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Herbivory by Leaf Miners on Florida Scrub Oaks

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
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Department of Biology
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DEDICATION

“Eu não sou daqui também marinheiro
Mas eu venho de longe
E ainda
do lado de trás da terra
além da missão cumprida
Vim só dar
despedida

Filho de sol poente
Quando teima em passear
desce de sal nos olhos
doente da falta de voltar
Filho de sol poente
Quando teima em passear
desce de sal nos olhos
doente da falta que sente do mar

vim só dar despedida
vim só dar despedida”

This dissertation is dedicated to my parents Anthonius Whilhelmus Gerardus Cornelissen and Wanda Garabini Cornelissen. Their unquestionable love, friendship and constant support inspired each and every one of my days over the past years.

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Herbivory by Leaf-miners on Florida Scrub Oaks

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ABSTRACT

This study investigated effects of plant quality and natural enemies on the abundance and survivorship of several leaf miner species on Florida scrub oaks over several ecological scales. Three oak species (*Quercus laevis, Q. geminata,* and *Q. myrtifolia*) and four leafminer species (*Acrocercops albinatella*, *Brachys tesselatus*, *Stilbosis quadripustulatus*, and *Cameraria* sp. nova) were the main focus of five separate studies, addressing effects of bottom-up and top-down factors at regional, local, and individual scales. At the regional scale, it was observed that *Cameraria* sp. nova was aggregated into sites, and sites closer to each other exhibited similar densities of mines than sites farther apart. None of the bottom-up and top-down factors studied were spatially structured, but did influence the variation in *Cameraria* abundance over the range of the host plant *Q. myrtifolia.* At the local scale, all leaf miners studied were aggregated between and within plants, and variation in bottom-up factors among individual plants explained variation in abundance for some of the leaf miners studied. Intra-specific competition was identified as an important factor influencing mine survivorship, but inter-specific competition among leaf miners and gall-formers

did not shape the community structure of oak herbivores. Experimental manipulation of bottom-up and top-down factors via fertilization and natural enemy removal showed that bottom-up effects were important determinants of leaf miner abundance, as fertilized plants supported 2 to 5-fold more herbivores than control plants. The removal of natural enemies, on the other hand, did not significantly impact the abundance and/or the survivorship of leaf miners and other guilds studied. At individual scales, it was demonstrated that two leaf miner species responded to random variations in leaf morphology, by increasing in abundance in individual host plants with more asymmetric leaves and/or higher levels of fluctuating asymmetry. These results offered support for the plant stress hypothesis and differences in host plant quality were again partially responsible for the results found.

Chapter 1

INTRODUCTION

Leaf Miners

Leaf mining is a feeding habit defined by consuming "live foliage while simultaneously dwelling inside it" (Connor & Taverner 1997). In practice, a leaf mine can be distinguished from most other forms of herbivory by the presence of at least partially intact epidermal layers on both surfaces of a leaf at the site of damage (Hering 1951). Leaf mines are usually visible on the exterior of the leaf as serpentine paths, blotches, or other characteristic shapes of discolored tissue. Mines may be occupied throughout an insect's feeding life, or may be abandoned for other feeding habits at some point in development. Some leaf miners, especially larger leaf-mining lepidopterans, excavate more than one mine during the course of development. Most, however, develop completely inside a single mine. In comparison to external folivores, leaf miners are relatively small insects, physically constrained by the thickness and area of leaves they occupy. The number of generations per year varies widely from univoltine species to facultative and obligate multivoltine ones. Within the insects, there are approximately 10,000 described species of leaf miners (Faeth 1991), but density estimates and demographic data are available for only 1% of these species (Auerbach *et al.* 1995). The habit is known from taxa in 51 families, but only in larvae of the holometabolous orders Coleoptera, Diptera, Hymenoptera, and Lepidoptera (Connor & Taverner 1997).

Lepidoptera include more leaf mining families and species than any other order (Hespenheide 1991) and leaf mining appears to be derived from other feeding habits, both external and internal forms of herbivory.

Leaf mining insects attack herbaceous and woody plants in both terrestrial and aquatic habitats (Auerbach *et al.* 1995) and are important pests of agricultural crops, greenhouse plants and orchard trees. Approximately 25% of the described species have been observed at high, eruptive densities but most species typically occur at low densities. Several ecological hypotheses have been proposed to explain the adaptive significance of leaf mining habit (reviewed by Connor & Taverner 1997), such as escape from natural enemies (such as predators, parasites and pathogens), protection from the physical environment (desiccation, UV radiation, dislodgment by weather), and avoidance of plant defenses (selective feeding to maximize intake of most nutritious/least noxious tissues). Recent analyses of mortality for leaf miner species revealed that parasitoids and, in early life stages, plant quality effects can be the most important factors that influence leaf miner abundance and survivorship (Cornell & Hawkins 1995, Hawkins *et al*. 1997).

Leaf miners are ideal for the study of the effects of bottom-up and top-down factors on insect abundance and survivorship. The fact that these herbivores live out most of their life histories within the confines of a mine, as well as the persistence of the record of the mine, allows an ecologist to observe and reconstruct their life history, to measure the effect of host plant, and to observe associated faunas such as predators and parasitoids (Hespenheide 1991). The ease with which leaf mines can be studied has made them a popular system for ecological research and for addressing questions related to population dynamics and community structure.

Bottom-up and Top-Down effects on insect herbivores

Understanding what influences herbivore abundance is a major challenge for insect ecologists. For insect herbivores, their occurrence and feeding is influenced by plant geographical range and local abundance (Doak 2000, Carson & Root 2000, Kery *et al.* 2001), size and structural complexity (Strong *et al.* 1984, Tinney *et al.* 1998, Masumoto *et al.* 2000), nutritional quality (Cooke *et al.* 1984, Fischer & Konrad 2000, Denno *et al.* 2000), secondary chemistry (Bernays & Chapman 1994, Johnson *et al*. 1996, Lindroth *et al.* 2000) and phenology (Leather 2000, Jarzomski *et al.* 2000, Masters *et al.* 2001), and influence of competitors (Norris 1997, Fisher *et al.* 2000) and higher trophic levels, such as predators and parasitoids (Hawkins *et al.* 1997). The effects of plant quality and natural enemies on the attack rates of herbivorous insects on plants have been extensively studied and many hypotheses have been proposed to explain both within and between population-level variations of herbivory rates among and between different plant species. Studies of herbivory on natural plant populations have figured prominently in the ecological literature for decades and have contributed to a general framework of ecological theory (Hairston *et al*. 1960, Oksanem *et al.* 1981, Polis & Strong 1996).

Although much information has accumulated for insect-plant interactions over the past years, many questions remain unanswered such as the interactions of plant quality, abiotic factors and effects of the third trophic levels on herbivores and plants. For example, are species commonly limited by the same factors over all parts of their ranges? Do bottom-up and top-down factors interact to shape responses of herbivore communities? If so, do all species respond equally or can we detect inter- and intra-guild variation in responses?

Most species are embedded in complex food webs and studying a single guild in related plant species at different scales can lead to a more complete understanding of how different factors influence the abundance, survivorship and interactions of species with other members of the community. Although much has been done to understand what factors influence insect herbivore occurrence and survival, to my knowledge there are very few studies in which multiple scales of observation have been used in ways to better understand what governs insect herbivore feeding, abundance and survivorship. I believe that the use of multiple levels of organization create the opportunity to better understand plant-herbivore interactions and to investigate how factors that influence the survival and performance of herbivorous insects are affected by scale. The purpose of this study was 1) to determine the influence of spatial scale on the occurrence of a leaf miner over its host plant range and examine how differences in plant quality and demographic rates influence the abundance of this particular insect when the whole range of distribution is considered; 2) to examine how natural variations in plant quality influence the distribution of leaf miners and other herbivores both between and within host plants and how mine survivorship is affected by the presence of intra- and inter-specific competition; 3) to examine how experimentally induced variation in bottom-up and top-down factors influence both the abundance and the survivorship of leaf miners and to detect any species-specific variation in response to altered plant quality and third trophic level effect, and 4) to examine how individual variation in leaf morphology influences leaf miner abundance and survivorship.

These questions were addressed in five separate chapters.

Chapter 2

LARGE VARIATIONS OVER LARGE SCALES: BOTTOM-UP AND TOP-DOWN EFFECTS ON THE ABUNDANCE OF AN OAK-LEAF MINER.

SYNOPSIS

Many plant and animal species have higher densities at the centre of their distribution, with a gradual decline in abundance towards the edge of the range, though reasons for this pattern is not well known. We examined the abundance structure of the leaf miner *Cameraria* sp. nova over the range of its host plant *Quercus myrtifolia* in Florida and addressed how bottom-up and top-down factors varied over its whole distribution. Leaf miner densities as well as plant quality and effects of natural enemies on mine survivorship were evaluated in 40 sites that covered the whole distribution of the plant. Spatial indices of intraspecific aggregation and spatially structured models were used to determine the effects of spatial location on the abundance of *Cameraria* and effects of both bottom-up (tannin concentration, foliar nitrogen, soil nitrogen, and leaf area) and top-down (larvae parasitism and predation) on abundance and survivorship. *Cameraria* mines were, on average, three times more abundant on coastal compared to inland sites and did not support the hypothesis of higher abundance on the centre of the distribution. Differences in plant quality, larvae parasitism and successful emergence of mines on edge versus central sites might be partially responsible for this finding. Correlograms constructed for the abundance data indicated a significant spatial structure

of *Cameraria* mines over the range: mines were positively spatially autocorrelated at small distances (≈122Km), indicating that sites close to each other tend to have similar mine densities compared to sites further apart. Partial regression analyses indicated that only a small variation on *Cameraria* abundance was explained by variation in bottom-up and top-down effects after the effects of spatial position were taken into account.

INTRODUCTION

It has been widely demonstrated that species tend to vary in abundance across their distributional ranges: many plant and animal species have higher densities at the centre of their distribution, with a gradual decline in abundance towards the edge of the range (e.g., Whittaker 1971, Hengeveld & Haeck 1982, Brown 1984, Curnutt *et al.* 1996, Mehlman 1997, Sorte & Hofmann 2004). Many biogeography texts have described this ‘abundant centre’ distribution and it has even been called a ‘general rule’ of biogeography (Sagarin & Gaines 2002). Several mechanisms have been proposed to explain the abundant centre distribution, and most of them are variations of the idea that species abundance distributions are coupled to environmental gradients. According to Brown (1984), habitat quality for a species is determined by a combination of many abiotic and biotic factors and local abundance is a reflection of how well a particular site meets the needs of a species along many niche axes. These axes include the physiological characteristics of the species (e.g., temperature tolerance, soil quality) as well as ecological factors (e.g., response to competitors and mortality imposed by predators and parasites). Brown (1984) and Brown *et al.* (1995) assumed that these parameters are spatially autocorrelated and increasing the distance from an optimal site decreases the probability of a site meeting the multidimensional needs of a species, with a consequent decline in population abundance. The processes behind these patterns are controversial, and some exceptions such as higher abundance on the edges of the distribution have been found (e.g., Prince *et al.* 1985, Blackburn *et al.* 1999), but the pattern holds true for some species (examples in Sagarin & Gaines 2002). However, among the 22 studies reviewed by Sagarin & Gaines (2002) that directly addressed the abundant centre distribution hypothesis, only 8 studies included data collected throughout the *entire* range of the species, raising concerns as to how well other studies reflect the patterns of change in local abundance across entire geographical ranges (Brewer & Gaston 2002). Little is known about the relationship between spatial variation and abundance of insects throughout the host plant range, except for the extensive studies conducted for the Holly leaf miner *Phytomiza ilicis* (Diptera: Agromyzidae) in Europe (Brewer & Gaston 2002, 2003, Klok *et al.* 2003, Gaston *et al.* 2004). Leaf miner insects can be ideal models to address the abundant centre distribution hypothesis, because they are generally restricted to a single host plant species and their sessile habit allows sampling in different parts of the range as well as the collection of life-history data in the field.

The edges of the range of the host plant species are supposed to be subject to the most stressful conditions, and environmental stresses such as water deficit and/or nutrient imbalances that affect host plant physiology and quality can be quite important to herbivores, as proposed by the Plant Stress Hypothesis (White 1984). The Plant Stress Hypothesis argues that herbivore abundance is higher on stressed host plants due to an increased availability of nutrients, a decreased concentration of defensive compounds and/or changes in the ratio of nutrients to chemical defenses. In a recent meta-analysis of the effects of water-stress on insect herbivores, Huberty & Denno (2004) found that stressed plants showed a tendency to exhibit elevated foliar nitrogen and there was a non-significant trend for leaf miners (mainly Lepidoptera and Diptera) to achieve higher densities on stressed plants over control plants. As argued by Huberty & Denno (2004) leaf miners are thought to benefit from water-stressed plants because they can take advantage of elevated leaf nitrogen and yet avoiding feeding on compartmentalized allelochemicals, and thus benefit from elevated nitrogen arising from intermittent stress. In this sense, stressed plants at the edges of the range might be more heavily attacked and exhibit larger populations of insect herbivores. Also, herbivores might develop faster in stressed plants, in accordance with predictions of the slow-growth, high-mortality hypothesis (Clancy & Price 1987), decreasing thus the mortality pressure exerted by natural enemies, such as predators and parasitoids.

How effects of plant quality and natural enemies interact to determine herbivore abundance and performance is well understood, but virtually all efforts to quantify the relative contributions of top-down and bottom-up regulatory factors on herbivore populations have been performed over relatively *local* scales, with very few exceptions (e.g., Brewer & Gaston 2002, Gaston *et al.* 2004). Plant quality features such as the concentration of secondary compounds as well as the abundance and composition of natural enemy communities might vary according to environmental factors such as soil type, temperature and rainfall patterns (Price 1997). As a consequence, both the quality of herbivore host plants and the pressure exerted by the third trophic level might differ between different parts of the distributional range of the species (Gaston *et al.* 2004).

Previous studies of oak leaf miners have revealed high variation in density both between and within plants (e.g., Sato 1991, Brown *et al.* 1997, Forkner & Hunter 2000) and similar results were found for scrub oaks in Florida at local spatial scales (e.g., Stiling *et al.* 1987, Cornelissen & Stiling 2005, Cornelissen & Stiling 2006a,b). In this study we investigated the abundance and survivorship of the leaf miner moth *Cameraria* sp. nova throughout the range of its host plant distribution *Quercus myrtifolia* (Fagaceae). This system provides an ideal model to test both the abundant centre hypothesis as well as the effects of top-down and bottom-up factors at larger scales: the sessile larva is easily identified by the shape of the mine, larval abundance and development might be strongly influenced by aspects of plant quality and mines leave a permanent record of larval fate, which provides insights on their population dynamics and effects of the third trophic level. Moreover, *Q. myrtifolia* has a geographic range 99.8% contained within the state of Florida (Nixon 1997, Price *et al.* 2004). This enabled us to sample the full distributional range of *Cameraria* mines and contrast abundance, survivorship, and demographic features of this leaf miner in all parts of its range.

STUDY SYSTEM

The Florida scrub is one of its most distinctive ecosystems found in coastal and ancient inland dunes throughout the state. The major scrub groupings in Florida are in the coastal panhandle, coastal peninsula and inland peninsula (for details see Marshall *et al.* 2000). *Quercus myrtifolia* (Fagaceae) is a semi-deciduous oak that often grows in dense stands in scrub ecosystems. Leaves are dark green, shiny and have a leathery appearance.

Plants are found in dry, sandy soils usually associated with sand dunes, sand hills and scrubs in Florida (Wunderlin & Hansen 2000). Although present in small, patchy distributions in Alabama, Georgia and South Carolina (Nixon 1997), most of the distribution of *Q. myrtifolia* is in Florida, although the plant is absent in approximately 21 Florida counties in the northern edge-of-range (Wunderlin & Hansen 2000).

Cameraria sp. nova (Lepidoptera: Gracillariidae) is the most common leaf miner on *Q. myrtifolia* and mines are abundant in late spring and early summer. Mating, oviposition and egg hatch occur between April and May and the upper surface blotch mines appear on *Q. myrtifolia* leaves by mid-May and early-June (Figure 2.1). Mines might reach 4cm in length and occupy 50-80% of the upper leaf surface (T Cornelissen, pers. obs.). The larvae go through 5 instars, taking up to 4 weeks to complete development and pupae form into a silken cocoon inside the mine. Although *Cameraria* sp. nova is the most common leaf miner on *Q. myrtifolia*, similar mines occur in low densities on the chapman oak *Q. chapmanii* and very low densities on the sand live oak *Q. geminata*, though whether they belong to the same species is not known. However, we suggest that *Cameraria's* geographical range is primarily limited by the availability of myrtle oaks, as densities on both chapman and sand live oaks are extremely low (average ± SD number of *Cameraria* mines on *Q. chapmanii* 0.058 ± 0.06 per 200 leaves and on *Q. geminata* 0.015 ± 0.02 per 200 leaves).

METHODS

Data Collection

Between July 1st and September 5th of 2005, data on the abundance and demographic parameters of *Cameraria* sp. nova as well as data on aspects of plant quality were determined at 40 sites in Florida, encompassing the whole distribution of *Q. myrtifolia*, its primary host. Sites included many state parks, state forests and reserves (Table 2.1), and sites were selected based on the presence of pure scrub patches, consisting mainly of *Q. myrtifolia*, *Q.chapmanii*, *Q. geminata* and *Q. inopina*. In a few sites (3 out of 40) the turkey oak *Quercus laevis* was also present, but not sampled in this study. Plants were sampled in 36 Florida counties, covering approximately 170,000 Km^2. At each site, we recorded geographic position (latitude-longitude coordinates expressed as decimal degrees) and elevation using a GPS positioned where the first individual of *Q. myrtifolia* was found.

Leaf miner density was estimated at each site by counting the number of *Cameraria* sp. nova mines on 200 randomly chosen leaves of 15 *Q. myrtifolia* individual plants per site (n=3,000 leaves per site). Leaves were sampled randomly, all around the canopy, and leaf miners were identified and recorded. We also recorded the abundance of other leaf miners (mainly *Stigmella* sp., *Bucculatrix* sp., *Stilbosis* sp. and *Brachys* sp.) and other herbivores belonging to several guilds, such as stem-gallers, leaf-gallers, leaf-tiers, chewers and leaf rollers (Appendix 1).

Brown (1984) argued that local abundance is a reflection of how well a particular site meets the needs of a species along many niche axes and plant quality features such as the concentration of secondary compounds and foliar nitrogen, as well as the abundance and the composition of the natural enemy community, might vary according to how environmental factors vary at different parts of the host plant range. Differences in plant quality were assessed by collecting 50 undamaged leaves (collected from at least 10 trees) on each site. Leaves were immediately placed on ice, returned to the laboratory and dried in an oven at 50^{o}C for 3-4 days. Digital pictures of all leaves were taken in the lab to estimate mean leaf area of *Q. myrtifolia* on each site. Leaves were then milled to a fine powder and tannin and foliar nitrogen concentration were determined from 10 leaves per site. Tannins were extracted from 50 mg of dry tissue, and tannin concentration was quantified using the radial diffusion assay with three replicates per leaf (for details see Hagerman, 1987). Nitrogen content was determined using a CE Instruments NC2100 CN Analyzer (CE Elantech, Incorporated, Lakewood, New Jersey, USA). We also collected 3 soil samples at each site, by taking 3 random cores near 3 different plants and sampling soil 5-10 cm deep. Soil samples were analyzed for the percent nitrogen and carbon using a CHN Analyzer.

Because top-down factors, such as the pressure exerted by natural enemies, might also influence leaf miner abundance over the range, and studies have shown that there can be systematic changes in particular demographic rates across a species' geographical range (e.g., Taylor *et al.* 1980, Sanz 1997), at each site we also collected between 50 and 100 mined leaves to assess survivorship rates and sources of mortality (n= 3,026 mines collected). Successfully emerged larvae of *Cameraria* exit the mine as flying adults by cutting the edges of the silky cocoon. Mines that were preyed upon are usually found open and the larva is missing. Larval parasitism is easily recognized by the presence of parasitoids inside the mine, by the presence of parasitoid holes on the external surface of mines and by the presence of parasitoid pupae attached to the leaf miner larvae. Larvae killed by host plant resistance are usually found dead inside the mine, but intact and flat and mine walls are usually blackened. Demographic rates for this particular leaf miner consist only of larval survivorship and mortality data, because assessment of oviposition for *Cameraria* sp. nova is not easily done, as no scar is left after female oviposition and eggs were no longer found when censuses started.

Data Analysis

Although data on several other leaf miners and other herbivore guilds on *Q. myrtifolia* were collected during this study, species association, community composition as well as competitive interactions over the host plant range will be addressed in another study. This study focused on the spatial structure of *Cameraria* sp. nova across its distributional range and effects of variation in host plant quality and pressure exerted by natural enemies on its distribution and abundance at the regional scale.

Spatially indices of intraspecific aggregation were calculated using SADIE techniques (Spatial Analysis by Distance Indices) (Perry 1995). Briefly, SADIE operates by comparing the spatial arrangement of the observed sample with other arrangements derived from it, such as those where the individuals are as crowded as possible, those in which they are arranged at random and those in which they are as regularly spaced as possible. Specifically, intra-specific aggregation indices compare the spatial arrangement of the observed distance to regularity (the total number of moves which individuals in each sample must make so that all sample units have the same number of individuals) with the permuted distances to regularity derived from randomization procedure (Perry 1995, Perry *et al.* 1999). An index of aggregation (I_a) and randomization tests were calculated as $I_a = 1$, indicating a random distribution and $I_a > 1$ an aggregated distribution of individuals across sample units. Additionally, the index *v* provides a measure of clustering for each sampling unit (site), with subscript *i* for patches and subscript *j* for gaps. A cluster is defined as a region of relatively large counts close to one another in a two-dimensional space (i.e., a patch) or of relatively small counts (i.e., a gap). Significant positive mean v_i values indicate spatial clustering of leaf mines into patches, whereas significant negative mean v_j values indicate the presence of gaps in the spatial distribution of the species. Details of SADIE techniques can be found in Perry *et al.* (1999). These indices were then tested under the null hypothesis of random arrangement of observed densities using formal randomization tests (Perry *et al.* 1999, McGeoch & Price 2004). All indices were calculated using SADIEShell version 1.22 .

The analysis of spatial pattern is of prime interest to ecologists because most ecological phenomena investigated by sampling geographic space are structured by forces that have spatial components (Legendre & Legendre 1998). To determine whether local population densities of *Cameraria* exhibit any spatial structure across the range of *Q. myrtifolia*, spatial correlation analysis was performed using the mean density of *Cameraria* mines at each site on *Q. myrtifolia*. Spatial structure was determined by calculating Moran's I spatial autocorrelation statistics as:

$$\mathrm{I}\,(d) = \frac{1/W\sum_{h=1}^{n}\sum_{i=1}^{n} w_{hi}(y_h - \overline{y})(y_i - \overline{y})}{1/n\sum_{i=1}^{n}(y_i - \overline{y})^2} \quad \text{for } h \neq i$$

where y_h's and y_i's are the values of the observed variables at sites *h* and *i*. Before computing spatial autocorrelation coefficients, a matrix of geographic distances $D = [D_{hi}]$ was created using Geodesic distances, i.e., distances that take into account the curvature of the earth's surface. Distance values were then grouped into distance classes following Sturge's rule (Nclasses=1+3.3logn, Legendre & Legendre 1998) and Moran's I was calculated for 7 equally spaced classes. Spatial correlograms were then created by plotting autocorrelation coefficients for the various distance classes d. The weights w_{hi} in the above equation are Kronecker deltas (Legendre & Legendre 1998) of a binary form, i.e., the weights take the value $w_{hi} = 1$ when sites *h* and *i* are at distance d (same class) and $w_{hi} = 0$ when sites h and I are at different classes. Finally, W is the sum of the weights w_{hi} for the given distance class, i.e., the number of pairs used to calculate the coefficient. Moran's I usually takes values in the interval [-1,+1] and positive autocorrelation in the data translate into positive values of I and negative autocorrelation produces negative values. Spatial autocorrelation coefficients were tested for significance by calculating confidence intervals and the significance of the overall correlograms were assessed using Bonferroni's correction for multiple comparisons.

Because not only leaf miner abundance might be spatially autocorrelated, but also aspects of plant quality and mortality rates might show spatial structure, i.e., closer sites are more similar than more distant sites, we also used Moran's I to describe any spatial structure in bottom-up (tannin concentration, foliar nitrogen, soil nitrogen and leaf area) as well as top-down (mortality imposed by predators and mortality imposed by parasitoids) factors. All analyses were conducted using PASSAGE (Pattern analysis, spatial statistics, and geographic exegesis) software version 1.0 (Rosenberg 2001).

The use of partial regression analysis is of great interest when one wants to partition the effects of biotic and abiotic factors on the abundance of a species and to model data showing any type of spatial dependence. Following Legendre & Legendre (1998), Brewer & Gaston (2002) and McGeoch & Price (2004) we used partial regression analysis to estimate how much variation in *Cameraria* abundance can be attributed to bottom-up and top-down factors once the effects of spatial location have been taken into account. We first ran a multiple stepwise regression to determine the best-fit combination of spatial and non-spatial variables that contributed to significantly explain the variability of *Cameraria* densities. Thereafter, significant terms were retained to construct a matrix of biotic (bottom-up and top-down factors) and abiotic (latitude, longitude, elevation) variables to be used in subsequent analyses. The combined effects of both top-down/bottom-up and spatial variables on *Cameraria* densities were calculated by multiple regression of *Cameraria* abundance onto both sets of predictive variables combined. Environmental variables and abiotic variables were then removed at two separate steps and the explanatory potential of the biotic variables, after correcting for spatial dependence, was calculated by measuring the change in deviance accounted for the regression model after the biotic variables were removed. Statistical significance of these fractions was tested using F-tests.

At the end of partial regression analyses, variation in *Cameraria* abundance was then partitioned into 4 fractions (a, b, c, d) representing: a) non-spatial environment (fraction that can be explained by biotic variables independent of spatial structure); b) spatially structured environment (spatial structure shared by *Cameraria* and biotic variables measured); c) non-environmental spatial (spatial structure in *Cameraria* densities not explained by the measured biotic variables), and d) unexplained residual variation. Original data were log (for linear measurements) or angular transformed (for percentages) to meet normality assumptions. All analyses were conducted using Systat 9.0 for Windows.

RESULTS

Cameraria mines were found in all 40 sites sampled in Florida, although large variations were observed at larger scales: mines were, on average, three times more abundant on coastal compared to inland sites (mean number of *Cameraria* mines on coastal sites 99.1± 8.99, inland sites 33.65 ± 4.99, $F_{1,38}$=41.58, P<0.00001, Figure 2.2). As a consequence of Florida's peninsular characteristic, sites on the edges of the range of *Q. myrtifolia* coincided with most coastal sites and exhibited significantly higher numbers of mines than sites at the center of the distribution of the host plant (mean number of *Cameraria* mines on edge sites 95.9± 8.07, center sites 26.23 ± 2.84, $F_{1,38}$=53.27, P<0.00001).

Cameraria mines were significantly intra-specifically aggregated on sites, as demonstrated by a significant index of aggregation (I_a = 1.936, P <0.005). The clustering index *v* also indicated significant spatial clustering of mines into patches (v_i=1.708, P=0.015) and significant gaps in the spatial distribution of this species (v_j= - 2.358, P=0.013).

Correlograms constructed for the abundance data indicated a significant spatial structure of *Cameraria* mines over the range: mines were positively spatially autocorrelated at small distances (≈122Km), indicating that sites close to each other tend to have similar mine densities compared to sites further apart ($P<0.05$ after Bonferroni's correction, Figure 2.3). The size of the patches, i.e., the distance between zones of high and low densities, is indicated in the correlogram by the distance in which the first negative autocorrelation was found, at approximately 487Km. A significant positive autocorrelation was also observed between sites separated by longest distance (≈850Km), which might represent coastal sites at opposite edges of the geographical range. No spatial structure was detected on the bottom-up (Figure 2.4) as well as top-down factors (Figure 2.5). The only demographic parameter that showed spatial structure was mine successful emergence (Figure 2.5), indicating that sites near to each other exhibited similar levels of mine survivorship. At very long distances, successful emergence was also negatively spatially autocorrelated, indicating that sites very far apart are dissimilar, although these results should be interpreted with caution, as in the last distance classes Moran's I is calculated from a relatively low number of pairs of sites (9 in this case). Successful emergence showed a similar pattern of autocorrelation to *Cameraria* abundances at small distances, i.e., sites that were apart in ≈122km were more similar in the number of mines and rates of mine survivorship than sites farther apart.

Regression analyses indicated that both biotic and abiotic variables contributed to variation in the density of *Cameraria* mines over the range: 67.6% of the variation in *Cameraria* abundance was explained by the combined effects of latitude, longitude, elevation, foliar nitrogen, successful emergence of mines and parasitism rates of larvae ($r^2=0.676$, $P<0.0001$). Partial regression analyses using these significant terms revealed that, once spatial position was taken into account (fraction c on model), only 23.1% of the variation in *Cameraria* abundance could be attributed to biotic variation alone (fraction a on model: $r^2=0.231$, $P<0.05$). The combined effects of biotic variables and spatial position (fraction b) explained 13.3% of the variation in *Cameraria* abundance over the range (Table 2.2). Although only 23.1% of the variation in *Cameraria* abundance was explained by environmental variation after taking into account the effects of spatial variables, this small fraction was still significant, indicating that a significant effect of bottom-up (successful emergence and foliar nitrogen) as well as top-down (larvae parasitism) factors was detected.

TABLE 2.1- List of sites where *Cameraria* sp. nova mines were collected in Florida. (St= State).

Site Number	Site Name	County
1	Fort Cooper St Park	Citrus
2	Withlacoochee St Forest	Citrus
3	Oscar Scherer St Park	Sarasota
4	Little Manatee River St Park	Hillsborough/Manatee
5	Paines Creek Historic St Park	Hardee
6	Silver River St Park	Marion
7	Alligator Creek Preserve	Charlotte
8	UCF Arboretum	Orange
9	Archbold Biological Station	Highlands
10	Lake June-in-winter St Park	Polk
11	Lake Wales Ridge St Forest	Polk
12	Hobart Park	Indian River
13	Bluefield Ranch	St Lucie
14	Jonathan Dickson St Park	Martin
15	Yamato Scrub	Palm Beach
16	Crystal Lake Sand Scrub Nature Reserve	Palm Beach
17	Rookery Bay Preserve	Collier
18	Estero Scrub Preserve	Lee
19	Lake Manatee St Park	Manatee
20	Blue Springs St Park	Osceola
21	Washington Oaks St Park	St Johns
22	UNF Scrub	Duval
23	Etoniah Creek St Forest	Putnam
24	Jay B. Starkey Wilderness Park	Hernando
25	Enchanted Forest	Brevard
26	Big Lagoon St Park	Escambia
27	Naval Live Oaks Nature Trail	Santa Rosa
28	Top Sail St Park	Walton
29	St Andrews St Park	Bay
30	St Joseph Peninsula St Park	Gulf
31	Tate’s Hell St Forest	Franklin
32	Route 51 Scrub Steinhatchee	Taylor
33	Cedar Key Scrub St Preserve	Levy
34	Swift Creek Conservation Area	Columbia
35	Arcadia Scrub	DeSoto
36	Ridge Manor Scrub County Park	Hernando
37	USF Ecoarea	Hillsborough
38	Paynes Prairie St Park	Alachua
39	Tenoroc Fish management Area	Polk
40	Boyd Hill Preserve	Pinellas

Table 2.2- Results of partial regression analyses for *Cameraria* densities and the combined effects of biotic and spatial variables. (* indicates statistically significant differences at P=0.05 and ** at P=0.001 after F-tests).

