

2005

Bottom-up and top-down effects on insects herbivores along a natural salinity gradient in a florida salt marsh

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Bottom-Up and Top-Down Effects on Insects Herbivores Along a Natural Salinity
Gradient in a Florida Salt Marsh

by

Maria Teresa Albarracin

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science
Department of Biology
College of Arts and Science
University of South Florida

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Date of Approval:
March 24, 2005

Keywords: gall makers, sap suckers, *Borrchia frutescens*, fertilizer, sticky traps,
parasitoids, herbivory.

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Acknowledgments

I would like to thank my adviser Peter Stiling for all his help and guidance during this process. For their valuable comments I am thankful with the member of my comittee Susan Bell and Gary Huxel.

I also want to thank the Stiling's lab (Tatiana Cornelissen, Mark Barrett, Amanda Baker, Laura Altfield and Rebecca Forkhner) for their friendship and all their help reviewing this thesis. For their help in the field I want to thank Tatiana, Celina Bellanceau, Gwen Oberholtzer, Ivan Gualtero, Samuel Albarracin and Claudia Bernal.

Thank you to my family in special my sister Claudia and German for their support and to my parents for inspired me. Finally I want to thank Ivan for all this years of friendship and love. To all of you Gracias!

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Bottom-Up and Top-Down Effects on Insect Herbivores Along a Natural Salinity Gradient in a Florida Salt Marsh

Maria Teresa Albarracín

ABSTRACT

I compared the strength of bottom-up and top-down effects on insect herbivores along a natural salinity gradient in salt marsh communities in West-Central, Florida. I used a 2x2 factorial design with plots divided into four different treatments: 1) fertilizer applied to increase plant quality 2) sticky traps added to remove natural enemies (parasitoids) 3) fertilizer applied and sticky traps added and 4) control plots. These plots were placed on 7 different sites containing the salt marsh plant *Borrichia frutescens* along a natural stress salinity gradient. In each plot I determined the abundance of the sap sucker *Pissonotus quadripustulatus*, the gall maker *Asphondylia borrichiae*, spiders and the number of chewed leaves and bored stems. I also recorded leaf area, plant density, plant height and foliar nitrogen. Plants in fertilized plots exhibited increased height, density and leaf area. In fertilized plots, galls were more abundant than in non-fertilized plots (approximately 63% increase), and the same pattern was observed for *Pissonotus* (55% increase). For chewed leaves and spiders there

were non significant increases on fertilized as compared to unfertilized plots. There were no significant effects of natural enemy (parasitoid) removal. Gall density and *Pissonotus* density were both significantly more abundant in the sites with lower soil salinity, but there was no interaction of either treatment with salinity level. My results suggest that in this system bottom up effects are stronger than top down effects but there is no change in the strength of bottom up and or top down effects along an environmental salinity gradient.

Introduction

The understanding of which factors influence the densities of herbivore populations has been pursued by ecologists for decades. Hairston *et al.* (1960) argued for the logical primacy of top-down forces in their widely known “world is green” hypothesis (also known as the HSS hypothesis), suggesting that herbivores are limited by their natural enemies and not by their host plants. Fretwell (1977) and Oksanen *et al.* (1981) extended the HSS theory, suggesting the idea that productivity controls the dynamics of trophic levels in the food chain. They predicted that bottom-up forces may often limit herbivore abundance in food webs which two trophic levels and that top-down forces could control herbivore densities only in food webs with three trophic levels.

In contrast, Menge and Sutherland (1976, 1987) proposed a model in which the strength of biotic factors, such as herbivory or predation, would be strongly influenced by environmental stress. In stressful environments, such as a wave battered shore, natural enemies may not be present so that herbivores would be more influenced by competition or by abiotic factors. In more benign conditions, natural enemies would be more common and have greater effects.

This study aims to investigate effects of both top-down and bottom-up factors on herbivores along a natural salinity gradient in a salt marsh community

in West –Central Florida. Salt marshes are considered stressful ecosystems because they are found in highly saline intertidal zones in which nitrogen levels can be low (Adam 1990). Salinity and nitrogen can induce changes in plant quality that in turn affect the strength of top-down and bottom-up factors. Stiling and Moon (2005 b) performed an extensive factorial experiment in a salt marsh whereby they altered the strength of top-down and bottom-up factors along an experimentally generated salinity gradient (by adding salt pellets) in a large *Borrchia* monoculture. Bottom-up effects, added fertilizer, were greater in more saline plots because nitrogen was more limiting than in less saline plots, and increases of herbivores were greater. The strength of top-down effects also varied, but not in a predictable way. For gall makers, top-down effects from parasitoids were greater in more saline areas because gall growth was limited enabling parasitoids greater access to fly larvae inside and increasing parasitism rates. For sap suckers, which laid their eggs inside stems, top-down effects were less in saline conditions because stems were tougher and less easy to penetrate by parasitic mymarid wasps. Thus, in this system environmental stress did appear to change the effects of top-down and bottom-up factors, but not in a predictable way. However, in nature *Borrchia* occurs in many different clones and in many different sites, each with varying salinity (Stiling and Rossi 1996). We wanted to know if results from an experimentally generated range of salinities in a large *Borrchia* monoculture held true under natural changes of salinity between different sites containing different clones.

Rationale and specific tests

This study used a 2X2 factorial design (Figure 1) along a natural salinity gradient to address the importance of the combined effects of plant nutrients and natural enemies on herbivores on *Borrichia frutescens*.

The following hypotheses were tested:

1. Herbivore densities should decrease along a natural salinity gradient due to the negative impacts of salinity on plant quality and its subsequent effects on herbivore populations.
2. Herbivore densities should be higher in fertilized plots compared to unfertilized plots due to the positive effects of fertilization on *Borrichia* nutritional quality.
3. Herbivore densities and survivorship should be higher in plots in which natural enemies are excluded due to the strong impacts of parasitoids on survivorship of herbivore eggs and larvae.
4. Bottom-up effects should be greatest at more saline sites where nitrogen is more limiting as evidenced by an interaction of fertilizer and salinity on herbivore densities.
5. Top-down effects should vary in strength along a salinity gradient according to the type of herbivore involved. Thus, there should be an interaction between parasitoid removal and salinity on herbivores densities

but the direction of change in herbivore populations along the salinity gradient would be different for galls than for sap suckers.

Treatments															
Fertilizer	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	
Parasites Excluded	Y, N	Y, N	Y, N	Y, N	Y, N	Y, N	Y, N	Y, N	Y, N	Y, N	Y, N	Y, N	Y, N	Y, N	
	1	2	3	4	5	6	7								
	<i>Low</i>			<i>Salinity Gradient</i>							<i>High</i>				

Figure 1. Factorial design to test the effects of bottom-up and top-down forces on *Borrichia frutescens*. Treatments are fertilization (Y = yes, N = no) and exclusion of parasitoids (Y = yes, N = no). Numbers 1 to 7 represent the sites over the salinity gradient.

