especially notable given the increase in mean *Iva* gall diameter, which likely accounts for the overall decline in total parasitism rates. Gall diameter affects gall midge resistance to parasitism. The much longer ovipositor of *T. umbilicatus* in comparison to its fellow guild members (>2×) affords it much greater opportunity to parasitize *A. borrichiae* for more of the gall midge's development time. The smaller *Iva* galls may never become too large for *T. umbilicatus* to parasitize.

Barbosa and colleagues (2009) found in their review of the literature that associational susceptibility, not associational resistance, was a more important phenomenon in plant–insect interactions, while AR was important when the herbivores were mammals. Similarly, in a study which simultaneously considered the effects of both mammalian and insect herbivores, Axelsson and Stenberg (2012) found that damage to plants from moose (*Alces alces*) was more extensive and more important than was herbivory from two beetles (*Bromius obscurus* and *Altica* sp., Chrysomelidae) and also found that AR affected the degree of mammal–induced damage on meadowsweet



a.



b.

Figure 2 a. Change in mean (±1 SE) *Iva* gall parasitism rates between the original and current studies according to *Borrichia* abundance treatment groups. b. Change in mean (±1 SE) *Torymus*–specific parasitism rates between the original and current studies among *Borrichia* abundance treatment groups

(*Filipendula ulmaria*, Rosaceae) in the presence of fireweed (*Epilobium angustifolium*, Onagraceae), but that plant association did not alter the degree of insect damage. However, a trapping study of the pine processionary moth, *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae), caught fewer males and found fewer nests among *Pinus pisaster* when cut plant material from *Betula pendula* was placed near the bases of the pines (Jactel et al. 2011), offering additional evidence that AR can affect insect herbivory. The reduction in *T. pityocampa* was attributed to the semiochemical diversity hypothesis, wherein the volatile compounds of non–focal species interfere with the ability of insect herbivores to orient properly to their focal host.

Our study is unusual in that few studies have demonstrated natural enemies as a mechanism of AR (Barbosa et al. 2009, but see Root 1973, Russell 1989). AR by natural enemies is the most likely factor influencing population size of *A. borrichiae* on *Iva*. Given the fact that *A. borrichiae* attacks both *B. frutescens* and *I. frutescens*, repulsion of *A. borrichiae* by an increase in *B. frutescens* abundance is unlikely and therefore the repellent plant hypothesis can be safely discounted as an explanatory mechanism. Similarly, another possible mechanism of AR, attractant decoy, is unlikely despite not being directly tested here, based on the results of a separate study which found significant host–associated genetic divergence in *A. borrichiae* (Stokes et al. 2012). That study and an earlier host choice experiment (Rossi et al. 1999) concluded that female *A. borrichiae* were exhibiting natal host fidelity in oviposition, which is inconsistent with the predictions of the attractant decoy hypothesis.

This study shows that AR mediated by natural enemies can be important for influencing insect populations and community structure within the confines of small offshore islands separated by about 1 km of open water. More work is needed to determine how the relative strength of AR varies over a range of different distances separating *Iva* and *Borrichia* patches and variation in AR with the relative size of each species patch. More studies of different systems are also needed to determine if parasite–mediated AR is responsible for affecting population and community dynamics in other plant–insect interactions. Studies should be designed to take into account the possibility of other mechanisms generating AR, or incorrect conclusions may result from an excessively reductionistic program of research (Brigandt and Love 2012).

Chapter 3:

Effects of relative host plant abundance, density and inter-patch distance on associational resistance to a coastal gall-making midge, *Asphondylia borrichiae* (Diptera: Cecidomyiidae)

Abstract

Associational resistance (AR) is an emergent property of ecological communities and may play an important role in their assembly and structuring. Gall densities of the midge *Asphondylia borrichiae* Rossi & Strong (Diptera: Cecidomyiidae) on the coastal plant *Iva frutescens* L. (Asteraceae) are reduced in the presence of a second host, *Borrichia frutescens* (L.) (Asteraceae). In this system associational resistance is mediated by parasitoid natural enemies that emerge from *B. frutescens* galls and attack galls on *I. frutescens*, thereby reducing gall densities on *I. frutescens*. I quantified distances between patches of *I. frutescens* and *B. frutescens* and the relative abundances of both plant species and predicted that gall densities would be reduced and parasitism rates elevated on *I. frutescens* closer to *B. frutescens* compared with more distant patches and on *I. frutescens* occurring with relatively greater *B. frutescens* abundance in comparison to reduced ratios of *Borrichia* to *Iva* abundance. Although gall densities were elevated on *I. frutescens* more distant from *B. frutescens,* as compared with *I. frutescens* adjacent to *B. frutescens*, parasitism rates were unaffected by patch distance in this system. Increasing relative abundance of *B. frutescens* was found to significantly reduce gall densities on *I.*

frutescens, though parasitism rates on *I. frutescens* galls were unaffected by *B. frutescens* abundance. These results suggest other factors, e.g. environmental quality, host plant genotype, etc., may swamp out the effects of parasitoid–mediated AR in this system as *I. frutescens* becomes more distant from *B. frutescens* or as the abundance of *B. frutescens* relative to *I. frutescens* is reduced.

Introduction

Associational resistance (AR) occurs when there is a reduction in herbivore load or attack rate on a plant species growing in a mixed community relative to the herbivore load on that species when grown in monoculture. The converse phenomenon is known as associational susceptibility (AS) and has been found to occur more frequently in plant-insect interactions than AR (Barbosa et al. 2009). A number of mechanisms for AR have been hypothesized, among them the resource concentration hypothesis, the enemies hypothesis, the semiochemical diversity hypothesis, the repellent plant hypothesis and the attractant decoy hypothesis (Tahvahnainen and Root 1972, Root 1973, Atsatt and O'Dowd 1976, Russell 1989, Jactel et al. 2011). Parasitoid–mediated AR has previously been observed in the interactions of the gall midge *Asphondylia borrichiae* Rossi & Strong (Diptera: Cecidomyiidae) with two of its host species, *Borrichia frutescens* (L.) DC. (Asteraceae) and *Iva frutescens* L. (Asteraceae) (Stiling et al. 2003). The relatively larger *A. borrichiae* galls on *B. frutescens* produce relatively larger parasitoids. The parasitoids produced in such galls are more capable of parasitizing the relatively smaller *A. borrichiae* galls produced on *I. frutescens*, which are susceptible by dint of a size effect.

On small offshore spoil islands near the west coast of central Florida, *I. frutescens* gall parasitism rates were higher and galling rates lower in the presence of neighboring *B. frutescens*, but the converse effect was not observed (Stiling et al. 2003). A follow–up study on the same islands, subsequent to large–scale additions and reductions of *B. frutescens* from different spoil islands supported earlier observational studies (Stokes and Stiling 2013). Because *I. frutescens* gall densities were reduced and parasitism rates were higher where *B. frutescens* was added, while the reverse was observed where *B. frutescens* was removed, spillover of parasitoids from *B. frutescens* to *I. frutescens* is likely the most important mechanism of AR in this system. The parasitoid *Torymus umbilicatus* is likely the member of the parasitoid guild most responsible for the AR effect observed, given that this species' specific parasitism rate increases in the presence of or increased abundance of *Borrichia*, which is facilitated by its possession of an ovipositor over 2× longer than those of other guild members, permitting hyperparasitism later in gall development (Stiling and Rossi 1994). The attractant decoy hypothesis and other explanatory mechanisms requiring host switching by the gall midge are unlikely, given that host choice experiments showed minimal cross-genus oviposition by *A. borrichiae* (Rossi et al. 1999) and a molecular population genetic study supported the results of the host choice experiment and provided evidence in favor of genetic divergence among host-species-associated populations at two locales (Stokes et al. 2012). Thus, *I. frutescens* and *B. frutescens* are host to distinct populations with little migration between hosts.

Here I present data from three additional studies where I seek to quantitatively model response variables (i.e., galling rate and parasitism rate on *I. frutescens*) with factors that are likely to affect parasitoid-mediated AR: distance separating host plant species, relative abundance of host plant species, and gall densities and parasitism rates on *B. frutescens*. I expect gall densities to increase and parasitism rates to decrease on *I. frutescens* with increasing distance from *B. frutescens*. Further, I predict the relationship between the ratio of *B. frutescens* to *I. frutescens* abundance and *I. frutescens* gall densities to be negative, while the host plant relative abundance ratio should have a positive relationship on *I. frutescens* gall parasitism rates.

