Effects of Genetic Variability and Habitat of *Qualea parviflora* (Vochysiaceae) on Herbivory by Free-feeding and Gall-forming Insects

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**Background and Aims** Differences in the chemical and physical traits of plants caused by both genetic and habitat characteristics may influence attack by herbivores. Leaves of *Qualea parviflora* (Vochysiaceae), a common tree of different habitats in the Brazilian Neotropical savannas (cerrado), are susceptible to severe attack by herbivorous free-living and gall-forming insects. Attack by free-living and gall-forming insects within and between populations of *Q. parviflora* were examined and it was determined to what extent genetic variability (detected by RAPD markers), phenotypic characteristics of the plants and habitat traits influence the number of free-living and gall-forming insect species and individuals attacking the plants, and the intensity of attack.

**Methods** On four occasions in 2000, leaves were sampled from ten individual trees in each of three types of vegetation in the cerrado: campo sujo, cerrado *sensu stricto* and cerradão at the Ecological Station of Pirapitinga (ESP), in Três Marias, north-western Minas Gerais, Brazil. Genetic variability was detected by RAPD markers, and concentrations of nutrients, phenols and tannins, sclerophyll and pre-dawn water potential of leaves were measured. Water and nutrient contents in the soil below each tree characterized the habitat. The free-living and gall-forming herbivorous insects were determined.

**Key Results** Of 69 RAPD markers analysed, 41 were polymorphic and were used for analyses of genetic variability of *Q. parviflora*. Most of the variability occurred within habitats, accounting for 97.65% of the genetic variability. Plants in the cerrado *sensu stricto* and campo sujo were the most similar. There were no significant associations between genetic similarity and the chemical and physical traits of *Q. parviflora*, or with habitat, nor was there a significant correlation between phenotypic and habitat traits. Increasing concentrations of tannins and sulphur, C : N ratio and sclerophyll correlated with increasing percentage of leaf area damaged by herbivores. Decreased sclerophyll, concentration of tannins and C : N ratio, and increased concentration of nutrients in leaves correlated with increased severity of attack by gall-forming insects.

**Conclusions** Nutrient concentration in the soil had more influence, indirectly, on free-feeding insects than did composition of *Q. parviflora* leaves. However, gall-forming insects are affected more by leaf quality, attacking fewer sclerophyllous leaves, with larger nutrient but smaller tannin concentrations.

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**Key words:** Cerrado, genetic variability, gall attack, herbivory, insect galls, plant quality, *Qualea parviflora*, RAPD, Vochysiaceae.

**INTRODUCTION**

Variation in the resistance of individual plants to attack by herbivorous insects may be caused by differences in genotype and in the plant’s environment (Mutikainen *et al*., 2000). Intra-population genotypic differences in resistance have been observed in several species (e.g. Anderson *et al*., 1989; Fritz, 1990; Karban, 1992; Zangerl and Berenbaum, 1997). These differences in the host plant’s resistance can exert selective pressures on the ability of insects to determine variations in the plant’s characteristics favourable to them (Anderson *et al*., 1989). Conversely, differences in herbivory can affect plant fitness, often having negative effects by decreasing growth rate, reproductive success and competitive ability, all of which can affect the genetic structure of populations (Coley, 1983; Coley and Barone, 1996).

The susceptibility of plants to attack by herbivores may be influenced by the plant’s quality, which in turn is linked to factors such as soil nutrients, light intensity and past herbivory (Bazzaz *et al*., 1987). Intraspecific variation in defence against herbivory may be influenced by spatial and temporal variation in resource availability, and to the physiological condition of the plants, such as size and age (Chapin *et al*., 1987). Some habitat features, such as composition and distribution of host plant species and composition and abundance of natural enemies, may also influence the activity of herbivores by modifying their patterns of search and colonization (Bazzaz *et al*., 1987).

Although the acceptance of a site for oviposition (as in gall-forming insects) or the amount of food ingested (as in free-feeding herbivorous insects) also depends on the physiological status of the individual insect (such as food supply and deprivation, egg load, age) and its experience (Schoonhoven *et al*., 1998), the effect of the host-plant on insect performance (growth, survival and fecundity) is the major determinant of choice (Hartnett and Abrahamson, 1979). For example, the adults of cabbage white butterfly,
Pieris rapae, selectively laid eggs on Brassica oleracea (Brassicaceae) plants with higher nitrogen and phosphorous content, on which their caterpillars grew faster and attained a heavier weight (Myers, 1985). Stein and Price (1995) also observed that females of two gall-forming tenthredinid sawflies, Eura sp. and Pontania sp., avoid shoots of very young ramets of Salix lasiolepis Benth. (Salicaceae) on which survival of their offspring is decreased.