Methods

Study System

Borrichia frutescens, commonly known as sea oxeye daisy, is a rhizomatous shrub typically less than a meter in height. It is common in the intertidal zones of salt marshes, and can be found in association with *Salicornia virginica*, *Batis maritima* and *Distichlis spicata* (Antlfinger 1981). An important characteristic of *Borrichia* is that it can be found over a wide range of salinities (Richards *et al.* 2004).

Asphondylia borrichia (Diptera, Cecidomyiidae) is a gall-making fly that oviposits in the apical meristems of *Borrichia* stems, inducing the formation of galls. Each gall has on average four chambers and each chamber has one larva (Rossi and Stiling 1995). *Asphondylia* is multivoltine and has around five to seven overlapping generations in a year. It occurs on *Borrichia* throughout the year, but highest densities are usually found in spring and early summer (Rossi *et al.* 1992). *Asphondylia* larvae have four species of hymenopteran parasitoids: *Rileya cecidomyiae* and *Tenuipetiolus teredon* (Eurytomidae), *Torymus umbilicatus* (Torymidae), and *Galeopsomyia haemon* (Eulophidae). Parasitism is the greatest cause of mortality for *Asphondylia* and is relatively easy to observe

in the field by examining the emergence holes of the galls. The parasitoids usually make small round holes, while the emergence hole of *Asphondylia* is bigger with the puparia attached (Stiling and Rossi 1997).

Pissonotus quadripustulatus (Delphacidae) is a phloem feeder, monophagous on *Borrchia* (Denno 1978, Stiling 1994). The adults are brachypterous, a characteristic that makes these insects easy to observe and survey. They are also multivoltine with overlapping generations, and their eggs are laid in the stems of *Borrchia*. Eggs are attacked by the fairy fly *Anagrus* sp. nr *armatus* (Hymenoptera: Mymaridae) and this constitutes by far the greatest cause of mortality (Moon and Stiling 2000). The dryinid parasitoid *Pseudogonatopus arizonicus* also attacks *Pissonotus*, although levels of attack by this parasitoid are generally less than 1% (Stiling and Moon 2005 a).

Another herbivore feeding on *Borrchia* is the stem borer *Argyresthia* sp. (Lepidoptera: Argyresthiidae). In the immature stages this stem borer feeds on the mesoderm beneath the epidermis of the stems. The damage produced is recognizable because the stems have a papery appearance (Moon and Stiling 2002 a). Spiders and damselflies are also found in natural patches of *Borrchia* and may be predators that feed on herbivores present in this system.

Experimental Design

This study was conducted in seven separate salt marshes around Tampa Bay, Florida. Study sites were located in Hillsborough (Simmons Park-Ruskin, Upper Tampa Bay) and Pinellas Counties (Fort de Soto, St. Petersburg, Honeymoon Island and two islands in the intracoastal waterway, called CW6 and CW5). These sites were chosen for their variability in *Borrichia* size and location along a natural salinity gradient. The areas with high salinity tend to have shorter *Borrichia* plants (Richards *et al.* 2004) with smaller leaf area and lower densities of herbivores (Honeymoon Island, Simmons Park, Upper Tampa Bay), while the areas with low salinity have taller *Borrichia* with higher densities of herbivores (CW5, CW6, Fort de Soto). In Fort de Soto there were two distinctly different sites, one with relatively high soil salinity, and one with low soil salinity.

At each site, top down effects were manipulated by reducing parasitism levels using 13x8 cm sticky traps, which are yellow cards, covered with tanglefoot adhesive (Gempler's, Belleville, Wisconsin, USA). Five traps were placed per plot (four in each corner and one in the middle). Moon and Stiling (2002 a) demonstrated that five sticky traps on each plot significantly reduced parasitism levels of both gall makers and sap suckers. Bottom up effects were manipulated by adding urea-based nitrogen fertilizer (46:0:0 NPK) poured over the entire area of the plot. Traps were changed monthly and 50g of fertilizer was added every two months, to avoid burning the plants. Each experimental site

contained three replicates of each treatment with the exception of the islands CW5 and CW6 in which I placed just two replicates, due to their small size, for a total of 72 plots. Surveys of herbivores were made twice every month from March through October 2003. The following treatments were assigned randomly to each plot: 1) control plots; 2) plots with sticky traps added (to reduce top-down effects); 3) plots with fertilizer added (to increase bottom-up effects); and 4) plots with both traps and fertilizer added.

Plant and soil response variables

At the beginning and end of the experiment I measured plant height in each plot by measuring the height of 10 plants and plant density, by randomly placing a 0.25 m² quadrant in each plot and counting the stems inside this area. Because increase salinity can change plant morphology, inducing tough woody stems, and this can affect herbivory density, percentage of green stems, was measured by counting 100 stems in each plot and scoring them as “green” and soft or “woody” and hard (Stiling and Moon 2005 b). Leaf area was measured by randomly collecting 5 leaves from each plot, digitalizing pictures of each leaf and using a software UTHSCA Image tool (Wilcox *et al.* 1996). Foliar nitrogen of *Borrchia* plants were quantified using a CHN analyzer. The five collected leaves were oven dried, ground in a Wiley Mill, and analyzed for foliar nitrogen (% dry mass) using CE Instruments NC2100 CN Analyzer (CE Elantech, Lakewood, N.J., USA). To measure soil salinity a technique from Pennings and Moore (2001) was

used. Samples of soil were collected from every plot, taken into the laboratory and weighed. Samples were then air dried and reweighed, then placed into an Erlenmeyer flask with ten ml of distilled water. Then, an amount of distilled water equal to the amount of water originally in the sample, was added. After another day, when the sediments settled down, the salinity was recorded using a refractometer (VEE GEE A366ATC, salinity 0-100‰).

Herbivore and parasitoid responses

In order to study how bottom-up and top-down factors affect the herbivores of this system, all herbivores were counted monthly. *Asphondylia* galls were counted on 200 stems on each plot, *Pissonotus* on 20 stems, chewed leaves on 50 stems, stem borers (*Argyresthia* sp.) on 50 stems, and spiders on 50 plants. Parasitism levels of *Pissonotus* eggs were estimated by sub-sampling 10 stems per plot in June and dissecting them under a microscope. Parasitized eggs can be distinguished because they appear black or orange while non-parasitized eggs are white with red spots (Moon and Stiling 2000). Parasitism levels of *Asphondylia* galls were estimated by counting the number of fly and parasitoid emergence holes on galls on each plot at every census.