Methods

Effect of host patch distance on coastal islands.

This study was conducted on 6 spoil islands, part of a series of spoil islands spaced at intervals no less than 0.8 km off the west Florida coast of the Gulf of Mexico near Clearwater and Tarpon Springs, Florida (Fig. 3). The islands are the result of a dredging operation by the U.S. Army Corps of Engineers in the 1960s and have subsequently been naturally colonized by coastal plants and their insect herbivores. All islands had two patches of *I. frutescens*, one adjacent to (<1 m) *B. frutescens* in island centers and one distant from *B. frutescens* (typically >10 m) at the edges of islands, near water and susceptible to tidal input. Monthly counts of galls were conducted from April 2009 through January 2010 to estimate galling rates in patches of *I. frutescens* adjacent to and distant from patches of *B. frutescens*, as well as on *B. frutescens*. During each sampling period, gall counts were taken of 100 haphazardly-selected leaf buds on each of five different *I. frutescens* bushes in patches near and far from *B. frutescens* patches, as well as gall counts on 400 haphazardly-selected *B. frutescens* leaf buds. Mature galls

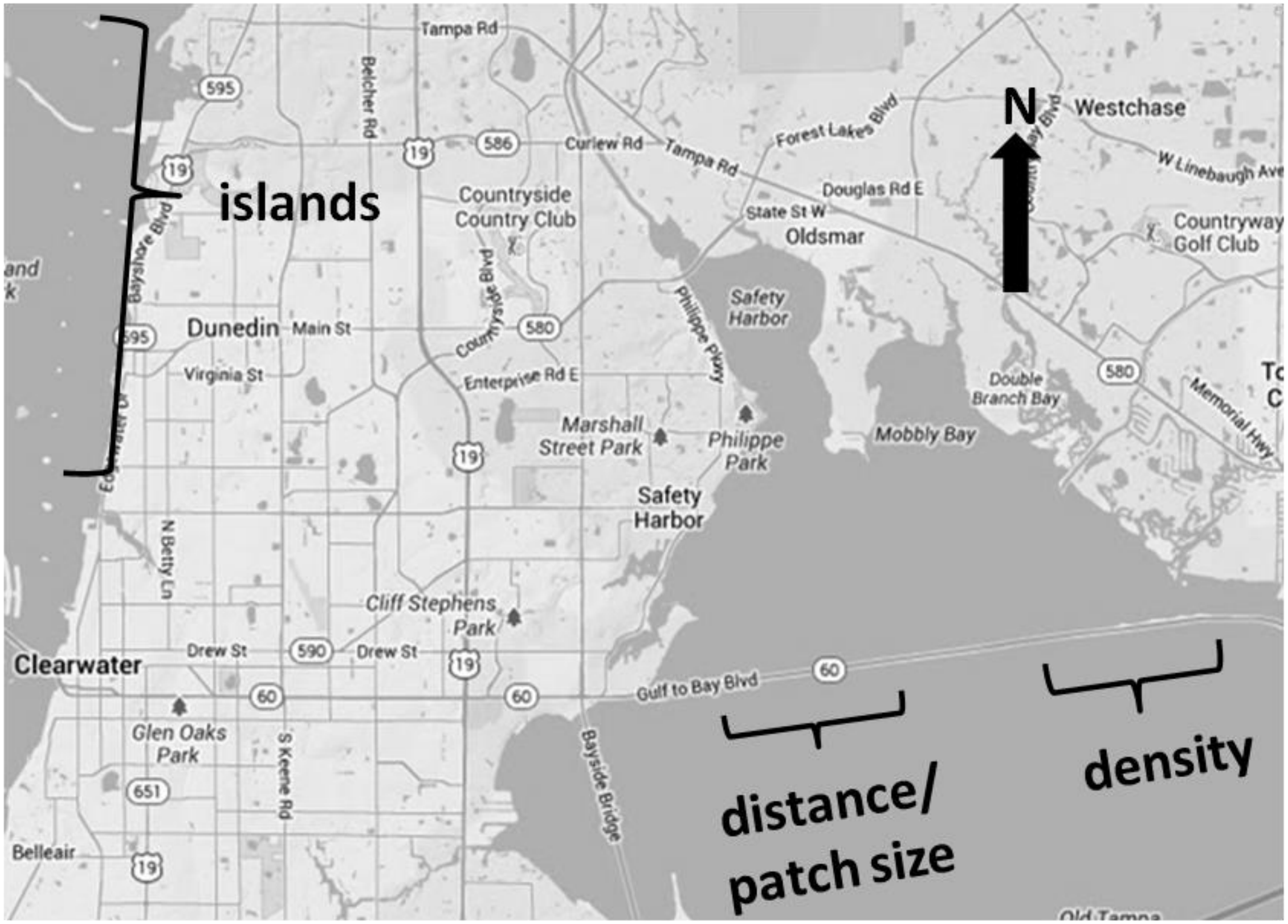


Figure 3 Map showing overview and relative location of each of the three studies. Tampa (Hillsborough County), Florida is to the east.

were collected from near and distant *I. frutescens* patches on all islands and returned to the lab for culturing of the gall community to determine parasitism rates.

Aggregate mean differences in galling rate, total parasitism rate, and *T. umbilicatus*–specific parasitism rates between patches of *I. frutescens* near to and far from *B. frutescens* were compared using paired–sample *t*–tests, using islands as replicates. I predicted galling rates on *I. frutescens* close to *B. frutescens* to be lower, and parasitism rates higher, than on *I. frutescens* distant from *B. frutescens*.

Effect of *B. frutescens* abundance on AR – Courtney Campbell Causeway East.

The Courtney Campbell Causeway (CCC) is a roadway which extends across northern Tampa Bay from Tampa, Hillsborough County, Florida to Clearwater, Pinellas County, Florida, On the east side of the CCC bridge is an extensive area of *B. frutescens* forming a continuous ground cover matrix with *I. frutescens* shrubs emerging intermittently, such that the distance between the host species is zero; moreover, relative host plant abundances vary with varying *B. frutescens* stem density. Monthly gall counts were performed from January 2009 to September 2009 to determine gall densities on *I. frutescens* and *B. frutescens*. Additionally, areal density of potential oviposition sites (leaf buds) on *B. frutescens* on both sides of each *I. frutescens* shrub in the study was measured and the local average determined. Gall emergence holes were counted concurrently on both species and classified according to whether they were the result of emerging gall midges or parasitoids (Stiling and Rossi 1997). Linear regression analysis was performed using *I. frutescens* gall density and parasitism rate as response variables and *B. frutescens* gall density, oviposition site areal density and parasitism rate as independent variables influencing *I. frutescens* gall density. I predicted *I. frutescens* gall density to be lower and parasitism rate higher as *Borrichia* gall density, oviposition site density and parasitism rate increased.

Effect of host patch distance and relative abundance on AR – Courtney Campbell Causeway West.

On the western side of the CCC bridge, discrete patches *B. frutescens* and *I. frutescens* of various relative abundances occur in a narrow band at random intervals, in effect forming a linear transect, approximately 4 km distant at the nearest sampling point to the CCC-East density study (Fig. 3). These patches were used to study both the effects of relative host species abundance and the effects of inter–patch distance on gall densities and parasitism rates on *I. frutescens*. Monthly censuses were performed from February 2009 to October 2009 to determine gall densities on *I. frutescens* and *B. frutescens*. Gall parasitism rates were determined by counting emergence holes and classifying them according to whether they were the result of emerging gall midges or parasitoids. Additionally, both *I. frutescens* and *B. frutescens* patch sizes and areal densities of potential oviposition sites (i.e., leaf buds) were determined to estimate the total number of potential oviposition sites per patch (*B. frutescens*) or per bush (*I. frutescens*) and the ratios of the abundances were log-transformed to standardize the scale as the host which is more abundant switches from one host species to the other. Distances between edges of adjacent patches were measured to obtain a minimum inter-patch distance for each pair of patches. To quantitatively model the effects of *Iva–Borrichia* inter–patch distance and relative oviposition site abundance on AR, linear regressions were conducted on three *I.*

frutescens response variables for parasitoid–mediated AR: galling rate, total parasitism rate and *T. umbilicatus*–specific parasitism rate.