Dependent Variable	% Variation in mine density explained by				
	Total	a (env)	b (env x space)	c (space)	d (residual)
Cameraria abundance	67.6	23.1*	13.3	31.2**	32.4

Figure 2.1- *Cameraria* sp nova mines on *Quercus myrtifolia*.

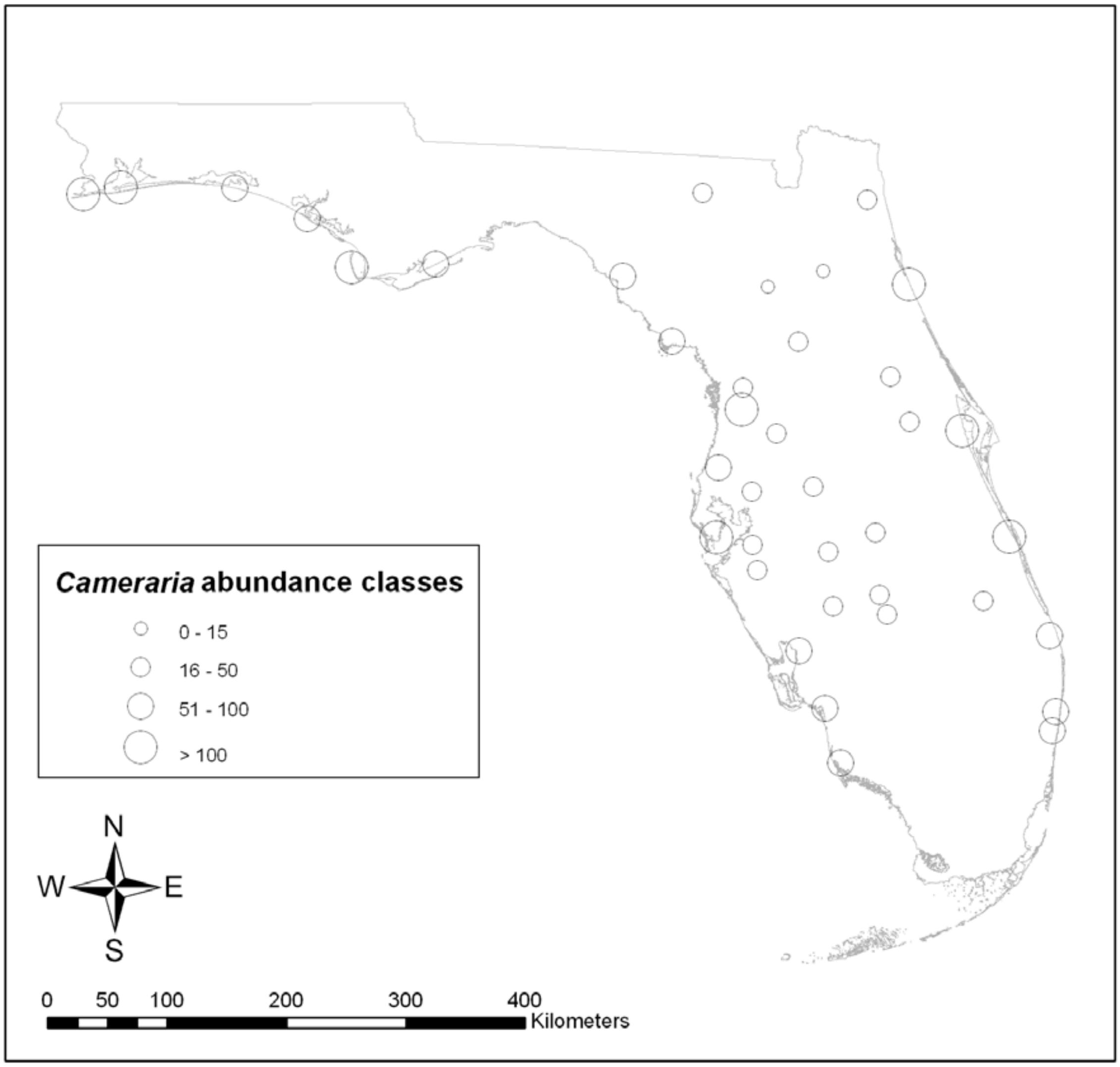

Figure 2.2- Distribution of *Cameraria* sp nova mines on 40 sites sampled in scrub patches in Florida. Sites were plotted using latitude and longitude coordinates converted into decimal degrees and the size of symbols is proportional to the abundance of *Cameraria* (average number of individuals per 200 leaves on 15 plants) at each site.

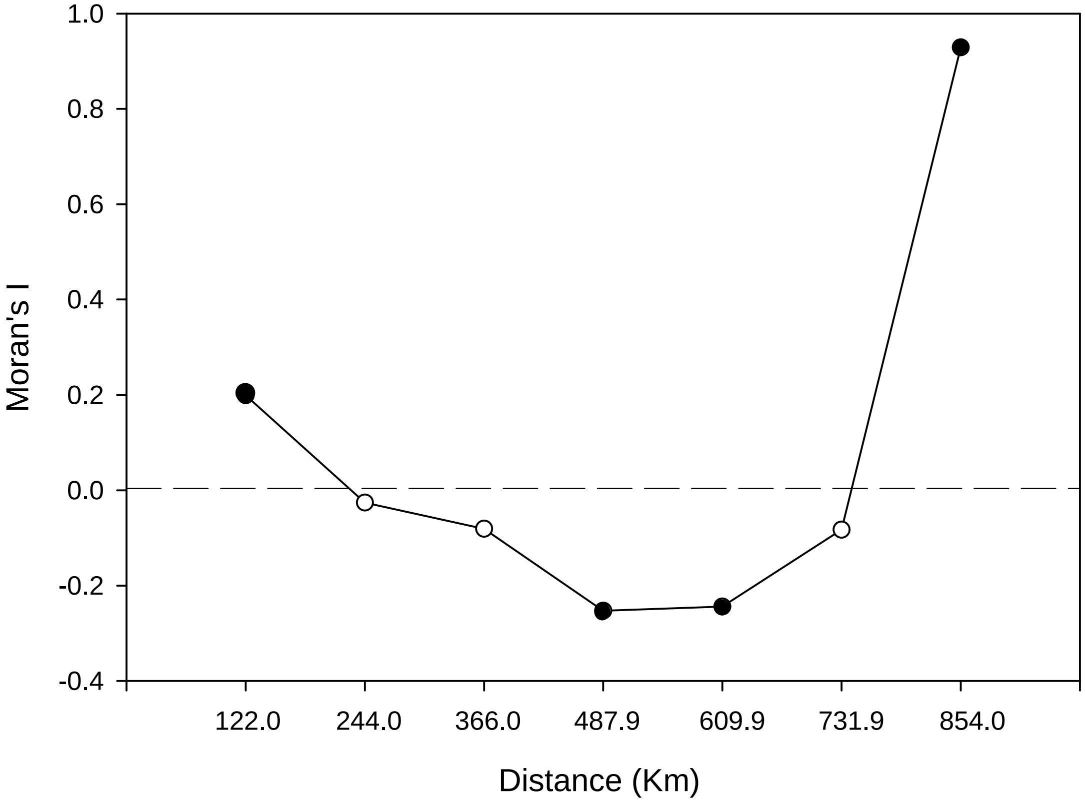

Figure 2.3- Spatial correlogram of the abundance of *Cameraria* mines from across the geographical range of *Q. myrtifolia*. Solid circles identify autocorrelation statistics that remain significant after progressive Bonferroni's correction (α = 0.05) and empty circles are non-significant values.

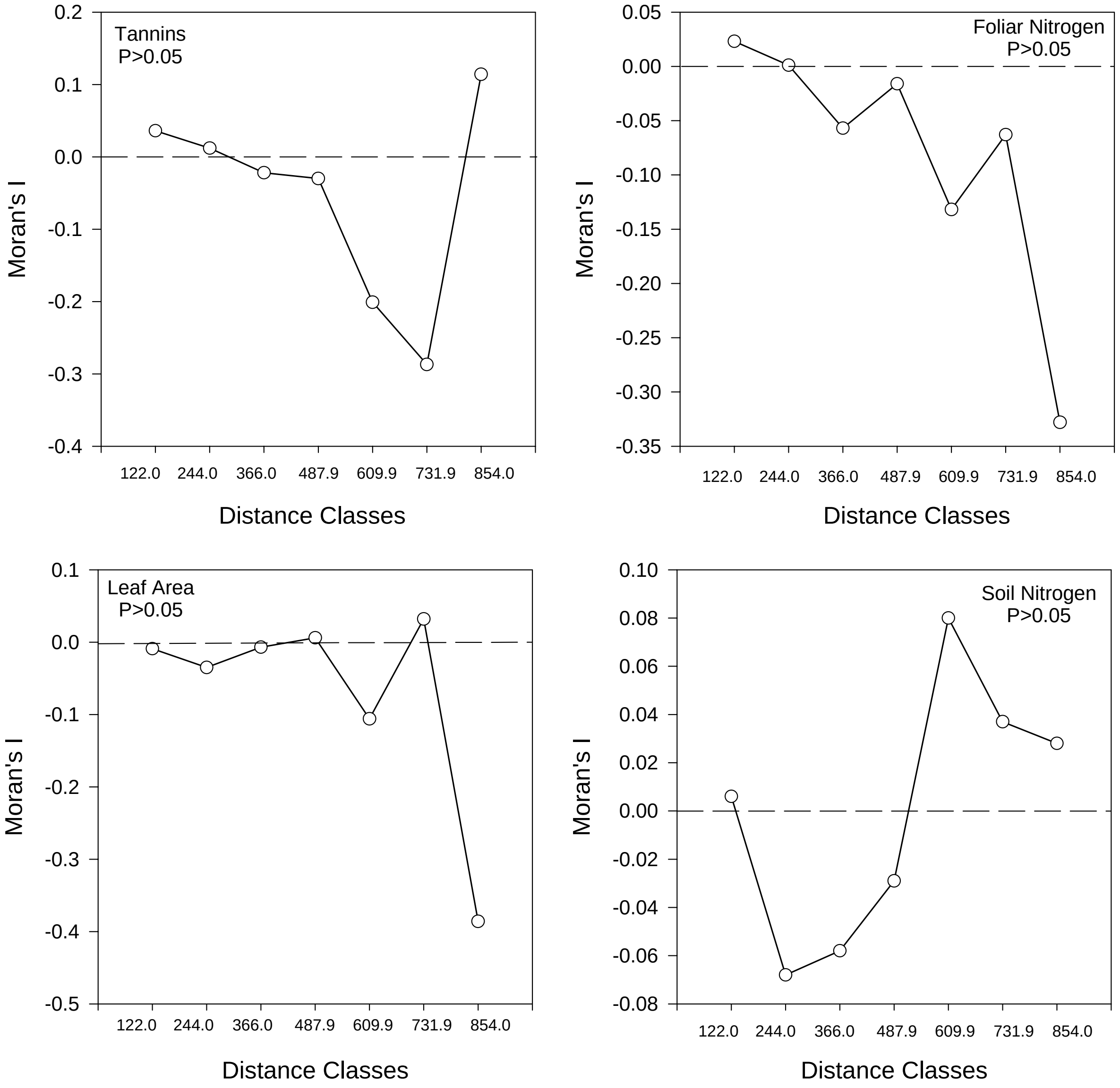

Figure 2.4- Spatial correlograms of bottom-up factors that might influence *Cameraria* abundance over the range of *Q. myrtifolia*. All correlograms were statistically non-significant after Bonferroni's correction (all P>0.05), indicating that variation in bottom-up factors over the range of *Q. myrtifolia* are independent of spatial structure.

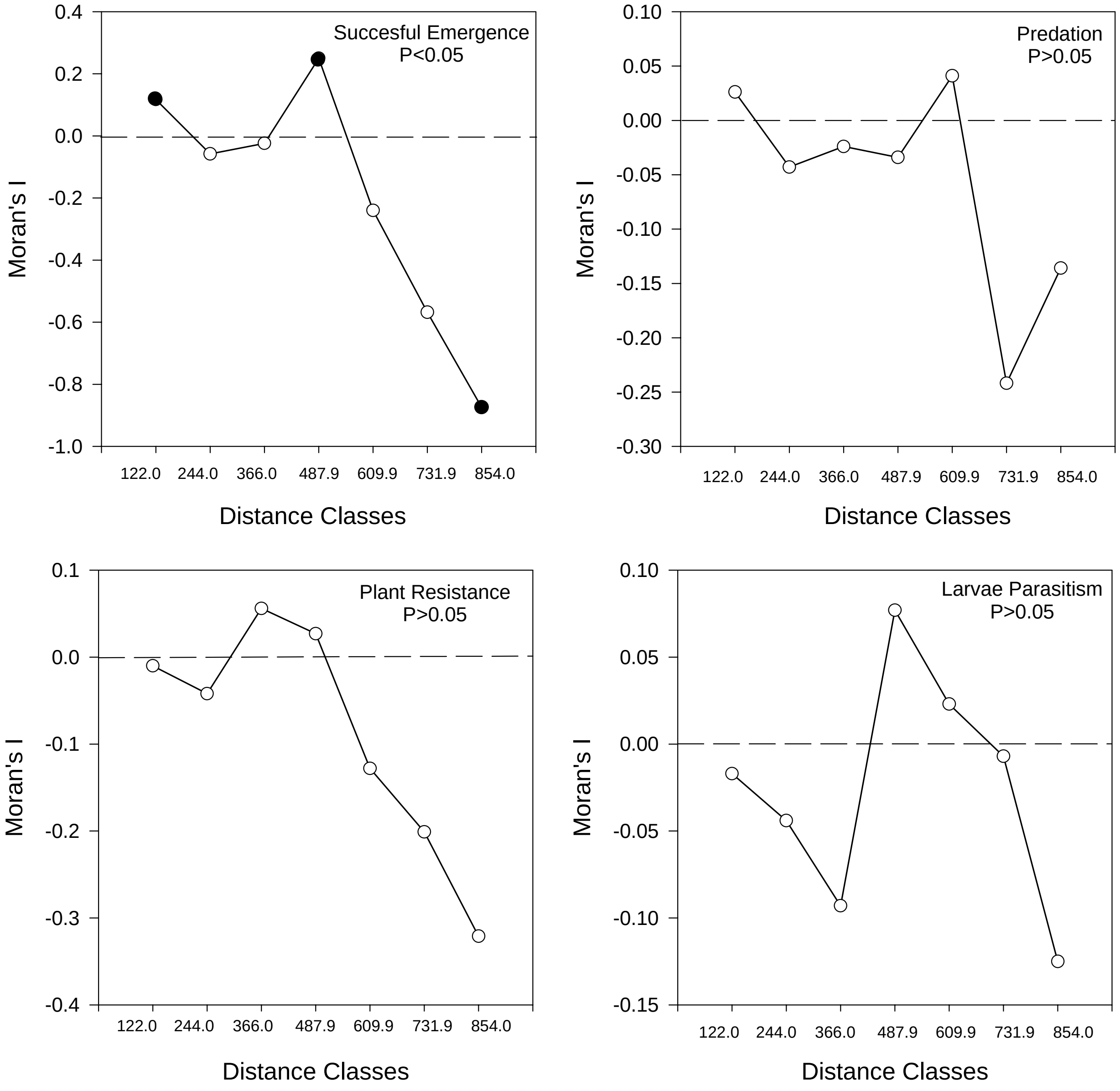

Figure 2.5- Spatial correlograms of demographic rates and top-down factors that might influence *Cameraria* abundance over the range of *Q. myrtifolia*. Successful emergence was the only demographic parameter spatially structured after Bonferroni's correction, indicating that mine survivorship is spatially structured ($P<0.05$).

DISCUSSION

Almost without exception, individual species exhibit profound spatial variation in the local densities which they attain (Blackburn *et al.* 1999). They are entirely absent from most places, at relatively low densities in the majority of those at which they occur, and at relatively high densities in a small proportion of occupied sites. A significant spatial structure was observed for *Cameraria* mines over the range of *Q. myrtifolia*, and positive spatial autocorrelations indicated that sites in closest proximity to each other were similar in both the abundance of mines as well as successful emergence, an indicator of mine survivorship. This patchy structure was also corroborated by the aggregation and clustering indices, a form of distribution typically resulting from one or more dispersal, intra- or inter-specific interactions, biotic or abiotic environmental factors (Legendre 1993). However, none of the top-down and bottom-up factors analyzed in this study were spatially structured, as demonstrated by the non-significant spatial correlograms. Similar results were found by Gaston *et al.* (2004) addressing variation in quality of *Ilex aquifolium* leaves over its range in Europe. Values of Moran's I for that study were very low and non-significant, indicating little similarity in most measures of host-plant quality used. Our data suggests that variation in mine survivorship (i.e., successful emergence) across sites might contribute to the variation in *Cameraria* densities over the range. Our analyses of tannin concentration over the range of *Q. myrtifolia* indicated that tannins were not spatially structured and did not contribute to the variation in *Cameraria* abundance. Genotypic differences among individuals that are not influenced by spatial position might explain these results. The amounts of foliar nitrogen were also not spatially structured, but did contribute to a small amount of variation in leaf miner abundance, a finding previously recorded in other plant-herbivore systems (reviewed by Waring & Cobb 1992, Kyto *et al.* 1996). The lack of spatial structure in the bottom-up and top-down factors analyzed in our study and the presence of spatial structure in *Cameraria* abundance indicates that there is no simple covariance between these two (Gaston *et al*. 2004). Although we have shown that there is variation in the amount of tannins and foliar nitrogen among sites, as well as the amount of larvae mortality inflicted by natural enemies, there is little evidence that this variation assumes any marked spatial structure. Although factors that influence host-plant quality such as light and soil nutrients might vary with environmental conditions, there may be no consistent trends in these factors with changes in latitude and/or longitude (Gaston *et al.* 2004), as shown in the present study. Also, other aspects of plant quality not addressed in this study, as well as climatic conditions such as temperature and radiation, might affect leaf miner abundance and might explain the spatial structure found for *Cameraria* population. Although the bottom-up and top-down factors analyzed in this study were not spatially structured, some factors still contributed for a fraction of the variation in *Cameraria* abundance over the range of *Q. myrtifolia*, independently of spatial position, a finding previously recorded for other sessile insects (e.g., McGeoch & Price 2004, Gaston *et al.* 2004). In previous studies, we have demonstrated that leaf miners on oaks respond to both natural as well as experimentally elevated levels of foliar nitrogen, and mine mortality tends to be relatively low and an important determinant of mine abundance in subsequent seasons (Cornelissen & Stiling 2005, 2006a,b).

Contrary to what was predicted by the abundant-centre hypothesis, *Cameraria* densities did not peak in the center of the distribution of *Q. myrtifolia*: higher abundances were actually observed at the edges of the distribution, coinciding with coastal sites in Florida. It has been suggested that the most favorable conditions are found at the centre of a species' distribution, and these conditions in turn, support the highest population density across the distributional range. Our results do not support this hypothesis, as central sites exhibited lower densities. Brown *et al.* (1995) suggested that spatial correlograms of species typically show a bowl-shape pattern, with positive spatial autocorrelations at both short and very long distances, and argued that high autocorrelations at very long lags (≈850km in our study) might indicate the similarly low levels of density found at opposing range edges. Although the correlogram for *Cameraria* shows the typical bowl-shape, we suggest that strong and significant positive autocorrelations found at the largest distance class actually indicates edge sites with high density of mines, at the opposite edges of the distribution, such as the Florida Panhandle and the Atlantic coast. A comparison of plant quality features between central and edge sites revealed that edge sites exhibited significantly higher foliar nitrogen ($F_{1,38}$=4.834, P=0.034) and a tendency for reduced tannin concentration ($F_{1,38}$=3.248, P=0.08). These results are in accordance with predictions from the Plant Stress Hypothesis, and reinforce the idea that leaf miner populations might benefit from increased nitrogen concentration in stressed plants and might peak in abundance on the distribution edges, instead of center or interior populations. Although a recent meta-analytical review (Huberty & Denno 2004) showed limited support for the plant stress hypothesis, leaf miners were an underrepresented group, and these authors suggested that future studies addressing this hypothesis should focus on herbivore guilds for whom substantial examples were lacking, such as leaf miners and stem borers. Our study provided evidence that, at the largest scale of distribution of this particular leaf miner species, plant stress seems to be an important determinant of the spatial structure of this species.

Organisms' abundance and fitness levels are predicted to peak in the species' range center and decline towards its range edges when environmental optimums are found at the centre of the distribution, which doesn't seem to be the case for *Cameraria* populations. Few studies have addressed edge effects on intra-specific abundance and demographic rates of populations, although differential survivorship, oviposition rates and effects of natural enemies on overall mortality might determine the spatial structure of some populations (e.g., Fagan *et al.* 1999, McGeoch & Gaston 2000). McGeoch & Gaston (2000), however, found higher abundance of the holly leaf miner *P. ilicis* on edge compared to interior habitats in England. Explanations for this pattern included lower bird predation and pupal parasitism in edge versus interior populations. In the present study, parasitism rates of *Cameraria* larvae on centre sites were approximately 35% higher than parasitism rates on edge sites (mean parasitism centre sites: 21.2% ± 2.5, edge sites: 15.3% ± 1.9), although no significant differences were observed for mine predation (mean larvae predation on centre sites 27.9% ± 2.4, edge sites: 25.2% ± 1.2). Overall, larvae mortality was higher on centre (62.0% ± 3.2) compared to edge populations (57.1% ± 3.3) indicating that demographic parameters that determine species abundance and population dynamics might differ between peripheral and central populations of *Cameraria*.

Recent studies have pointed out the importance of using spatially explicit models when studying population dynamics, especially when nearby populations share more similarity than would be expected by chance. Scrub oaks are critically endangered habitat in Florida, and our results have shown that coastal populations support higher densities of leaf miners and leaf mine survivorship in these coastal areas is greatest, indicating the importance of the edges of the distribution for the population dynamics of this species. The Florida peninsula is famous for being flat (Marshall *et al.* 2000) but because of its low-lying topography, Florida underwent a series of inundations during the glacial period, and during interglacial events, currents deposited sand along what were shorelines, creating inland sand ridges. These ridges represent the highest altitudes in Florida, and their existence has been shown to be important for several other species, such as spiders (Marshall *et al.* 2000), beetles (Hubbell 1954), and grasshoppers (Deyrup 1996). Our results indicate that central populations of *Q. myrtifolia*, which coincide with Florida's most prominent ridges (e.g., Brooksville Ridge, Lake Wales Ridge, Deland-Crescent City Ridge) and highest elevations (41 to 65 meters) do not support large populations of *Cameraria*, suggesting that either isolation, elevation or even genotypic differences between host plants on center scrub islands might have influenced the patterns found. The limited dispersal ability of leaf miners might be responsible for the existence of spatial autocorrelation in *Cameraria* populations, as demonstrated for other insects with limited dispersal that exhibit metapopulation dynamics (e.g., Gonzalez-Megias *et al.* 2005).

After controlling for spatial location, the amounts of foliar nitrogen and the percentage of larvae parasitism were statistically significant predictors of *Cameraria* abundance over the range of its host plant. The importance of foliar nitrogen for insect herbivores has been extensively discussed (Mattson 1980) and higher foliar nitrogen on coastal edge plants, where mine densities were higher, offer additional support for the plant stress hypothesis and its effects on herbivores. Drought stress has been shown in a number of studies to significantly impact the top-down and bottom-up influences on insect herbivores (Mattson & Haack 1987, Fay *et al.* 1993, Levine & Paige 2004) and coastal barrier islands in Florida exhibit considerably low rainfall than mainland areas (U.S Department of Interior, Florida). Parasitoids have also been long suggested as potential forces in the dynamics of leaf miner populations, as miners tend to support richer parasitoid communities then free-living herbivores (Connor & Taverner 1997). We are unaware of studies that addressed levels of natural enemy attack over the range of other leaf miner species and their importance to mine abundance and survivorship. Lower parasitism rates of *Cameraria* larvae on edge, coastal sites also offer support to the slow-growth, high-mortality hypothesis (Clancy & Price 1987). According to this hypothesis, herbivores feeding on plants of low nutritional quality do not necessarily increase damage on their host by overcompensatory feeding if increased development time due to poor host quality increases the window of vulnerability of herbivores to natural enemy attack. In this sense, leaf miners developing on edge populations where plant quality was higher might develop faster and decrease the window of vulnerability to the attack of parasitoids. Future studies evaluating mine development time, growth and survivorship on edge versus central populations will address this point and offer a better test of the slow-growth, high-mortality hypothesis.

The abundance structure of a species is clearly the compound outcome of several processes, such as climate, history, resource quality and biotic interactions (McGeoch & Price 2004). If species have population dynamics driven strongly by resource quality, then abundance structures are likely to be determined by the distribution and quality of resources across the landscape. Although at local scales it has been demonstrated that leaf miners respond strongly to bottom-up factors (Moon & Stiling 2004, Cornelissen & Stiling 2006b), resources themselves were not spatially structured in our study, indicating that at larger spatial scales, other factors might dictate the abundance structures found for the *Cameraria* population studied. Host plant quality and natural enemy effects may be strong and identifiable only at fine, and not landscape, scales (Bevers & Flather 1999), whereas dispersal abilities and other abiotic variables, such as latitude and altitude, might determine leaf miner abundance and structure at the landscape scale. Within patches, however, plant quality might be a strong determinant of mine density, as demonstrated by our previous studies in similar oak systems.

Chapter 3

CLUMPED DISTRIBUTION OF OAK LEAF MINERS BETWEEN AND WITHIN PLANTS

SYNOPSIS

Leaf miners typically show non-random distributions both between and within plants. We tested the hypothesis that leaf miners on two oaks species were clumped on individual host trees and individual branches and addressed whether clumping was influenced by aspects of plant quality and how clumping and/or interactions with other oak herbivores affected leaf miner survivorship. Null models were used to test whether oak herbivores and different herbivore guilds co-occur at the plant scale. Twenty individual *Q. geminata* plants and 20 *Q. laevis* plants were followed over the season for the appearance of leaf miners and other herbivores, and foliar nitrogen, tannin concentration, leaf toughness and leaf water content were evaluated monthly for each individual tree. The survivorship of the most common leaf miners was evaluated by following the fate of marked mines in several combinations that involved intra- and inter-specific associations. We observed that all leaf miners studied were clumped at the plant and branch scale, and the abundance of most leaf miner species was influenced by plant quality traits. Mines that occurred single on leaves exhibited significantly higher survivorship than double and triple mines and leaves that contained a mine and a leaf gall and a mine and damage by chewers exhibited lowest survivorship. Although leaf miners were clumped at individual host trees, null model analyses indicated that oak herbivores

do not co-occur significantly less than expected by chance and there was no evidence for biological mechanisms such as inter-specific competition determining community structure at the plant scale. Thus, despite co-occurrence resulting in reduced survivorship at the leaf scale, such competition was not strong enough to structure separation of these oak herbivore communities.

INTRODUCTION

Patterns of distribution of insects on plants are strongly determined by host plant variation. A question of great interest in herbivory-related studies is how plant traits affect attack rates by phytophagous insects and how variation in plant quality affects the distribution and performance of herbivores both within and among host plants. Recently, it has become clear that differences between individuals can have profound effects upon the kind of dynamics exhibited by herbivorous populations, their stability and their mean levels of abundance (Crawley & Akhteruzzaman, 1988) and the study of individual variation an its consequences for population dynamics has become a priority. For folivorous insects, it is not unusual for populations to be aggregated on their host plants (Stanton, 1983; Faeth, 1990). For leaf mining insects, oviposition site selection by females may be highly influenced by variation in leaf structure (Reavey & Gaston, 1991), leaf age and size (Faeth, Mopper, & Simberloff, 1981; Faeth, 1991), leaf chemistry (Stiling, Brodbeck, & Strong, 1982) and effects of the third trophic level (reviewed by Connor & Taverner, 1997) and leaf miners usually show non-random distributions among plants and among leaves within an individual tree (Stiling, Simberloff, & Anderson, 1987; Shibata, Ishida, Soeya, Morino, Yoshida et al., 2001). One recurring explanation for clumped patterns of leaf miners in particular is that insect distribution reflects variation in nutritional quality and/or secondary chemistry among and within the host plants (Faeth, 1990). Because leaf quality is a major determinant of host choice by many herbivores (Strong, Lawton, & Southwood, 1984), variations in leaf quality are expected to influence leaf miner distribution, abundance and survivorship.

Besides variations in leaf quality, interactions with other herbivores, including external and other internal feeders may affect the distribution and abundance of leaf-mining insects. The distribution of endophagous insects, such as gall-formers and leaf miners, is typically aggregated and when selection for tissues of better nutritional quality does occur, competition between insects that utilize the same type of resource can arise. The role of intra- and inter-specific competition among insects in ecological theory has changed throughout the years, from the argument that competition was weak and infrequent on phytophagous insect communities in the early 80's, to the resurrection of the importance of competition between phytophagous insects in the 90's (Denno, McClure, & Ott, 1995; Reitz & Trumble, 2002). For leaf miners, competition may arise from the presence of con-specifics and/or from the presence of other feeding guilds, such as gall-formers and free-feeding herbivores. The quality of resources available to leaf miners, and hence their performance, may be modified by the feeding of other folivores (Faeth, 1992). Leaf chewers, in particular, can affect the foliage on which leaf miners are feeding by changing plant quality and by altering or redirecting plant resources (Shibata et al., 2001). Damage caused by leaf chewers can, for example, lead to an increase in secondary compounds, such as phenolics, and a decrease in nitrogen concentration

(Hartley & Lawton, 1987). Gall-formers also have the potential to modify plant quality by physically and/or chemically modifying plant vasculature, architecture and nutritional quality (Johnson, Mayhew, Douglas, & Hartley, 2002), and some studies have demonstrated that galled leaves exhibit higher nutritional quality than neighboring non-galled leaves (e.g., Abrahamson & Weis, 1986, but see Hartley & Lawton, 1992). For sessile insects and free-feeding herbivores in general, most of the studies that indirectly addressed interspecific competition by analyzing species co-occurrence have used presence-absence distributional data as a surrogate for competition (e.g., Stiling, Rossi, Catell, & Bowdish, 1999; Kagata & Ohgushi, 2001, and examples in Denno et al., 1995), although more recent studies advocate for the use of more refined statistical tests based on random distributions such as null models (e.g., Gotelli & Graves, 1996; Ribas & Schoereder, 2002; Zwolfer & Stadler, 2004).

The purpose of this study was to investigate how differences in plant phenology and nutritional quality influence the distribution and abundance of leaf miners on *Quercus geminata* (Fagaceae) and Q. *laevis*, both between and within plants. We also aimed to examine the effects of other herbivores co-occurring on the same host plants on both the abundance and survivorship of the most common leaf miners on both oak species. The specific aims of this study were: 1) to examine variation in the spatial distribution of leaf miners among plants, within plants and according to canopy position, 2) to determine how leaf miner abundance was affected by plant nutritional quality in terms of foliar nitrogen, water content, tannin concentration and leaf toughness, 3) to observe how the presence of other herbivore guilds such as chewers and gall-formers affect the abundance and survivorship of leaf miners on oaks, and 4) to determine whether herbivore guilds and herbivore species co-occur significantly less or more than expected on oak host plants using null models.