During the search for a host plant, herbivorous insects behave in particular ways, such as repetitive contacts of legs, antennae, mouthparts or ovipositor with the plant surface, or even take a test bite; these serve to evaluate the physical and chemical characteristics of the leaf and determine acceptance or refusal (Schoonhoven et al., 1998). Physical features of plant organs or tissues which affect feeding by insects include the presence of trichomes and wax crystals on the surface, and the thickness and toughness (sclerophylly and high silica content) of the tissue (Ernest, 1989). For example, Woodman and Fernandes (1991) showed that Verbascum thapsus (Scrophulariaceae) leaves varied in density of hairs and demonstrated that generalist herbivores selected the least pubescent leaves as opposed to those which are very pubescent. In addition, cicadellids (Homoptera) and tingids (Hemiptera) chose to feed on leaves of the tropical tree Tabebuia ochracea (Bignoniaceae) with small concentrations of calcium oxalate crystals (Ribeiro et al., 1994). Coley (1983) found that leaf toughness was the most important factor determining leaf palatability for herbivores on persistent and pioneer trees of the tropical rainforest in Panama.

Nutrient concentration and type, and concentration of compounds such as tannins and other phenolics (generally regarded as secondary defence metabolites) in leaves are examples of chemical characteristics that influence insect behaviour (Whittaker and Feeny, 1971; Schoonhoven et al., 1998). Marquis et al. (2001) found that phenolics have an important role as defences against both pathogens and insects in many plant species of the Brazilian cerrado. Cornelissen and Fernandes (2001) also suggested that the area of leaf damaged by herbivores in Bauhinia brevipes (Leguminosae) can be influenced by, for example, water content, sugars, toughness, and nitrogen content of the leaves.

Environmental factors affecting the plant and its response to herbivory are extremely variable, and poorly understood. The effects of these factors were examined on Qualea parviflora (Vochysiaceae), which is a deciduous cerrado tree species that accumulates aluminium (to leaf Al concentrations above 10 000 mg kg⁻¹; Haridasan, 1982), and is distributed in different ecological regions of Brazil. There the cerrado (Neotropical savanna) is the second most important biome, covering about 20 % of the country (Felfili and Silva, 1993). Three floristic types (called physiognomies) are commonly found: (1) campo sujo, a savanna-like vegetation, with scattered trees and shrubs and canopy cover of <2 %; (2) cerrado sensu stricto, a savanna-like vegetation with a higher density of trees and shrubs (canopy cover of about 20 %); (3) cerradão, a xeromorphic forest vegetation with a fairly continuous canopy tree, which ranges from 15 to 85 % cover (Eiten, 1972; Felippe and Dale, 1990). Qualea parviflora commonly occurs in these three types of vegetation, where free-living herbivorous and gall-forming insect species severely attack the leaves.

The following were studied: (a) variation in the number of species of herbivorous free-living and gall-forming insects; (b) the intensity with which the insects attack Q. parviflora leaves; and (c) the extent to which genetic variability [detected by random amplified polymorphic DNA (RAPD) markers], and plant end environmental characteristics influence the insects. The following questions were addressed: How is the genetic variability of Q. parviflora distributed within and among populations in the three types of cerrado vegetation? How are genetic variability, phenotype and habitat of the host plant related? Which factors best explain variations in the number of individuals of herbivorous free-living and gall-forming insect species and the intensity with which they attack host plants?

MATERIALS AND METHODS

Study site

The study was carried out at the Ecological Station of Pirapitinga (ESP), 100 ha of cerrado biome in south-east Brazil (18°20′S to 18°23′S and 45°17′W to 45°20′W), where three types of vegetation occur: campo sujo, cerrado sensu stricto and cerradão. The distribution of Q. parviflora Mart. (Vochysiaceae) was continuous over the area but plant density varied in each vegetation type: 0-12 trees m⁻² in cerradão, 0-34 trees m⁻² in campo sujo and 0-39 trees m⁻² in cerrado sensu stricto. The distances between the populations in cerrado sensu stricto and campo sujo and cerradão were approx. 142 and 202 m, respectively, and cerradão and campo sujo were about 172 m apart.