Results

Effect of host patch distance on coastal islands.

Although differences in mean galling rates between near and distant patches of *I. frutescens* were statistically significantly different, with galling rates much higher on *I. frutescens* bushes distant from *B. frutescens* (t = 4.261, df = 5, P < 0.01; Fig. 4a), overall parasitism rates were not significantly different (t = 1.816, df = 5, P > 0.05; Fig. 4b). Furthermore, *T. umbilicatus*–specific parasitism rates were significantly greater on *I. frutescens* patches distant from *B. frutescens* in comparison to near *I. frutescens* patches (t = 3.548, df = 5, P < 0.01; Fig. 4c).

Effect of *B. frutescens* abundance on AR – Courtney Campbell Causeway East.

Of the four comparisons performed by linear regression (*B. frutescens* galling rate on *I. frutescens* galling rate, *B. frutescens* leaf bud areal density on *I. frutescens* galling rate, *B. frutescens* parasitism rate on *I. frutescens* parasitism rate, and *B. frutescens* parasitism rate on *I. frutescens* galling rate), none were statistically significant (Table 1).

Effect of Host Patch Distance and Relative Abundance – Courtney Campbell Causeway West

Linear regressions were performed to measure the effect of each of three variables (*B. frutescens* galling rate, distance between host species patches, and relative host

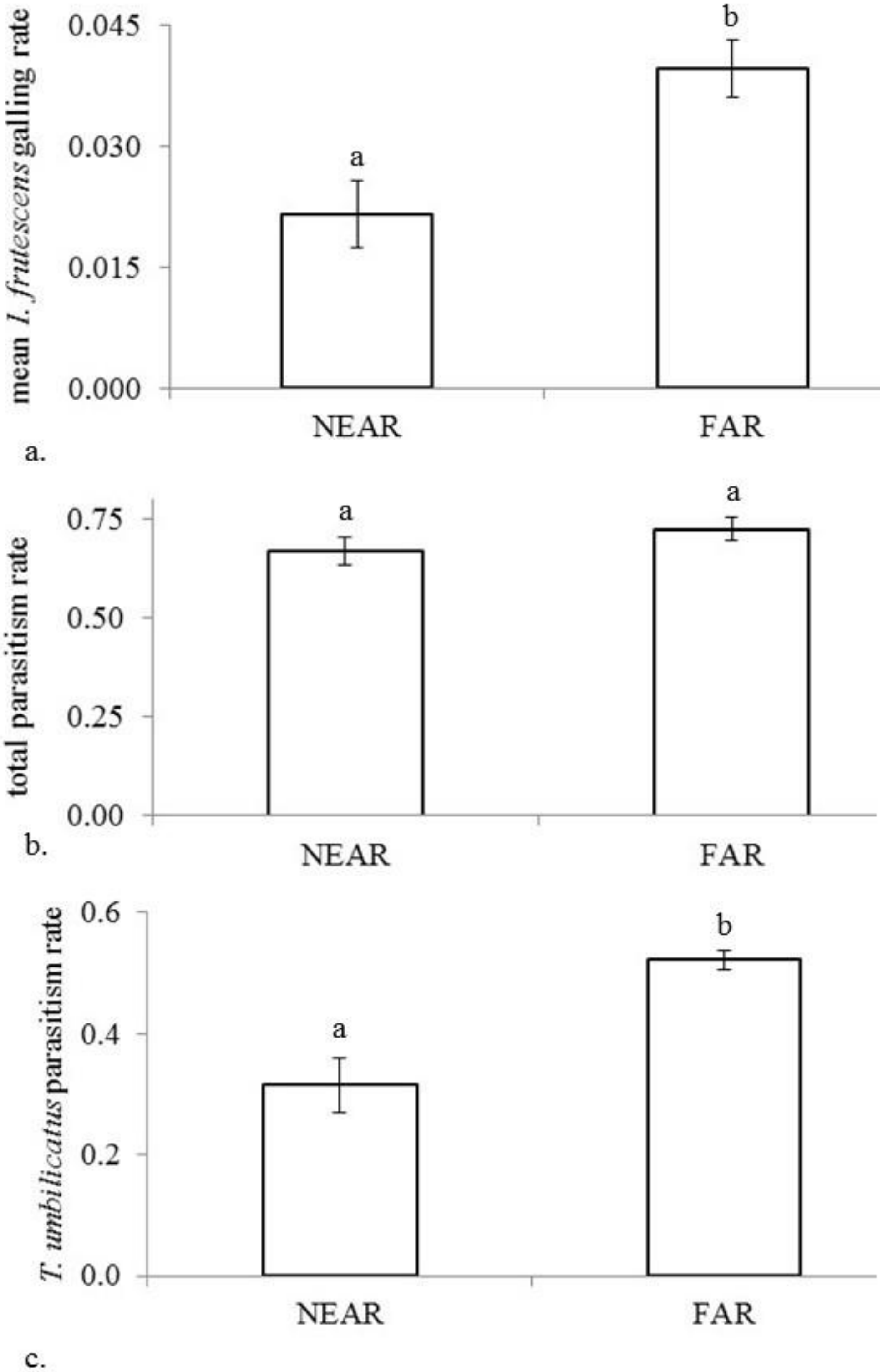


Figure 4 Comparison of (a.) mean galling rates (±1 SE) (b.) mean total parasitism rates and (c.) mean *Torymus umbilicatus*–specific parasitism rates (±1 SE) on *Iva frutescens*