STUDY SYSTEMS

The sand live oak, *Quercus geminata* (Fagaceae), is a semi-evergreen oak and, typically, old leaves abscise and new leaves appear in late April or early May, reaching full size in approximately 2 weeks. *Stilbosis quadripustulatus* (Lepidoptera: Cosmopterygidae) is a moth whose larvae induce mines on the adaxial surfaces of *Q. geminata*. *S. quadripustulatus* is a univoltine species, whose adults emerge in early summer (from May to June) from pupae that overwinter in soil and litter. Oviposition occurs approximately in early June, when females oviposit at the junction of the midvein and a major lateral vein. Larvae take from 60 to 90 days to complete their 5 instars and mines may reach 3.0 cm in length (Simberloff & Stiling, 1987). Many other herbivores compose the insect community associated with *Q. geminata* (Figure 3.1). Leaves are frequently found damaged by chewing insects such as the eastern buck moth *Hemileuca maia* (Lepidoptera: Saturniidae), and at least 4 cynipid species (Hymenoptera: Cynipidae) of galling insects are commonly observed on sand live oak leaves and stems: *Andricus quercusfoliatus*, *Disholcaspis quercussuccinipes*, *Callirrhytis quercusbatatoides*, and *Belonocnema quercusvirens. A. quercusfoliatus* induces white flower-like galls on sand live oak stems, whereas *D. quercussuccinipes* wasps induce clusters of 5-20 yellowish brown galls usually crowded around a terminal oak twig. *C. quercusbatatoides* wasps induce abrupt swellings of twigs, varying in form and size and *B. quercusvirens* induces tan, globular pea-like galls on the underside of *Q. geminata* leaves. Galls are unilocular and occur in large numbers during the fall. Eyespot galls (Diptera: Cecidomyiidae) are recognized as circular spots, usually 8-10 mm in diameter. The adults emerge from the soil in the spring and lay eggs in the upper leaf surface. As the larva grows, the leaf tissue surrounding it swells slightly and red rings are seen around the galls. Larvae complete their development in 8-12 days and pupate in the soil. This is the most common gall found on sand live oak leaves, often reaching densities of 5 galls per leaf.

The turkey oak *Quercus laevis* is one of the characteristic trees associated with the sand hill community over much of Florida. *Q. laevis* is a moderately fast to fast-growing tree and presents deciduous simple leaves, alternately arranged with usually 5 lobes, although this number may vary from 3 to 7. *Acrocercops albinatella* (Lepidoptera: Gracillaridae) is a microlepidopteran species whose larval stages feed on young leaves, creating distinct linear-blotch mines on the lower surface of *Q. laevis* leaves. Larvae typically feed on the palisade parenchyma cells and deposit frass throughout the mine, completing their development in approximately 10 days. Larvae emerge from the blotch mine and usually pupate on the same leaf from which they emerge (T Cornelissen, pers. obsv.). *Brachys tesselatus* (Coleoptera: Buprestidae) is a univoltine species that also forms distinct blotch mines in *Q. laevis* leaves. The adults emerge in Mid-March to Mid-April, coinciding with budburst of turkey oak. Adults initially feed on the early leaves and flowers until mating and oviposition. Eggs are deposited singly on the upper surface of the leaves and after hatching the larvae mine into the mesophyll creating distinct, characteristic damage. *Brachys* in our study sites go through two generations and the first mines appear in early April and remain active until late June, when larvae complete their development and exit mines to pupate on the soil. New adults emerge in early July and oviposit to form new *Brachys* mines that remain active until September-October. Pupation and overwintering of this second generation occurs within the leaves after they have senesced and abscised from the tree. New adults emerge from the leaf litter in the following spring (Waddell, Fox, White, & Mousseau, 2001). Turkey oak leaves are also attacked by a vast array of herbivores (Figure 3.2), such as the leaf roller weevil *Homoeolabus analis* (Coleoptera: Atellabidae), the eastern buck moth *H. maia*, the white tussock moth *Orgyia leucostigma* (Lepidotpera: Lymantriidae) and other leaf miners such as *Stigmella* (Lepidoptera: Nepticulidae) and *Cameraria* (Lepidoptera: Gracillariidae). Eyespot galls and an unidentified cynipid gall are the most common galls observed in turkey oak leaves in our field sites and no stem galls have been recorded.

METHODS

Data Collection

This study was conducted between February and November of 2003 at the University of South Florida Botanical Garden. To examine effects of plant phenology and nutritional quality on the abundance of leaf miners and other herbivores, 20 individuals of *Q. geminata* and 20 individuals of *Q. laevis* were marked in February, just before budbreak and leaf flush. *Q. geminata* trees ranged from 1.9 to 2.5 m in height and *Q. laevis* trees ranged from 2.5 to 3.2 m in height. On each individual plant, 5 upper-canopy (above 2.0m) and 5 lower-canopy (below 1.5m) branches were selected and all leaves on each branch were individually numbered using a permanent marker. A total of 6,489 marked leaves on *Q. geminata* and 2,243 marked leaves on *Q. laevis* were followed over the season. Leaves were marked at the time of leaf flush when all leaves were still intact, and examined monthly for the appearance of leaf miners, gall-formers, damage by chewers, and leaf abscission.

To assess variation in host plant quality among trees, water content, foliar nitrogen concentration, tannin concentration and leaf toughness were evaluated monthly for each individual plant. On each collection date, 10 undamaged leaves (5 from the upper-canopy and 5 from the lower-canopy) were sampled from each tree, placed immediately on ice, and leaf toughness was evaluated using an Effegi FT-011 penetrometer (International Ripening Co, Italy). Water content was quantified by the difference between leaf wet and dry weights and leaves were further oven-dried and milled to a fine powder. Tannins were extracted from 50 mg of dry tissue, and tannin concentration was quantified using the radial diffusion assay with three replicates per leaf (for details see Hagerman, 1987). Nitrogen content was determined using a CE Instruments NC2100 CN Analyzer (CE Elantech, Incorporated, Lakewood, New Jersey, USA).

To assess the effects of conspecifics and other herbivores on the survivorship of the most common leaf miners on both oak species, on each individual plant, we noted leaves that exhibited each of the following combinations: *Quercus geminata*: 1) one *Stilbosis* mine, 2) two *Stilbosis* mines, 3) three or more *Stilbosis* mines, 4) one *Stilbosis*, one *Brachys* mine, 5) one *Stilbosis* and one or more eyespot galls, 6) one *Stilbosis* mine and leaf damage by chewers. Although *Brachys* mines occur mainly on *Q, laevis,* some mines have been observed on *Q, geminata* where both plant species co-occur (T Cornelissen, pers. obs). *Acrocercops* was never recorded in *Q. geminata* in our field sites. For *Q. laevis* the following combinations were investigated: 1) one *Acrocercops* mine, 2) two or more *Acrocercops* mines, 3) one *Brachys* mine, 4) two or more *Brachys* mines, 5) one or more *Acrocercops* and one or more *Brachys* mine, 6) one or more *Acrocercops* mine and damage by chewers, 7) one or more *Brachys* mine and damage by chewers. The fate of leaf miners on each combination was followed over the season, with observations conducted at bi-weekly intervals (n=598 marked leaves for *Q. geminata* and 708 leaves for *Q. laevis*). Leaf miner survivorship and mortality, as well as date of leaf abscission were scored for each leaf combination throughout the season. Leaf miners offer a great opportunity to assess population survivorship and mortality factors since a record of the miner success is clearly observed on the leaves: parasitized mines have tiny circular exit holes on mine's surface and/or pupae within the mine, and predated mines are usually found ripped open and the larva is absent. Successfully emerged larvae of *Acrocercops* cut open the mines and pupate usually on the same leaf where the mine developed. *Brachys* larvae pupate inside mines and/or cut circular holes on the underside of the leaf. *Stilbosis* mines cut semi-circular holes at the mine edge and larvae exit to pupate in the soil.

Data Analysis

To examine the spatial distribution of leaf miners both between and within individual plants, the distribution of leaf miners were compared to random (Poisson) and clumped (negative binomial) distributions using a X^2 analysis (Ludwig & Reynolds, 1988), with individual plants and branches within plants used as sampling units. We also calculated indexes of dispersion (variance-to-mean ratio) and Green's modified index of clumping (Green, 1966) for each data set. The variance and mean are equal in a theoretical Poisson distribution and the index of dispersion was calculated as ID= $s^2/\ \bar{x}$, where s^2 and $\bar{x}$ are the variance and sample mean, respectively. Significant departures of ID from the value of 1.0 were tested using a X^2 statistic with n-1 degrees of freedom. Green's index of clumping was calculated as GI = $[(s^2/\ \bar{x}) - 1/\ n\text{-}1]$, where n = sample size. GI varies between 0 (for random) and 1 (for maximum clumping).

To test for differences in nutritional quality among plants and between leaves at different canopy positions, we averaged toughness, water, nitrogen and tannin contents for each plant over the season and regressed these values with the abundance of the most common leaf miners and other herbivores, summed over the season. Differences in leaf quality and herbivore abundance between low and high canopy were tested using a One-Way ANOVA. All the variables analyzed were first submitted to Lilliefor's test for data normality and log transformations were employed to stabilize variances and normalize the data. However, for the sake of clarity, figure axes and means (+1SEM) show untransformed data.

To examine the community effects of herbivores on oaks, we first analysed data on mine survivorship among all the leaf categories as described in the methods. Single (n=457 leaves) and double mines (n= 377) were the most frequent combinations, followed by mines and damage by chewers (n=228), and mines and galls (n= 149). Differential survivorship among categories was tested using a X^2 and proportional survivorship among classes was tested using an ANOVA with individual plants as replicates. Because previous studies have shown non-random distribution of leaf miners and other herbivores on oaks, we also asked whether clumped distributions among individual plants differed among herbivores and among guilds, i.e., if a plant already heavily occupied by an herbivore in particular is avoided or preferred by other herbivores.

To test whether herbivores on oaks co-occur significantly more or less than expected at the plant scale we used a null models analysis. Here, observed patterns are randomly generated and a null model is then used to randomize the occurrence of species and to compare the patterns in these "artificial" communities with those in real communities (Ribas & Schoereder, 2002). Data on species distribution among the oak plants were transformed into presence/absence matrices, in which columns are individual plants and rows are herbivore species. Analyses were conducted separately for *Q. geminata* and *Q. laevis*. We used the C-score index (Stone & Roberts, 1990) as a metric to quantify the pattern of co-occurrence of leaf miners and gall-formers within a presence-absence matrix, as follows: $C = (r_i - S)(r_j - S)$, where r_i and r_j are the row totals, and S is the number of sites occupied by both species. The C-score measures the average number of "checkerboard units" (Gotelli & Entsminger, 2001) and is an index negatively correlated to species co-occurrence. The null hypothesis in this case is that the presence of a given herbivore species does not influence the occurrence of other species and if the index of co-occurrence falls within the 95% frequency distribution of the randomized matrices, the null hypothesis is accepted and the hypothesis of biological mechanisms conditioning the species co-occurrence is rejected (Ribas & Schoereder, 2002). To test these distributions against randomized matrices, we used a fixed-fixed model, with 5,000 iterations, in which the row and column sums of the original matrix are preserved. This algorithm was chosen for the fact that it has a low frequency of Type I and Type II errors (Gotelli & Ellison, 2002) and random matrices were created using a swapping algorithm, in which the original matrix is shuffled through repeated swapping of random submatrices. All analyses were conducted using EcoSim (Gotelli & Entsminger, 2001). Analyses were conducted only for leaf miners and gall-formers due to their sessile habit and for the fact that leaf chewers move freely among plants and should be less influenced by the clumped distribution of endophagous herbivores.

RESULTS

Testing the data against the null hypothesis of a Poisson (random) distribution indicated that all leaf miner species studied were not randomly distributed among plants (*Acrocercops*: $X^2=52.68$, df=11, $P>0.05$; *Brachys*: $X^2=43.86$, df=10, $P>0.05$; *Stilbosis*: $X^2=68.45$, df=13, $P>0.05$) and within branches on individual plants (*Acrocercops*: $X^2=711.72$, df=5, $P>0.05$; *Brachys*: $X^2=135.11$, df=5, $P>0.05$; *Stilbosis*: $X^2=744.3$, df=6, $P>0.05$). The distribution of all leaf miners, however, did fit a negative binomial distribution, suggesting clumped distributions both among plants (*Acrocercops*: $X^2=52.68$, df=11, $P>0.05$; *Brachys*: $X^2=43.86$, df=10, $P>0.05$; *Stilbosis*: $X^2=68.45$, df=13, $P>0.05$) and within branches on individual plants (*Acrocercops*: $X^2=14.88$, df=14, $P<0.05$; *Brachys*: $X^2=7.11$, df=9, $P<0.05$; *Stilbosis*: $X^2=9.37$, df=13, $P<0.05$). Indices of dispersion and Green's index corroborated the clumped distribution of leaf miners on both *Q. laevis* (*Acrocercops*: ID=8.03, $X^2=152.58$, $P>0.05$, GI=0.741; *Brachys*: ID=4.40, $X^2=83.62$, $P>0.05$, GI=0.853) and *Q. geminata* (*Stilbosis*: ID=5.19, $X^2=1008.3$, $P>0.05$, GI=0.727).

No significant differences in nutritional quality were observed between leaves from the lower- and upper-canopy for both plant species (all $P>0.05$), although *Q.*

geminata leaves tended to be softer in the lower canopy (average toughness lower-canopy leaves: 0.71± 0.023, average toughness high canopy leaves: 0.84± 0.072), and *Q. laevis* upper-canopy leaves tended to exhibit higher tannin concentration (average tannin concentration 0.286±0.091) than lower-canopy leaves (average tannin concentration 0.223±0.11). Although leaf miner abundance tended to be higher in lower-canopy leaves compared to upper-canopy leaves for both oak species, these differences were not statistically significant (all $P>0.05$).

For both oak species, we observed seasonal trends in plant quality, with a decrease in nitrogen concentration over the season, as well as an increase in toughness and tannin concentration (Figure 3.3). For *Q. geminata*, we observed higher *Stilbosis* density in plants with more nitrogen and softer leaves (Figure 3.4), whereas *Q. laevis* plants with higher nitrogen and lower tannin concentration supported significantly higher densities of the first generation of *Brachys* (Figure 3.5). None of the plant quality variables analyzed in this study affected the abundance of *Acrocercops* mines on *Q. laevis* (Figure 3.6), as well as eyespot galls, *Andricus* galls and chewed leaves on *Q. geminata*. For *Q. laevis*, however, a higher percentage of damage by chewers was observed in individual plants with softer leaves ($r^2=0.21$, $P=0.04$).

Survivorship of leaf miners was highest when mines were single on leaves, compared to double mines or mines occurring on leaves that were also chewed ($X^2=$ 14.69 to 19.55, all $P<0.05$). Lowest survivorship rates were observed for *Stilbosis* mines occurring on *Q. geminata* leaves with one or more eyespot galls ($F_{1,18}= 16.44$, $P=0.032$). For the community of herbivores on both oak species, null models indicated that both leaf miners and gall-formers co-occurred at the plant scale (Figure 3.7) and there was no evidence for competitive exclusion between and within guilds, as indicated by C-score indices falling within the 95% limits of frequency distribution of the randomized matrices (Table 3.1).

Table 3.1- C-score indices of the randomised and observed matrices for leaf miners on *Q. laevis* and *Q. geminata* and gall-formers on *Q. geminata*. Table shows the minimum and maximum values of the indices calculated for 5,000 randomised matrices pre data set, together with the observed index and P-values in two-tailed tests (Obs. Observed, Exp. Expected).

Guild	Randomized matrix		Obs. matrix	P-values	
	Minimum	Maximum		Obs.>Exp.	Obs.<Exp.
Leaf miners *Q. geminata*	11.1	14.7	12.70	0.107	0.921
Gall formers *Q. geminata*	7.59	10.16	7.50	0.987	0.631
Leaf miners *Q. laevis*	10.46	12.86	11.01	0.759	0.314

Figure 3.1 – Examples of some of the herbivores on *Quercus geminata* (from left to right): *Hemileuca maia* (Lepidoptera: Saturniidae), Leaf rollers on tips of leaves, old *Disholcaspis* (Hymenoptera: Cynipidae) galls on stems, new *Disholcaspis* galls on stems associated with ants, *Andricus quercusfoliatus* (Hymenoptera: Cynipidae) galls on stems, *Stilbosis quadripustulatus* (Lepidoptera: Cosmopterygidae) mines on expanded leaves, *Phigalia* sp. (Lepidoptera: Geometridae), banded tussock moth *Halysidota tesselaris* (Lepidoptera: Arctiidae), and eyespot galls on leaves.

Figure 3.2 – Examples of some of the herbivores on *Quercus laevis* (from left to right): unidentified Geometridae (Lepidoptera), Leaf rollers on new leaves, unidentified stem gall, *Homoeolabus analis* (Coleoptera: Attelabidae), *Brachys tesselatus* (Coleoptera: Buprestidae) mines, *Acrocercops albinatella* (Lepidoptera: Gracillaridae) mines, adult *Brachys* on leaves, *Tischeria* sp. mine (Lepidoptera: Tischeriidae), and whitemarked tussock moth *Orgyia leucostigma* (Lepidoptera: Lymantriidae).

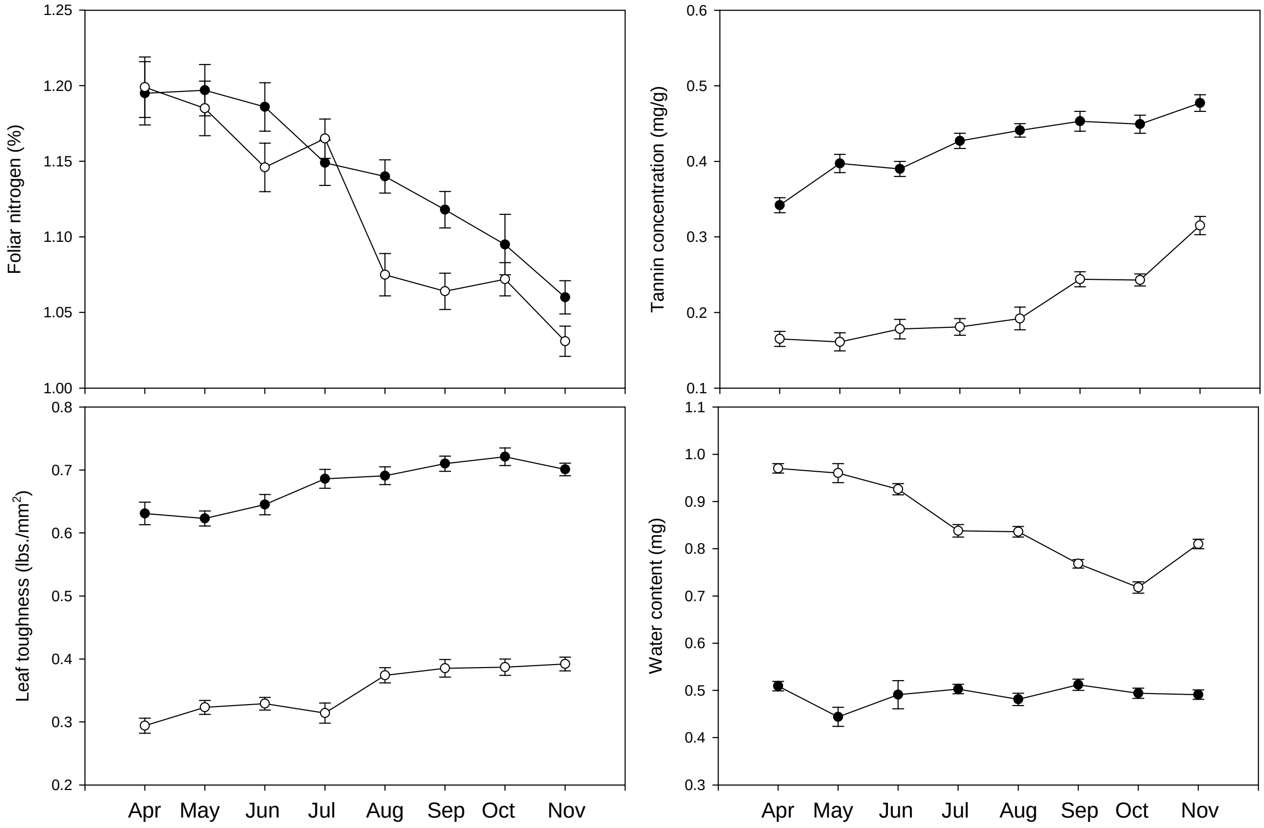

Figure 3.3- Temporal variation on the concentration of foliar nitrogen, tannins, leaf toughness and leaf water content for *Q. geminata* (solid circles) and *Q. laevis* (open circles). Data are means (+1SE) of 10 undamaged leaves per individual tree, with lower- and upper-canopy leaves combined.

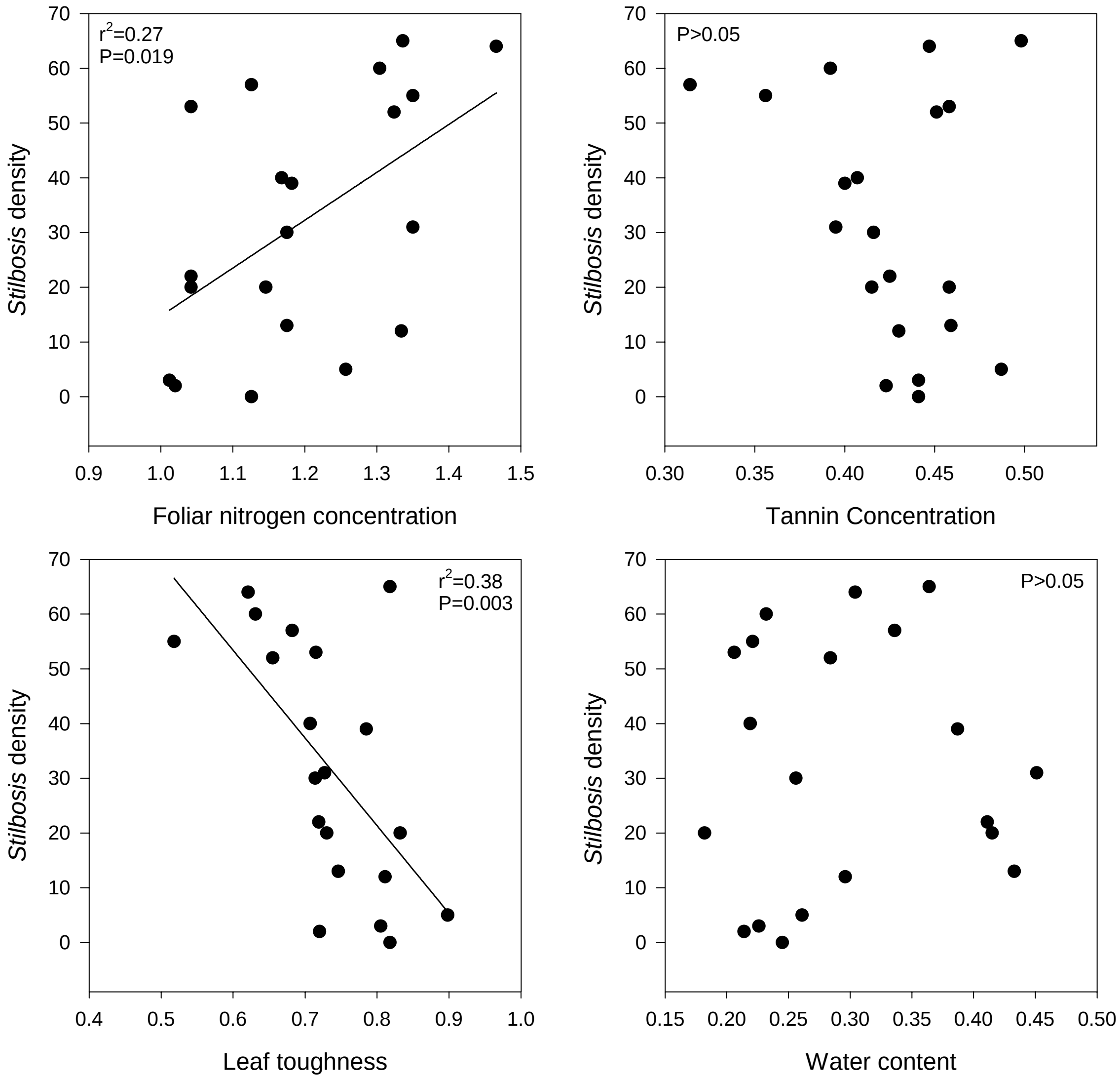

Figure 3.4- Relationship between the abundance of *Stilbosis* mines and variation in *Q. geminata* nutritional quality. Data on *Stilbosis* abundance are summed over the season for each individual plant and data on plant nutritional quality are averages over the season.

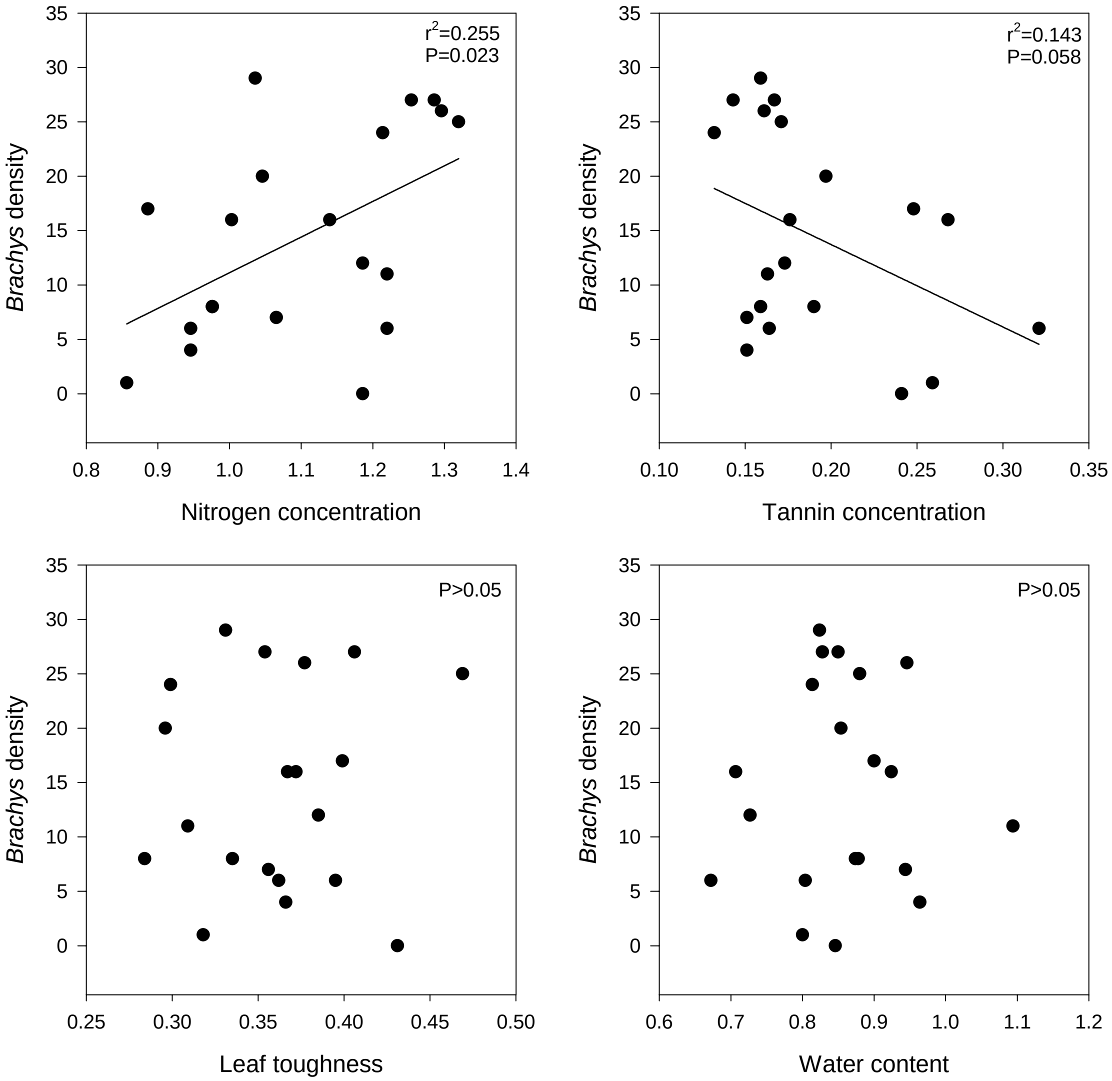

Figure 3.5- Relationship between the abundance of *Brachys* mines and variation in *Q. laevis* nutritional quality. Data on *Brachys* abundance are summed over the season for each individual plant and data on plant nutritional quality are averages over the season.

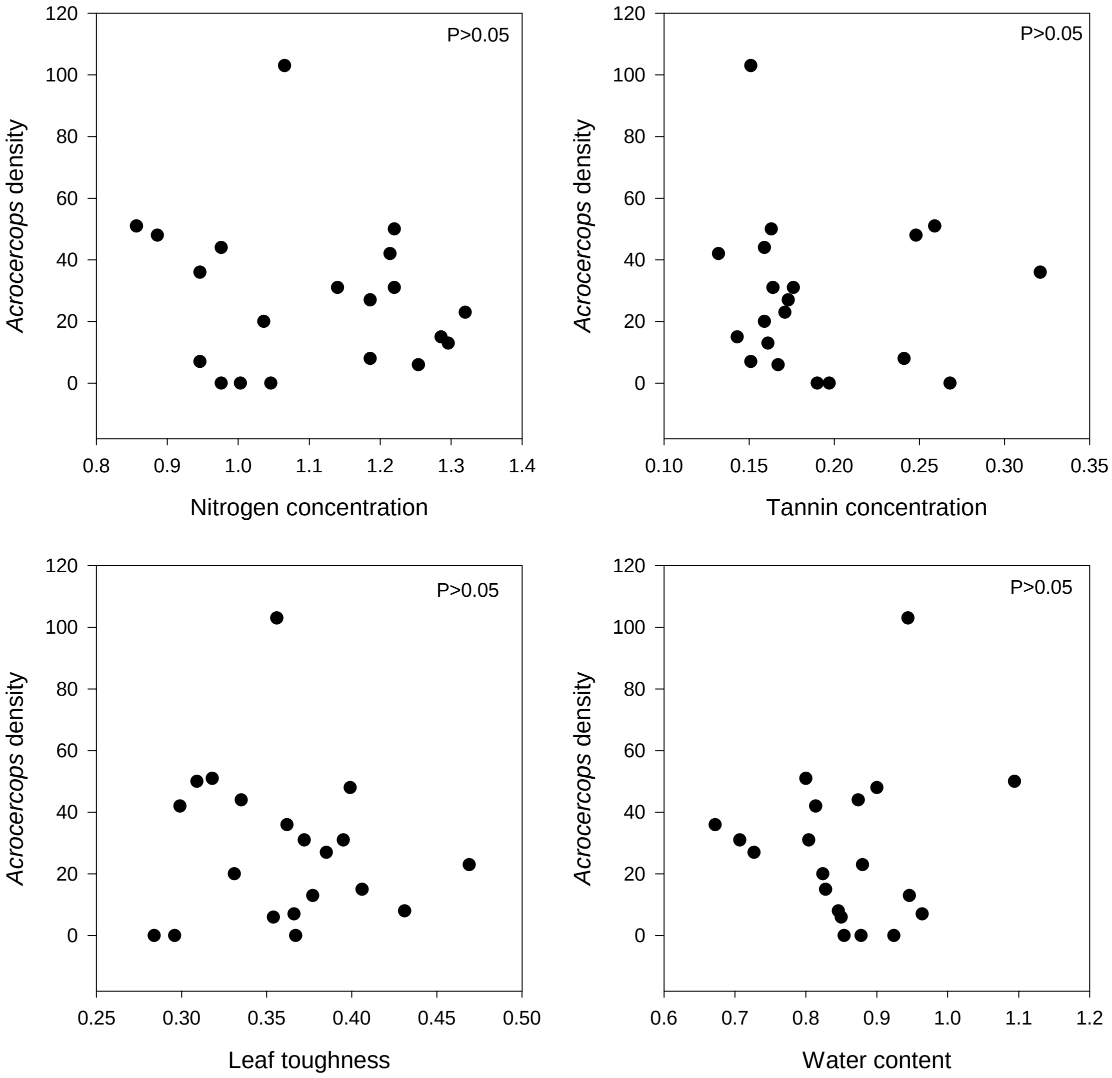

Figure 3.6- Relationship between the abundance of *Acrocercops* mines and variation in *Q. laevis* nutritional quality. Data on *Acrocercops* abundance are summed over the season for each individual plant and data on plant nutritional quality are averages over the season.