Data Analysis

Treatments effects on total herbivore densities summed over the year were tested using a 3-way factorial ANOVA with site, fertilizer addition and parasitoid removal as factors. Data was transformed using $\log_{10}(x+1)$ when necessary. When data could not be transformed to meet normality assumptions, a Kruskal–Wallis one–way Anova or Mann – Whitney was used. Means and standard errors are reported untransformed. In addition Pearson's correlations were sometimes used to correlate herbivore densities to environmental data using all 72 plots. All statistical analysis were performed using SYSTAT 9.0 (Wilkinson 1999)

Results

Plant and soil variables

Soil salinity among the seven studied sites ranged between 4 and 18 ppt with a mean value of 10.33 ± 0.827 (Figure 2), and were significantly different between sites (Kuskall-Wallis = 58.564, $P < 0.001$).

Most of the treatments and interactions between treatments had no effect on plant variables (Table 2, Figures 3-6). The two exceptions were that fertilizer significantly increased both leaf area and percentage foliar nitrogen. In contrast site had a significant effect on all plant variables both pre and post treatment (Table 1). Total mean density of *Borrchia* stems, plant height and leaf area were not significantly different between fertilizer and removal of parasitism treatments. However, site had a significant effect on all plant variables (Figures 3-6). Correlations revealed that in general most plant variables significantly decreased with increases in soil salinity, except stem density, which increased (figure 7).

June data on percentage of green stems could not be transformed to meet ANOVA assumptions, so the three factors were analyzed separate using a Kruskal -Wallis test. Site was again the only factor that had a significant effect on

the mean percentage of green stems (Kruskal-Wallis= 45.759, $P < 0.001$, (Figure 6).

Herbivore and parasitoid responses

Herbivore densities were significantly different among sites (Tables 3-4, Figures 8-14). Furthermore the effect of site differed according to the type of herbivore so that one site was best for galls (CW6), another for *Pissonotus* (Fort de Soto), another for stem borers (CW5) and yet another for chewed leaves (Simmons and Honeymoon island). Site also significantly affected gall size (K-W=31.276, d.f.=6, $P < 0.001$), gall parasitism ($F_{6,40} = 19.148$, $P < 0.001$) and *Pissonotus* parasitism ($F_{6,40} = 4.855$, $P = 0.001$). Significant negative correlations were observed for *Pissonotus* abundance ($P = 0.001$), stem borer abundance ($P = 0.008$), gall size ($P = 0.054$) and gall parasitism ($P = 0.028$) with an increase in soil salinity (Figure 14). The only guild with a positive correlation with increasing salt was the chewers ($P < 0.001$) (Figures 16).

Site had a significant effect, ($P < 0.001$) on the density of both the spiders and the damselflies. However, none of the other treatments had a significant effect on the densities of these predators. I was not expecting differences between treatments with these organisms, because they are predators that move easily among plots, and the sticky traps may be not efficient enough to trap this class of predators.

Fertilizer had a significant effect on gall abundance and gall size (Mann-Whitney $U= 754.5$, $P=0.028$). In general, galls on the fertilized plots exhibited bigger sizes than the ones on the control plots, with the exception of the ones found at Upper Tampa Bay. As a result, fertilizer significantly reduced gall parasitism. On the other hand because of an increase in green stems, fertilizer significantly increased parasitism rates of *Pissonotus* eggs. The amount of this increase in egg parasitism on fertilized plots varied among sites resulting in a significant site x fertilizer interaction ($P=0.018$). Despite this, *Pissonotus* densities showed an increase on fertilized plots. There were no other effects of fertilizer or other interactions with other treatments.

Traps failed to influence herbivore abundance or parasitism levels of either *Asphondylia* or *Pissonotus*. There were also no significant interactions between traps and any other treatment (Tables 3-4).

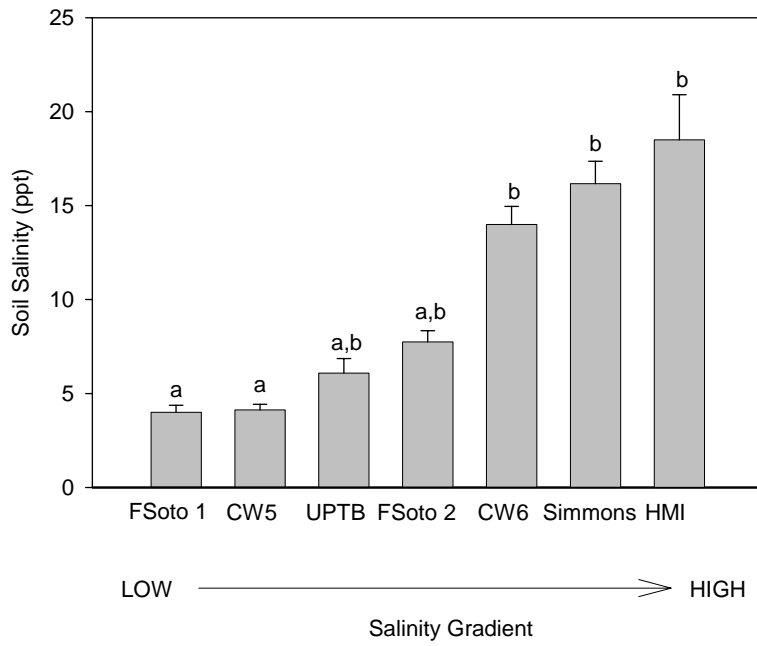


Figure 2. Soil salinity (mean and standard error) of the 7 sites surveyed. Sites are arranged from the lowest to the highest soil salinity. Means with the same letters are not significant different.

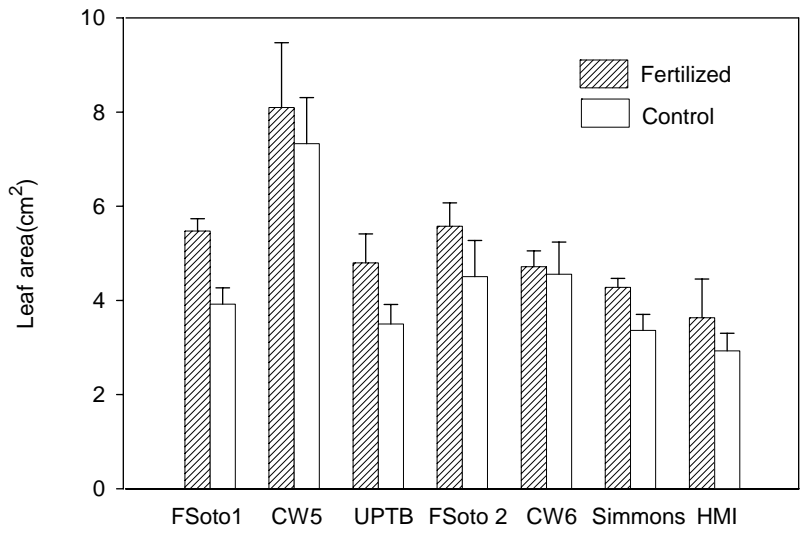


Figure 3. Mean (\pm SE) leaf area of *Borrchia* plants in fertilized and control plots among the 7 sites surveyed.