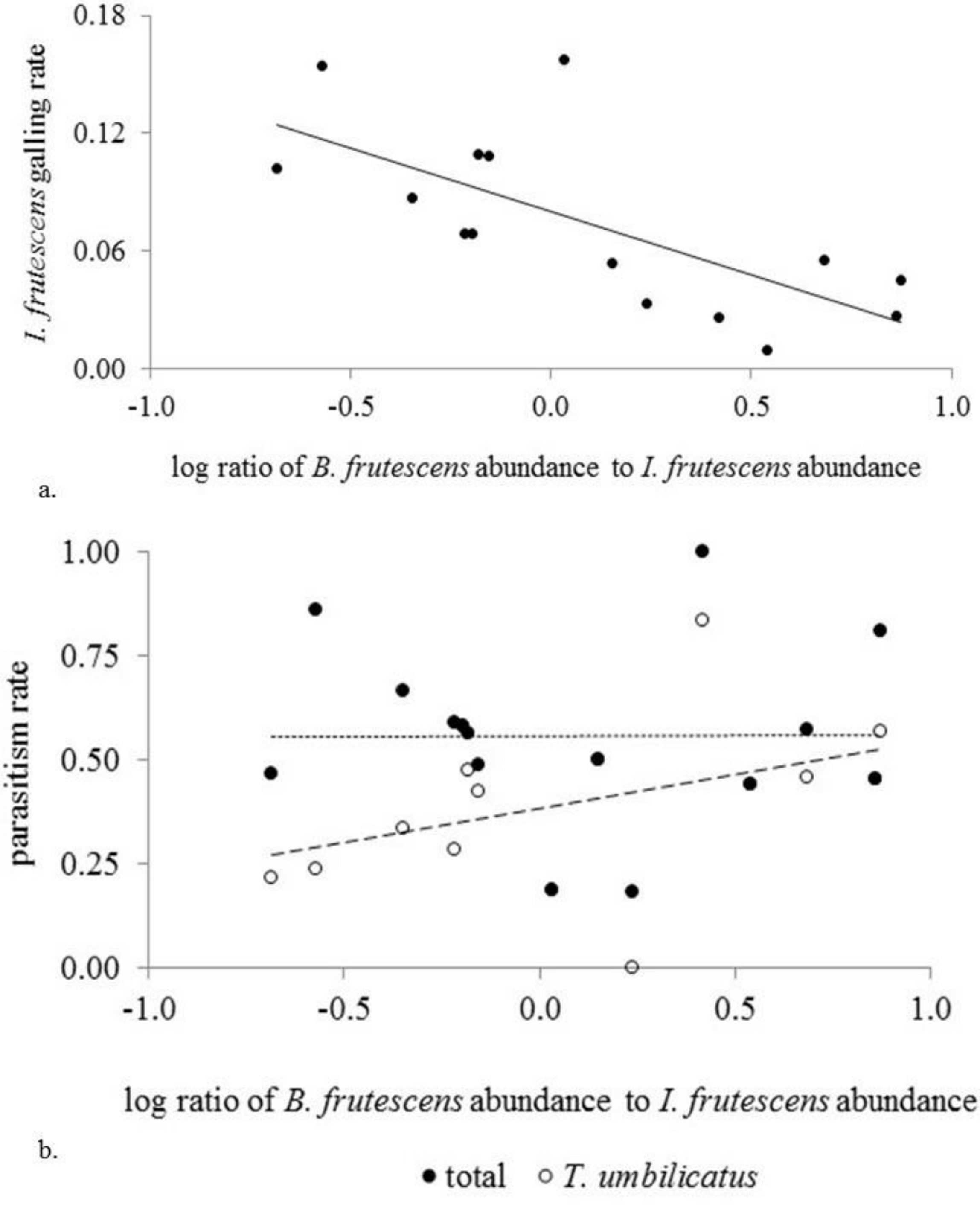


Figure 5 Relationship between log-10-transformed *B. frutescens* to *I. frutescens* relative abundance and (a.) *Iva frutescens* galling rate and (b.) total and *Torymus umbilicatus*–specific parasitism rates.

Table 1 Results of linear regressions in the density study at Courtney Campbell Causeway-East. No significant results were detected.

Predictor variable	Response variable	R	*P*
B. frutescens galling rate	*I. frutescens* galling rate	0.39	0.09
B. frutescens leaf bud areal density	*I. frutescens* galling rate	0.22	0.36
B. frutescens parasitism rate	*I. frutescens* galling rate	< 0.01	0.99
B. frutescens parasitism rate	*I. frutescens* parasitism rate	0.34	0.20

Table 2 Results of linear regressions between 3 predictor and response variables at Courtney Campbell Causeway-West. Values provided are the regression coefficient and the *p*-value of the model, respectively. * statistically significant relationship.

	Predictor Variable		
Response variable	*B. frutescens* galling rate	Host patch distance	Log relative host plant abundance
I. frutescens galling rate	0.33, 0.24	0.07, 0.82	0.71, <0.01*
Total *Iva* parasitism rate	0.04, 0.88	0.02, 0.95	<0.01, 0.99
T. umbilicatus-specific parasitism rate on *Iva*	0.10, 0.73	< 0.01, 0.99	0.41, 0.13

abundance) on three response variables: *I. frutescens* galling rate, overall parasitism rate on *I. frutescens* and *T. umbilicatus*–specific parasitism rate on *I. frutescens*. Of these nine comparisons (Table 2), the only significant relationship was a negative correlation between *I. frutescens* galling rate and log-transformed relative host abundance, which explained approximately 50% of the observed variation (F = 13.017, r = 0.7073, P < 0.01; Fig. 5).

Discussion

In a prior study, Stiling and colleagues (2003) found a strong effect of *B. frutescens* galls on *I. frutescens* gall densities, which they attributed to parasitoids, especially *T. umbilicatus*, from *B. frutescens* spilling over and attacking *A. borrichiae* galls on *I. frutescens*. A follow–up study involving large scale experimental additions and removals of *B. frutescens* on coastal spoil islands found additional support for the existence of parasitoid–mediated AR in this system (Stokes and Stiling 2013). However, the data presented here cannot further clarify the effects of inter-patch distance or patch sizes on the strength of AR operating in the system. Although *I. frutescens* galling rates were significantly lower on spoil islands when *I. frutescens* was adjacent (<1 m) to *B. frutescens* in comparison to when *I. frutescens* was located distant (>10 m) from it, parasitism rates showed no such pattern and *T. umbilicatus* parasitism rates were significantly higher on patches of *I. frutescens* distant from *B. frutescens*. One possible explanation for these observed patterns is that there may be a high dispersal rate of both gall makers and parasitoids from islands. Following a flight of at least 0.8 km over open water, all immigrating insects, gall makers and parasitoids alike, may oviposit extensively on the first appropriate plants encountered, which happen to be the *I. frutescens* bushes occurring at a distance from *B. frutescens*, on island edges.

It was expected that distance between patches would affect the strength of AR along the western side of the Courtney Campbell Causeway. Such an effect would possibly weaken quickly, with AR rapidly dropping off after short distances, as in the case of the repellent plant hypothesis because of diffusion of noxious odors (Tahvanainen and Root 1972, Atsatt and O'Dowd 1976). Surprisingly, no effect of distance between patches on AR was detected in the study, possibly because the study design did not exceed the cruising range of the natural enemies, which is unknown. However, *B. frutescens* gall densities were generally low in the area examined, reducing the ability to detect distance effects on parasitoid–mediated AR.

Despite the inability to detect an effect of inter–patch distance on AR mediated by natural enemies, *I. frutescens* galling rates strongly declined (by >5×) with increasing relative abundance of *B. frutescens*, corresponding to general predictions of AR and accounting for 50% of the variation in *I. frutescens* gall density. However, overall parasitism rates and *T. umbilicatus*–specific parasitism rates were unaffected by relative abundance of *B. frutescens*. An increase in *B. frutescens* oviposition site availability may indicate conditions favorable to *B. frutescens* and, possibly, less favorable for *I. frutescens*. Decreased microenvironmental favorability for *I. frutescens* may, in turn, decrease *I. frutescens* plant quality and thereby depress galling rates.

In summary, these results shed little light on the effects of inter-patch distance and relative host abundance on the strength of parasitoid–mediated AR in the *Asphondylia borrichiae–Borrichia frutescens–Iva frutescens* system. Genotype of *B. frutescens* and local environmental conditions are both known to affect gall densities on *B. frutescens* (Stiling and Rossi 1996). *Iva frutescens* gall densities are also likely to be influenced by environment and plant genotype. A mosaic of *I. frutescens* and *B. frutescens* genotypes on coastal islands and along the Courtney Campbell Causeway, together with a varied range of microenvironmental factors could obscure distance and relative abundance effects in this system. More detailed studies are needed to disentangle the relative strengths of the effects of *I. frutescens* and *B. frutescens* relative abundance, plant quality, genotypic susceptibility and environmental quality to galling and inter–patch distance on parasitoid–mediated AR in this system.

Chapter 4:

Effects of Competition from Stemborers on a Gall Community

Abstract

Interspecific competition between phytophagous insects using the same host plant occurs frequently and strongly affects populations of competing species. Competition between gallmakers and stemborers could be especially intense because both types of herbivore are unable to avoid competition by relocation during their immature stages. For apical meristem gallmakers the main result of competition is likely to be the interruption of resources to the gall by the stemborers' devouring of stem contents. The proximate effect of such competition could be to reduce gall size, increase the number of chambers per gall unit volume and to reduce the size and potential reproductive output of the gallformer. Additionally, smaller galls may be more susceptible to attack from size–limited parasitoids, resulting in a second indirect effect of competition. Using a community of galling and stemboring insects on the saltmarsh shrub *Iva frutescens*, I measured the varied indirect effects of competition. I examined the primary indirect effect of competition on gall midge crowding and the secondary effects on parasitism and parasitoid guild composition. Results indicated that galls co-occurring with stemborers were smaller, crowding of gall inhabitants was 22% greater, and the composition of the parasitoid guild was altered relative to galls on unbored stems. The overall parasitism rate was not different on galls between bored and unbored stems. These results show that

competition resulting from the presence of stemborers has the potential to affect the gall midge *Asphondylia borrichiae* and secondarily affects its guild of hymenopteran parasitoids.