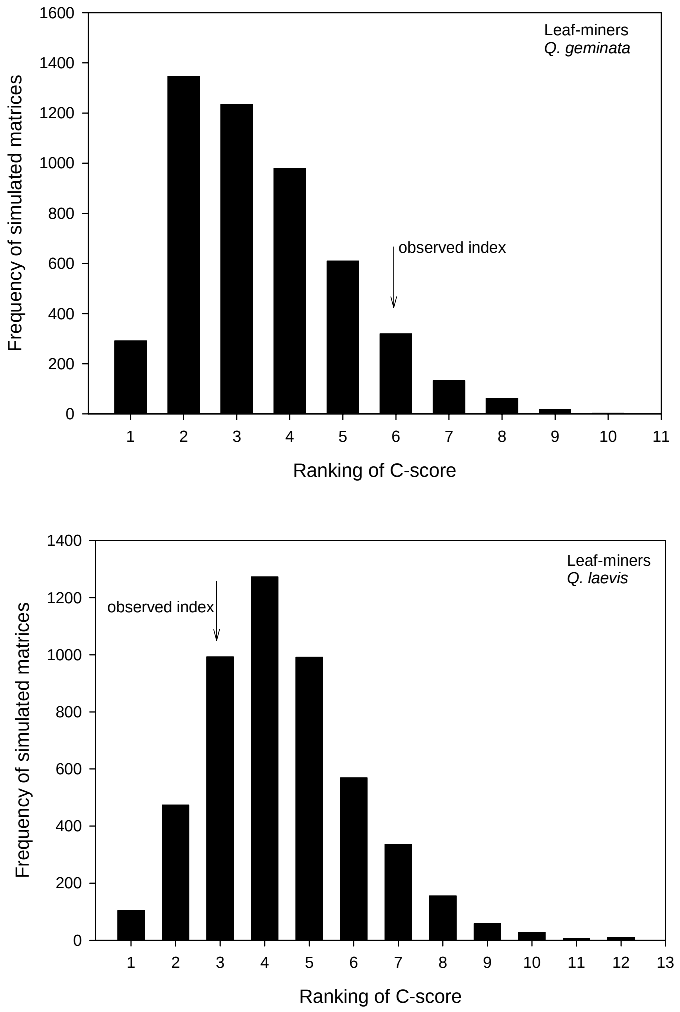

Figure 3.7- Co-occurrence patterns of leaf miners and gall-formers at the plant scale. The histograms give the frequencies of simulated C-scores using a fixed-fixed model. Ranking of C-scores are frequency classes of randomized matrices represented by numbers to facilitate scaling. Refer to Table 1 for minimum and maximum values on each class. Arrows represent the observed C-score indices for each data set. Indices falling within the 95% limits of frequency distribution of randomized matrices indicate co-occurrence, whereas higher C-scores represent smaller co-occurrence than expected by chance and lower C-scores indicate higher co-occurrence than expected by chance.

DISCUSSION

Our data showed that leaf miners on both oak species were not randomly distributed, but clumped among host plants and within branches on individual plants. Several factors could potentially select for non-random distribution of leaf miners. Bottom-up factors such as foliar nitrogen concentration and secondary chemistry have been frequently invoked as potential factors affecting the distribution, abundance and survivorship of phytophagous insects and leaf miners in particular (e.g., Stiling *et al.*, 1982; Faeth, 1991; Eber, 2004). Our data corroborates the hypothesis that leaf miners are strongly affected by bottom-up factors, as plants with higher nitrogen concentration, lower leaf toughness and lower tannin concentration exhibited significantly more mines than plants with lower nutritional quality. Variation in nitrogen levels, especially, both among and within plants has been demonstrated to affect leaf miners choice for oviposition sites and larval development (Mattson, 1980; Scheirs, DeBruyn, & Verhagen, 2001, 2002). Faeth (1990), on the other hand, observed that larvae of the leaf miner *Cameraria* sp. on *Quercus emoryi* were highly clumped at various spatial scales among and within trees, but his study did not support the hypothesis that leaf miners cluster because of variation in plant nutritional quality. Although our study showed that leaf miners respond to bottom-up factors, responses to plant quality varied among the leaf miners studied. *Stilbosis* mines were mainly affected by foliar nitrogen concentration and leaf toughness, whereas *Brachys* abundance was influenced by nitrogen and tannin concentration, and none of the plant quality variables affected the abundance of *Acrocercops*. These differential responses might be explained by differences in life-history traits of these leaf miners. *Acrocercops albinatella* causes relatively small linear-blotch superficial mines just under the leaf epidermis of turkey oak leaves and development times do not exceed 10 days. These mines are unlikely to be strongly affected by variations in plant quality due to their fast development rates and the fact that they create limited depth mines in young leaves with higher nitrogen content and lower concentrations of defensive chemicals. *Brachys* and *Stilbosis* mines, on the other hand, are more likely to be affected by host quality, having longer developmental times and full depth mines.

Another factor operating at the leaf scale that potentially selects for non-random distribution of leaf miners is resource or interference competition. Insects that are relatively immobile seem particularly susceptible to competitive influences because they cannot easily escape from neighboring individuals (Stiling *et al*., 1987) and results of competitive interactions should then be manifested in their distribution patterns. In a previous study of the distribution of *Stilbosis* mines on *Q. geminata* and *Q. nigra*, Stiling et al. (1987) have shown that fewer mines were found together on the same side of the leaf mid-vein than expected by chance. Intra-specific competition among leaf miners has mostly been neglected as a regulatory mechanism on their population dynamics (Eber, 2004), even though leaf miners are restricted to small "resource islands" represented by individual leaves (Janzen, 1968). Our results have shown that mines usually occurred single on leaves, but lower survivorship was observed on leaves with double or triple mines. In these categories, 34% of the leaf mines dissected exhibited dead and dry larvae inside the mine. Similar results have been found for other systems (e.g., Bultman & Faeth, 1986; Auerbach & Simberloff, 1989) and interference competition or indirect exploitative competition with conspecifics has been suggested as a dominant source of mortality for many leaf miner species (reviewed by Auerbach, Connor, & Mopper, 1995). We also observed that leaf miners developing on leaves that were also damaged by chewers experienced lower survivorship than mines developing on intact leaves. Previous or concurrent feeding by other phytophages may alter physical and chemical aspects of the leaf or reduce leaf size so that insufficient area remains for development, especially for sessile herbivores such as leaf miners and gall-formers. Faeth (1985), for example, observed that *Stilbosis juvantis* mines developing on leaves that were artificially an/or naturally damaged by chewer herbivores also experienced significantly lower survivorship than did miners on intact leaves due to increased parasitism levels. The higher attack rate of parasitoids on mines that were developing on damaged leaves was probably attributable to physical and/or chemical alterations caused by chewing herbivores. The exact mechanism by which damage and intensified parasitism levels interact is unclear, although physical, visual, and chemical cues associated with damaged leaves may influence parasitoid searching behavior and oviposition preferences (Faeth, 1985).

Variation and changes in resource quality can lead to different patterns of distribution of insect damage, and herbivores might become positively or negatively associated, both intra- and inter-specifically (Fisher, Hartley & Young, 1999). For the silver birch *Betula pendula*, several studies have demonstrated a negative association between generalist herbivores and the leaf miner *Eriocrania*, possibly due to direct interference and resource removal (e.g., Bylund & Tenow, 1994; Valladares & Hartley, 1994). In our systems, although intraspecific competition was an important source of mortality for the leaf miners studied, we did not find evidence suggesting that interspecific competition structured distributional patterns on individual host trees. Although leaf miners were clumped among host trees, and at the leaf scale competition might reduce leaf mine survivorship, at the plant scale both leaf miners and gall-formers co-occur. These results might be explained by the fact that although leaf miners were clumped among plants, they occurred in low densities in our field sites (mean ± 1SE per 200 leaves in 35 plants: *Acrocercops*: 36.7 ± 3.49; *Brachys* 1st generation: 21.1 ± 2.03; *Brachys* 2nd generation: 8.55 ± 1.91; *Stilbosis*: 35.64 ± 3.25) and patterns of inter-specific repulsion might be detected only during outbreak seasons.

In conclusion, our results indicated that leaf miners show non-random patterns of distribution both among and within plants and these differential distribution might be determined by variation in several aspects of plant quality. Intra-specific competition is suggested as a regulatory mechanism in the population dynamics of the species studied at the leaf scale, although at the plant scale inter-specific associations do not seem to be important mechanisms determining the community structure of these oak herbivores.

Chapter 4

RESPONSES OF DIFFERENT HERBIVORE GUILDS TO NUTRIENT ADDITION AND NATURAL ENEMY EXCLUSION

SYNOPSIS

We experimentally investigated the effects of plant quality and natural enemies on the abundance of different herbivore guilds on oak trees. Two oak species (*Quercus laevis* and *Q. geminata*) and four guilds of leaf herbivores (leaf miners, gall-formers, leaf-rollers and chewers) were studied using a factorial design that manipulated predation/parasitism pressure and plant nutritional quality. Forty plants of each species were divided into 4 treatments: 1) control plants (nutrients and natural enemies unaltered); 2) nutrients added, natural enemies unaltered; 3) nutrients unaltered, natural enemies removed; and 4) nutrients added and natural enemies excluded. Fertilized plants exhibited significantly higher foliar nitrogen for both oak species and tannins tended to increase over time and decrease with fertilization, but only for *Q. geminata* this trend was significant. Fertilized plants supported significantly higher densities of all herbivore guilds than unfertilized plants, but exclusion of natural enemies did not significantly affect herbivore abundance for any guild studied. Our results demonstrated that all herbivores on oaks, regardless of guild type, respond more strongly to bottom-up effects such as host plant quality, and less to top-down effects caused by natural enemies.

INTRODUCTION

A central question in community ecology is the degree to which populations are limited by both top-down and bottom-up forces (Hunter, 2001). Previous studies have suggested that, for herbivores, top-down and bottom-up forces commonly interact to influence herbivore populations, but bottom-up forces set the stage on which top down forces act, in a way that enemy pressure will vary with plant growth and quality (e.g., Hunter & Price, 1992; Price, 2002). Although individual studies investigating the importance of top-down or bottom-up factors on insect herbivores have been extensively performed, relatively few studies have attempted simultaneous manipulations of plant quality and natural enemies pressure in terrestrial plant systems. Some previous studies recognize that herbivore identity and life history could be an important variable in determining the relative strength of top-down and bottom-up forces (e.g., Forkner & Hunter, 2000; Moon & Stiling, 2002; Denno *et al.*, 2002). For ectophagous lepidopterans in upland forest communities, for example, it has been demonstrated that bottom-up forces dominate oak-herbivore-natural enemy interactions and top-down forces such as predation by birds on chewers and several herbivore guilds are relatively weak (Forkner & Hunter, 2000). In salt marsh communities, however, both plant quality and natural enemies can significantly impact the abundance of gall-makers and sap-suckers of *Borrichia frutescens* (Stiling & Moon, 2005) and vegetation complexity mediate the impact of natural enemies of plant hoppers in *Spartina alterniflora* (Denno *et al*., 2002). However, Gruner (2004) suggests that, in general, few terrestrial studies have attempted to partition the relative impacts of top-down and bottom-up forces among trophic levels or feeding guilds.

The aim of this research was to investigate the combined effects of plant quality and natural enemies on the abundance of different guilds of herbivores utilizing the same host plants by using a factorial manipulation of predation/parasitism pressure and plant nutritional quality. Invertebrate predation and attack by parasitoids were chosen as focal top-down effects in our system and manipulation of plant nutritional quality by fertilization was chosen as the bottom-up effect based on previous studies of the importance of nitrogen content and plant secondary chemistry to insect herbivores (Strong *et al.*, 1984). To our knowledge, this is the first study simultaneously manipulating top-down and bottom-up forces for several oak herbivore guilds with within-guild variation in species life history patterns. For leaf miners, previous studies have stressed the importance of plant quality on abundance and survivorship (Faeth & Simberloff, 1989; Connor, 1991), but have also shown how indirect effects of plant quality can affect natural enemy performance (Bultman & Faeth, 1986) and we expected strong bottom-up effects for this oak guild. For gall-makers, the nutrition hypothesis (Stone & Schönrogge, 2003) states that gall-formers should be less influenced by the nutritional status of the host plant, as they have the ability to manipulate galled tissues to make them more nourishing and less well defended than non-galled tissues on the same plant and we expected top-down effects to be strong. For chewers, experimental studies (e.g., Strauss, 1987) have demonstrated heterogeneous responses to increased plant quality caused by fertilization depending on the herbivore species, but based on Forkner and Hunter's (2000) and Gruner's (2004) recent studies, we expected bottom-up effects to be strong.

Specifically, we aimed to investigate the following questions: 1) What are the impacts of plant quality and natural enemy pressure on *Quercus* herbivore densities?; 2) How do plant quality and natural enemies interact to affect the abundance of different guilds?; 3) Does the strength of top-down forces change with plant quality, i.e., are parasitism rates higher/lower in fertilized plants?, and 4) Does the strength of top-down forces change with herbivore guild and identity?

STUDY SYSTEMS

Sand live oak, *Quercus geminata* (Fagaceae), is a semi-evergreen oak which, typically, supports many different herbivore species. *Stilbosis quadripustulatus* (Lepidoptera: Cosmopterygidae) is a univoltine leafminer whose larvae induce mines on the adaxial leaf surfaces and leaves are also frequently chewed by the eastern buck moth *Hemileuca maia* (Lepidoptera: Saturniidae). At least 4 cynipid galling insects (Hymenoptera: Cynipidae) are commonly observed on sand live oak leaves and stems: *Andricus quercusfoliatus*, *Disholcaspis quercussuccinipes*, *Callirrhytis quercusbatatoides*, and *Belonocnema quercusvirens. Andricus quercusfoliatus* induces white flower-like galls on sand live oak stems and it the most common stem gall in our study site. Eyespot galls (Diptera: Cecidomyiidae) are also very abundant and are recognized as circular spots on the leaves, usually 8-10 mm in diameter. This is the most common foliar gall found on sand live oaks, often reaching densities of 5 galls per leaf.

Turkey oak, *Quercus laevis*, has deciduous simple leaves, alternately arranged with usually 5 lobes, although this number may vary from 3 to 7. *Acrocercops albinatella*

(Lepidoptera: Gracillaridae) is a microlepidopteran species whose larval stages feed on young leaves, creating distinct linear-blotch mines on the lower leaf surface, with larvae completing their development in approximately 10 days. *Brachys tesselatus* (Coleoptera: Buprestidae) is a leaf miner species that also forms distinct blotch mines in *Q. laevis* leaves and goes through two generations in our study sites. Turkey oak leaves are also attacked by an array of other herbivores, such as the leaf rolling weevil *Homoeolabus analis* (Coleoptera: Atellabidae), the eastern buck moth *H. maia*, the white tussock moth *Orgyia leucostigma* (Lepidotpera: Lymantriidae) and other leaf miners such as *Stigmella* spp. (Lepidoptera: Nepticulidae), *Bucculatrix* spp. (Lepidoptera: Bucculatricidae) and *Cameraria* spp. (Lepidoptera: Gracillariidae).

Generalist predatory ants (Hymenoptera: Formicidae) and the green lynx spider *Peucetia viridans* (Araneae: Oxyopidae) are among the most common arthropod predators on both oak species in our study sites. The leaf miners studied are also attacked by several parasitoid species, including *Zagrammosoma multilineatum* (Hymenoptera: Eulophidae) and *Chrysonotomyia* sp. (Hymenoptera: Eulophidae).

METHODS

Data Collection

This study was conducted between February and November of 2003 on natural stands of scrub oak vegetation in Tampa, Florida. Forty *Quercus laevis* trees ranging between 2.0 and 2.5 meters in height were marked at the University of South Florida ECOAREA and forty *Q. geminata* trees ranging between 1.7 to 2.0 meters in height were marked at the USF Botanical Garden. We investigated effects of plant quality and pressure exerted by natural enemies on the abundance of three different leaf miners (*Acrocercops albinatella*, *Brachys tesselatus*, *Stilbosis quadripustulatus*), on the abundance of one leaf roller on *Q. geminata*, and on the abundance of chewing herbivores and gall-formers on both *Q. laevis* and *Q. geminata*. Using a 2 x 2 factorial design, the forty trees of each plant species were randomly divided into 4 treatments: 1) –F,+P: control plants with nutrients and natural enemies unaltered ; 2) +F,+P: nutrients added, natural enemies present (unaltered); 3) –F,-P: nutrients unaltered, natural enemies excluded, and 4) +F,-P: nutrients added and natural enemies excluded. This design allowed 10 replicates per treatment combination per plant species. Plant quality was manipulated by adding, bi-weekly from April to June, 150 g of 46:0:0 NPK fertilizer to assigned *Q. laevis* trees and 100 g of the same fertilizer to assigned *Q. geminata* trees. A previous study with the same systems indicated that predation by spiders was a negligible mortality factor for the leaf miners studied (Cornelissen & Stiling, 2005), and only mortality caused by predatory ants and parasitism caused by microhymenopterans were manipulated as the top-down factor in our systems. Ants were removed by placing masking tape covered with Tanglefoot® around the base and twigs of assigned oak trees at the beginning of the experiments and by handpicking ants already present on the trees. Parasitoid removal was performed by adding between 12 and 15 yellow 13X8 cm Sticky Strip traps (Gempler’s, Belleville, Wisconsin, USA) per assigned tree. These traps are plastic cards coated with Tanglefoot adhesive and hymenopteran parasitoids are attracted to the yellow color and get stuck in the adhesive, effectively reducing parasitism levels (Moon & Stiling, 2002). Previous studies conducted in salt marsh and hammock communities have shown the efficiency of yellow sticky traps to reduce parasitoid abundance and parasitism rates of gall-formers and leaf-miners (e.g., Moon & Stiling, 2002; 2004). Traps were placed all around the tree canopy using binder clips and replaced bi-weekly for *Q. laevis* and every three weeks for *Q. geminata*. Old traps were individually wrapped in plastic and refrigerated for further analyses under a microscope, where the densities of all parasitic microhymenopteran and other invertebrate species were determined by counting the number of microhymenopterans and other invertebrates caught on the traps on three 4 X 4 cm quadrats on 2 traps per plant, at the beginning and at the end of the season (n= 240 samples per treatment per plant species).

To assess variation in host plant quality among treatments, tannin concentration and foliar nitrogen concentration were evaluated monthly for each plant by haphazardly sampling 8 undamaged leaves from each tree, all around the canopy. Leaves were oven-dried and milled to a fine powder and tannins were extracted from 50 mg of dry tissue. Tannin concentration was analyzed as foliar protein binding capacity using the radial diffusion assay with three replicates per leaf (for details see Hagerman, 1987) and tannic acid was used as a standard. Nitrogen content was determined using a CE Instruments NC2100 CN Analyzer (CE Elantech, Incorporated, Lakewood, New Jersey, USA). Variation in herbivore densities among treatments were quantified monthly on each plant by haphazardly counting the number of mines, chewed leaves, gall-formers, and other herbivores on 200 leaves of *Q. laevis* and 300 leaves of *Q. geminata* on each sampling date. All leaf miners including *Acrocercops, Brachys, Stilbosis, Cameraria, Stigmella,* and *Bucculatrix* were identified and counted. *Andricus* galls and leaf rollers on *Q. geminata* were counted at the beginning and end of the season. To test for effects of treatments on herbivore mortality, we assessed survivorship of selected leaf miner species using 5 mines of each of the 3 most common leaf miner species, namely *Acrocercops, Brachys*, and *Stilbosis*. Mines were permanently marked (n=50 mines per species per treatment combination) on each individual plant using a Sharpie® pen as soon as the eggs hatched and larvae initiated mine formation. After mine termination, all leaves were inspected under a stereomicroscope for assessment of leaf miner survivorship and identification of mortality factors. Parasitized mines have tiny circular exit holes left by the parasitoid on mine's surface and/or pupae within mine, and preyed upon mines are usually found ripped open and the larva is absent. Successfully emerged larvae of *Acrocercops* cut open the mines and pupate usually on the same leaf where mine developed, *Brachys* larvae pupate inside mines and/or cut circular holes on the underside of the leaf and *Stilbosis* mines cut semi-circular exit holes on the mine underside towards the apex (Simberloff & Stiling, 1987; Stiling *et al.*, 1999).

Data Analysis

All the variables analyzed were first submitted to Lilliefor's test for data normality and Levene's test for homogeneity of variances. Transformations (angular, log-transformation and arcsine square rooted) were employed when necessary to stabilize variances and normalize the data. To test for differences in plant nutritional quality among treatments two-way repeated measures Analysis of Variance (ANOVA) was used to assess effects of treatment on plant quality. Between-subject factors were fertilizer addition (yes, no), natural enemies exclusion (yes, no) and their interaction, and time (6 sampling dates) was the within-subject factor. The sphericity assumption of repeated-measure designs is less likely to hold for experiments with more than two-treatments and univariate F-tests were adjusted using the Greenhouse-Geisser epsilon (ε)(Quinn & Keough, 2002). To test for differences in the abundance of leaf miners and other herbivores among treatments we performed two-way multivariate analysis of variance (MANOVA) based on the mean density of each herbivore over the season, for each oak species separately. For both MANOVAs, F-tests were based on values using Wilk's lambda, provided by the GLM procedure on SPSS 12.0.2 (SPSS, 2003). Significant differences in herbivore abundance among treatments detected with MANOVAs were further analyzed using two-way repeated-measures ANOVA for each herbivore species, with F-tests based on type III sum of squares. Differences in leaf miner survivorship among treatments were tested using two-way ANOVAs.

Because we detected strong bottom-up effects for most herbivores studied (see results), we examined the magnitude of fertilization effects on the abundance of different herbivore species and different herbivore guilds, by calculating effect sizes using the log of the response ratio (Hedges *et al.*, 1999). The response ratio is the ratio of some measure of outcome in the experimental group to that of the control group (Rosemberg *et al.*, 2000), and it has the advantage of estimating the effect as a proportionate change resulting from experimental manipulation. We obtained least square means and standard deviations by averaging monthly counts for each herbivore species and used the 10 plants per treatment as replicates to calculate effect sizes. We contrasted herbivore abundances under the treatment +F,-P vs. herbivore abundance under the treatment –F,-P, which gives effect size on herbivore densities of bottom-up effects in the absence of natural enemies. We calculated the natural log of the response ratio as $lr=\ln(\bar{X}_{+F-P}/\bar{X}_{-F-P})$, where X_{+F-P} is the mean herbivore abundance on plants that were fertilized and traps were added and X_{-F-P} is the mean herbivore abundance on plants that were trapped only. Negative proportional changes indicate a decrease in herbivore abundance compared to trapped plants and positive values indicate an increase in abundance due to fertilization. We also contrasted herbivore abundances under the treatment –F,+P vs. –F,-P, which gives the difference in herbivore densities owing to predation in the absence of a bottom-up effect. However, because we did not detect significant effects of natural enemy exclusion on herbivore abundance on both oak species (see results), these analyses are not shown. To estimate the cumulative effect size (E_{++}) for the 10 replicates per treatment combination, effect sizes were combined using a weighted average (Rosemberg *et al.*, 2000) and we used a mixed-model effect to examine the effects of herbivore guild and dietary breath in shaping responses to bottom-up effects in the absence of natural enemies. Herbivores were grouped into guilds (leaf-miners, gall-formers, leaf rollers, and chewers) and further classified into specialists and generalist based on the literature. *Acrocercops*, *Brachys* and *Stilbosis* mines, as well as *Andricus* galls were considered as specialists, whereas leaf rollers, chewers and eyespot galls were considered generalists. It is important to point out, however, that we are using the percentage of chewed leaves as a surrogate for the abundance of chewers on both oak species. We calculated the total heterogeneity (Q_T) as well as heterogeneity within (Q_W) and between groups (Q_B) and the significance of these statistics was evaluated using a Chi-square distribution. Effect sizes were considered significant if bootstrapped (3,000 iterations) 95% confidence intervals did not overlap zero.

RESULTS

Treatment Effectiveness

Application of Tanglefoot® on assigned oak trees proved effective in keeping ants away from treated trees compared to control trees at the beginning (control trees: 10.3 ± 1.36 ants, treated trees: 0.71 ± 0.12 ants, $F_{1,28} = 87.1$, $P<0.001$) and end of the season (control trees: 18.90 ± 0.84 ants, treated trees: 1.13 ± 0.33 ants, $F_{1,28} = 91.3$, $P<0.001$). Sticky traps also proved efficient in capturing microhymenopterans and other small invertebrates. Although individual parasitoid identification was not possible, we observed that microhymenopterans were much more abundant on the traps than other insects such as ants, ladybugs, and flies (microhymenopterans: 32.3 ± 1.56, other invertebrates: 11.24 ± 0.19) and no significant differences were observed in the abundance of parasitoids caught on traps from plants treated with traps only (29.66 ± 0.75 parasitoids per trap) and plants that were fertilized and trapped (29.47 ± 1.05, $F_{1,38}=0.22$, $P=0.74$). However, the number of parasitoids caught per trap significantly increased from the beginning (mean parasitoid number 29.47±0.73) to the end (mean parasitoid number 31.61± 0.65) of the season (paired t-test, $t=-2.96$, $P=0.033$).

Treatment effects on host plant quality

Fertilization tended to decrease tannin concentration for both species, but only for *Q. geminata* was this decrease significant ($F_{3,36} = 3.07$, $P<0.05$, Figure 4.1). For both plant species, we observed a significant effect of time on tannin concentration (*Q. geminata* $F_{5,180}= 108.75$, $P = 0.0001$; *Q. laevis* $F_{5,180}= 40.49$, $P = 0.0001$), indicating a significant increase in tannin concentration from the beginning to the end of the season, but no time x treatment interaction was observed for either tree species (*Q. geminata*: P=0.106 and *Q. laevis*: P = 0.370). Fertilization also significantly increased the amount of foliar nitrogen for *Q. geminata* by approximately 18.5% compared to control plants and by 10.1% for fertilized *Q. laevis* plants (*Q. geminata* $F_{3.36}$=46.27, P =0.0001, *Q. laevis* $F_{3.36}$=3.05, P =0.041), but foliar nitrogen concentration significantly decreased over the season for both plant species (significant time effects, P = 0.0001 for both plant species, Figure 3.1). For *Q. laevis* there was also a significant time x treatment interaction ($F_{4,12}$=6.08, P<0.001), indicating that changes in nitrogen concentration over the season varied among treatments.

For both oak species, natural enemy removal did not affect host plant quality (natural enemies effect, all P>0.05) and no significant interactions between fertilization and natural enemy exclusion were observed for the host plant parameters investigated.

Treatment effects on the abundance of leaf miners and other herbivores

Fertilization significantly increased the abundance of herbivores on *Q. geminata* trees (Table 4.1), although different species were impacted at different magnitudes. *Stilbosis* mines, for example, increased by approximately 5-fold on fertilized plants and by 4-fold on fertilized and trapped plants compared to control plants, whereas other mines such as *Cameraria* and *Stigmella* increased by approximately 2-fold on fertilized compared to control plants (Figure 4.2). Leaf rollers were the only herbivores not significantly affected by fertilization and chewers were the only herbivores significantly affected by top-down effects (Table 4.2). For all herbivores studied on *Q. geminata*, we observed a significant time effect (all P = 0.0001) indicating seasonal changes in herbivore density. For *Stilbosis* and other mines, we also observed a significant time x bottom-up effects, indicating that the effects of fertilizer changed over the season.

For herbivores on *Q. laevis*, we also observed significant bottom-up effects on herbivore densities (Table 4.1), although univariate analyses indicated that only leaf miners increased in density with the addition of fertilizer (Table 4.2). *Brachys* mines, for example, approximately doubled in density on fertilized or fertilized and trapped plants compared to control plants (Figure 4.3), and even stronger impacts of fertilization were observed for the second generation of *Brachys* mines on turkey oaks. *Acrocercops* mines increased by approximately 40% on fertilized compared to non-fertilized plants, but the abundance of eyespot galls, chewers and other leaf miners were not affected by the addition of fertilizer (all P >0.05). None of the herbivores on turkey oaks were affected by the removal of natural enemies or the combined effects of bottom-up and top-down manipulations (Table 4.2, all P >0.05), but we observed a significant time effect for all herbivores studied, indicating seasonal changes in herbivore densities from April to September.

Treatment effects on leaf miner survivorship

Neither bottom-up nor top-down manipulations significantly impacted mine survivorship, as no significant differences in the proportion of mines that survived and/or were killed by natural enemies (predators and parasitoids combined) were observed among treatments (Two-Way ANOVAs, all P>0.05). Survivorship rates of the leaf miners studied were relatively high (range 46.3 to 76.0%) and mortality caused by natural enemies was relatively low (range 3.5 to 24.0%), regardless of experimental manipulation. Parasitism rates for *Brachys* mines, for example, developing on control plants averaged 9.2 ± 0.02%, whereas parasitism rates on mines developing on trapped plants averaged 7.8±0.06%. For *Acrocercops* mines, predation rates by ants on mines developing in control plants averaged 30.1± 0.08%, whereas predation by ants on mines developing on trapped plants averaged 24.6±0.09%.

Strength of bottom-up forces

Quantitative synthesis of our results using the log of the response ratio revealed strong and positive responses of herbivore density to plant fertilization, although different responses were observed both between and within guilds. Among leaf miners, stronger bottom-up effects were observed for *Stilbosis* mines (E++ = 1.32, bootstrapped CI= 0.837 to 1.83, Figure 4.4A), followed by mines induced by the second generation of *Brachys*. Grouping herbivores into guilds revealed that bottom-up manipulations caused changes in density that significantly differed among guilds (Q_B=27.97, P = 0.0001) and increased abundance caused by fertilization was stronger for leaf miners (E++=0.780, bootstrapped CI = 0.406 to 0.946) compared to other herbivore guilds. Leaf rollers were not significantly affected by fertilization and weakest bottom-up effects were observed for chewers (Figure 4.4B). Specialists were significantly more strongly influenced by bottom-up effects than generalists (Q_B=34.09, P = 0.0001), as specialists were 95.8% more abundant on plants that were fertilized and trapped (E++=0.9582, bootstrapped CI = 0.661 to 1.25, n=45), whereas generalists increased in abundance by only 28.7% on the same experimental plants (E++ = 0.2877, bootstrapped CI = 0.122 to 0.47, n=63).