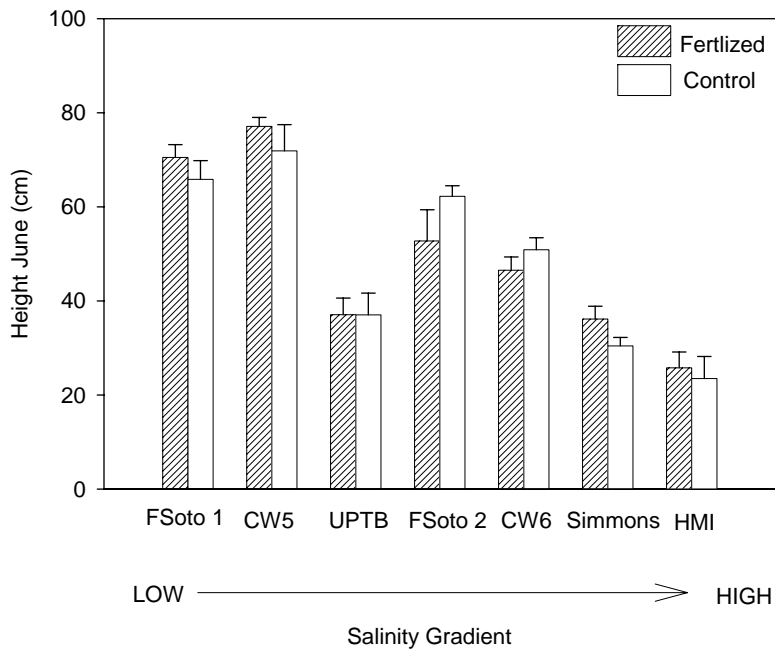


Figure 4. Mean (\pm SE) stem height of *Borrchia* plants in fertilized and control plots among the 7 sites surveyed.

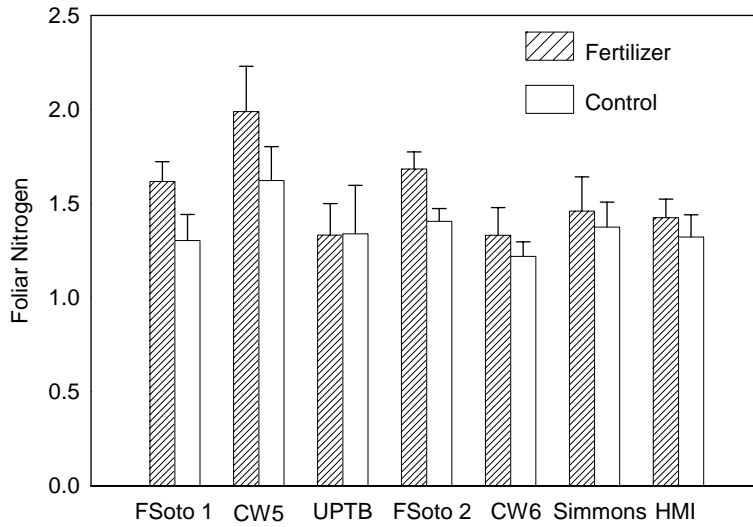


Figure 5. Mean (\pm SE) foliar nitrogen of *Borrichia* plants in fertilized and control plots among the 7 sites surveyed.

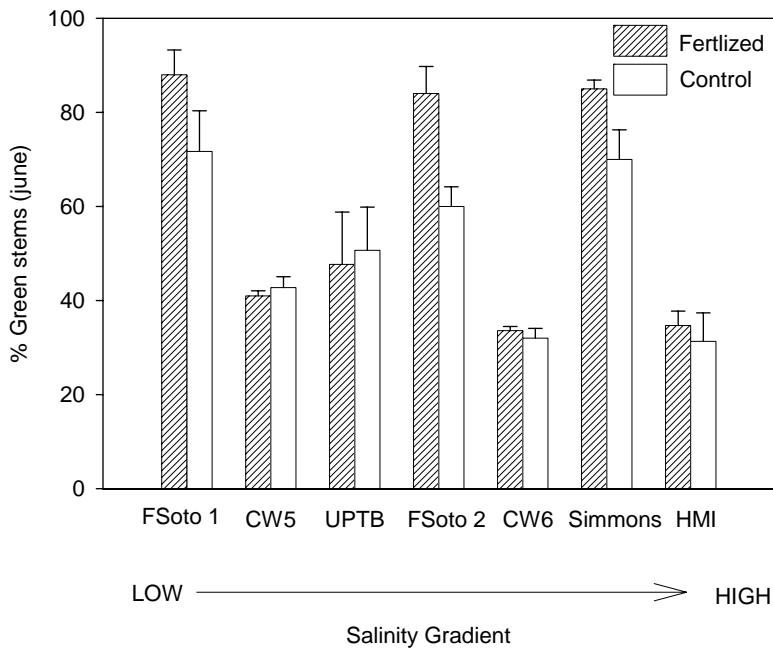


Figure 6. Mean (\pm SE) percentage of green stems of *Borrichia* plants in fertilized and control plots among the 7 sites surveyed.