Introduction

Ecological communities are structured by interactions among organisms, including predation, competition and facilitation (Chase et al. 2002, Callaway and Walker 1997, Stachowicz 2001). Competition has long been assumed to be important in regulating ecological communities, though theory has predicted its effects to vary by trophic level (Hairston et al. 1960, Oksanen et al. 1981). Literature reviews of field experiments by Schoener (1983) and Connell (1983) found that competition frequently operates in natural systems. Competition seems to be especially important in phytophagous insects, negatively affecting body size, abundance and fecundity (Kaplan and Denno 2007). Much of this competition is mediated by the host plants themselves. For example, gall forming aphids occurring on the leaf midrib of *Pistacia palaestina* plants are strong nutrient sinks, diverting resources away from other leaflet margin gall formers and causing death rates of 84% (Inbar et al. 1995).

The coastal shrub marsh elder (*Iva frutescens* L.) is attacked by the gall midge *Asphondylia borrichiae* Rossi & Strong (Diptera: Cecidomyiidae) and at least two species of stemboring insects, a lepidopteran (*pers. obs.*) and *Neolasioptera* sp. (Diptera: Cecidomyiidae) (Stiling et al. 1999, R. Gagne 2012, *pers. comm.*). *Asphondylia* oviposition results in an ambrosia gall, a tumor–like structure with multiple fungus–lined chambers, each chamber containing a developing midge. The immature stages of the midge are sessile in the gall and are vulnerable to attack by a guild of four hymenopteran parasitoid species: *Torymus umbilicatus* (Gahan) (Torymidae), *Tenuipetiolus teredon* (Walker) (Eurytomidae), *Rileya cecidomyiae* Ashmead (Eurytomidae) and *Galeopsomyia haemon* (Walker) (Eulophidae) (Stiling et al. 1992). The first, *T. umbilicatus*, is the largest guild member and has the longest ovipositor at nearly 4.00 mm; the other guild members possess ovipositors less than half that length on average (Stiling and Rossi 1994). Stiling et al. (1999) found a highly significant negative co–occurrence between *A. borrichiae* and *Neolasioptera* sp. and hypothesized that the gall-former/stemborer interaction might be so asymmetrical as to be an amensalism, given the improbability of *Asphondylia* having a negative effect on the stemborer. Because of that result, I hypothesized that when stemborers and gallers inhabit the same stem, the activity of stemborers might interfere with the transport of water and nutrients to the gall and that competition might result in reduced gall size and increased number of chambers per gall unit volume (i.e. crowding factor). In addition, because parasitoids are size–limited, competition might increase the gall formers' susceptibility to parasitism. Further, species of the guild of parasitoid hymenopterans which attack *A. borrichiae* differ in mean ovipositor length, which could result in shifting guild composition if gall diameter decreases in response to the presence of stemborers. Specifically, I predict gall diameters will decrease on stems containing stemborers and that crowding factor and total parasitism rate will be greater, while *Torymus umbilicatus*–specific parasitism rate will be reduced.

Methods

To test the effects of competition from stemborers on gallmakers and their parasitoids, *Iva* stems with and without *Asphondylia* galls were collected from seven spoil islands near Tarpon Springs, Florida and Clearwater, Florida. These spoil islands, built from dredged material by the U.S. Army Corps of Engineers during the early 1960s, were roughly equivalent in area and located approximately at 1 km intervals. The islands were subsequently colonized by locally occurring species. Twenty galled stems were detached at the point of branching from a more proximal stem; at the same time, an adjacent, similar ungalled stem from the same *Iva* bush was also collected for each galled stem collected. Collections were performed once each month during April, May, October, November and December of 2010 when gallers are most common. Stems and galls were inspected for emergence holes and gall diameters recorded before placement in plastic bags for culturing of insects developing in the plant tissue. Emergent insects were stored in 99% v/v isopropanol for identification. I compared diameters of galls on stems with and without stemborers using a two–way ANOVA with stemborer presence and island as main effects. Parasitism rates were compared on bored and unbored stems by means of a *t*–test using aggregate island parasitism rates summed across all collection dates as replicates and eliminating islands with fewer than 10 gall emergences in culture with which to estimate parasitism rate. I also tested for a relationship between aggregate stemborer frequency and parasitism rate by site, using islands as replicates. Knowing that gall crowding, which is partially dependent on gall size, can influence size of female *A. borrichiae* and their potential fecundity (Rossi et al. 1999), I estimated crowding for each gall. First, the number of emergence holes on each gall at the point of collection and culturing, together with the insects emerged during culturing, were used to determine the minimum number of chambers (=oviposited *Asphondylia* eggs) per gall. Second, gall diameters were measured across the widest plane perpendicular to the axis of the stem and gall volumes were estimated using the formula $4/3\pi r^3$, using half of the diameter measurement as the radius and assuming an approximate spherical shape for the galls. Finally, the chamber–to–volume ratio was used as a measure of crowding (following Rossi et al. 1999). Because parasitism rates were high (frequently exceeding 80%), too few females were obtained to directly test the effect of stemborer presence on *A. borrichiae* fecundity. Instead, I tested for differences in crowding factor between bored and unbored stems as a proxy test of the hypothesis using Welch's approximate *t*-test.

Results

Significant differences were detected in gall diameter between bored and unbored stems ($F = 17.115$, df = 1, $P < 1\times10^{-4}$) and among sites ($F = 26.328$, df = 1, $P < 1\times10^{-6}$). Gall diameters were greater on unbored stems for four of the seven collection sites and the mean gall diameter on unbored stems over all sites was 6.94 mm, compared to 6.42 mm for galls on bored stems (Fig. 6), a decrease in estimated volume of approximately 21%. There was no interaction effect between factors ($F = 0.072$, df = 1, $P \approx 0.79$), indicating that stemborer presence had the same proportionate effect on all islands.

No difference was detected in parasitism rates between galls on bored and unbored stems ($F = 0.787$, df = 4, 6, $P = 0.5734$; $t = 0.494$, df = 10, $P = 0.6319$). A linear regression of total gall parasitism rate against aggregate stemborer frequency on each island found no relationship between the factors ($r = 0.2328$, $P \approx 0.60$). However, a comparison of gall parasitoid guild composition did show that the presence of stemborers resulted in a marginally significant alteration of parasitoid guild composition ($\chi^2 = 9.23$, df = 4, $P \approx 0.056$, Fig. 7). In larger galls, where stemborers were absent, *Torymus* parasitoids, which have ovipositors twice the length of those of other parasitoid guild members (Stiling and Rossi 1994), were more common. In smaller galls, where stemborers were present, smaller species of the guild increased in frequency.

Galls on bored stems were significantly more crowded than on unbored stems (F=1.61, df = 295, 194, P<0.001; t' = 2.46, df = 489, P<0.02); stemborer presence increased crowding by 22% on average (Fig. 8)

Discussion

Among phytophagous insects, gall forming insects have been viewed as superior competitors because of their ability to molecularly manipulate their host plants, diverting resources from other species. For example, invasive populations of an oak gall wasp, *Neuroterus saltatorius,* in British Columbia reduce foliage quality and decrease biomass production of a specialist butterfly (Prior and Hellman 2010). However, stemborers can also be strong competitors. Stiling and Strong (1983) showed strong direct effects between five species of *Spartina* stemborer, with some species murdering others. Rathcke (1976) also detected some competition between a guild of stemborers in prairie plants. Here I have shown strong effects of *Iva* stemborers on a gall community, mediated by the plant, since stemborers rarely enter the galls. Co-occurrence of gallers with stemborers resulted in significantly smaller galls and increased crowding of midges within galls. Crowding was significantly higher (by 22%) in galls on stems containing stemborers, suggesting resource limitation resulting from stemborers caused a reduction in gall size and a concomitant increase in crowding of midges within galls. I believe this is possibly only the second demonstration of plant–mediated competition between stemborers and other phytophagous insects. Previously, Moon and Stiling (2002, 2005) reported a negative relationship between stemborer density and leafhopper density on *Borrichia*.