Table 4.1- Results from multivariate analyses of variance for mean herbivore density on *Q. geminata* and *Q. laevis*. F-tests were based on Wilk's lambda and bottom-up effects refer to fertilization and top-down effects refer to natural enemy removal. Bottom-up x Top-down refers to the combined effects of fertilization and natural enemy exclusion.

Plant species	Source	df	Wilk's λ	F	P
Q. geminata	Bottom-up	6,31	0.266	14.25	<0.001
	Top-down	6,31	0.768	1.56	0.191
	Bottom-up x Top-down	6,31	0.692	2.29	0.06
Q. laevis	Bottom-up	5,32	0.327	13.19	<0.001
	Top-down	5,32	0.981	0.123	0.986
	Bottom-up x Top-down	5,32	0.867	0.982	0.444

Table 4.2- Effects of treatments (bottom-up and top-down manipulations) on herbivore abundance on *Q. geminata* and *Q. laevis*. Bottom-up effect refers to fertilizer addition, top-down effect refers to natural enemy removal and top-down x bottom-up effect refers to combined effects of fertilization and natural enemy exclusion. Time effect refers to subsequential samplings (*Andricus* galls and leaf rollers on *Q. geminata* were counted only at the beginning and end of season and analyzed using a Two-Way Anova, with no time effect). Table shows ε (Greenhouse-Geisser Epsilon) corrected P values for within-subject factors and their interactions.

Herbivore sp	Bottom-up effect		Top-down effect		Bottom-up x Top-down effect		Time		Time x bottom-up effects		Time x top-down effects	
Q. geminata	$F_{1,36}$	P	$F_{1,36}$	P	$F_{1,36}$	P	$F_{5,180}$	P	$F_{5,180}$	P	$F_{5,180}$	P
Stilbosis	73.63	0.001	0.65	0.425	2.01	0.165	113.93	0.001	37.13	0.0001	0.54	0.599
Leaf rollers	0.77	0.384	1.08	0.304	2.32	0.137	-	-	-	-	-	-
Eyespot galls	17.51	0.001	0.002	0.960	1.55	0.220	48.80	0.001	0.41	0.615	1.04	0.343
Andricus galls	8.69	0.006	0.39	0.845	5.80	0.021	-	-	-	-	-	-
Chewed Leaves	4.20	0.048	5.05	0.031	5.54	0.024	52.49	0.001	0.95	0.384	1.08	0.337
Other mines	5.37	0.026	1.71	0.200	2.99	0.092	49.12	0.001	3.32	0.041	1.13	0.333
Q. laevis	$F_{1,36}$	P	$F_{1,36}$	P	$F_{1,36}$	P	$F_{5,180}$	P	$F_{5,180}$	P	$F_{5,180}$	P
Brachys 1st	15.20	0.0001	0.009	0.978	1.57	0.217	136.94	0.0001	14.30	0.0001	1.53	0.227
Brachys 2nd	21.30	0.0001	0.17	0.682	0.12	0.728	78.94	0.0001	17.37	0.0001	1.27	0.281
Acrocercops	7.25	0.011	0.19	0.666	0.66	0.422	93.58	0.0001	0.83	0.376	0.013	0.920
Eyespot galls	1.12	0.298	0.34	0.564	0.55	0.465	71.40	0.0001	4.47	0.018	4.30	0.020
Chewed leaves	1.95	0.170	0.15	0.700	0.99	0.326	3.66	0.038	0.05	0.925	0.16	0.813
Other mines	0.11	0.980	0.12	0.734	0.28	0.602	31.44	0.0001	0.26	0.697	0.54	0.523

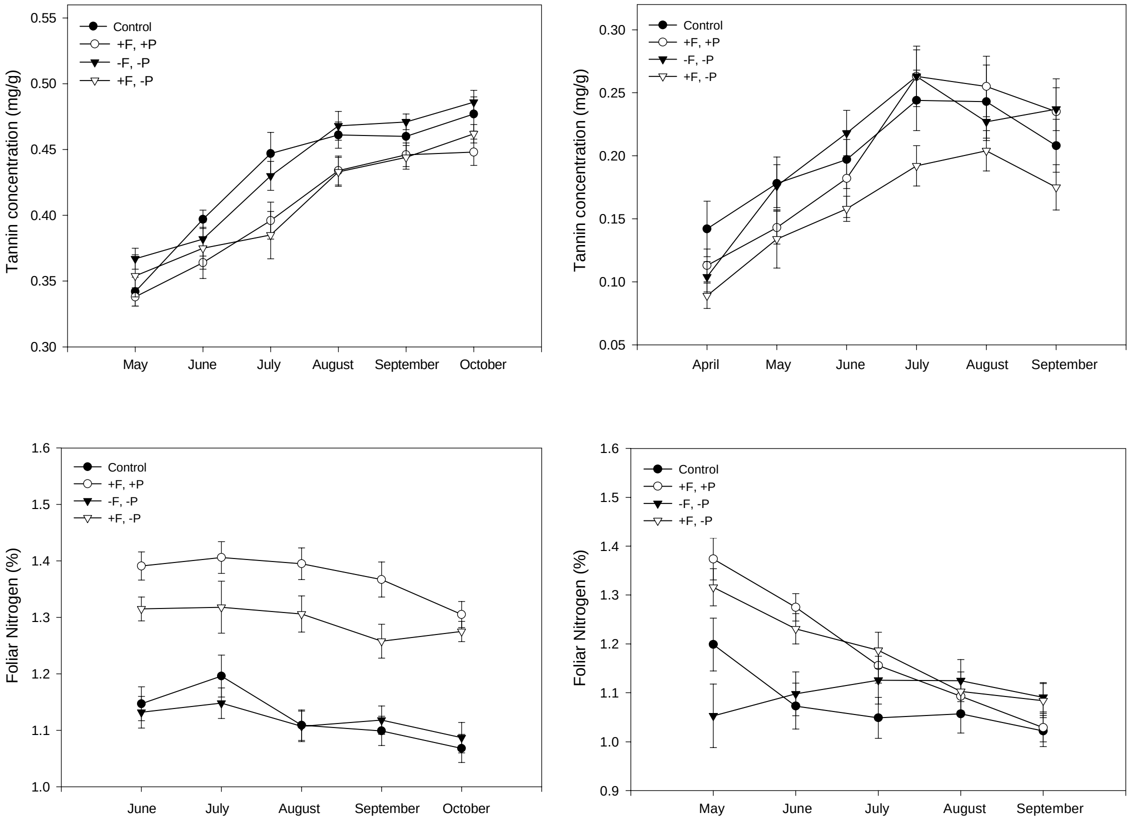

Figure 4.1- Treatment effects on the concentration of tannins and foliar nitrogen of *Quercus geminata* (left panels) and *Q. laevis* (right panels) over the season. See text for explanation of treatment symbols.

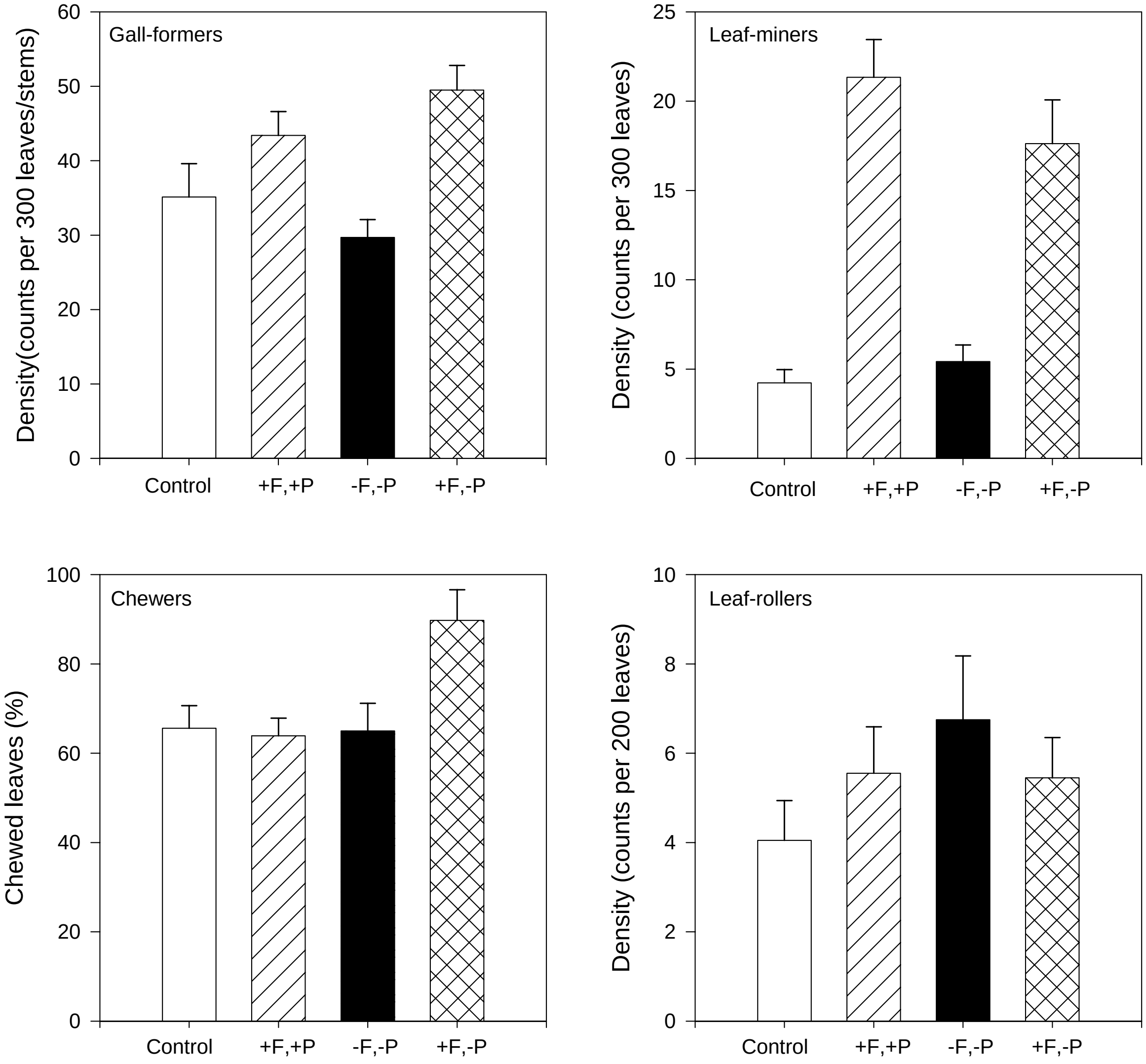

Figure 4.2- Treatment effects on the abundance of different herbivores guilds on *Q. geminata*. Bars (± 1SE) show average of monthly counts for all herbivores belonging to the same guild.

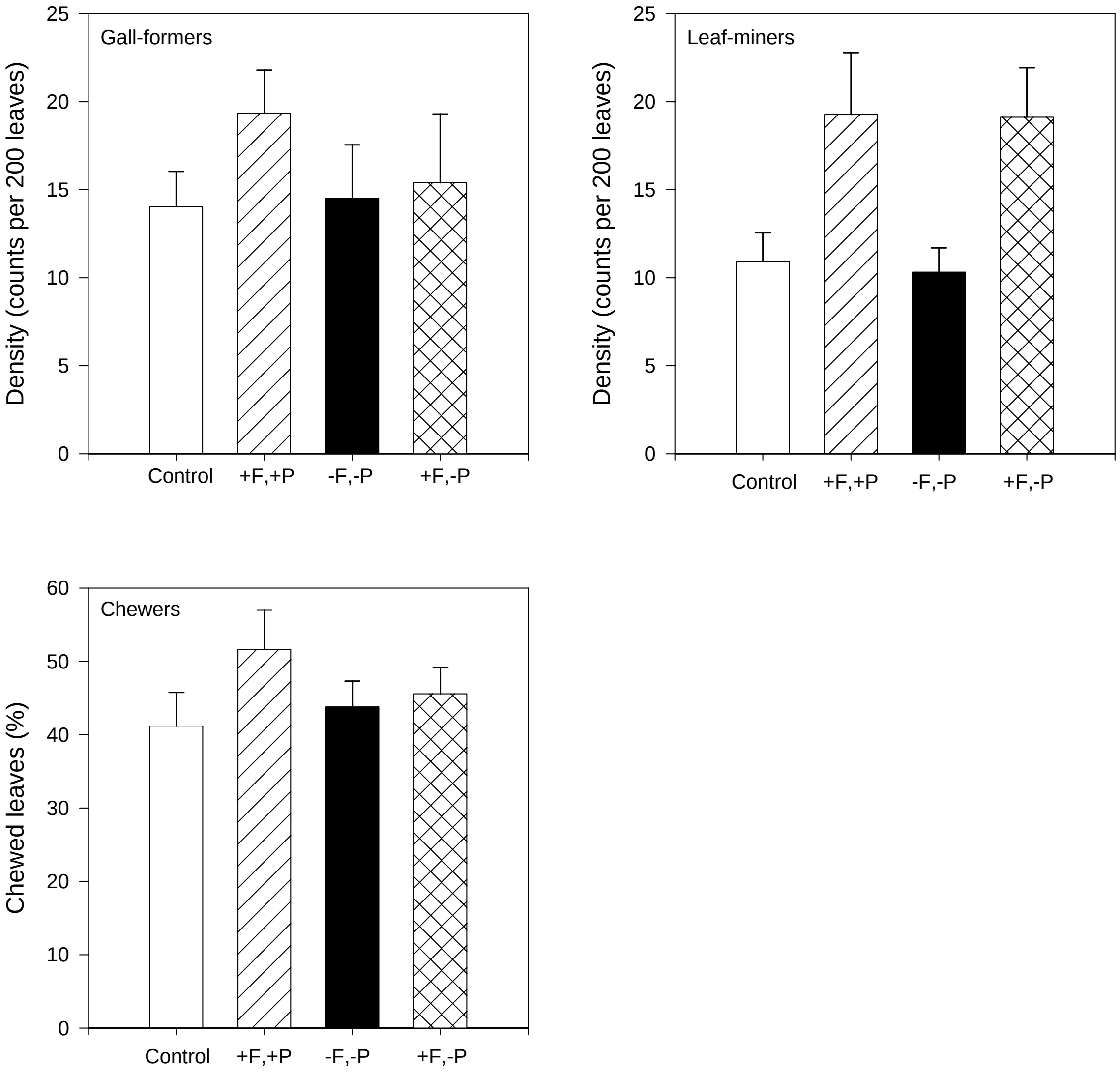

Figure 4.3- Treatment effects on the abundance of different herbivores guilds on *Q. laevis.* Bars (± 1SE) show average of monthly counts for all herbivores belonging to the same guild.

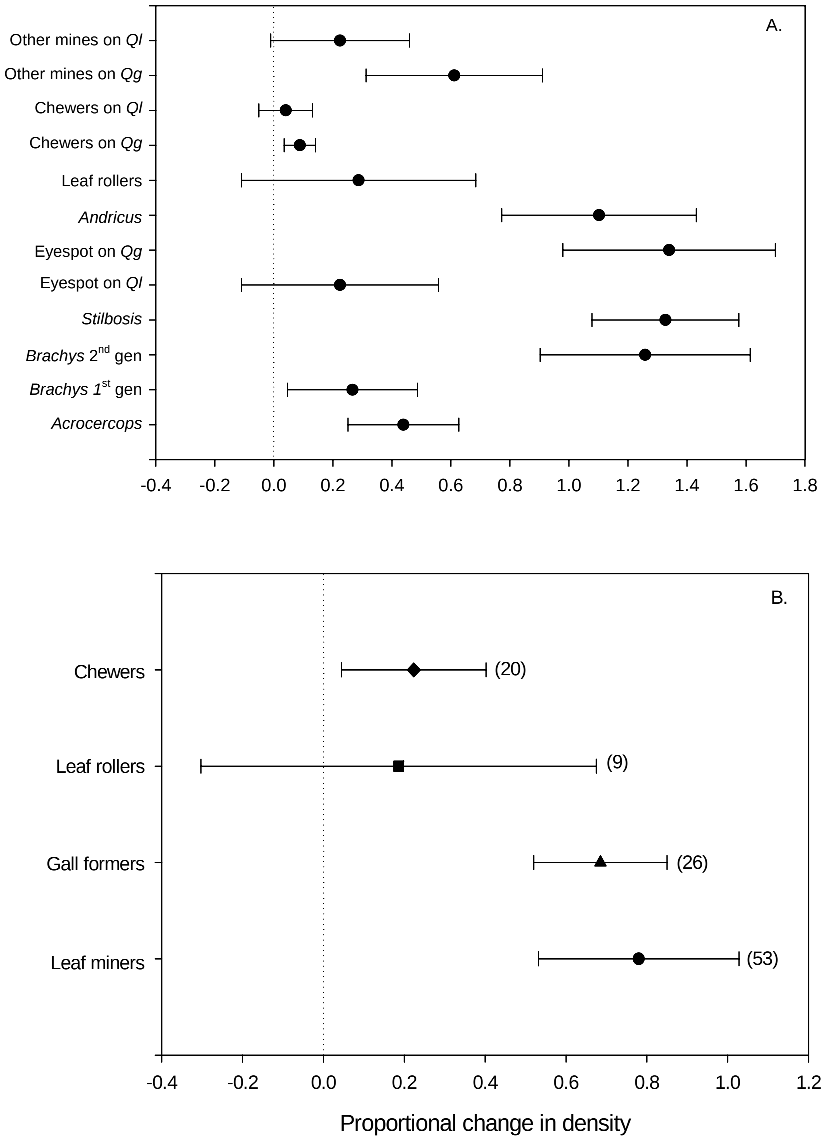

Figure 4.4- A) Strength of bottom-up manipulations on the abundance of herbivores on both *Q. geminata* and *Q. laevis* in the absence of natural enemies. Bottom-up effects refer to fertilizer addition and herbivore densities under the treatments –F,-P and +F,-P were contrasted. The cumulative effect size (ln ratio) is reported for each herbivore with its 95% confidence interval (*Q.g* = *Quercus geminata* and *Q.l* = *Quercus laevis*); B) Strength of bottom-up manipulations according to herbivore feeding guild. Numbers in parentheses indicate the number of independent comparisons for each effect and effects are significant if confidence intervals do not overlap with zero.

DISCUSSION

Using field experiments, the abundance of different herbivore guilds and the survivorship of oak leaf miners were compared among different combinations of natural enemy pressure and host plant quality. Our results show that fertilization successfully increased the availability of nitrogen for herbivores feeding on both plant species. We also observed a decrease in tannins on fertilized trees, which has been previously demonstrated for oaks (e.g., Forkner & Hunter, 2000) and for other unrelated plant species (Waring & Price, 1988; reviewed by Koricheva *et al.*, 1998, Haukioja *et al.*, 1998). Overall, fertilization tended to increase the quality of oak foliage in our studied systems, and variation in host quality among and within plants has been demonstrated to affect different herbivore guilds (reviewed by Waring & Cobb, 1992; Kyto *et al.*, 1996).

Higher host quality of fertilized plants offer support for the higher densities of leaf miners and most other herbivores on fertilized compared to control plants. Increases in the biomass and nutritional quality of fertilized vegetation may enhance the overall abundance of herbivorous insects and, as a consequence, could result in increased densities of natural enemies and increased rates of predation and parasitism. In our systems, however, natural enemy pressure did not increase with productivity, as no significant differences in the proportion of mines killed by natural enemies were observed among treatments. Also, analyses of natural enemies caught on sticky traps showed no difference in the abundance of microhymenopterans on plants that were trapped and plants that were fertilized and trapped. These results are contrary to other studies in which predation and parasitism rates were higher in fertilized plants (e.g., Stiling &

Rossi, 1997; Moon & Stiling, 2004), but in agreement with other studies with oaks (e.g., Forkner & Hunter, 2000) and are in accordance with the suggestion that top-down factors such as natural enemy pressure might have weak impacts on the abundance and performance of the oak herbivores studied.

Contrary to our results, other field studies have shown the strong effect of parasitoids in leaf miner survivorship and performance (Auerbach & Simberloff, 1988; Hawkins, 1994). In a factorial manipulation of plant quality and parasitism pressure for five different plant species and 15 herbivores including 7 leaf miner species, Moon & Stiling (2004) found that fertilization strongly increased the density of the most common herbivores and fertilization also increased the effects of parasitism for herbivores of two of the host plants studied. Despite the evidence for the importance of natural enemies on herbivore abundance and performance (reviewed by Hawkins *et al.*, 1997), removing predators and parasitoids in our systems did not strongly increase herbivore abundance on oak trees. Low rates of egg parasitism and high rates of egg survivorship have been demonstrated for some of the leaf miners studied (e.g., Mopper *et al.*, 1984; 1995) and negligible effects of predators and parasitoids on egg mortality might be responsible for the absence of a positive response by herbivores to the removal of natural enemies. Cornell & Hawkins (1995) reviewed the evidence for survival patterns and mortality sources of herbivorous insects and found that natural enemies emerged as the most frequent source of mortality, although endophytic species such as leaf miners and gall formers sustained higher survival rates in the latest stages than free-feeding herbivores. Additionally, although enemies were the most frequent cause of death at all life stages, their effects are smallest in the egg and early larval stages (Cornell & Hawkins, 1995;

Price, 1997). *Stilbosis* females, for example, deposit eggs on the lower leaf surface, at the junction of a midvein and major lateral vein. Mopper *et al.* (1995) have shown that *Stilbosis* eggs and early stage larvae usually suffer little natural enemy attack, resulting in low mortality rates, as females place eggs among the dense trichomes on the ventral leaf surface. For *Brachys* mines, eggs are deposited on the upper surface of turkey oak leaves, but secured and protected by a waxy secretion (Waddell & Mousseau, 1996). Data on mortality rates of *Acrocercops* eggs caused by predation or parasitism are not available, but fast egg hatching and fast larval development might reduce the window of vulnerability to the attack of natural enemies. Alternatively, although application of Tanglefoot and use of sticky traps have been efficient in reducing parasitism rates in other plant-herbivore-natural enemy systems, it might have had limited efficiency in our oak trees, especially for leaf miners. The fact that the percentage of chewed leaves significantly increased in plants that were fertilized and natural enemies were excluded suggest that reduction of ant abundance might have influenced the abundance of chewers on these plants, increasing leaf consumption, as demonstrated by other studies (e.g., Marquis & Whelan, 1994).

Moran & Scheidler (2002) manipulated both top-down and bottom-up forces in a successional plant community and observed that, although fertilizer addition caused changes in plant biomass, many herbivore species were unaffected by experimental manipulation. This result stresses the importance of examining many components of communities when addressing trophic interactions, as some species might not strongly respond to either bottom-up or top-down processes, as suggested by studies in terrestrial diverse communities such as successional fields (Moran & Scheidler, 2002) and temperate forests (Forkner & Hunter, 2000). Our results are in accordance with this suggestion, as the strength of bottom-up forces varied among and within guilds. Species-specific and differential guild responses to bottom-up and top-down manipulations might be explained by differences in life-history traits, such as growth and development, of each herbivore and leaf miner species studied. While our results show that the strength of bottom-up forces varied among and within guilds, bottom-up effects on herbivore abundance were usually strong, and strongest effects of bottom-up manipulations were observed for leaf miners and gall-formers. In a similar analysis for herbivores inhabiting *Spartina* marshes, Denno *et al.* (2003) observed that the relative effect of nitrogen addition was greater than the impact of spider predation on 5 out of 6 sap-feeders and, overall, bottom-up effects dominated over top-down impacts. Our results also showed that amongst the leaf miners, *Stilbosis* exhibited the greatest change in abundance caused by bottom-up effects such as increased plant quality. This particular mine species may be most likely to be affected by host quality because *Stilbosis* mines have the longest developmental times, full depth mines, and, therefore, a higher likelihood of being affected by spatial and seasonal variation in host plant quality.

Our study has simultaneously manipulated top-down and bottom-up factors for a community of herbivores on scrub oaks and our analyses of the relative strengths of plant quality and natural enemies on herbivore abundance and performance have shown that bottom-up forces dominate our oak-herbivores-natural enemies system and top-down effects such as the impact of predators and parasitoids do not significantly impact herbivore abundance and the performance and survivorship of leaf miners.

Chapter 5

DOES LOW NUTRITIONAL QUALITY ACT AS A PLANT DEFENCE? AN EXPERIMENTAL TEST OF THE SLOW-GROWTH, HIGH-MORTALITY HYPOTHESIS

SYNOPSIS

The slow-growth-high-mortality hypothesis was experimentally tested in this study by investigating the effects of plant quality and natural enemies on leaf miner growth, performance and survivorship. Two leaf miners (*Acrocercops albinatella* and *Brachys tesselatus*) occurring on the turkey oak *Quercus laevis* were studied using a factorial design that manipulated predation/parasitism pressure and plant nutritional quality. Forty trees were randomly divided into four treatments: 1) control plants (nutrients and natural enemies unaltered); 2) nutrients added, natural enemies unaltered; 3) nutrients unaltered, natural enemies reduced, and 4) nutrients added and natural enemies reduced. Water content, leaf toughness, tannin concentration, and foliar nitrogen were quantified monthly for each plant and mine growth and survivorship were assessed by tracing mines on a 2 to 3-day interval and by following the fates of 50 mines per species per treatment combination. Fertilized plants exhibited significantly higher amounts of nitrogen, but no significant differences among treatments were observed for water content, leaf toughness and tannin concentration. These results only partially support the slow-growth-high-mortality hypothesis, as mines were significantly smaller and developed faster on fertilized plants, but neither fertilization nor natural enemy

exclusion significantly affected mine survivorship or mortality caused by natural enemies.

INTRODUCTION

Resources and natural enemies, as well as the interaction between them, have the potential to impact herbivore abundance, distribution, performance and survivorship. The effects of both resources and natural enemies on herbivory rates experienced by plants have been widely discussed (e.g., Moran & Hamilton, 1980; Augner, 1995; Williams *et al*., 2001) and low plant quality can affect insect performance directly, by reducing growth rate, fecundity and survival (Slansky, 1993; Haggströn & Larsson, 1995) or indirectly by affecting the risk of mortality caused by natural enemies (Feeny, 1976; Price *et al.*, 1980; Clancy & Price, 1987). The interaction between variation in host plant quality and risk of attack by natural enemies was formalized into the slow-growth, high-mortality hypothesis (hereafter SGHMH; Clancy & Price 1987). According to this hypothesis, herbivores feeding on plants of low nutritional quality (e.g., low nitrogen, high levels of secondary compounds, high toughness and/or lignin content) do not necessarily increase damage on their host by overcompensatory feeding if increased development time due to poor host quality increases the window of vulnerability of herbivores to natural enemy attack. Specifically, the SGHMH proposes that the nutritional quality, allelochemistry, and/or morphology of the host plant can act as a sublethal plant defence by prolonging development of herbivorous insects and subsequently increasing mortality inflicted by predators and parasitoids (Benrey &

Denno, 1997). Clancy and Price (1987) wrote, “many more individual case studies are needed to determine if the SGHMH should be generally rejected or accepted”. Since then, relatively few tests of the SGHMH have been performed, with mixed results. In a factorial manipulation of plant quality and predation pressure, Loader and Damman (1991) found that *Pieris rapae* larvae growing on low-nitrogen plants developed more slowly and were more likely to be killed by predators than conspecifics growing on high-nitrogen plants. Parasitism rates, however, were higher in herbivores feeding on high-nitrogen plants. For galling sawflies on arroyo willows, Clancy and Price (1987) also observed higher parasitism rates in fast-developing *Pontania* galls and slow-growing galls were less vulnerable to attack from parasitoids. Benrey and Denno (1997), on the other hand, observed that slow-developing larvae of *Pieris rapae* were more heavily parasitized by *Cotesia glomerata* than fast-developing larvae reared on artificial diets, but slow-growth did not translate into increased parasitism when variation in larval growth was achieved with the use of natural variation of plant quality caused by interspecific differences. Williams (1999) reviewed the evidence for the SGHMH, and found that, usually, slow-growing, surface-feeding herbivores were at less risk from parasitism but greater risk from predation, than faster-growing herbivores. For concealed herbivores, such as gall-formers and leaf miners, the SGHMH was rejected in approximately half of the cases reviewed, regardless of natural enemies being predators or parasitoids.

Most of the data used to test the SGHMH has been observational in nature, and only a few studies have experimentally manipulated aspects of plant quality that potentially affect herbivore growth and development and might consequentially impact natural enemy attack (see Loader & Damman, 1991; Benrey & Denno, 1997; Lill &

Marquis, 2001). Furthermore, among the 41 studies reviewed by Williams (1999), only five studies were conducted with leaf-miners, and none of these studies were specifically designed to test the SGHMH, as most of them assessed leaf miner survivorship under natural variations in host plant quality (i.e., differences in host plant species, within-host variation in quality, effects of plant hybrids) and none of these studies manipulated natural enemies to assess their effects on leaf miner survivorship and performance. The current study aimed to test the SGHMH for two very distinct species of leaf miners, *Acrocercops albinatella* (Lepidoptera: Gracillaridae) and *Brachys tesselatus* (Coleoptera: Buprestidae), feeding upon the turkey oak *Quercus laevis* (Fagaceae), with a factorial manipulation of both plant quality and natural enemy pressure. Manipulation of the third trophic level was achieved by reducing invertebrate predators and parasitoids and manipulation of plant nutritional quality was achieved by plant fertilization. Specifically, the following predictions were tested: 1) Low tissue quality cause herbivores to feed for longer periods of time and lengthens larval development time. Therefore, leaf miners feeding on fertilized plants should exhibit smaller mines and shorter development times than leaf miners on control plants; 2) Lengthened development time or increased feeding results in increased mortality caused by natural enemies. Therefore, leaf miners feeding on fertilized plants should exhibit higher survivorship/lower mortality caused by predators and parasitoids than leaf miners on control plants; 3) If natural enemies are a significant source of mortality for leaf miners on turkey oaks, mines feeding on plants in which natural enemies were reduced should exhibit higher survivorship than mines on control plants. Because both plant quality and natural enemies were manipulated in the factorial design, here it was also predicted that 4) leaf miners feeding on fertilized plants in which natural enemies were excluded, should exhibit the highest survivorship amongst all treatments.

STUDY SYSTEM

The turkey oak, *Quercus laevis*, is one of the characteristic trees associated with the sand hill community throughout Florida. *Q. laevis* is a moderately fast to fast-growing tree and presents deciduous glabrous leaves, alternately arranged with usually five lobes, although this number may vary from three to seven (Nixon, 1997). Although a common tree in Florida native vegetation, there are relatively few studies concerning herbivory in this plant species. *Acrocercops albinatella* (Lepidoptera: Gracillaridae) is a microlepidopteran species whose larval stages feed on young leaves, creating distinct linear-blotch mines on the lower surface of *Q. laevis* leaves. Larvae typically appear in early spring (late February or early March) and feed on the palisade parenchyma cells, completing their development in approximately ten days. Larvae emerge from the blotch mine and usually pupate on the same leaf from which they emerge (T. Cornelissen, pers. obs.). *Brachys tesselatus* (Coleoptera: Buprestidae) is a beetle species that also forms distinct blotch mines in *Q. laevis* leaves. The adults emerge from mid-March to mid-April, coinciding with budburst of turkey oak. Adults initially feed on the early leaves until mating and oviposition. Eggs are deposited singly on the upper surface of the leaves and after hatching the larvae mine into the mesophyll creating distinct, characteristic damage. Contrary to what happens in South Carolina (Waddell *et al*., 2001) *Brachys* in the study sites here go through two generations, instead of just one. The first mines appear in early April and remain active until late June, when larvae complete their development and pupate inside the mines. New adults emerge in early July and oviposit to form new *Brachys* mines that remain active until September-October. Pupation and overwintering of this second generation occurs within the leaves after they have senesced and abscised from the tree. New adults emerge from the leaf litter in the following spring (Waddell *et al*., 2001). Turkey oak leaves are also attacked by many other herbivores, such as the leaf roller weevil *Homoeolabus analis* (Coleoptera: Atellabidae), the eastern buck moth *Hemileuca maia*, the white-marked tussock moth *Orgyia leucostigma* (Lepidotpera: Lymantriidae) and, less commonly, other leaf miners such as *Stigmella* spp. (Lepidoptera: Nepticulidae) and *Cameraria* spp. (Lepidoptera: Gracillariidae).