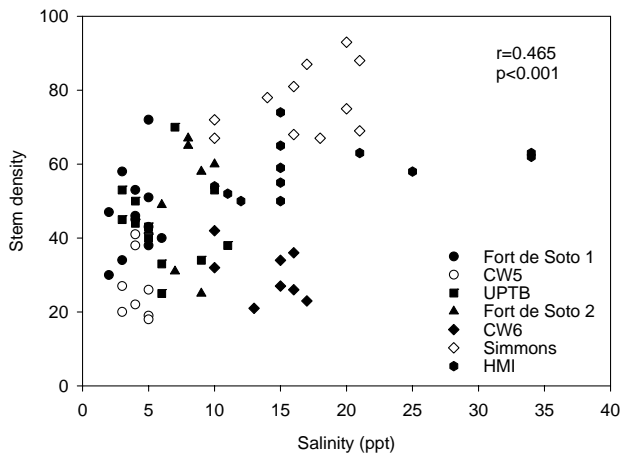
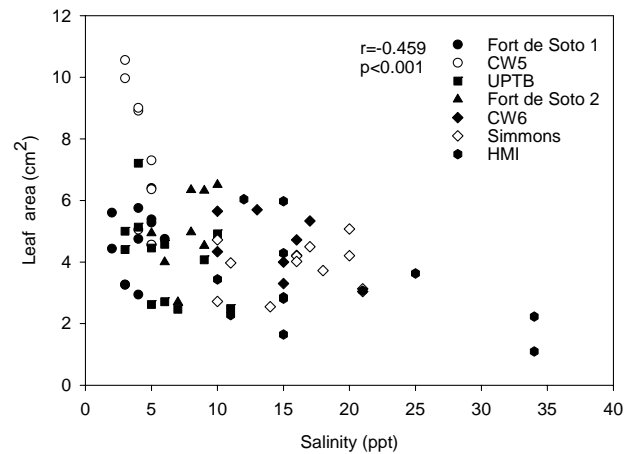
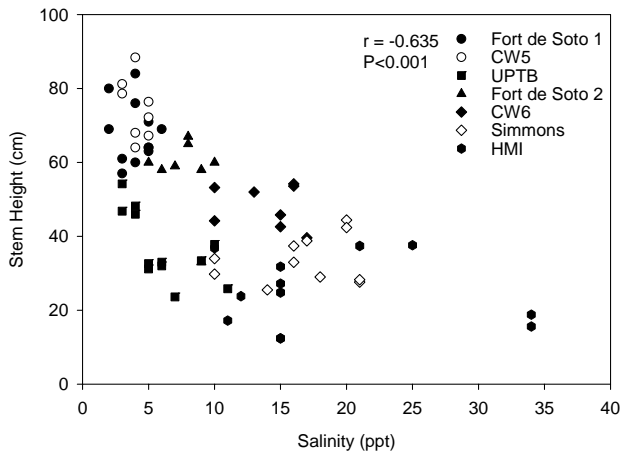
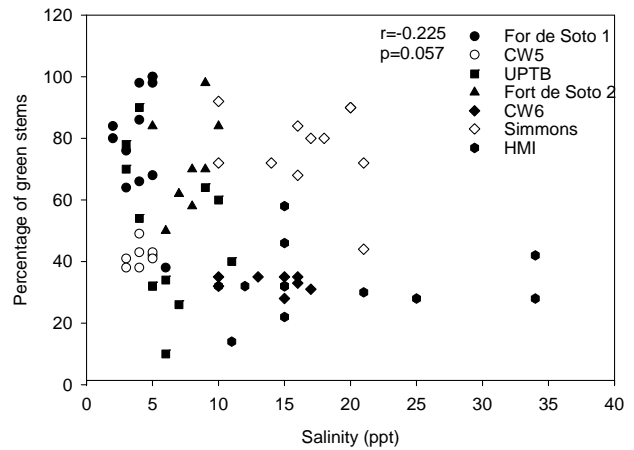
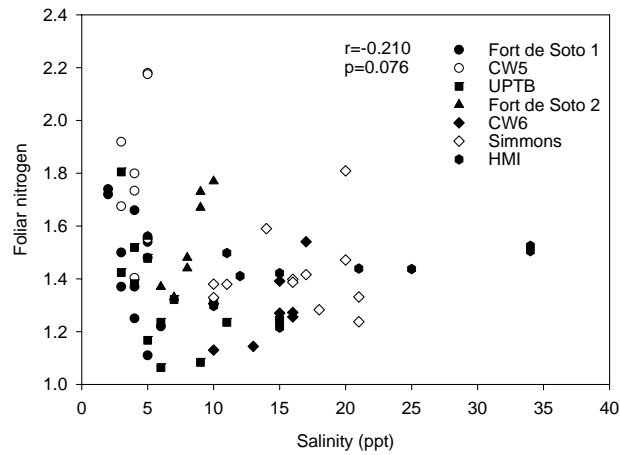


Figure 7. Pearson's correlations for plant variables measured on *Borrchia* among the 7 sites studied. Each symbol represents a different site ($n=72$).

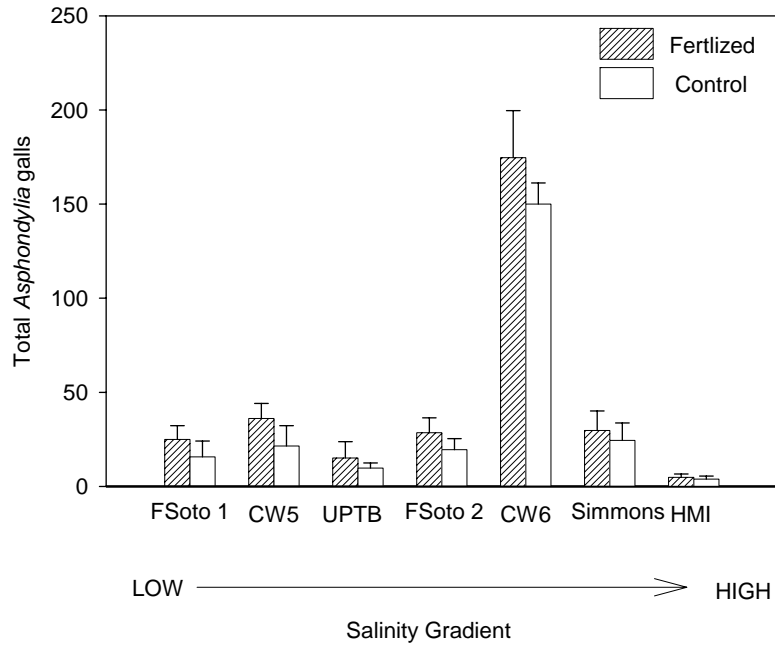


Figure 8. Mean (\pm SE) gall abundance in fertilized and control plots among the 7 sites surveyed.

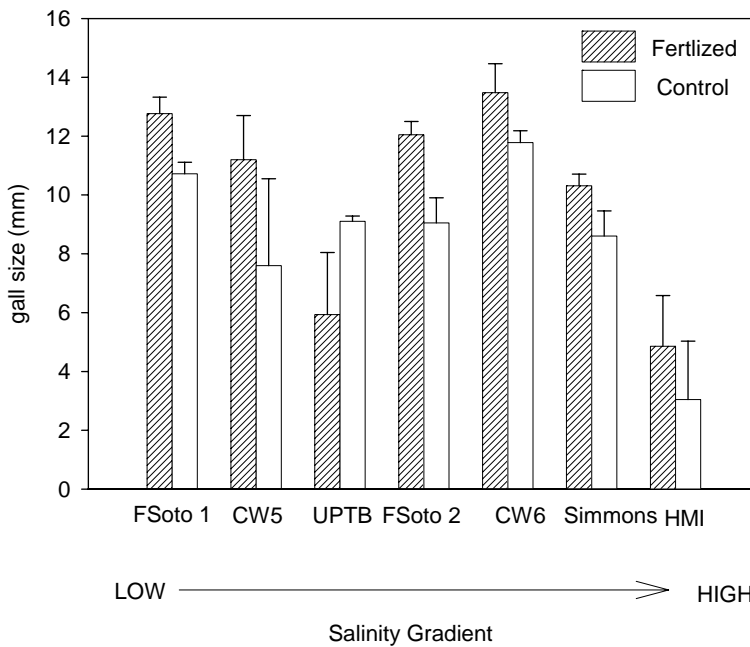


Figure 9. Mean (\pm SE) gall size (mm) measured in fertilized and control plots among the 7 sites surveyed.