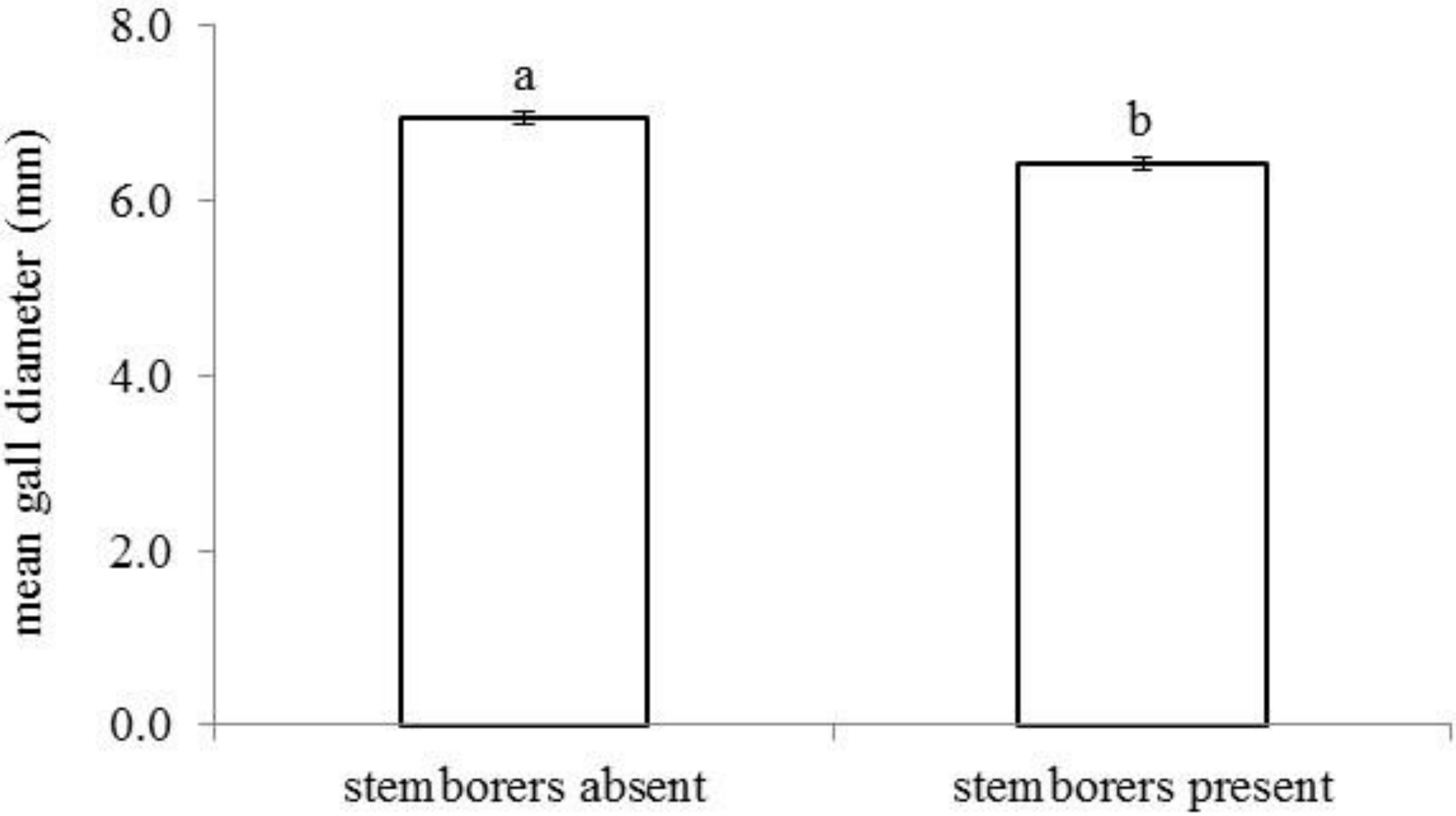


Figure 6 Mean gall diameters (±1 SE) on stems with and without emergent stemborers were significantly different, as designated by letters.

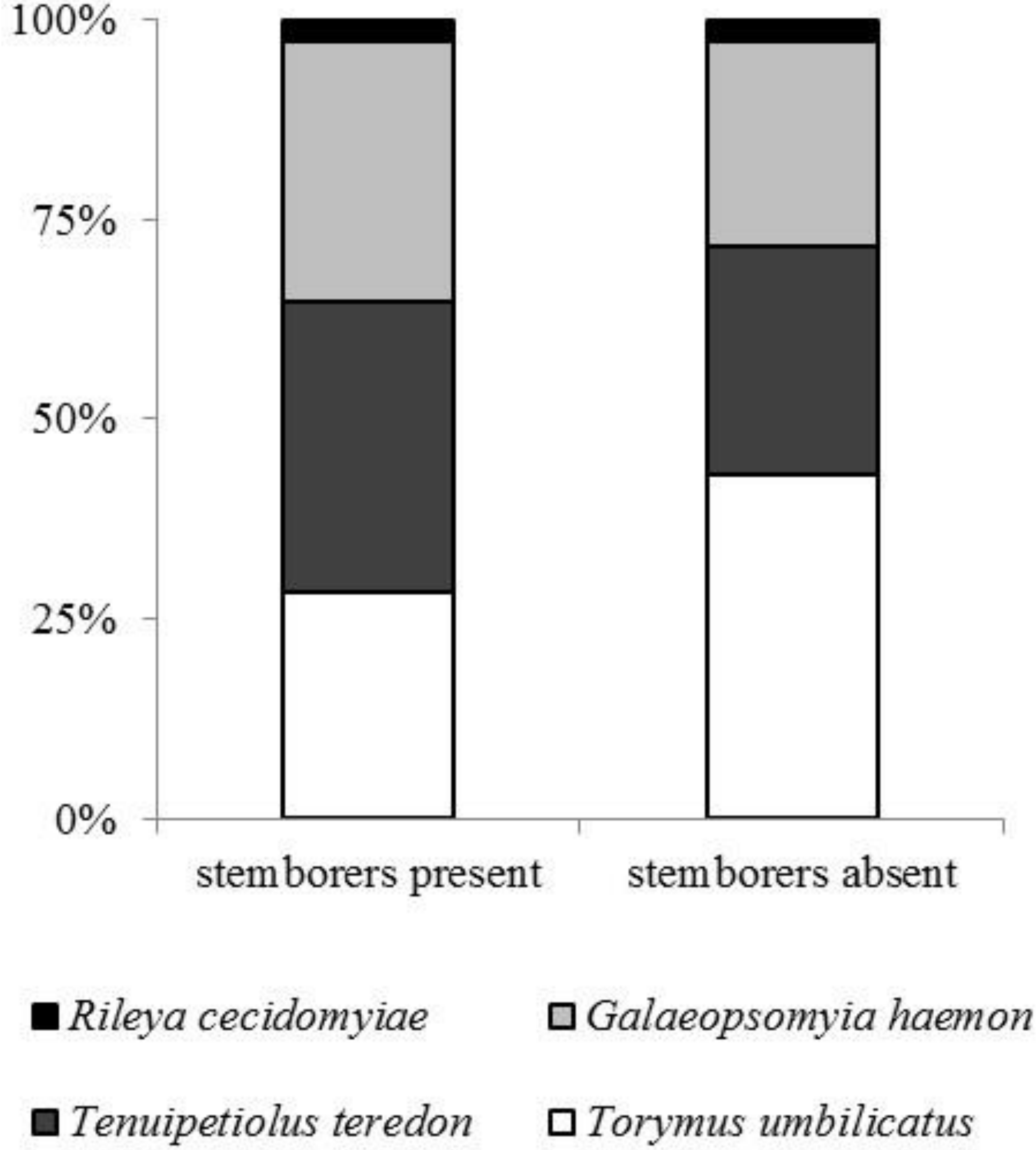


Figure 7 Stemborer presence alters parasitoid guild composition ($P \approx 0.056$).