Generalist predatory ants (Hymenoptera: Formicidae) and the green lynx spider *Peucetia viridans* (Araneae: Oxyopidae) are among the most common arthropod predators in these study sites. The leaf miners studied are also attacked by several microhymenopteran parasitoid species, including *Zagrammosoma multilineatum* (Hymenoptera: Eulophidae) and *Chrysonotomyia* sp. (Hymenoptera: Eulophidae).

METHODS

Data collection

This study was conducted between February and November of 2003 on natural stands of scrub oak vegetation in Tampa, Florida. Forty *Quercus laevis* trees ranging between 1.5 and 2.5 meters in height were marked at an unburned patch at the University of South Florida ECOAREA (for a description of the study site see Mushinsky *et al*., 2003).

The effects of plant quality and pressure exerted by natural enemies on the abundance, performance, and survivorship of two different leaf miners (*Acrocercops albinatella* and *Brachys tesselatus*) on *Q. laevis* were investigated using a 2 X 2 factorial design. The forty trees were randomly divided into four treatments, allowing ten replicates of each treatment, as follows: 1) –F,+P: control plants with nutrients and natural enemies unaltered ; 2) +F,+P: nutrients added, natural enemies present (unaltered); 3) –F,-P: nutrients unaltered, natural enemies reduced, and 4) +F,-P: nutrients added and natural enemies reduced. Plant quality was manipulated by the addition of 150 g of 46:0:0 NPK fertilizer to assigned *Q. laevis* trees bi-weekly from April to June. Unfertilized plants had soil around the tree slightly disrupted, but no granular fertilizer was added. Plants did not significantly differ in height, number of leaves on ten shoots and leaf area (five leaves per tree) before treatments were assigned (One-Way ANOVAs, all $P > 0.05$). A previous study with the same system indicated that predation by spiders was a negligible mortality factor for the leaf miners (Cornelissen & Stiling, 2005), hence only mortality caused by predatory ants and parasitism caused by microhymenopterans were manipulated. A preliminary count of green spiders on all marked plants in March of 2003 showed that spider abundance was very low (average 0.8± 0.2) and did not differ among plants assigned to the four treatments. Ants were excluded by placing masking tape covered with Tanglefoot ® around the base and twigs of assigned oak trees at the beginning of the experiments and by handpicking ants already present on the trees. Efficiency of ant trapping was checked monthly by counting ants during five minutes per tree on all treatment combinations. Tanglefoot® was reapplied on assigned turkey oak trees in May 2003. Parasitoid reduction was achieved by adding between 12 and 15 yellow 13X8 cm Sticky Strip traps (Gempler's, Belleville, Wisconsin, U.S.A.) per assigned tree. Previous studies with gall-formers and leaf miners have shown that yellow traps are efficient at capturing parasitoids in other systems such as salt marshes and oak hammock communities (e.g., Moon & Stiling, 2002, 2004). Traps were placed throughout the tree canopy using binder clips and were replaced bi-weekly. Old traps were collected and individually wrapped in plastic and frozen for further analyses. To assess sticky trap efficiency, the number of microhymenopterans and other invertebrates caught on the traps were counted on three 4 X 4 cm quadrats on two traps per plant at the beginning and at the end of the season.

To assess variation in host plant quality among treatments, water content, foliar nitrogen concentration, tannin concentration and leaf toughness were evaluated monthly for each plant, between April and September of 2003. On each sampling date, eight undamaged leaves were collected from each tree and placed immediately on ice. Leaf toughness was evaluated using an Effegi FT-011 penetrometer (International Ripening Co, Italy) and water content was quantified by the difference between leaf wet and dry weights. Leaves were further oven-dried and milled to a fine powder. Tannins were extracted from 50 mg of dry tissue, and tannin concentration was quantified using the radial diffusion assay with tannic acid as a standard (for details see Hagerman, 1987). Nitrogen content was determined using a CE Instruments NC2100 CN Analyzer (CE Elantech, Incorporated, Lakewood, New Jersey, U.S.A.).

To test for differences in mine growth and survivorship among treatments, five mines of each leaf miner species (n = 50 mines per species per treatment combination) were permanently marked on each plant using a permanent black ink marker as soon as the eggs hatched and larvae initiated mine formation. *Acrocercops* mines were measured at 2-day intervals by tracing the numbered mines using acetate sheets. *Brachys* mines were traced at 3-day intervals. At the end of the experiment, mine drawings were digitized and mine size was measured using the software UTHSCSA Image Tool (University of Texas, USA), with digital pictures calibrated to the nearest 0.01mm. We compared final mine size (cm^2), developmental time (days to pupation) and mine growth rate among treatments for each leaf miner species. Mine growth rate on each leaf was calculated as Growth rate = (Final mine size – Initial mine size) / number of days mine was growing. After mine termination, all leaves were inspected under a stereomicroscope to assess leaf miner survivorship and identification of mortality factors. Leaf miners offer a great opportunity to assess population survivorship and mortality factors since a record of the miner success is clearly observed on the leaves: parasitized mines have tiny circular exit holes on mine’s surface and/or pupae within the mine, and predated mines are usually found ripped open and the larva is absent. Successfully emerged larvae of *Acrocercops* cut open the mines and pupate usually on the same leaf where the mine developed. *Brachys* larvae pupate inside mines and/or cut circular holes on the underside of the leaf.

Data analysis

All the variables analyzed were first submitted to Lilliefor’s test for data normality and transformations (log-transformation and arcsine square rooted) were employed to stabilize variances and normalize the data. However, for the sake of clarity, figure axes and means (+1SEM) show untransformed data. Seasonal variation in turkey oak quality was described elsewhere (Cornelissen & Stiling, 2006b) and on this study only variation in plant quality among treatments is emphasized. To test for differences in plant nutritional quality among treatments, we first averaged tannin concentration, nitrogen content, water, and toughness using all eight leaves sampled for each plant on each collection date and two-way ANOVAs were used to assess effects of treatment on host plant quality. A paired *t*-test was used to assess differences in leaf area of *Q. laevis* before and after fertilization and Pearson correlations were used to examine the relationship between tannin and nitrogen concentration and between water and toughness on each individual plant. To test for differences in leaf miner growth and survivorship among treatments, differences in mine size, days to pupation, and growth rate of mines that survived to pupation were analyzed using a Two-Way ANOVA with fertilization and natural enemies as main factors. Differential survivorship among treatments as well as the proportion of mines killed by natural enemies (parasitoids and predators) and proportion killed by unknown factors (e.g., plant resistance, larvae dead inside mine) on each treatment were also analyzed using a Two-Way ANOVA. All statistical analyses were performed using Systat 9.0 for Windows (Wilkinson, 1999).

RESULTS

Treatment effectiveness

Application of Tanglefoot® on assigned turkey oak tress proved effective in keeping ants away from treated trees compared to control trees (control trees: 14.6 ± 1.1 ants, treated trees: 0.92 ± 0.22 ants, $F_{1,28} = 137.6$, $P < 0.001$). Sticky traps also proved efficient in capturing microhymenopterans and other small invertebrates. Microhymenopterans were much more abundant on the traps than other insects such as ants, ladybugs, and flies (microhymenopterans 32.3 ± 1.56, other invertebrates 11.24 ± 0.19). No significant differences were observed in the abundance of parasitoids caught on traps from plants treated with traps only (29.66 ± 0.75 parasitoids per trap) versus plants that were fertilized and had sticky traps added (29.47 ± 1.05, $F_{1,38} = 0.22$, $P = 0.74$).

Treatment effects on host plant quality

Fertilization significantly increased the amount of foliar nitrogen on *Q. laevis* trees ($F_{1,36} = 8.993$, $P = 0.005$) and fertilized trees showed a tendency for decreased tannin concentration (Figure 5.1), although differences in tannins among treatments were not statistically significant (Fertilizer effect: $F_{1,36} = 2.82$, $P = 0.092$). No significant differences among treatments were observed for leaf toughness and leaf water content (all $P > 0.05$). Linear regressions revealed that, for *Q. laevis*, no significant relationship was observed between tannin and nitrogen concentration per individual plant ($P = 0.361$), or between water and leaf toughness ($P = 0.688$). Paired *t*-tests revealed no significant difference in leaf area before and after fertilization ($t = -0.778$, $P = 0.441$).

As expected, natural enemy removal did not affect host plant quality (natural enemies effect, all $P > 0.05$) and no significant interactions between treatments were observed.

Treatment effects on leaf miner performance

Fertilization significantly decreased mine size of *Acrocercops* ($F_{1,126} = 12.71$, $P = 0.001$; Fig. 5.2a) and of both generations of *Brachys* (1st generation $F_{1,96} = 21.74$, $P < 0.0001$; 2nd generation: $F_{1,66} = 4.85$, $P = 0.031$; Fig. 5.2d). Analysis conducted only for mines that survived showed that *Acrocercops* mines on fertilized plants were approximately 56% smaller than mines growing on control plants, whereas *Brachys* mines on fertilized plants were approximately 16% smaller than mines on control plants. Removal of natural enemies did not significantly impact the size of *Acrocercops* mines ($F_{1,126} = 2.97$, $P = 0.09$) or the size of *Brachys* mines (1st generation: $F_{1,96} = 2.23$, $P = 0.138$; 2nd generation: $F_{1,96} = 31.30$, $P = 0.583$).

Fertilization also significantly affected the development of leaf miners. *Acrocercops* mines growing on fertilized plants pupated earlier ($F_{1,126} = 9.69$, $P = 0.002$) and developed faster ($F_{1,126} = 16.74$, $P < 0.001$) than mines growing on control plants (Fig. 5.2b, 5.2c). For the first generation of *Brachys* mines, significant effects of fertilizer on performance was also observed, as mines required fewer days to pupate ($F_{1,96} = 4.19$, $P = 0.043$, Fig. 5.2e) and developed faster ($F_{1,96} = 5.83$, $P = 0.018$, Fig. 5.2f) than mines developing on control plants. For the second generation of *Brachys* mines, fertilization significantly decreased the number of days required to pupation ($F_{1,66} = 20.15$, $P = 0.001$), but no significant effects were observed on mine growth rate ($F_{1,66} = 2.35$, $P = 0.07$). No significant interactions between fertilization and removal of natural enemies were observed for either leaf miner (all $P > 0.05$).

In general, high survivorship was observed for all the leaf miners studied (Fig. 5.3) and for both *Acrocercops* and *Brachys,* survivorship was not affected by experimental manipulation (fertilizer and natural enemy effects: all $P > 0.05$). For *Acrocercops*, however, miners experienced lower mortality inflicted by predators when developing on fertilized plants compared to control and/or trapped plants ($F_{1,36} = 12.72$, $P < 0.001$), but no significant effects of fertilizer were observed when natural enemies were parasitoids ($F_{1,36} = 2.34$, $P = 0.159$). For both generations of *Brachys*, no significant effects of fertilizer addition or natural enemy removal were observed on the proportion of mines killed by predators or parasitoids (all $P > 0.05$).

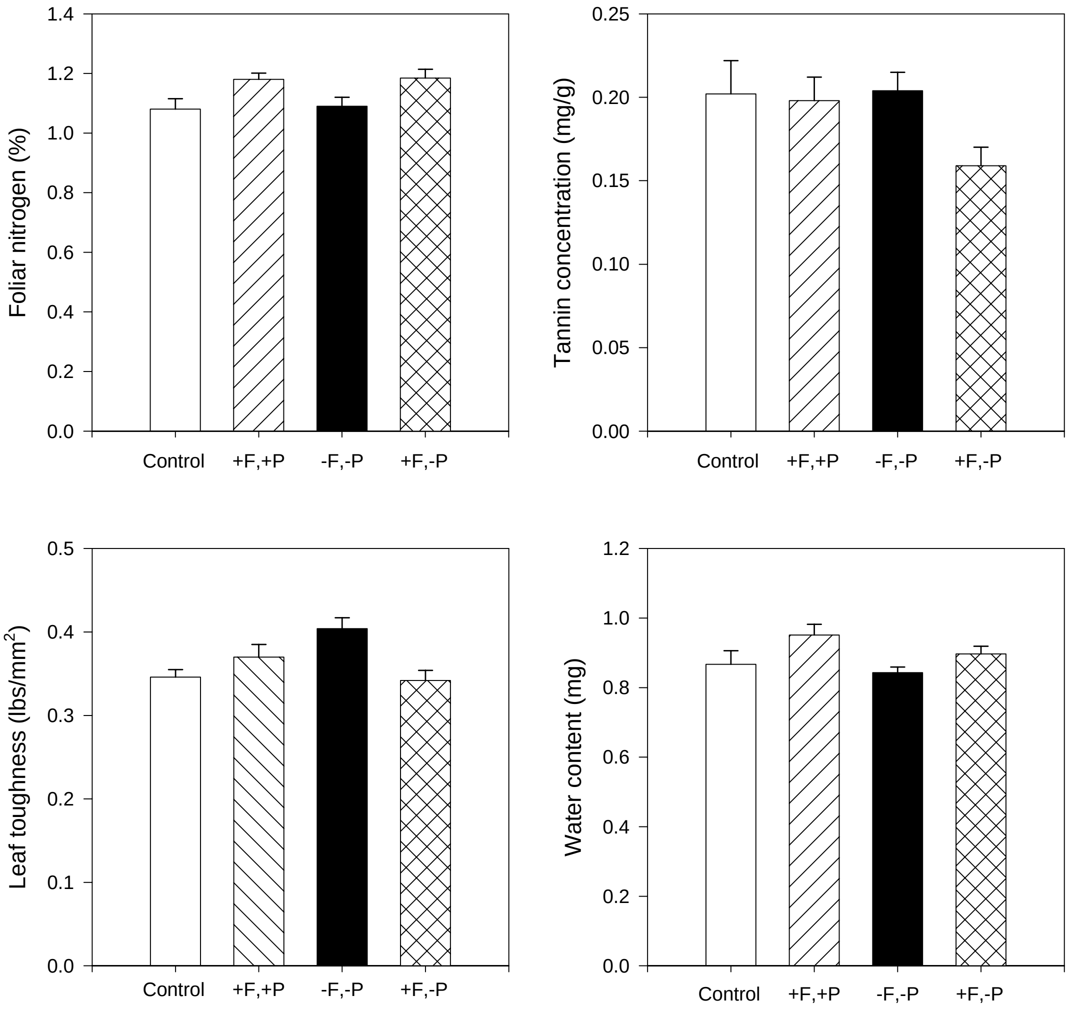

Figure 5.1- Treatment effects on the concentration of foliar nitrogen, tannin concentration, leaf water and foliar toughness of *Quercus laevis*. Bars shown mean (+1SE) of averaged monthly samplings (Control: plants with nutrients and natural enemies unaltered; +F,+P: nutrients added, natural enemies present (unaltered); –F,-P: nutrients unaltered, natural enemies reduced; +F,-P: nutrients added and natural enemies reduced).

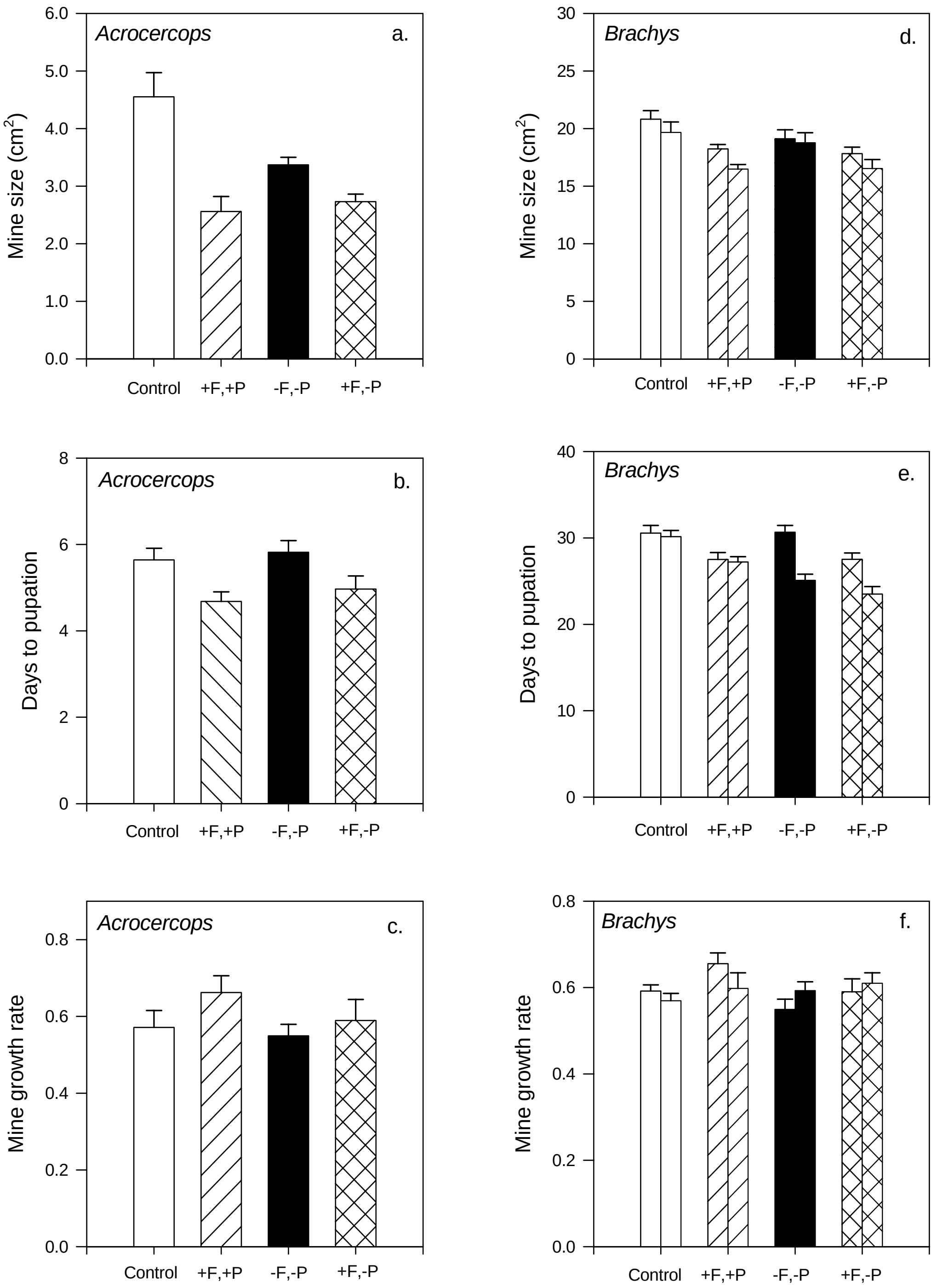

Figure 5.2- Treatment effects on the size and development of the leaf miners *Acrocercops albinatella* and *Brachys tesselatus*. For *Brachys*, bars to the left indicate data for the first generation and bars to the right indicate data for the second generation. Bars show means (±1SE) and treatment legends are as on Fig. 1.

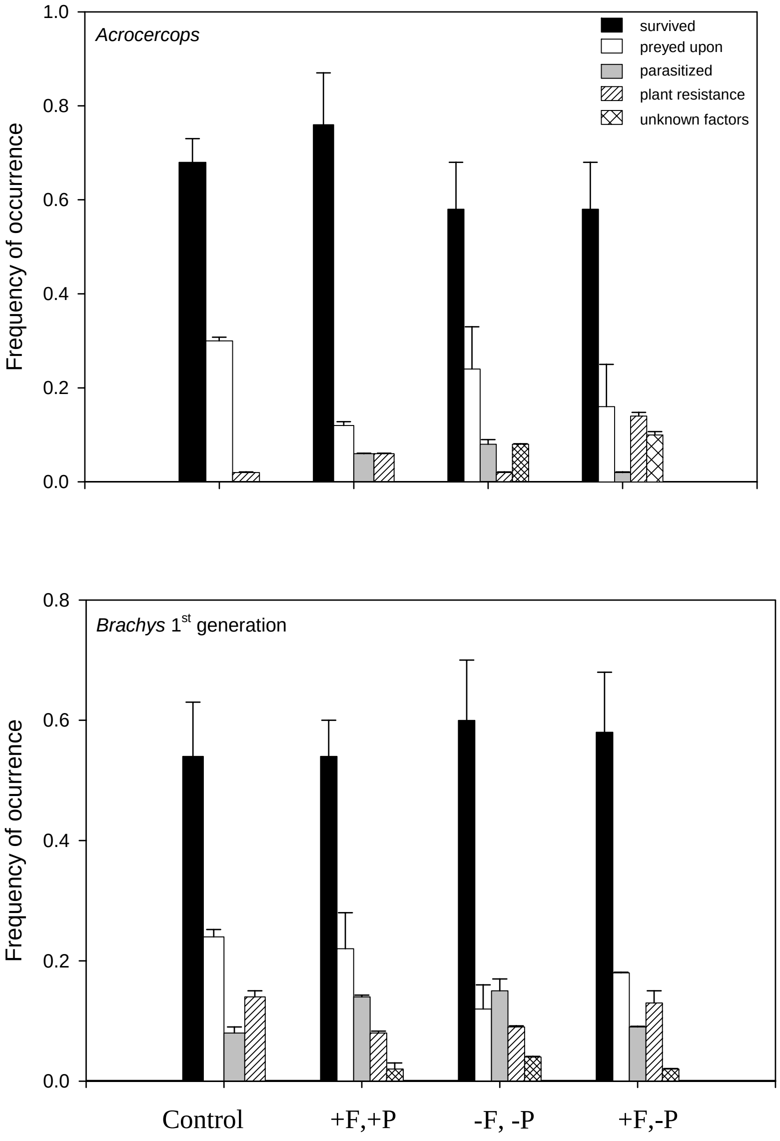

Figure 5.3- Frequency of occurrence of mortality factors for leaf miners growing under four different treatments. Bars show means (±1SE) and treatment legends are as in Fig.1.

DISCUSSION

Herbivorous insects often suffer reduced growth rates when feeding upon suboptimal hosts and, although poor diets are not lethal in themselves, it is assumed that they nevertheless increase the mortality rate by prolonging the vulnerable stages of herbivores (Rostas & Hilker, 2003), which might lead to higher levels of enemy attack (Moran & Hamilton, 1980; Clancy & Price, 1987). Plant nitrogen concentration influences important interactions between herbivorous insects and plants (Kyto *et al.*, 1996) and low nitrogen supplies might result in increased total plant consumption through increased consumption rates and/or prolonged periods of feeding, digestion, and development (Mattson, 1980). Kyto *et al.* (1996) reviewed the evidence for the effects of soil fertilization on phytophagous insects and concluded that enhanced nitrogen availability benefited herbivores by improving host plant quality, but also affected population-regulating processes such as predation, parasitism, and competition. Our results have demonstrated the beneficial effects of plant fertilization in terms of increased availability of foliar nitrogen and a tendency for decreased concentration of tannins. Previous studies in the same oak system indicated that several herbivore guilds respond to improved plant quality caused by fertilization with an increase in density (Cornelissen & Stiling, 2006b). In this study, it has been shown that leaf miners also respond to improved plant quality by altering development and reducing the length of the larval stage. Mines were significantly smaller on fertilized plants, developed faster and pupated earlier than mines growing on control plants. Similar responses to improved plant quality have been reported for other leaf miners (e.g, DeBruyn *et al.*, 2002,), gall-formers (e.g., Clancy & Price, 1987; Stiling & Moon, 2005) and free-feeding herbivores (e.g., Myers, 1985; Loader & Damman, 1991).

Although the experiments here have demonstrated that enhanced nutritional quality caused by plant fertilization allowed mines to develop faster and pupate earlier, no significant effects of improving plant quality and/or removing natural enemies were observed on mine survivorship and mortality imposed by the third trophic level. Therefore, the shorter window of larval vulnerability did not translate into higher survivorship or escape from natural enemy pressure. Interestingly, these results demonstrated high survivorship rates for both leaf miners studied, regardless of experimental treatments. Other studies with leaf miners occurring in oaks (e.g, Faeth, 1980; Auerbach & Simberloff, 1988; Stiling & Simberloff, 1989; Connor & Beck, 1993) have reported much lower survivorship and emergence rates (range: ≈0.6% to 42%) and higher mortality inflicted by natural enemies (range: ≈21% to 38%). Adding sticky traps and treating experimental plants with Tanglefoot® proved efficient at removing natural enemies on experimental trees, although effects of the third trophic level on mine performance and survivorship do not seem to be relevant in our studied systems. Alternatively, the results reported here might also show that, although application of Tanglefoot and use of sticky traps have been efficient in reducing natural enemy impact in other plant-herbivore-parasitoid systems, (e.g., Moon & Stiling, 2004), it might have had limited efficiency in our oak trees, compared to other systems such as salt marshes (e.g., Moon & Stiling, 2002). Although Hawkins (1994) reported high parasitism rates for leaf miners compared to other feeding guilds in biological control studies, the results reported here suggest that, under natural conditions, the concealment provided by the leaf-mining habit might actually offer an advantage to larval stages of *Acrocercops* and *Brachys*. The hypothesis that the leaf mine might be adaptive is part of the broader concept that concealed feeding strategies serve as defences against natural enemies (for a review see Connor & Taverner, 1997) and seem to hold true at least for the leaf miners studied here.

Variation in host plant nutrition and chemistry is often a primary cause of differences in feeding efficiency, growth rate and pupal mass of herbivores feeding on different host plants and different parts within plants. These results generally demonstrated that leaf miners feeding on sub-optimal hosts tended to consume more tissue and develop for longer periods of time, although attack rates by natural enemies and survivorship did not differ among optimal and sub-optimal hosts. Lower predation rates of *Acrocercops* mines on optimal hosts suggest that faster development did promote escape from natural enemies such as predatory ants. For *Brachys* mines, however, these results reinforce the idea that sub-lethal plant defences remain a paradox (Clancy & Price, 1987; Leather & Walsh, 1993). *Brachys* mines develop much slower than *Acrocercops* mines, may exhibit mines that damage more than 70% of the leaf in which they develop (T. Cornelissen, pers. obs.) and are, therefore, “apparent” to natural enemies for longer periods of time. Moran and Hamilton (1980) suggested two other scenarios in which poor nutritive quality of foliage could evolve as an adaptation to insect herbivory: 1) if herbivores are able to detect differences in the nutritive quality of individual plants and if they preferentially feed upon more nutritious host individuals, then low nutritive quality of leaves is advantageous and 2) if successive herbivore generations tend to feed upon the same host individual, then low nutritive quality will prevent future build up of herbivore numbers, thereby increasing plant fitness. Previous studies with leaf miners have shown high degrees of discrimination among hosts with varying degrees of nutritional quality (e.g, Faeth *et al.*, 1981; Auerbach & Alberts, 1992; Fox *et al*,. 1997) and high fidelity to individual natal hosts (e.g., Mopper *et al.*, 1995; Mopper *et al.*, 2000), corroborating both scenarios proposed as explanations for the adaptive significance of low nutritional quality of hosts. Alternatively, low densities of both *Acrocercops* and *Brachys* mines in our study site (mean ± 1SE per 200 leaves: *Acrocercops*: 25.9 ± 2.26; *Brachys* 1st generation: 11.1 ± 1.02; *Brachys* 2nd generation: 7.71 ± 0.97) might indicate that the detrimental effects of leaf miners on plant fitness are realized only during outbreak years.

Previous studies have demonstrated that the support for the SGHMH is mixed, varying among taxa and natural enemy guilds (Williams 1999; Fordyce & Shapiro, 2003). In this study, low nutritional quality does not act as a plant defence and our results reinforce the idea that sub-lethal plant defences remain a paradox, although alternative explanations such as those provided by Moran and Hamilton (1980) might be sustained in the studied system.

Chapter 6

PERFECT IS BEST: LOW LEAF FLUCTUATING ASYMMETRY REDUCES HERBIVORY BY LEAF MINERS

SYNOPSIS

Fluctuating asymmetry (FA) represents small, random variation from symmetry and can be used as an indicator of plant susceptibility to herbivory. We investigated the effects of FA of two oaks species, *Quercus laevis* and *Q. geminata,* and the responses of three herbivore guilds: leaf miners, gallers, and chewers. To examine differences in FA and herbivory between individuals, 40 leaves from each tree were collected, and FA indices were calculated. To examine differences in FA and herbivory within-individuals, we sampled pairs of mined and unmined leaves for asymmetry measurements. Differences in growth of leaf miners between leaf types were determined by tracing 50 mines of each species on symmetric leaves and asymmetric leaves. Asymmetric leaves contained significantly lower concentrations of tannins and higher concentrations of nitrogen than symmetric leaves for both plant species. Both frequency of asymmetric leaves on plants and levels of asymmetry positively influenced the abundance of *Brachys*, *Stilbosis* and other leaf miners, but no significant relationship between asymmetry and herbivory was observed for *Acrocercops.* *Brachys* and *Stilbosis* mines were smaller on asymmetric leaves, but differences in mine survivorship between symmetric and asymmetric leaves were observed only for *Stilbosis* mines. This study indicated that leaf

miners might use leaf FA as a cue to plant quality, although differential survivorship among leaf types was not observed for all species studied. Reasons for the different results between guilds are discussed.

INTRODUCTION

Studies on the effects of plant quality on the attack rates of herbivorous insects have been performed extensively and many hypotheses have been proposed to explain within and between variations in herbivory rates among different plant species. A frequently invoked factor influencing herbivory levels is stress, since stressors may affect plant nutritional quality for herbivores. The plant stress hypothesis (PSH) proposed by White (1984) argues that herbivore abundance is higher on stressed host plants due to an increased availability of nutrients, a decreased concentration of defensive compounds and/or changes in the ratio of nutrients to chemical defenses. Evidence supporting the prediction that moderate stress benefits herbivores due to increased nutritional quality are abundant (e.g., McClure 1980, Lewis 1984, Mattson & Haack 1987) and positive relationships between insect herbivory and plant stress have been found for some tree species, crops and herbaceous plants (e.g., Mattson & Haack 1987, Heinrichs 1988). Nevertheless, some authors claim that experimental tests of the PSH have generated conflicting results (e.g., Bultman & Faeth 1987, Louda & Collinge 1992, Koricheva *et al.* 1998, DeBruyn *et al.* 2002), and many authors (e.g., Larson 1989, Koricheva *et al*. 1998) have championed the abandonment of the PSH and the search for alternative hypotheses. DeBruyn *et al*. (2002) argued that a major cause of the inconsistent support for the PSH is an inconsistency in the measurements of stress used. Frequent measures of stress in plants include different estimates of productivity, plant growth, biomass, shoot:root ratios and physiological parameters, such as leaf water deficit and plant secondary chemistry. As suggested by Moller (1995), an objective definition of environmental stress would advance our understanding of the relationship between plant stress and herbivory.