Genetic variability in Q. parviflora

For genetic variability analysis, undamaged, young leaves from each of 30 plants were collected in October 2000. Leaves were washed in distilled water and stored in a freezer at −70 °C. DNA was extracted from about 100–150 mg of leaf tissues using the standard method of Doyle and Doyle (1987).

Initially, 24 primers of Operon Technologies Inc. (CA, USA) were screened for polymorphism and amplification quality using eight randomly chosen individuals from the three populations. RAPD amplifications were performed in a 13 µL volume containing 1× reaction buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂), 1 unit Taq DNA polymerase (Invitrogen, MD, USA), 200 µM of each dNTP, 30 ng of primer, 3-25 µg bovine serum albumin and 9.0 ng of template DNA. Amplifications were performed using a GeneAmp PCR System 9700 (Applied Biosystems, CA, USA) under the following conditions: 94 °C for 5 min (1 cycle); 94 °C for 1 min, 35 °C for 1 min and 72 °C for 2 min (40 cycles); and 72 °C for 7 min (one cycle). Polymorphism was detected using 1.5 % agarose gels stained with ethidium bromide (1.0 mg mL⁻¹) and sized by comparison to a 1 kb Plus DNA ladder standard (Invitrogen).

Primers that amplified polymorphic and clearly interpretable fragments were selected to amplify the individuals
from the three populations (Table 1). RAPD amplifications followed the protocols described, and the products were electrophoresed for 4 h in 1.5 % agarose gels, stained with ethidium bromide (10 mg mL⁻¹) and the size of the products determined by comparison to a 1 kb Plus DNA ladder standard (Invitrogen). Gels were photographed using Electrophoresis Documentation and Analysis System 120 (Kodak Digital Science™) and analysed visually, on a computer screen, for presence or absence of bands. Only bands of high intensity were included in the analyses. Poorly amplified or very sporadic bands were not considered.

**Table 1.** The five Operon primers selected, their nucleotide sequences, total number of markers, and mean number of polymorphic markers per population obtained from the amplification of 30 individuals of *Q. parviflora* from the Ecological Station of Pirapitinga

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Total number of polymorphic markers</th>
<th>Mean number of polymorphic markers per population</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPL-04</td>
<td>5'-GACTGCACAC-3'</td>
<td>12</td>
<td>11.3</td>
</tr>
<tr>
<td>OPL-13</td>
<td>5'-ACCGCTGCT-3'</td>
<td>05</td>
<td>5.0</td>
</tr>
<tr>
<td>OPW-05</td>
<td>5'-GGCCGAAG-3'</td>
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<td>6.0</td>
</tr>
<tr>
<td>OPW-13</td>
<td>5'-CACACCGAACA-3'</td>
<td>11</td>
<td>9.3</td>
</tr>
<tr>
<td>OPX-17</td>
<td>5'-GACACCGACC-3'</td>
<td>07</td>
<td>5.7</td>
</tr>
</tbody>
</table>

Phenotypic traits

To study phenotypic traits of *Q. parviflora*, 53 fully expanded and undamaged leaves were collected from each of the 30 plants in January, April, July and November in 2000. To determine within- and between-population variation in phenotypic traits, the four samples taken at different dates were pooled.

The mean concentration of macro- and micronutrients, total phenols (percentage in dry matter) and tannins (percentage of dry matter) were measured for each of the 30 plants. A different number of leaves per individual plant was collected for nutrient analysis (number of leaves per plant *n* = 20), total phenols (*n* = 10), sclerophyll (*n* = 20) and tannins (*n* = 3) because of differences in methods. The macro- and micronutrients were analysed on 400 mg of dry plant tissue after wet digestion with HNO₃ and HClO₄. The products determined by comparison to a 1 kb Plus DNA ladder standard (Invitrogen). Gels were photographed using Electrophoresis Documentation and Analysis System 120 (Kodak Digital Science™) and analysed visually, on a computer screen, for presence or absence of bands. Only bands of high intensity were included in the analyses. Poorly amplified or very sporadic bands were not considered.