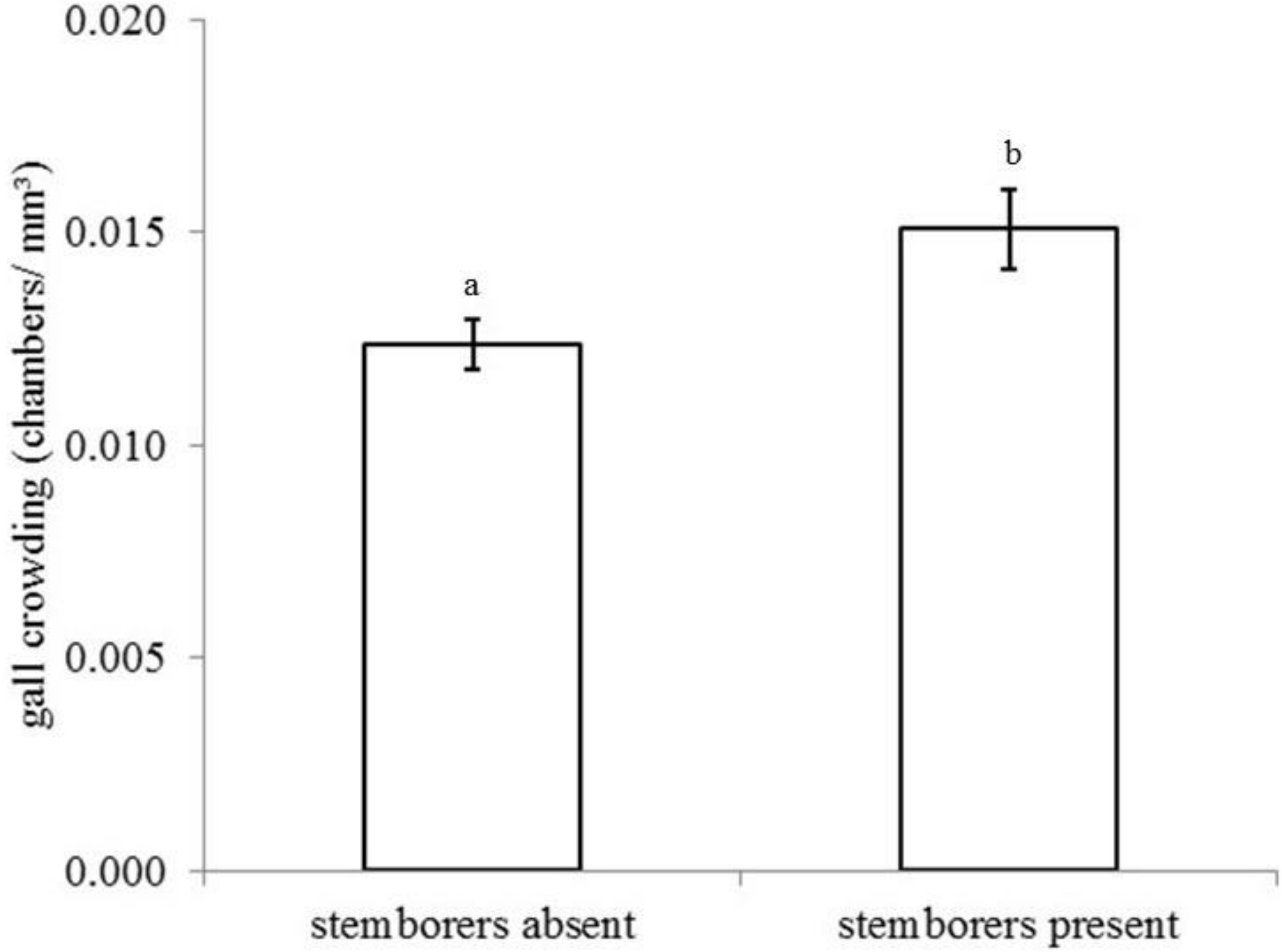


Figure 8 Gall crowding is 22% greater in galls on stems with stemborers than those without. Error bars are ±1 SE. Letters indicate statistically significant differences in crowding between galls on stems with and without emergent stemborers.

The secretive, endophytic nature of stemborers may make them difficult to study, yet their potential to completely devour stem contents makes them highly likely to be strong competitors of many other phytophagous species. High parasitism rates, which frequently exceeded 80%, prevented me from rearing sufficient females from galls on bored and unbored stems to directly test for size differences and differences in egg load. However, a previous study by Rossi et al. (1999) showed that higher crowding factors in *I. frutescens* galls resulted in smaller female midges with reduced fecundity. These results support the findings of Kaplan and Denno (2007), who, in a meta–analysis of competition among phytophagous insects, found frequent and significant effects of interspecific competition on a variety of response factors, including fecundity, body size and survival. Given the method of host plant utilization of each herbivore, the stemborers would be expected to be the dominant competitors, frequently intercepting host resources by virtue of their generally proximal occurrence to the main branch on host stems. It is possible that a more extensive study may detect even more frequent competitive effects of stemborers on gallmakers. Dissections of stems revealed boring rates of about 65%, suggesting that interactions between stemborers and gallmakers are likely frequent.

Parasitism rates were not significantly different between bored and unbored stems. This is probably because in smaller galls, one species of parasitoid is simply replaced by another. Of the four parasitoid species, the largest, *T. umbilicatus*, and the smallest, *G. haemon*, are both facultative hyperparasitoids. In smaller galls *T. umbilicatus* are likely outcompeted by *T. teredon*, *R. cecidomyiae* and *G. haemon*, all of which have ovipositors less than 2.0 mm in length but are more frequent in smaller galls (Stiling and Rossi 1994). In addition, *T. umbilicatus* in small galls are more likely to be hyperparasitized by *G. haemon*. Thus, competition by stemborers can cause a shift in the guild composition of the gall parasitoids, despite not changing parasitism rates overall. I believe this to be one of the first demonstrations of competition from one guild of phytophagous insects altering the parasitoid guild structure of another phytophagous species, mediated by plant traits. The importance of accounting for plant-trait-mediated nontrophic and indirect interactions has been emphasized by Ohgushi (2005), who demonstrated that including such interactions, which are common in natural communities and sometimes more common than direct trophic interactions, is essential for a holistic understanding of factors affecting community composition and assembly.

In summary, competition by stemborers reduces gall size and increases midge crowding within galls, which likely reduced midge size and fecundity. In addition, this competition does not affect gallmaker parasitism rates but does cause a shift in the gall parasitoid community.

Literature Cited

Altieri, MA. 1991. How best can we use biodiversity in agroecosystems? Outlook on Agriculture 20:15–23.

Atsatt, P.R. and D.J. O'Dowd. 1976. Plant defense guilds. Science 193:24–29.

Axelsson, E. P. and J.A. Stenberg. 2012. Associational resistance in a multiple herbivore system: differential effects of mammal versus insect herbivores. Ecological Research 27:1053–1058.

Barbosa, P., J. Hines, I. Kaplan, H. Martinson, A. Szczepaniec, and Z. Szendrei. 2009. Associational Resistance and Associational Susceptibility: Having Right or Wrong Neighbors. Annual Review of Ecology, Evolution and Systematics 40:1–20.

Brigandt, I and A. Love. "Reductionism in Biology", The Stanford Encyclopedia of Philosophy (Summer 2012 Edition), Edward N. Zalta (ed.), URL = <http://plato.stanford.edu/archives/sum2012/entries/reduction-biology/>.

Brown, P. and A.M. Rossi. 2013. Geographic and Host-Associated Size Variation in the Parasitoid Wasp *Torymus umbilicatus* (Hymenoptera: Torymidae) in Florida: Implications for Host Survival and Community Structure. Florida Entomologist 96:832-838.

Callaway, R.M., L.R. Walker. 1997. Competition and facilitation: a synthetic approach to interactions in plant communities. Ecology 78:1958–1965.

Cardinale, B.J., J.E. Duffy, A. Gonzalez, D.U. Hooper, C. Perrings, P. Venail, A. Narwani, G.M. Mace, D. Tilman, D.A. Wardle, A.P. Kinzig, G.C. Daily, M. Loreau, J.B. Grace, A. Larigauderie, D.S. Srivastava and S. Naeem. 2012. Biodiversity loss and its impact on humanity. Nature 486:59–67.

Chase, J.M., P.A. Abrams, J.P. Grover, S. Diehl, P. Chesson, R.D. Holt, S.A. Richards, R.M. Nisbet, T.J. Case. 2002. The interaction between predation and competition. Ecology Letters 5:302–315.

Connell, J. 1980. Diversity and the coexistence of competitors, or the ghost of competition past. Oikos 35:131-138.

Connell, J. 1983. On the prevalence and relative importance of interspecific competition: Evidence from field experiments. The American Naturalist 122:661–696.

Connor, E.F. and D. Simberloff. 1979. The assembly of species communities: Chance or competition? Ecology 60:1132-1140.