During recent years, it has been claimed that developmental instability reflects the inability of organisms to control developmental processes during ontogeny and to achieve a predetermined phenotypic optimal expression (Moller & Swaddle 1997). One measure of developmental instability is fluctuating asymmetry (FA) that represents small, random variations from symmetry in otherwise bilaterally symmetrical characters. Leaf fluctuating symmetry has been used as an objective measurement of the effects of environmental stress on plants (e.g., Martel *et al.* 1999, Roy & Stanton 1999, Alados *et al.* 2001). Individual- and population-levels of bilateral FA have been related to several biotic and abiotic stresses, including environmental factors, such as nutrition, temperature, radiation, and pollution, as well as genetic factors, such as mutation, inbreeding, and hybridisation. FA is then suggested as a reliable stress estimator and measures of FA could thus represent sensitive indicators of the developmental performance of organisms in their environment and biomonitors of how organisms are able to deal with deviant environmental and genetic conditions.

In addition to being an indicator of plant stress, some studies have shown correlations between FA and insect herbivore attack, suggesting leaf FA can be used not only as an indicator of plant stress, but also plant susceptibility to herbivory (e.g., Wiggins 1997, Zvereva *et al.* 1997). Plants with more asymmetric leaves or higher levels of leaf asymmetry should exhibit increased levels of herbivory due to higher nutritional quality of asymmetric leaves compared to symmetric leaves (Sakai & Shimamoto 1965, Lempa *et al.* 2000). Little is known as to how developmental disorders are connected to plant metabolism and the associated biochemical changes exhibited by asymmetric leaves (Lempa *et al.* 2000), but, since the left and the right sides of a bilaterally symmetrical trait develop under the control of the same genes, minor deviations from perfect symmetry actually represent developmental instability and may be responsible for differences in nutritional quality or secondary chemistry between asymmetric and symmetric leaves. Positive correlations between FA and herbivory indicate either that plants with asymmetric leaves are, on average, more susceptible to attack by herbivores, and/or that herbivory itself acts as a stressor and directly increases the level of leaf asymmetry. Although some authors favour the idea that herbivores themselves can act as stressors increasing leaf asymmetry (e.g., Zvereva *et al.* 1997), correlations between leaf FA and herbivory are not always likely to be causal (Lempa *et al.* 2000). Instead, chemical and nutritional differences between symmetric and asymmetric leaves may influence leaf selection by herbivores, which leads to positive correlations between herbivory and FA.

This study aimed to examine the relationship between leaf fluctuating asymmetry and herbivores on *Quercus geminata* and *Q. laevis.* We addressed the relationship between herbivory and FA by examining the community of herbivores attacking these two oak species and how they respond to random variations in leaf morphology. Leaf miners (*Stilbosis quadripustulatus, Brachys tesselatus,* and *Acrocercops albinatella*), leaf gallers (eyespot galls and *Belonocnema quercusvirens*) and leaf chewers (*Hemileuca maia, Orgyia leucostigma*) were studied. The following hypotheses were tested: 1)

Fluctuating asymmetry, plant stress and herbivory: fluctuating asymmetry in otherwise symmetrical bilateral traits is a surrogate of plant stress and asymmetrical leaves should differ in nutritional quality and herbivore susceptibility compared to symmetrical leaves; 2) Fluctuating asymmetry *between-individuals* and frequency of herbivory: if FA in leaves predicts plant susceptibility to herbivores, plants with more asymmetric leaves or higher degrees of asymmetry should be subject to higher levels of herbivory than individual plants with a lower incidence of foliar asymmetry; 3) Fluctuating asymmetry *within-individuals* and frequency of herbivory: frequency of herbivory in asymmetrical leaves should be higher than frequency of herbivory in symmetrical leaves or leaves with lesser degrees of asymmetry; 4) Fluctuating asymmetry and the slow-growth, high-mortality hypothesis (Clancy & Price 1987): herbivores feeding on leaves with lower nutritional quality or digestibility should take longer to develop and would be more susceptible to natural enemies. Therefore, insects feeding on asymmetrical leaves should exhibit higher survivorship than insects feeding on symmetrical leaves.

STUDY SYSTEMS

Sand live oak, *Quercus geminata* (Fagaceae), is a semi-evergreen oak and, typically, old leaves abscise and new leaves appear in late April or early May, reaching full size in approximately 2 weeks. The leaves are rounded and persistent with deeply revolute, conspicuous impressed veins on the underside and the bases and sides of the leaves are observed to be asymmetric in many instances. *Stilbosis quadripustulatus* (Lepidoptera: Cosmopterygidae) is a moth whose larvae induce mines on the adaxial surfaces of *Q. geminata*. *S. quadripustulatus* is a univoltine species, whose adults emerge in early summer (from May to June) from pupae that overwinter in soil and litter. Oviposition occurs approximately in early June, when females oviposit at the junction of the midvein and a major lateral vein. Larvae take from 60 to 90 days to complete their 5 instars and mines may reach 3.0 cm in length (Simberloff & Stiling 1987). Many other herbivores compose the insect community associated with *Q. geminata*. Leaves are frequently found damaged by chewing insects such as the eastern buck moth *Hemileuca maia* (Lepidoptera: Saturniidae), and at least 4 cynipid species (Hymenoptera: Cynipidae) of galling insects are commonly observed on sand live oak leaves and stems: *Andricus quercusfoliatus*, *Disholcaspis quercussuccinipes*, *Callirrhytis quercusbatatoides*, and *Belonocnema quercusvirens*. *A. quercusfoliatus* induces white flower-like galls on sand live oak stems, whereas *D. quercussuccinipes* wasps induce clusters of 5-20 yellowish brown galls usually crowded around a terminal oak twig. *C. quercusbatatoides* wasps induce abrupt swellings of twigs, varying in form and size and *B. quercusvirens* induces tan, globular pea-like galls on the underside of *Q. geminata* leaves. Galls are unilocular and occur in large numbers during the fall. Eyespot galls (Diptera: Cecidomyiidae) are recognized as circular spots, usually 8-10 mm in diameter. The adults emerge from the soil in the spring and lay eggs in the upper leaf surface. As the larva grows, the leaf tissue surrounding it swells slightly and red rings are seen around the galls. Larvae complete their development in 8-12 days and pupate in the soil. This is the most common gall found on sand live oak leaves, often reaching densities of 5 galls per leaf.

The turkey oak *Quercus laevis* is one of the characteristic trees associated with the sand hill community over much of Florida. *Q. laevis* is a moderately fast to fast-growing tree and presents deciduous simple leaves, alternately arranged with usually 5 lobes, although this number may vary from 3 to 7. Although a common tree in Florida native vegetation, there are relatively few studies concerning herbivory in this plant species. *Acrocercops albinatella* (Lepidoptera: Gracillaridae) is a microlepidopteran species whose larval stages feed on young leaves, creating distinct linear-blotch mines on the lower surface of *Q. laevis* leaves. Larvae typically feed on the palisade parenchyma cells and deposit frass throughout the mine, completing their development in approximately 10 days. Larvae emerge from the blotch mine and usually pupate on the same leaf from which they emerge (T Cornelissen, pers. obsv.). *Brachys tesselatus* (Coleoptera: Buprestidae) is a univoltine species that also forms distinct blotch mines in *Q. laevis* leaves. The adults emerge in Mid-March to Mid-April, coinciding with budburst of turkey oak. Adults initially feed on the early leaves and flowers until mating and oviposition. Eggs are deposited singly on the upper surface of the leaves and after hatching the larvae mine into the mesophyll creating distinct, characteristic damage. Contrary from what happens in South Carolina (Waddell *et al*. 2001) *Brachys* in our study sites go through two generations, instead of just one. The first mines appear in early April and remain active until late June, when larvae complete their development and pupate inside the mines. New adults emerge in early July and oviposit to form new *Brachys* mines that remain active until September-October. Pupation and overwintering of this second generation occurs within the leaves after they have senesced and abscised from the tree. New adults emerge from the leaf litter in the following spring (Waddell *et al*. 2001). Turkey oak leaves are also attacked by a vast array of herbivores, such as the leaf roller weevil *Homoeolabus analis* (Coleoptera: Atellabidae), the eastern buck moth *H. maia*, the white tussock moth *Orgyia leucostigma* (Lepidotpera: Lymantriidae) and other leaf miners such as *Stigmella* (Lepidoptera: Nepticulidae) and *Cameraria* (Lepidoptera: Gracillariidae).

METHODS

Data Collection

Patterns of leaf asymmetry, leaf quality and herbivory were examined for 30 individuals of *Q. geminata* and 30 individuals of *Q. laevis* from March to October 2002 at the University of South Florida Botanical Garden, Tampa, Florida. To verify the relationship between FA and leaf quality and to examine the frequency of occurrence of asymmetric leaves and levels of leaf asymmetry on each plant, 40 leaves were sampled from each individual plant in April 2002. Because herbivores themselves may act as plant stressors, these leaves were sampled before the beginning of mine initiation and before leaves were damaged by free-feeding herbivores. To quantify *Quercus geminata* fluctuating asymmetry, widths of all leaves were measured on both the right and the left side, from the leaf edge to the midrib, at the middle point of the leaf, which usually coincides with the widest part of the leaf. *Q. laevis* exhibited some variation in the number of leaf lobes, but 83% of the leaves we sampled exhibited 3 pairs of lobes and measurements were taken between the first and second pairs of lobes. These distances were measured after photographing each leaf with a digital camera at a standard distance of 30 cm in the laboratory and analyzing leaf length, leaf area, and right and left widths using the software UTHSCSA Image Tool (University of Texas, USA). All the digital pictures were calibrated to the nearest 0.01mm before measurements were taken and the resolution set to the software did not allow measurement errors greater than 1.0%.

Absolute asymmetry in leaf width was defined as the unsigned difference between right width (RW) and left width (LW) of a particular leaf as $FA_{width} = |RW - LW|$ (Figure 6.1). The absolute value of right-left traits is a good estimator of variance in FA among leaves assuming that there is no directional asymmetry (consistently larger left or right side) or antisymmetry (consistent lack of symmetry, but in no particular direction). To examine differences in nutritional quality between symmetric and asymmetric leaves, all the leaves sampled from each plant were analyzed for water, nitrogen content, and tannin concentration. Water content was quantified by the difference between leaf wet and dry weights after leaves were oven-dried. Leaves were then milled to a fine powder. Tannins were extracted from 50 mg of dry tissue, and tannin concentration was quantified using the radial diffusion assay (for details see Hagerman 1987). The average of three replicates per leaf was used for statistical analysis. Nitrogen content was determined using a CHN analyzer.

To verify the relationship between plant fluctuating asymmetry and herbivory *between-individuals*, we used data on asymmetry from the 40 leaves collected from each plant to calculate two indices of FA (Palmer & Strobeck 1986):

$$Index \quad 1 = \frac{\sum |R_i - L_i|}{N}$$

$$Index \quad 2 = \frac{\sum \left[\frac{|R_i - L_i|}{(R_i + L_i)/2} \right]}{N}$$

where R_i is the value of the right side, L_i is the value of the left side and N is the number of measurements taken. Index 1 is the absolute fluctuating asymmetry and it is the most intuitive asymmetry measurement (Roy & Stanton 1999). Index 2 is size-scaled, calculated as the absolute value of right (R_i) minus left (L_i) sides divided by the average ($R_i + L_i$) /2, to correct for the fact that asymmetry may be size-dependent. These indices were then correlated with the density of leaf miners, galls, and chewed leaves recorded on each individual plant. *Quercus* plants were monitored for the occurrence of herbivores and the number of *Acrocercops, Stilbosis,* and *Brachys* mines were quantified in October 2002 by recording the number of mines in 200 leaves on each plant. Leaf galls were counted in 200 leaves on each plant and stem galls were quantified by counting 100 twigs on each individual in September 2002. We also recorded the number of other leaf miners and chewed leaves on both plant species by counting 100 leaves of each plant in October 2002.

To examine the relationship between FA and herbivory *within*-individuals, 40 mined leaves from each leaf miner species were collected from each plant and the 40 nearest neighbouring leaves without mines were collected from the same individual plant. Leaves were oven-dried between sheets of filter paper, mounted as herbarium specimens and asymmetry measurements of mined and unmined leaves were calculated for each leaf miner species as described before. If the difference between right and left widths (RW-LW) was different from zero (either positive or negative) leaves were categorized as asymmetric, and if the difference between right and left widths was equal to zero leaves were categorized as symmetric. However, since we used only 2 decimal places when categorizing FA values and calculating FA indices, leaves with FA measurements ranging between 0.001 and 0.09 mm were rounded to zero and categorized as symmetric, in a more conservative approach. Leaves yielding absolute FA values equal or greater than 0.1mm (either positive or negative) were all classified as asymmetric since we believe the resolution of the equipment we used is quite accurate, as revealed by the small error range (1.00% or 0.001 mm) and high values obtained for the Index of Repeatability for both plant species (see Results). To examine herbivore distribution between symmetric and asymmetric leaves, for each species of leaf miner each pair of leaves collected on each plant was categorized as 1=mined leaf, symmetric: unmined, symmetric, 2= mined leaf, symmetric: unmined, asymmetric, 3= mined leaf, asymmetric: unmined, asymmetric and 4 = mined leaf, asymmetric: unmined, symmetric. To test the relationship between FA within plants and leaf gallers, we sampled 20 leaves galled by *Belonocnema* and 20 leaves with eyespot galls and the nearest 20 non-galled leaves and each pair of leaves was placed in one of the 4 categories as described above.

To test for differences in mine survivorship between symmetric and asymmetric leaves, 50 mines of each leaf miner species were marked in asymmetric leaves and another 50 in symmetric leaves. Mined leaves were classified as symmetric or asymmetric after photographing 300 mined leaves in the field and taking measurements as described before. To account for individual variation in leaf miner development related to individual oak plants, no more than 6 mines (3 on symmetric leaves, 3 on asymmetric leaves) were marked on each plant. All mines (n = 300) were permanently marked using a Sharpie® pen as soon as the eggs hatched and larvae initiated mine formation. *Acrocercops* mines were measured at 2-day intervals by tracing the numbered mines using acetate sheets, whereas *Brachys* mines were traced at 3-day intervals and *Stilbosis* mines were traced at 5-day intervals. Mine drawings were also digitalized and mine size was measured using the software UTHSCSA Image Tool, with digital pictures calibrated to the nearest 0.01mm. We compared mine size, developmental time (days to pupation) and mine growth rate on symmetric and asymmetric leaves for each one of the leaf miner species. Mine growth rate on each leaf type was calculated as Growth rate = (Final mine size – Initial mine size) / number of days mine was growing. After mine termination, all leaves were inspected under a stereomicroscope to assess leaf miner survivorship and identification of mortality factors. Leaf miners offer a great opportunity to assess population survivorship and mortality factors since a record of the miner success is clearly observed on the leaves: parasitized mines have tiny circular exit holes left by the parasitoid on mine's surface and predated mines are usually found ripped open. Successfully emerged larvae cut semi-circular exit holes on the mine underside towards the apex (Simberloff & Stiling 1987).

Data Analysis

Leaf characters demonstrate FA if signed right-minus-left values are normally distributed with a mean value of zero, reflecting randomly directed deviations from the optimal symmetrical phenotype. One sample t-tests and Lilliefor's tests (Wilkinson 1999) were used to test whether mean values of signed right-minus-left values differed significantly from zero. Asymmetry was calculated as the absolute difference between right and left widths of a particular leaf. However, asymmetry of undamaged leaves of both plant species increased with leaf size (*Q. laevis*: $r=0.412$, $P<0.01$; *Q. geminata*: $r=0.361$, $P<0.05$) and so measurements of asymmetry on leaf width were further corrected for leaf size according to the formula $FA_{width}=2*|RW-LW| / (RW+LW)$. According to Palmer (1996), an important consideration in asymmetry studies is measurement error, since errors may look like asymmetry, requiring that either the symmetry differences measured are larger than the measurement error, or that subsequent measurements taken on the same leaf are highly correlated. We estimated measurement error by remeasuring 10% of the leaves collected from each plant species 10 days after the first measurements were taken and the two measurements were correlated using an index of repeatability (Falconer 1981).

All the variables analysed were first submitted to Lilliefor's test for data normality and transformations (angular, log-transformation and centering) were employed to stabilize variances and normalize the data. However, for the sake of clarity, figure axes and means (+1SEM) show untransformed data. To test for differences in nutritional quality between symmetric and asymmetric leaves, we averaged water, tannin, and nitrogen content of all symmetric and asymmetric leaves within each individual plant (n = 30 for each species) and differences between the two leaf types were examined using one-way ANOVAs. To examine the relationship between plant fluctuating asymmetry and herbivory *between-individuals*, we regressed the percentage of asymmetric leaves and the two FA indices calculated for each plant species with the density of leaf miners (*Brachys*, *Acrocercops*, *Stilbosis,* other mines), galls, and percentage of leaves attacked by folivores. Other mines included *Cameraria*, *Buccalatrix*, and *Stigmella* mines that were present on the leaves, but in low abundance compared to the other leaf miners. A stepwise interactive multiple regression (Wilkinson 1999) was used to examine which factors predicted leaf miner abundance and predictors with low tolerance values (<0.10) and high collinearity were excluded from the model. To verify the relationship between FA and herbivory *within*-individuals we used One-Way ANOVAs to test for differences in FA between mined and unmined leaves of *Q. laevis* and Q. *geminata*. Pairs of mined-unmined leaves and galled-non-galled leaves were classified into 4 categories as described before (data collection) and differences in the frequency of these categories were tested using a chi-square test. Preference for each leaf type was assumed if mines/galls were found more frequently in a particular leaf type than would have been expected as a result of a simple chance encounter with leaves of both types (symmetric, asymmetric). To examine the relationship between leaf types and herbivore survivorship, differences in mine growth, days to pupation and growth rates of leaf miners in symmetric and asymmetric leaves were tested using One-Way ANOVAs.

Some authors have stated that herbivores themselves may cause leaf asymmetry due to their feeding activities. We hypothesized that if leaf miners themselves cause asymmetry, we would expect that mined sides of leaves would be larger/wider than unmined sides when the entire mine is encountered on a particular side (right or left) of the leaf. To test this hypothesis, we performed paired t-tests over two scales: plants and leaves. For the leaf scale, we conducted a one-tailed paired t-test with all the mines sampled that were located on a particular side of the leaf of *Q. laevis* (n = 314 *Brachys*) and *Q. geminata* (n = 1017 *Stilbosis* mines). For the plant scale, we also performed a paired t-test comparing measurements of asymmetry of 20 undamaged leaves collected before herbivory (April 2002) with 20 undamaged leaves collected from each plant after herbivory and after the second and partial flush of new leaves in July of 2002.

To verify whether increased herbivory in one year influences asymmetry in the following season, we compared levels of FA of 30 sand live oaks measured in 2002 with levels of FA of these same plants in 2003. Thirty individuals between 0.7 and 1.3 m in height were monitored from April 2002 to June 2003. Twenty undamaged leaves were sampled from each plant in August 2002 for FA measurements and plants were monitored for *Stilbosis* occurrence from May to October 2002, when we recorded the number of *Stilbosis* mines on 200 leaves on each plant. In June of 2003 we again sampled twenty new but fully developed undamaged leaves from each plant for fluctuating asymmetry measurements as described before. These measurements were further compared with leaf FA and herbivory rates recorded for the previous year.

RESULTS

Tests for asymmetry on Q. laevis and Q. geminata

Q. laevis and *Q. geminata* demonstrated similar patterns of leaf asymmetry before herbivory, as signed right-minus-left character values were normally distributed (Lilliefor's tests, P=0.14-0.61) and did not deviate significantly from zero in all data sets tested (One-sample t-tests, P= 0.29-0.74), showing no evidence of antisymmetry or directional asymmetry. The data set used to test the relationship between FA and herbivory within individuals also exhibit the normal distribution with a mean that does not significantly deviate from zero, except for leaves mined by *Acrocercops* (*Brachys*: mean RW-LW = 0.017, t=0.598, P= 0.55; *Stilbosis*: mean RW-LW = 0.029, t=1.016, P= 0.31; *Acrocercops:* mean RW-LW = -0.065, t=-2.777, P= 0.006). The mean repeatability of FA measurements was high for both *Q. laevis* (Index of repeatability = 0.905; $F_{119,120}$ = 19.46, P <0.0001) and *Q. geminata* (Index of repeatability = 0.966; $F_{119,120}$ = 21.57, P <0.0001), indicating small measurement errors and the reliability of FA measurements. *Q. laevis* leaves were, on average, more asymmetric than *Q. geminata* leaves. Values of FA index 1, for example, ranged between 0.065 and 0.882 (average: 0.469 ± 0.045) for turkey oaks and between 0.100 and 0.576 (average: 0.266 ± 0.020) for sand live oaks.

Fluctuating asymmetry and leaf quality

Asymmetric leaves of both plant species exhibited better nutritional quality for herbivores than symmetric leaves within the same individual plant (Figure 6.2). Asymmetric leaves contained significantly lower concentrations of tannins (*Q. laevis*: $F_{1,58}$=18.19; $P<0.0001$; *Q. geminata*: $F_{1,58}$=12.14; $P<0.001$) and higher nitrogen content (*Q. laevis*: $F_{1,58}$=4.50; $P<0.05$; *Q. geminata*: $F_{1,58}$=4.79; $P<0.05$) than symmetric leaves. No differences in water content were observed between symmetric and asymmetric leaves of either plant species ($P>0.05$). When we regressed tannin concentration and FA indices calculated for each plant, we observed that variation in FA index 2 explained 13.0% of the variation in tannin concentration of *Q. geminata* ($F_{1,28}$=8.12, $P<0.05$), and 16% of the variation in tannins of *Q. laevis* leaves ($F_{1,28}$=5.49, $P<0.05$), but no significant relationship was observed between the variation in FA indices and nitrogen for both plant species ($P>0.05$).

Fluctuating Asymmetry between individuals and herbivory

Herbivore abundance on the two oak species exhibited a tendency to vary with both the frequency and the levels of asymmetry on individual plants. *Q. laevis* plants with a higher percentage of asymmetric leaves were significantly more attacked by *Brachys* (r^2=0.279, $F_{1,28}$=10.84, $P<0.005$; Figure 6.3) and other leaf miners (r^2=0.259, $F_{1,28}$=9.81, $P<0.005$). Similar patterns were observed for herbivores attacking *Q. geminata*, and the percentage of asymmetric leaves explained 31.8% of the variation of *Stilbosis* ($F_{1,28}$=13.06, $P<0.001$; Figure 6.3) and other mines (r^2=0.125, $F_{1,28}$=11.46, $P<0.005$). No significant relationship was observed between the percentage of asymmetric leaves on turkey oaks and the abundance of *Acrocercops* mines and between the percentage of asymmetric leaves and chewers and stem gallers for both oak species (all $P>0.05$).

Variation in levels of asymmetry between plants also influenced leaf miners abundance. Plants with higher FA indices exhibited higher densities of *Brachys* and *Stilbosis* mines (Figure 6.4). Variation In FA index 2 (size-scaled) explained 38.8% of the variation in *Brachys* abundance between plants ($F_{1,28}=17.72$, $P<0.001$) and 37.8% of the variation in *Stilbosis* mines among individual sand live oak plants ($F_{1,28}=17.04$, $P<0.001$). Variations in levels of asymmetry also influenced the abundance of other leaf miners mines – mainly *Cameraria* and *Stigmella* - in both plant species (*Q. geminata*: $r^2 = 0.303$, $F_{1,28}=12.16$, $P<0.005$; *Q. laevis*: $r^2 = 0.263$, $F_{1,28}=9.96$, $P<0.005$). No significant relationship was observed between variation in FA indices and variation in densities of *Acrocercops* mines in *Q. laevis*, eyespot galls on *Q. geminata* and chewed leaves on both plant species (all $P>0.05$).

Although variation in both the percentage of asymmetric leaves and levels of asymmetry of *Q. geminata* tended to positively influence *Belonocnema* galls ($r^2=0.10$, $P=0.071$), no significant relationship was observed between variation in levels of FA and variations in densities of other leaf and stem galling species studied (*Andricus:* $r^2=0.09$, $P=0.11$; *Disholcaspis*: $r^2=0.06$, $P=0.32$; *Callirhytis*: $r^2=0.10$, $P=0.09$; eyespot galls: $r^2=0.04$, $P=0.42$).

A Pearson correlation matrix revealed high collinearity among predictors of leaf miner abundance between plants, such as the percentage of symmetric and asymmetric leaves in a plant (*Q. laevis*: $r= 0.98$, *Q. geminata*: $r=0.97$) and the two FA indices used (*Q. laevis*: $r= 0.974$, *Q. geminata*: $r=0.913$). These correlations generated multiple regressions coefficients with low tolerance and all correlated predictors were standardized by centering each value to generate a mean of zero for each predictor (Quinn & Keough 2002). An interactive stepwise multiple regression revealed that 67.4% of the variation in *Brachys* abundance among plants was explained by variation in FA index 1, the amount of tannins in leaves and variation in *Q. laevis* leaf area (r^2=0.674, $F_{5,24}$=9.902, P<0.0001). Variation in *Stilbosis* abundance was also influenced by variation in asymmetry among plants, since almost 62% of the variation was explained by variation in FA index 1, the percentage of asymmetric leaves in a plant and the presence of other leaf miners (r^2=0.619, $F_{4,25}$=10.16, P<0.0001). For *Acrocercops* mines asymmetry was not important because 38% of the variation among plants was explained by tannin concentration and the amount of nitrogen on leaves alone (r^2=0.380, $F_{2,27}$=8.288, P<0.005).

Fluctuating asymmetry within individuals and herbivory

Leaves attacked by both *Brachys* and *Stilbosis* were, on average, more asymmetric than unmined leaves within the same individual plant (Figure 6.5a). Leaves mined by *Brachys* were, on average, 4.3 times more asymmetric than neighboring unmined leaves ($F_{1,58}$= 39.67, P<0.0001) , while leaves mined by *Stilbosis* were approximately 2.6 times more asymmetric than unmined leaves ($F_{1,58}$= 43.39, P<0.0001). However, no significant differences in asymmetry were observed between neighbouring mined and unmined leaves attacked by *Acrocercops* (average absolute FA of mined leaves: 0.265mm ± 0.027, average absolute FA of unmined leaves: 0.276mm ± 0.035).

Leaf miners were more frequently encountered in asymmetric leaves compared to symmetric ones in both plant species (*Q. laevis*: $\chi^2 = 65.84$, $P < 0.0001$; *Q. geminata:* $\chi^2 = 60.31$, $P < 0.0001$; Figure 6.5b), although frequency of occurrence of categories of leaf types was different for each one of the leaf miner species. For *Brachys* mines, for example, in approximately 61% of the cases the mined leaf was asymmetric and the unmined leaf in the pair was symmetric, whereas for *Acrocercops* mines, a more even distribution of the mines was observed among the four possible categories (Figure 6.5b). For *Stilbosis* mines, in 61.4% of the cases, the mined leaf was asymmetric and the unmined leaf was symmetric, and in approximately 30% of the cases, both leaves in a pair were asymmetric. In cases where both leaves were asymmetric, for both *Brachys* and *Stilbosis*, mean asymmetry of the mined leaf was significantly higher than asymmetry of the nearest unmined leaf (*Brachys*: $F_{1,58} = 11.78$; *Stilbosis*: $F_{1,58} = 14.63$; both $P < 0.005$).

For both *Belonocnema* and eye spot galls, we observed no significant differences in frequency of occurrence among the 4 categories of galled-nongalled leaves (*Belonocnema:* $X^2 = 2.78$, $P > 0.05$; eyespot galls: $X^2 = 4.31$, $P > 0.05$) and no differences in asymmetry were found between the galled and the nearest non-galled leaf (*Belonocnema:* average FA galled leaf = 0.213mm ± 0.019, average FA of non-galled leaf =0.209mm ± 0.026; eyespot galls: average FA galled leaf = 0.236mm ± 0.023, average FA of non-galled leaf =0.239mm ± 0.025; all $P > 0.05$).

Fluctuating asymmetry and mine survivorship

Mines of all three species were, on average, smaller on asymmetric leaves than on symmetric ones (*Brachys*: $F_{1,98} = 5.90$, $P < 0.05$, *Stilbosis*: $F_{1,98} = 32.77$, $P < 0.0001$, *Acrocercops:* n.s.; Figure 6.6a) and *Brachys* and *Stilbosis* mines on asymmetric leaves exhibited significantly slower growth rates, consuming less plant tissue than mines growing on symmetric leaves (Brachys: $F_{1,49}$=13.89, P<0.001; *Stilbosis*: $F_{1,22}$=15.601, P<0.001; Figure 6.6b). Although *Brachys* and *Stilbosis* mines were smaller and consumed less tissue on asymmetric leaves, no significant differences in the number of days necessary to pupation were observed between the two leaf types (Figure 6.7a, all P>0.05). Also, no significant differences in mine size, growth rates or days to pupation were observed for *Acrocercops* mines growing on symmetric and asymmetric leaves of *Q. laevis.*

Mortality imposed by top-down or bottom-up factors differed among the leaf miners studied. *Acrocercops* mines exhibited the highest survivorship among the species studied, with 68% of the mines developing until pupation, compared to 51% of survivorship of *Brachys* mines and 49% of survivorship for *Stilbosis* mines. Parasitism by micro hymenopterans was responsible for mortality of 23% of *Brachys* mines, while predation and plant resistance (larvae found dead inside the mine) accounted for 38% of the mortality of *Stilbosis* mines. For the *Stilbosis* mines that survived to pupation, we observed a significantly higher survivorship of mines on asymmetric compared to symmetric leaves (X^2 = 24.51, P<0.05; Figure 6.7b). No significant differences in survivorship between symmetric and asymmetric leaves were observed for *Brachys* or *Acrocercops* mines (P>0.05).

Do herbivores cause asymmetry?

At the plant scale, paired t-tests revealed that the mean difference in relative FA before and after herbivory did not depart significantly from zero for both *Q. laevis* (mean difference before and after herbivory: 0.003 mm, 95% CI: = -0.005 to 0.011, t=0.689, P=0.469) and *Q. geminata* (mean difference before and after herbivory: 0.002 mm, 95% CI: = -0.001 to 0.006, t=1.271, P=0.214). Paired t-tests conducted at the leaf scale also revealed no significant differences in leaf width between mined and unmined sides of *Q. laevis* leaves attacked by *Brachys* (mean width of mined side of leaf = 7.429; mean width of unmined side of leaf = 7.412; t = 0.604, P = 0.550) or between mined and unmined leaves of *Q. geminata* leaves attacked by *Stilbosis* (mean width of mined side of leaf = 2.612; mean width of unmined side of leaf = 2.587; t = 1.192, P = 0.243). These results suggest that there was no direct relationship between the presence of these leaf miners and changes in width of the mined side of the leaves.

The number of *Stilbosis* mines per 200 leaves on the 30 plants studied between 2002 and 2003 ranged between 7 and 48 mines (mean: 26.23 ± 2.27) but higher attack rates in some plants did not influence FA measurements in the following year, since we observed a high correlation between FA measurements in 2002 and 2003 for each one of the 30 plants studied (r^2= 0.86, n =30). These results demonstrated that although herbivory rates varied among individuals, plants with higher number of mines in one year did not exhibit higher FA in the following year, demonstrating a consistency of variation in FA among individual plants during two years.