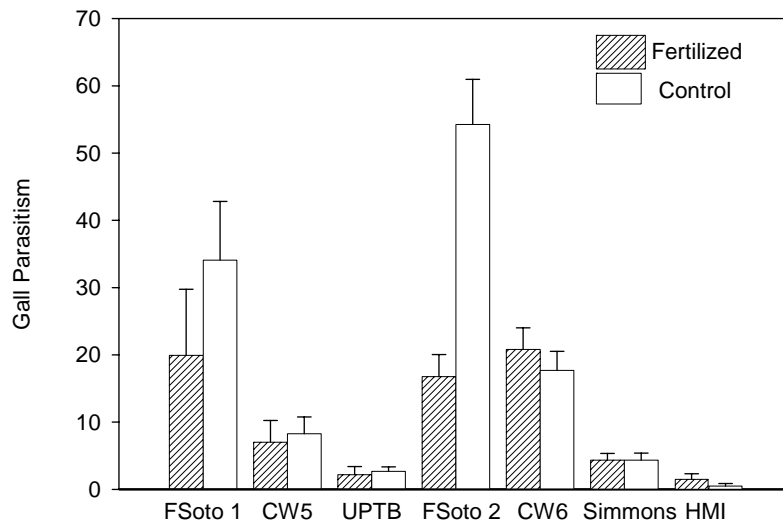


Figure 10. Mean (\pm SE) gall parasitism in fertilized and control plots among the 7 sites surveyed.

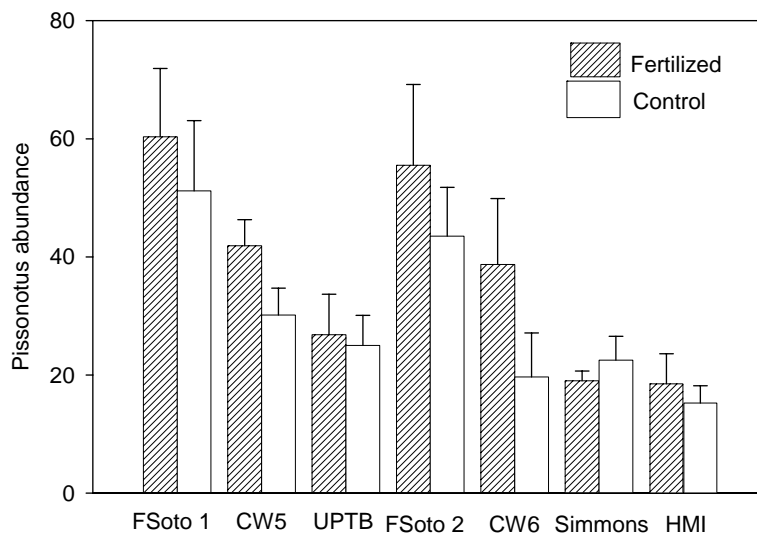


Figure 11. Mean (\pm SE) *Pissonotus* abundance (Number of *Pissonotus* counted on 20 stems/plot) found in fertilized and control plots on each of the 7 studied sites.

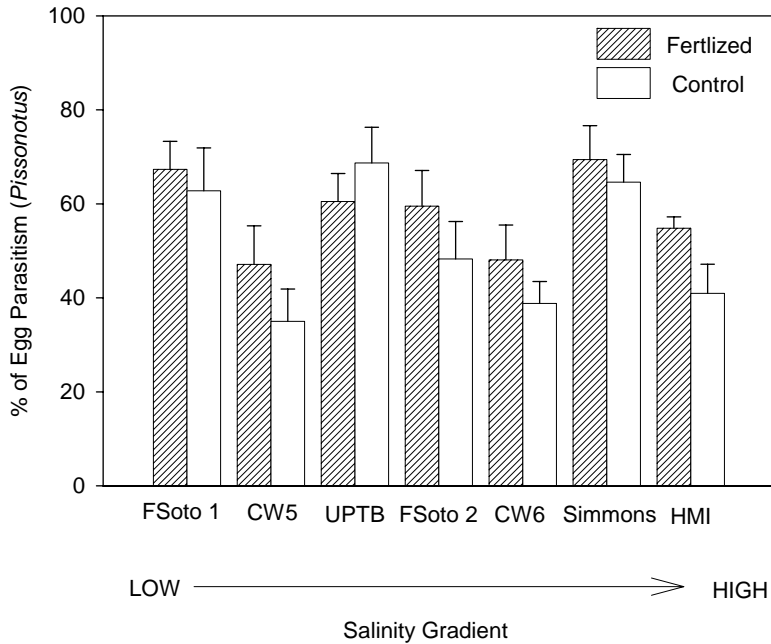


Figure 12. Mean (\pm SE) percentage parasitism on *Pissonotus* eggs in fertilized and control plots among the 7 sites surveyed.

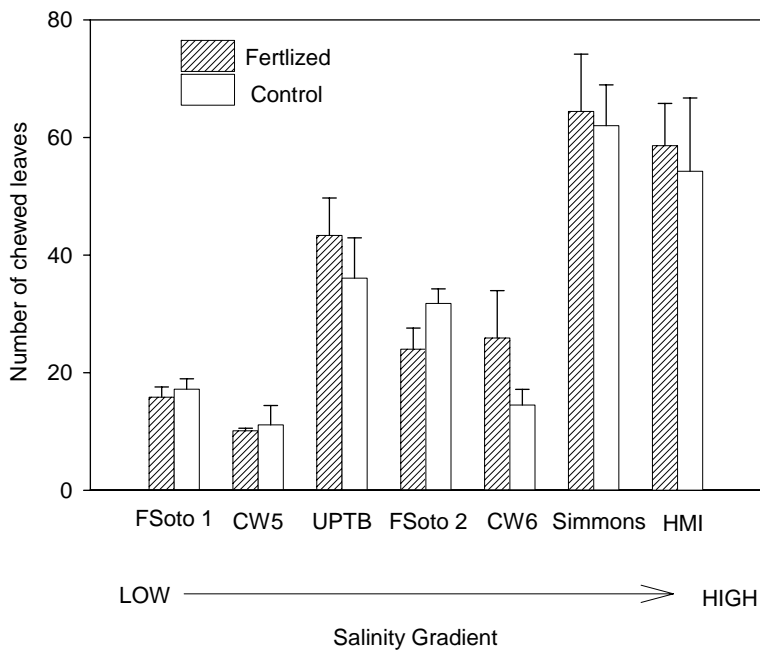


Figure 13. Mean (\pm SE) number of chewed leaves (number of chewed leaves/50 stems) counted on fertilized and control plots among the 7 sites surveyed.