Darwin, C.R. 1859. On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for Life. London: John Murray. 1st ed.

Diamond, J.M. 1975. Assembly of species communities. Ecology and Evolution of Communities. (M.L. Cody and J.M. Diamond, eds). Harvard University Press, Cambridge, MA

Gagne, R. 2012. (*Neolasioptera* sp., Diptera: Cecidomyiidae), Systematic Entomology Laboratory, Agricultural Research Service, U.S. Department of Agriculture.

Goldberg, D.E., T. Rajaniemi, J. Gurevitch, and A. Stewart-Oaten. 1999. Empirical approaches to quantifying interaction intensity: competition and facilitation along productivity gradients. Ecology 80:1118-1131.

Gurevitch, J., L.L. Morrow, A. Wallace and J.S. Walsh. 1992. A meta-analysis of competition in field experiments. The American Naturalist 140:539-572.

Gurevitch, J., J.A. Morrison, and L.V. Hedges. 2000. The interaction between competition and predation: A meta-analysis of field experiments. The American Naturalist 155:435–453.

Hairston, N.G., F.E. Smith, L.B. Slobodkin. 1960. Community structure, population control, and competition. The American Naturalist 94:421–425.

Hambäck, P.A., J. Ågren and L. Ericson. 2000. Associational resistance: Insect damage to purple loosestrife reduced in thickets of sweet gale. Ecology 81:1784–1794.

Holt, R.D. 1977. Predation, apparent competition, and the structure of prey communities. Theoretical Population Biology 12:197–229.

Inbar, M., A. Eshel and D. Wool. 1995. Interspecific competition among phloem-feeding insects mediated by induced host-plant sinks. Ecology 76:1506-1515.

Jactel, H., G. Birgersson, S. Andersson, and F. Schlyter. 2011. Non–host volatiles mediate associational resistance to the pine processionary moth. Oecologia 166:703–711. doi 10.1007/s00442-011-1918-z

Kaplan, I. and R.F. Denno. 2007. Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory. Ecology Letters 10:977–994.

Moon, D.C. and P. Stiling. 2002. Top-down, bottom-up, or side to side? Within-trophic-level interactions modify trophic dynamics of a salt marsh herbivore. Oikos 98:480-490.

Moon, D.C. and P. Stiling. 2005. Effects of nutrients and parasitism on the density of a salt marsh planthopper suppressed by within-trophic-level interactions. Ecological Entomology 30:642-649.

Nurse, P. 1997. The ends of understanding. Nature 387:657.

Ohgushi, O. 2005. Indirect interaction webs: Herbivore-induced effects through trait change in plants. Annual Review of Ecology, Evolution and Systematics 36:81-105.

Ohgushi, O. 2008. Herbivore-induced indirect interaction webs on terrestrial plants: the importance of non-trophic, indirect and facilitative interactions. Entomologia Experimentalis et Applicata 128:217-229.

Oksanen, L., S.D. Fretwell, J. Arruda, and P. Niemela. 1981. Exploitation ecosystems in gradients of primary productivity. The American Naturalist 118:240-261.

Ostfeld, R.S. 2013. A Candide response to Panglossian accusations by Randolph and Dobson: biodiversity buffers disease. Parasitology 1196–1198.

Prior, K.M. and J.J. Hellman. 2010. Impact of an invasive oak gall wasp on a native butterfly: a test of plant-mediated competition. Ecology 91:3284-3293.

Raffel, T.R., L.B. Martin and J.R. Rohr. 2008. Parasites as predators: Unifying natural enemy ecology. Trends in Ecology and Evolution 23:610–618.

Randolph, S.E. and A.D.M. Dobson. 2012. Pangloss revisited: a critique of the dilution effect and the biodiversity-buffers-disease paradigm. Parasitology 139:847–863.

Rathcke, B.J. 1976. Competition and coexistence within a guild of herbivorous insects. Ecology 57:76-87.

Root, R.B. 1973. Organization of a plant–arthropod association in simple and diverse habitats: the fauna of collards (Brassica oleracea). Ecological Monographs 43:95–124.

Rossi, A.M. and P. Stiling. 1995. Intraspecific variation in growth rate, size, and parasitism of galls induced by *Asphondylia borrichiae* (Diptera: Cecidomyiidae) on three host species. Annals of the Entomological Society of America 88:39–44.

Rossi, A.M., P. Stiling, M.V. Cattell, T.I. Bowdish. 1999. Evidence for host-associated races in a gall-forming midge: trade-offs in potential fecundity. Ecological Entomology 24:95-102.

Rossi, A.M., P. Stiling, D.R. Strong, and D.M. Johnson. 1992. Does gall diameter affect the parasitism rate of *Asphondylia borrichiae* (Diptera: Cecidomyiidae)? Ecological Entomology 17:149–154.

Russell, E. P. 1989. Enemies hypothesis: A review of the effect of vegetational diversity on predatory insects and parasitoids. Environmental Entomology 18:590–599.

Schoener, T.W. 1983. Field experiments on interspecific competition. The American Naturalist 122:240–285.

Stachowicz, J.J. 2001. Mutualism, facilitation, and the structure of ecological communities. BioScience 51:235–246.

Stiling, P., D. Moon, and D. Gordon. 2004. Endangered cactus restoration: mitigating the non-target effects of a biological control agent (*Cactoblastis cactorum*) in Florida. Restoration Ecology 12:605–610.

Stiling, P. and A.M. Rossi. 1994. The window of parasitoid vulnerability to hyperparasitism: template for parasitoid community structure. Parasitoid Community Ecology (eds. B. Hawkins and W. Sheehan). pp. 228 – 244. Oxford University Press.

Stiling, P. and A.M. Rossi. 1996. Complex effects of genotype and environment on insect herbivores and their enemies. Ecology 77:2212–2218.

Stiling, P. and A.M. Rossi. 1997. Experimental manipulations of top–down and bottom–up factors in a tri–trophic system. Ecology 78:1602–1606.

Stiling, P., A.M. Rossi and M.V. Cattell. 2003. Associational resistance mediated by natural enemies. Ecological Entomology 28:587–592.

Stiling, P, A.M. Rossi, D.R. Strong and D. Johnson. 1992. Life history and parasites of *Asphondylia borrichiae* (Diptera: Cecidomyiidae), a gall maker on *Borrichia frutescens*. Florida Entomologist 75:130-137.

Stiling, P., A.M. Rossi, M.V. Cattell and T.I. Bowdish. 1999. Weak competition between coastal insect herbivores. Florida Entomologist 82:599–608.

Stiling, P. and D.R. Strong. 1983. Weak competition among *Spartina* stemborers by means of murder. Ecology 64:770-778.

Stokes, K. 2008. Host-associated sympatric genetic divergence in the gall midge *Asphondylia borrichiae* Rossi and Strong (Diptera: Cecidomyiidae). Unpublished master's thesis. University of North Florida.

Stokes, K., and P. Stiling. 2013. Effects of large–scale host plant addition and removal on parasitoid–mediated associational resistance in the gall midge *Asphondylia borrichiae*. Ecological Entomology, 38:531-534.

Stokes, K., P. Stiling, M.R. Gilg, and A.M. Rossi. 2012. The gall midge *Asphondylia borrichiae* (Diptera: Cecidomyiidae): An indigenous example of host-associated genetic divergence in sympatry. Environmental Entomology 41:1246 – 1254.

Strauss, S.Y. 1991. Indirect effects in community ecology: Their definition, study and importance. Trends in Ecology and Evolution 6:206–210.

Strong, Jr. D.R., L.A. Szyska and D.S. Simberloff. 1979. Test of community-wide character displacement against null hypotheses. Evolution 33:897-913.

Tahvanainen, J.O. and R.B. Root. 1972. The influence of vegetational diversity of the population ecology of a specialized herbivore, *Phyllotreta cruciferaea* (Coleoptera: Chrysomelidae). Oecologia 10:321–346.

van Hezewijk, B.H. and J. Roland. 2003. Gall size determines the structure of the *Rabdophaga strobiloides* host–parasitoid community. Ecological Entomology 28:593–603.

Wootton, J.T. 1994. The nature and consequences of indirect effects in ecological communities. Annual Review of Ecology, Evolution and Systematics 25:443–466.