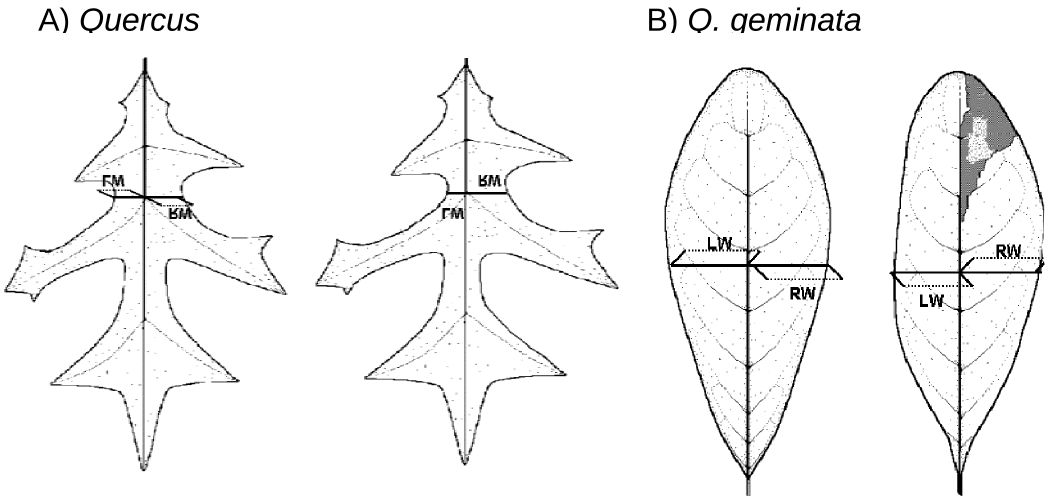

Figure 6.1- Schematic representation (not to scale) of measurements used to define fluctuating asymmetry in A) *Quercus laevis* and B) *Q. geminata*. RW = right width and LW = left width.

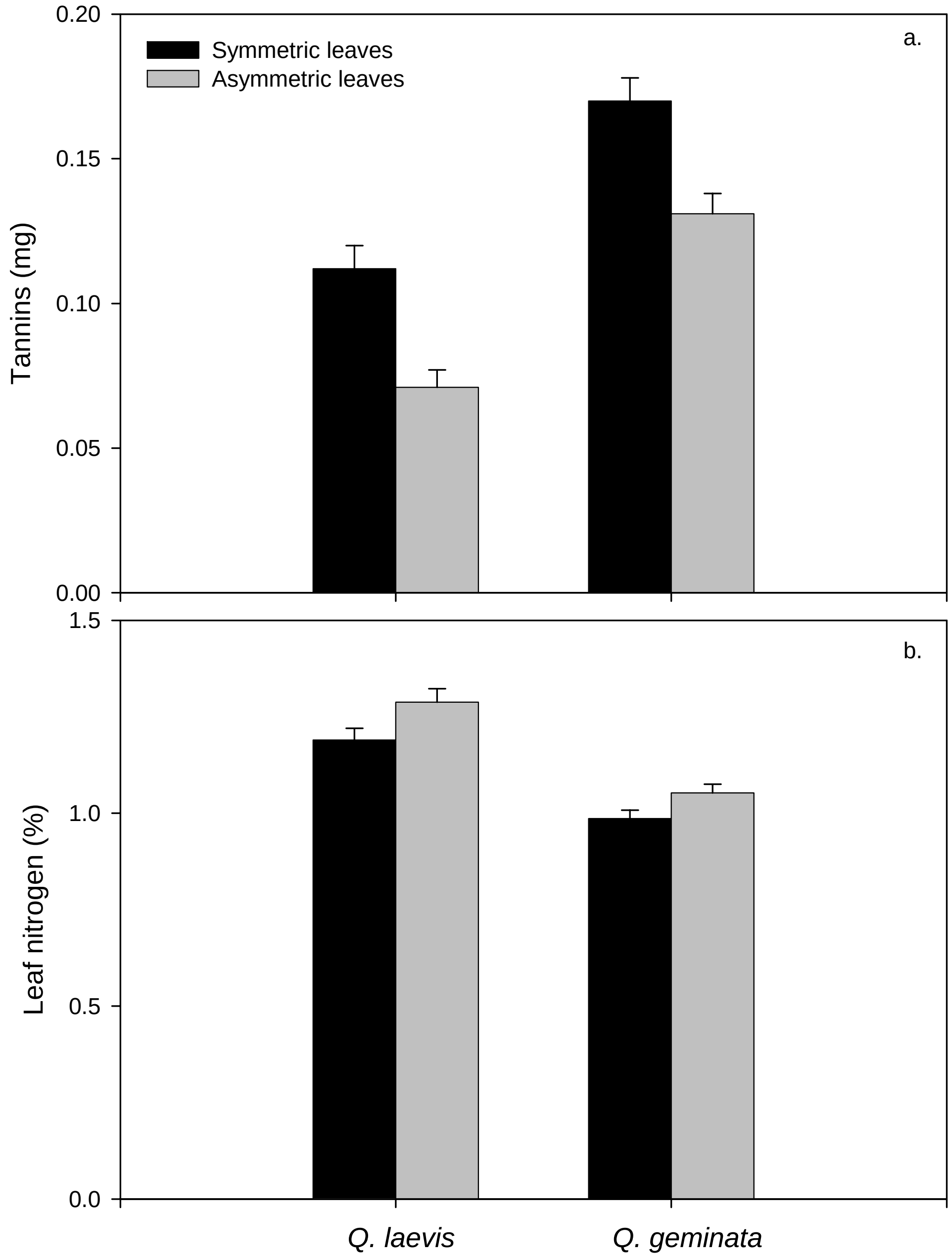

Figure 6.2- Differences in (a) tannin concentration and (b) nitrogen content between symmetric and asymmetric leaves of *Q laevis* and *Q. geminata*. Bars indicate mean ± 1SE.

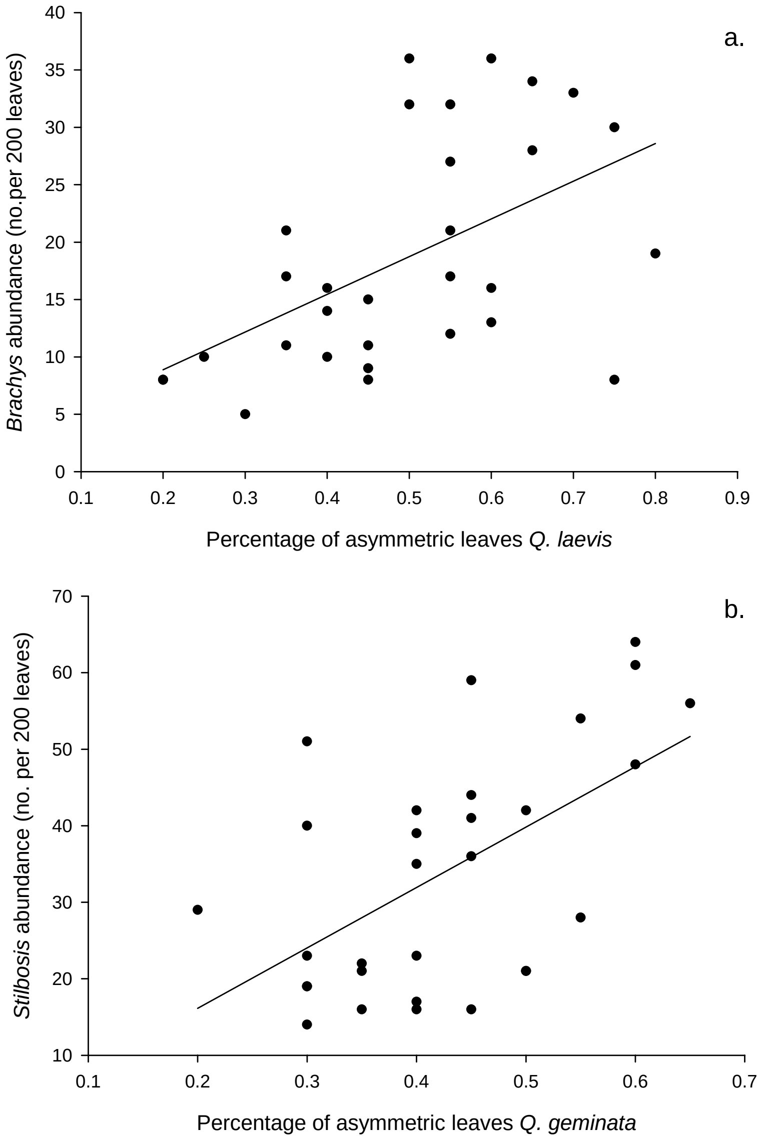

Figure 6.3-. Relationship between the abundance of a) *Brachys* mines and the percentage of asymmetric leaves on the host plant *Q.laevis* (r^2=0.279, P<0.005) and b) *Stilbosis* mines and the percentage of asymmetric leaves on *Q. geminata* (r^2=0.318, P<0.001). Mine abundance was determined by counting the number of mines of each leaf miner species on 200 leaves per plant.

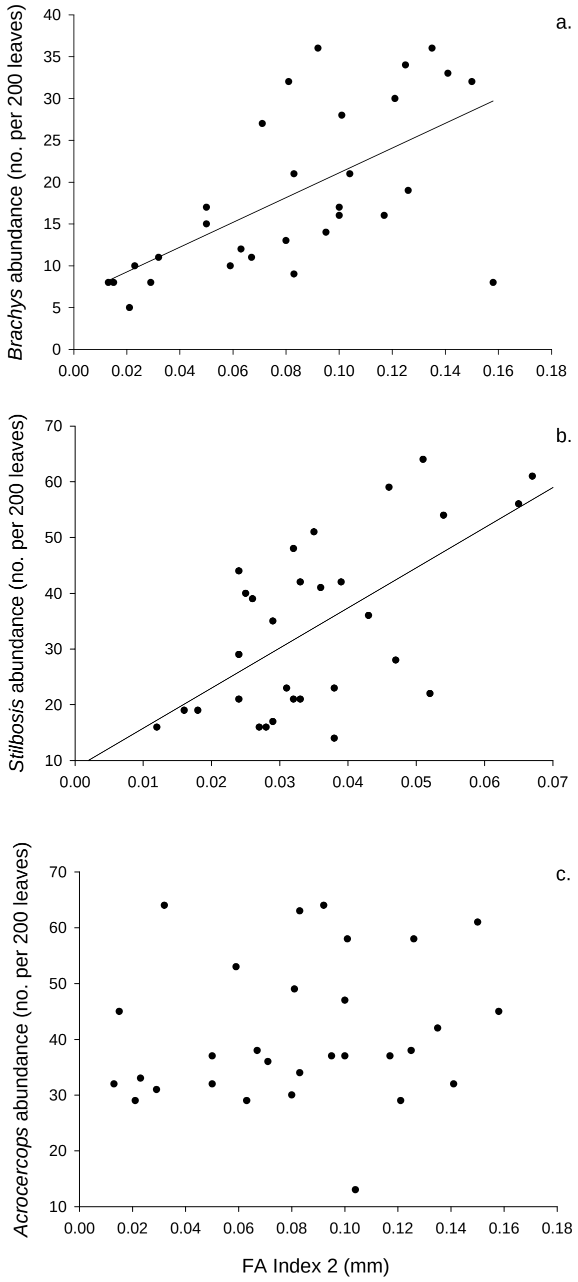

Figure 6.4- Relationship between the abundance of mines caused by a) *Brachys* (r^2=0.475, P<0.001), b) *Stilbosis* (r^2=0.394, P<0.001), and c) *Acrocercops* (r^2=0.018, P>0.05) and the levels of relative asymmetry (FA Index 2) on host plants. FA index 2 refers to the asymmetry index that is size-scaled, calculated as the absolute value of right (R_i) minus left (L_i) sides divided by its average ($R_i + L_i$) /2.

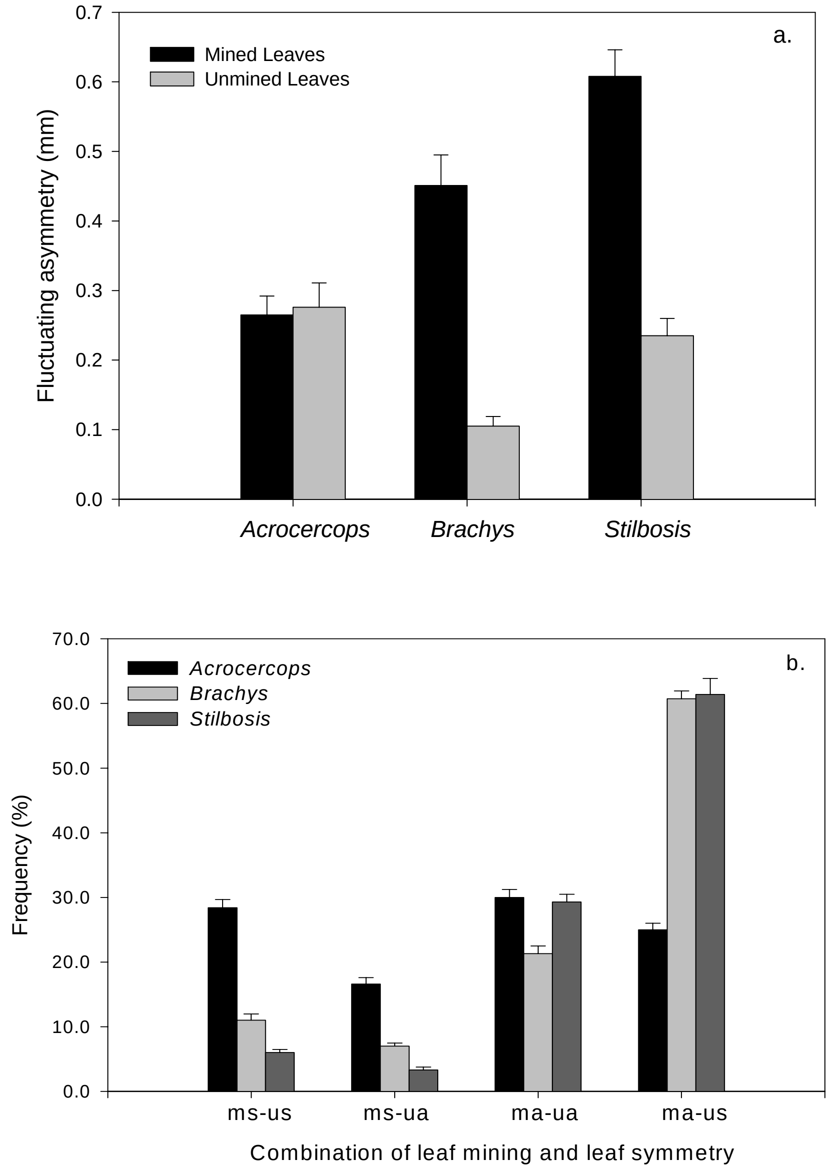

Figure 6.5- Differences in a) fluctuating asymmetry between mined and unmined leaves attacked by *Acrocercops, Brachys* and *Stilbosis* and b) frequency of occurrence of combinations of asymmetric and symmetric leaves on pairs of mined and unmined leaves for each species studied (ms-us= mined leaf, symmetric: unmined, symmetric, ms-ua= mined leaf, symmetric: unmined, asymmetric, ma-ua= mined leaf, asymmetric: unmined, asymmetric and ma-us= mined leaf, asymmetric: unmined, symmetric). Bars indicate mean ± 1SE.

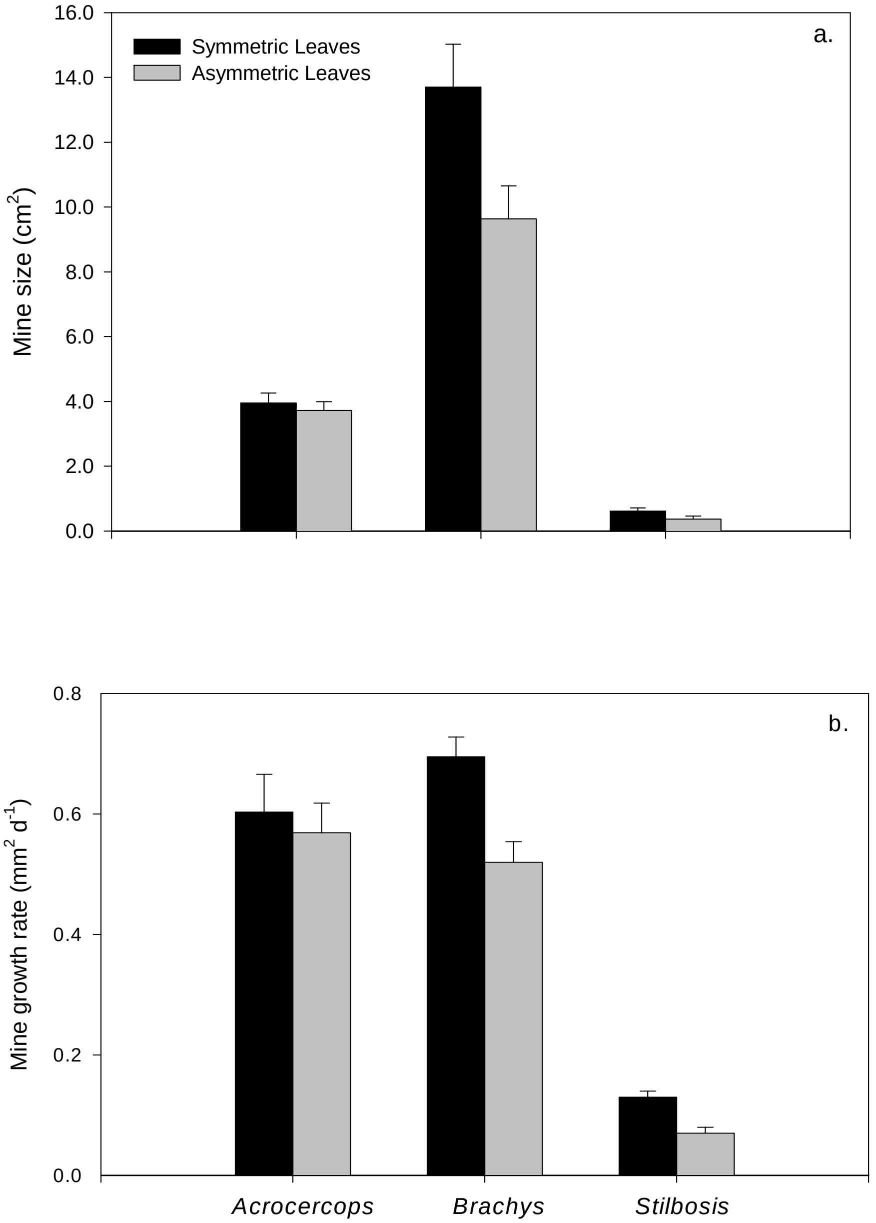

Figure 6.6- Differences in a) mine size and b) mine growth rate between symmetric and asymmetric leaves attacked by *Acrocercops*, *Brachys* and *Stilbosis* leaf miners. Bars indicate mean ± 1SE.

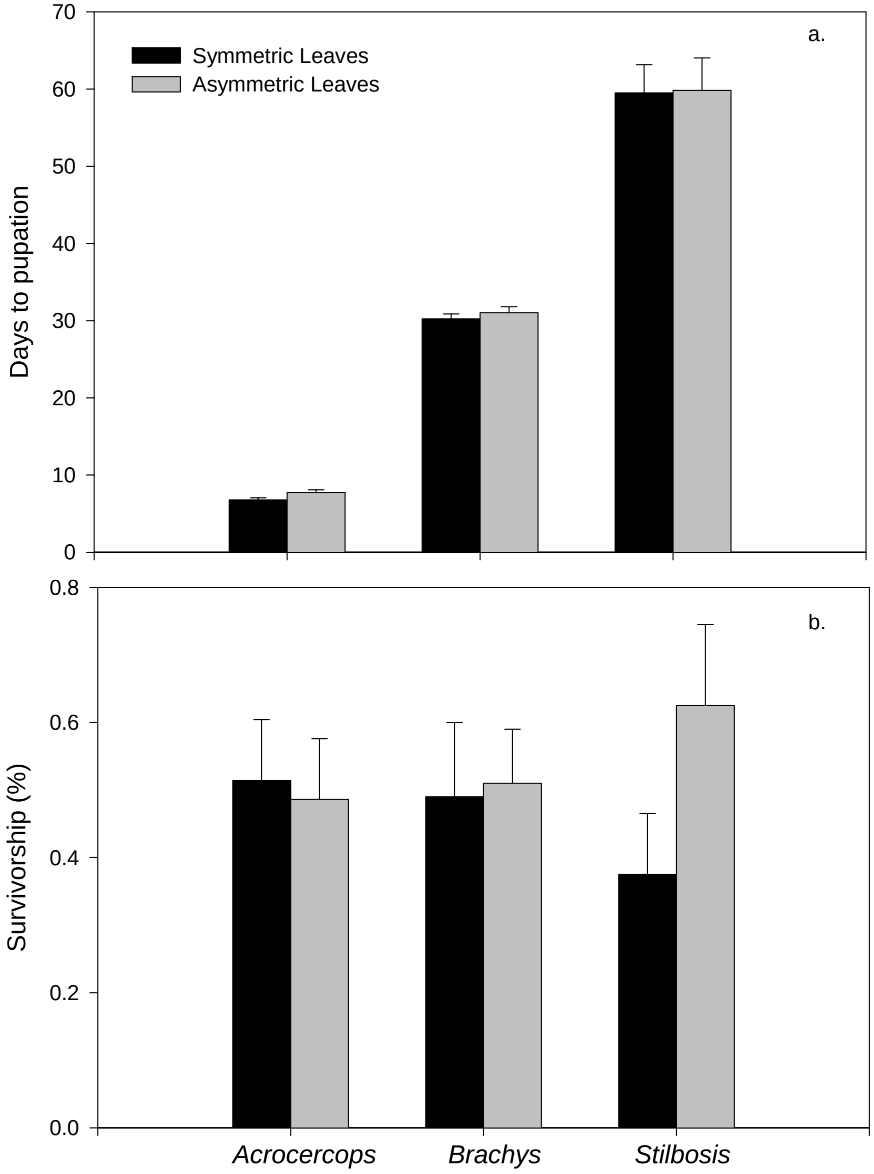

Figure 6.7- Differences in a) leaf miners developmental time and b) survivorship in symmetric and asymmetric leaves of oak species. Bars indicate mean ± 1SE.

DISCUSSION

Although literature relating the effects of plant quality to variation in herbivore abundance between and within plants is plentiful, few studies have related random variations in leaf morphology and its effects on leaf quality to variation in herbivory rates in individual plants (but see Wiggins 1997, Martel *et al.* 1999). We suggest that leaf fluctuating asymmetry may be used by herbivores as a predictor of plant quality, positively influencing insect abundance. Several findings of our study reinforce the hypothesis that herbivores may use asymmetry as a cue to plant quality: 1) Asymmetric leaves offered better nutritional quality for herbivores, such as lower tannin concentration and higher nitrogen content; 2) plants with more asymmetric leaves or higher levels of asymmetry were attacked more by both *Brachys* and *Stilbosis* leaf miners; 3) FA indices calculated *before* herbivory were reasonable predictors of leaf miners abundance at the end of the season; 4) Within a plant, leaf miners were more frequently found in asymmetric leaves and mined leaves exhibited higher levels of FA than unmined leaves; 5) Mines were smaller on asymmetric leaves compared to symmetric leaves, and *Stilbosis* mines exhibited higher survivorship on asymmetric leaves. Most of our findings reinforce the hypothesis that herbivores are not responsible for asymmetry and the relationship between herbivory and asymmetry is not causal: 1) individual oak plants were consistent in their levels of asymmetry before and after herbivory; 2) there was no direct relationship between the presence of a mine and changes on the width of the side of the leaf where the mine had developed; 3) naturally increased levels of herbivory on *Q. geminata* plants did not increase asymmetry in the following season. Since we have used natural variation in asymmetry between and within individuals, our study did not address the sources of physiological and developmental stress in the *Quercus* species studied, but it has been suggested that FA in leaves may be influenced by several abiotic and biotic factors, such as nutritional deficiencies, water shortage, pollution and plant competition (Palmer & Strobeck 1986).

Higher attack rates on plants with more asymmetric leaves and higher levels of asymmetry may be attributed to the observed differences in nutritional quality between symmetric and asymmetric leaves, although it is not known how these differences arise. Since the left and the right sides of a particular bilaterally symmetrical trait develop under the control of the same genes, deviations from perfect symmetry actually represent developmental instability, and the ability to develop symmetrical traits may be related to the ability to produce defensive chemicals (Moller 1995), if resource allocation to developmental control competes with allocation to production of defensive chemicals. Also, FA may be determined by the same genes as those affecting resistance to herbivores or genes giving rise to elevated levels of FA may have pleiotropic effects on plant resistance (Moller & Swaddle 1997). Although it is not well known how FA is associated with biochemical changes and plant metabolism, differences in nutritional quality between symmetric and asymmetric leaves arise and may be responsible for differential attack rates in these two leaf types. This was first demonstrated by Sakai & Shimamoto (1965) studying tobacco plants and further supported by Lempa *et al* (2000) studying several chemical compounds in birch plants. For birch plants, it was observed that plants with higher levels of FA contained significantly lower amounts of hydrolysable tannins, gallic acid, and flavonoid-glycosides. Laboratory feeding trials also showed that *Epirrita autumnata* consumed more from birch leaves collected from high FA trees compared to low FA trees (Lempa *et al.* 2001). We are unaware of other studies that have tested differences in nutritional quality between symmetric and asymmetric leaves and how these affect herbivore preference and performance. Differences in nutritional quality between asymmetric and symmetric leaves reinforce the idea that the relationship between asymmetry and herbivory is not causal, i.e., herbivory does not cause asymmetry in our study system. Instead, herbivores may use asymmetry as a cue to plant quality and suitable oviposition sites. Other studies that found a positive relationship between fluctuating asymmetry and herbivory rates have sampled only after herbivory has occurred, eliminating the possibility to assess whether asymmetric leaves were present before herbivory and whether herbivores preferentially attack these leaves. With our sampling design, we demonstrated that asymmetric leaves were present in both *Q. laevis* and *Q. geminata* before leaf miners attacked the leaves. Our results also showed that mines caused by *Brachys* and *Stilbosis* were smaller in asymmetric leaves compared to symmetric leaves and this may be explained by the lower consumption rates observed in asymmetric leaves compared to symmetric ones. Our results partially support the slow-growth, high-mortality hypothesis since *Brachys* and *Stilbosis mines* growing on symmetric leaves with reduced nutritional quality exhibited higher consumption rates resulting in bigger mines at the end of the season. Nevertheless, we found only limited support for higher mortality rates caused by natural enemies on mines growing on symmetric leaves, since survivorship of *Brachys* and *Acrocercops* mines did not differ between symmetric and asymmetric leaves of *Q. laevis* and only *Stilbosis* mines exhibited higher survivorship on asymmetric leaves of *Q. geminata*. These results indicate that although bottom-up factors, such as asymmetry and plant quality may influence the choice of oviposition sites by insects, the positive effects of better nutritional quality on mine survivorship are not always realized when top-down factors, such as predation and parasitism, are strong and other studies with leaf miners have demonstrated the strength of top-down pressures on insect survivorship (e.g., Auerbach & Simberloff 1988, Mopper *et al.* 1995, Hawkins *et al.* 1997, Forkner & Hunter 2000, reviewed by Connor & Taverner 1997).

Although variation in herbivory rates in both *Q. laevis* and *Q. geminata* may be related to fluctuating asymmetry and its consequential changes in plant quality, our study has also demonstrated idiosyncratic responses to FA both between and within guilds. Leaf chewers and leaf galls were not influenced by levels of FA in both plant species. Galling insects may not be as influenced by aspects of plant quality as leaf miners are, since their special mode of feeding within the gall allows them to manipulate plant characteristics, and possibly avoid defensive strategies of the host and circumvent differences in nutritional quality between symmetric and asymmetric leaves. Manipulation of host plants by gall-formers may extend to control over the chemical composition of gall tissues, and galling herbivores may alter the physiological state of host tissues, especially the tissues nearest to the developing larvae (Price *et al.* 1987, Shorthouse & Rohfritsch 1992, Hartley 1998). Among leaf miners, strong responses were found for both *Stilbosis* and *Brachys*, but *Acrocercops* mines on turkey oaks seem not to be influenced by variation in asymmetry between and within plants. This variation in leaf miner response to fluctuating asymmetry may be explained by differences in life-history traits of the species studied. *Acrocercops* cause relatively small linear-blotch superficial mines just under the leaf epidermis of turkey oak leaves and development times do not exceed 10 days. These mines are unlikely to be strongly affected by variations in plant quality due to their fast development rates and the fact that they create limited depth mines in young leaves with higher nitrogen content and smaller concentration of defensive chemicals. *Brachys* and *Stilbosis* mines may be more likely to be affected by host quality, having long developmental times, full depth mines, and a higher likelihood to be affected by spatial and seasonal variation in host quality.

CONCLUSION

Our study of leaf miners on Florida scrub oaks has demonstrated the diversity and peculiarity of responses of a single guild to effects of plant quality and natural enemies when several scales of organization are considered. At the largest scale, leaf miners were clustered into sites (Chapter 2) and sites separated by smaller distances were more similar in the abundance of mines than sites farther apart. However, at the regional, landscape scale, we observed that bottom-up and top-down factors were not spatially structured, demonstrating that other factors were probably more relevant in structuring the abundance of leaf miners when spatial position is taken into account. At local scales, we have demonstrated that leaf miners tended to be clustered into individual plants, and into individual branches within plants (Chapter 3). Natural inter-individual variation in bottom-up factors such as the concentration of secondary defenses and foliar nitrogen is partially responsible for the aggregation patterns found. Leaf miners also responded to experimentally elevated levels of foliar nitrogen, as fertilized plants supported significantly more herbivores than plants with unmanipulated levels of nitrogen (Chapter 4). Our studies have demonstrated that, although bottom-up factors affected both the abundance and performance of some leaf miner species, top-down factors tended to be weak and non-significant. Removing natural enemies from the plants, for example, did not change the abundance (Chapter 4) and/or the survivorship (Chapter 5) of leaf miners, indicating that, if top-down effects on leaf miners do occur, they were not detected with our experimental designs. At the individual scale, our studies of fluctuating asymmetry have indicated that leaf miners responded to natural and random variation in leaf morphology, an indicator of plant stress (Chapter 6). Individual plants with higher percentage of asymmetric leaves and/or higher levels of asymmetry supported higher densities of some leaf miner species and differences in plant quality between symmetric and asymmetric leaves partially explained this result. At the individual scale, however, we again did not detect top-down effects on the survivorship of leaf miners, as no differences in survivorship and mortality rates were observed between symmetric and asymmetric leaves.

Although species-specific variation in leaf miner response to plant quality and natural enemies were observed, bottom-up factors tended to be stronger than top-down factors, and significantly impacted the abundance, performance, and survivorship of leaf miners, especially at local and individual scales.

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APPENDICES

Appendix 1 – Some leaf and stem gall-formers sampled on *Quercus myrtifolia* and *Q. chapmanii* over the range of their distribution in Florida.

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Tatiana G. Cornelissen, daughter of Antonius W.G. Cornelissen and Wanda G. Cornelissen was born on August 1st 1975 in Belo Horizonte, Brazil. Between 1994 and 2000 she obtained a Bachelor's Degree in Ecology and a M.Sc. degree in Ecology and Conservation from the Federal University of Minas Gerais, Brazil. She moved to Florida in August of 2001, where she began a Ph.D. program in Ecology, under the supervision of Dr.Peter Stiling. Her work has been published on Global Change Biology, Oecologia, Oikos and Ecological Entomology. She is expected to obtain her Ph.D. degree in May of 2006.