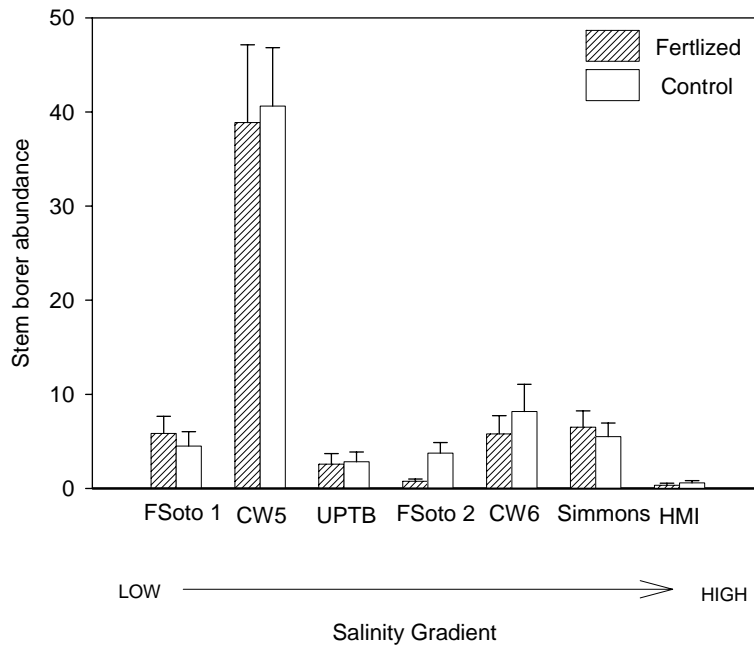


Figure 14. Mean (\pm SE) number of stem borers counted on fertilized and control plots among the 7 sites surveyed.

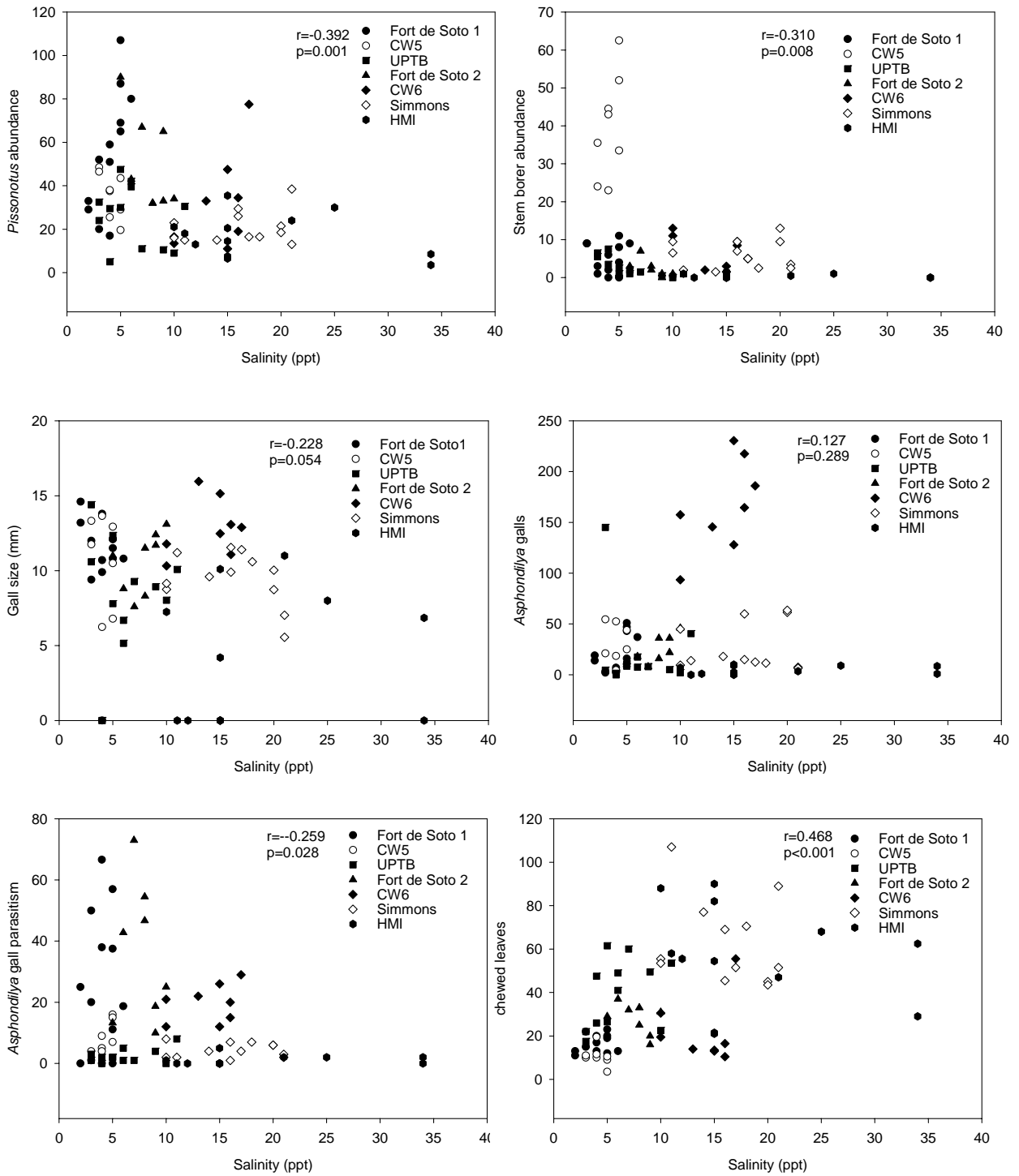


Figure 15. Pearson's correlations for herbivores abundances counted on *Borrichia* monoculture among the 7 sites studied. Each symbol represents a different site (n=72).

Table 1. Summary of ANOVA results (*df*, *F*-ratios and *P*-values) for the effects of site, fertilization and traps for plant variables measured on *Borrchia* in March 2003. Values in bold indicate statistically significant results.

Source	df	Leaf area		Stem density		% of green stems	
		F	P	F	P	F	P
Site	6,44	14.198	<0.001	5.686	<0.001	14.301	< 0.001
Fertilization	1,44	0.004	0.952	0.600	0.443	1.510	0.226
Traps	1,44	0.953	0.334	0.008	0.930	0.219	0.642
Site x Fertilization	6,44	0.568	0.754	0.376	0.890	0.453	0.839
Site x Traps	6,44	0.430	0.855	0.162	0.985	0.388	0.883
Fertilization x traps	1,44	0.025	0.874	0.00	0.997	0.383	0.539
Sitextrapsxfertilization	6,44	0.165	0.985	1.127	0.362	0.485	0.816

Table 2. Summary of ANOVA results (*df*, *F*-ratios and *P*-values) for the effect of site, fertilization and traps for plant variables measured on *Borrchia* in June 2003. Values in bold indicate statistically significant results.

Source	df	Leaf area		Stem density		Height		Foliar nitrogen	
		F	P	F	P	F	P	F	P
Site	6,44	8.530	<0.001	21.6	<0.001	39.564	<0.001	8.801	<0.001
Fertilization	1,44	6.671	0.013	0.749	0.392	0.035	0.852	19.05	<0.001
Traps	1,44	0.109	0.743	1.126	0.294	0.092	0.763	0.178	0.675
Site x Fertilization	6,44	0.144	0.989	0.887	0.512	0.666	0.677	1.570	0.179
Site x Traps	6,44	0.214	0.971	0.354	0.904	0.330	0.917	0.605	0.725
Fertilization x traps	1,44	0.675	0.416	2.042	0.160	0.034	0.855	0.255	0.616
Site x Fertilizer x Traps	6,44	0.317	0.925	0.528	0.784	0.446	0.844	0.992	0.443

Table 3. Summary of ANOVA results (*df*, *F*-ratios and *P*-values) for the effect of site, fertilization and traps on *Asphondylia* galls and gall parasitism, *Pissonotus* abundance and percentage of parasitism on *Pissonotus* eggs.

Values in bold indicate statistically significant results.

Source	df	Gall abundance		Gall parasitism		<i>Pissonotus</i> abundance		% of egg parasitism (<i>Pissonotus</i>)	
		F	P	F	P	F	P	F	P
Site	6,40	16.38	<0.001	19.19	<0.001	5.99	<0.001	4.855	<0.001
Fertilization	1,40	4.18	0.048	6.10	0.018	3.43	0.071	4.085	0.050
Traps	1,40	0.24	0.63	0.29	0.59	0.02	0.882	1.079	0.305
Site x Fertilization	6,40	0.39	0.88	3.89	0.004	0.55	0.768	0.450	0.841
Site x Traps	6,40	0.21	0.97	1.43	0.23	0.24	0.962	2.087	0.076
Fertilization x traps	1,40	0.00	0.99	0.06	0.81	0.00	0.996	0.117	0.734
Site x traps x fertilization	6,40	1.42	0.23	1.17	0.34	0.36	0.902	1.232	0.311

Table 4 Summary of ANOVA results (*df*, *F*-ratios and *P*-values) for the effect of site, fertilization and traps for number of chewed leaves and stem borers abundance. Values in bold indicate statistically significant results.

Source	df	Number of chewed leaves		Stem borers	
		F	P	F	P
Site	6,44	21.202	<0.001	5.581	<0.001
Fertilization	1,44	0.345	0.560	1.447	0.235
Traps	1,44	0.543	0.465	1.729	0.195
Site x Fertilization	6,44	0.344	0.910	0.936	0.479
Site x Traps	6,44	0.408	0.870	2.179	0.063
Fertilization x traps	1,44	1.057	0.309	0.038	0.846
Site x traps x fertilization	6,44	0.886	0.514	1.722	0.138

Discussion

In this study I found that plant morphology of the salt marsh plant *Borrchia frutescens* varied dramatically among sites. At the extreme, plants at CW5 were more than twice the height of those at Honeymoon Island and their leaves were also more than twice as big. *Borrchia* height is greatly influenced by interstitial soil salinity (Richards *et al.* 2004) at these Florida sites and plant height was significantly negatively correlated with soil salinity. Foliar nitrogen was also variable between sites, and was generally less in higher salinity plots.

Borrchia herbivores also varied dramatically in density between sites. Galls at one site, CW6, were over three times as abundant as at any other site, while densities of *Pissonotus* were twice as great at Fort de Soto as at Simmons and Honeymoon island. Stem borers were very common at CW5 but not at any other site. Such patterns could be influenced by competition such that if galls are common at CW6 then there are fewer resources available for other herbivores. Moon and Stiling (2002 b) showed that high densities of stem borers tended to reduce the density of *Pissonotus* eggs which they kill as they bore through the stems, but in the present study the number of *Pissonotus* was low at Simmons and Honeymoon Island despite there being few stem borers. In general, densities

of *Borrchia* herbivores are so low as to not influence the densities of one another and there is an abundance of unoccupied stems at each site (Stiling *et al* 1999).

Of the applied treatments, the only consistent effects were those due to fertilizer. Increased plant quality increased the density of galls and *Pissonotus* both directly, and in the case of *Pissonotus*, indirectly, by lowering egg parasitism. Significant trap effects were not detected in the present study. Nor were there many interactions between treatments. It appears bottom-up effects are much stronger at all sites than are top-down ones, a conclusion supported by previous experiments in this system (Moon and Stiling 2000, Stiling and Moon 2005 a, b). Previous, but recent, experimental manipulations of bottom-up and top-down effects along an experimentally generated salinity gradient showed significant interactions between salt level and both top-down and bottom-up effects. Bottom-up effects were much weaker in more saline plots possibly because increased salinity reduces the plant's ability to uptake additional nutrients (Jefferies and Perkins 1977). Secondly, plant toughness increases in saline plots and this reduces the strength of top-down effects of parasitoids which cannot so easily penetrate stems to parasitize *Pissonotus* eggs. For *Asphondylia*, on the other hand, more saline plots reduce gall size, and fertilization cannot increase gall size enough to reduce gall parasitism as it can on less saline plots. However these experiments were performed on large *Borrchia* monocultures at a single site which probably consisted of relatively few clones, or at least similar ones. Stiling and Rossi (1996) showed that clonal variation among sites may

alter the resistance of plants to herbivore attack. Under such uniform conditions and where clone was controlled for, it may have been easier to detect significant interactions of effects with salinity (Stiling and Moon 2005 b). In the “real world” of large site to site variation in soil salinity, soil nutrients, plant size, plant height and the clonal identity of plants, as experienced in this study, it is harder to detect these subtle interactions between treatments. Only the strongest effects are detected, in this case the effect of fertilizer. Thus, as previous studies have noted in this system (Moon and Stiling 2002 a,c, Stiling and Moon 2005 a,b) and in general (Hunter and Price 1992, Prezler and Boecklen 1996, Price 2002) the relative strength of bottom-up effects are strong. Only as evidenced by experimental manipulations in which top-down effects are not well controlled.

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