**Habitat features**

A sample of soil was removed to 20 cm in depth under each individual tree in each vegetation type (*n* = 30 trees). The soil samples were air-dried and the following analysed: N-ammonium (%), N-nitrate (%), P (mg dm⁻³), Ca (mg dm⁻³), K (mg dm⁻³), Mg (mg dm⁻³), organic matter (dag kg⁻¹), Si (mg kg⁻¹), Na (mg dm⁻³), Fe (mg dm⁻³), Cu (mg dm⁻³), Zn (mg dm⁻³), Mn (mg dm⁻³), Al (cmol dm⁻³), exchangeable acidity [H + Al (mg dm⁻³)], total and effective cation exchange capacities, aluminium saturation (m%), pH in water, and soil water content (%).

Soil water content was determined by difference between fresh and dry weight (dried for 72 h, or to constant weight, at 45 °C). Phosphorous was determined colorimetrically (Silva, 1999). Organic matter was determined indirectly by the Walkley and Black method (Walkely and Black, 1934). Inorganic nitrogen was analysed by the adapted methods of Mullin and Riley (1955) and Kempers and Zweers (1986). An air–acetylene flame was used for potassium (Johnson and Ulrich, 1959). Silica was analysed colorimetrically (Hallmark et al., 1982). Calcium, iron, copper, zinc, manganese and aluminium concentrations were measured by atomic absorption spectrophotometry (Meyer and Keliher, 1992).

**Sampling of adult gall-forming and free-living insects and leaf damage**

Adult herbivorous insects associated with *Q. parviflora* leaves were sampled in the morning (between 0800 and 1200 h) and in the afternoon (between 1400 and 1800 h), for 10 min per plant on each collection date, in each population. All the insects observed on the leaves were collected, and sent to specialists for identification.

For each tree, ten shoots were randomly collected around the circumference of each plant (*n* = 300 shoots). For each shoot and plant the total number of leaves, number of leaves attacked by insects, and gall abundance (new and hatched galls) were recorded, and means per plant were estimated. Free-living insect damage to the foliage was quantified in two mature leaves per shoot (20 leaves per plant). Leaves were drawn and digitized by a flat-bed scanner. The total leaf area and percentage of leaf area damaged were calculated using the Scion-Image software (1998). Only herbivore damage that resulted in lamina loss (leaf removal, chewing or mining) was included (see Moles and Westoby, 2000).
Data analysis

To verify how genetic variability of *Q. parviflora* is distributed within and between populations the raw data matrix (presence and absence of bands) was used to generate a similarity matrix based on the Jaccard coefficient (Sneath and Sokal, 1973), where \( J = 0 \) is considered total dissimilarity and \( J = 1 \) is total similarity. An UPGMA cluster analysis was performed based on the similarity matrix using the software NTSYS version 2.02k (NTSYS, 1997). Additionally, an analysis of molecular variance (AMOVA; Excoffier *et al*., 1992) was performed using the Arlequin Ver 2.0 software (Schneider *et al*., 2000).

To verify the relationship between genetic variability, phenotype and habitat characteristics, a Mantel test (Mantel, 1967) was made using NTSYS v. 2.02k (NTSYS, 1997). As phenotype and habitat variables had different units, before analysis all variables were standardized by the following formula: \( \frac{(x - M)}{SD} \), where \( M \) = mean and \( SD \) = standard deviation (NTSYS, 1997). A similarity matrix was obtained using the Jaccard coefficient for qualitative data (Sneath and Sokal, 1973).

Principal component analysis (PCA) was done using a correlation matrix of raw data to establish the relationship among genetic variability, and phenotypic and habitat traits of *Q. parviflora*, using Systat v. 8.0. Initial PCA showed that some variables did not have factor loadings greater than 0.50 on retained components. These variables were then taken out from the analysis and the PCA was repeated (see Marquis *et al*., 2001).

Multiple linear correlation and step-wise multiple linear regression were used to test which factors are significant in influencing variation in attacks by free-living herbivores and gall-formation on the host-plant (Zar, 1996). Herbivorous attack was estimated by the number of attacked leaves and percentage of leaf area damaged, while gall-forming insect attacks were estimated from the number of leaves with galls and number of galls per individual plant. Afterwards, simple and multiple linear regressions were used to verify the relationship between number of species and attack by free-living herbivores and gall-forming insects and the new axis obtained from PCA (see Collevatti and Schoereder, 1995; Marquis *et al*., 2001).

RESULTS

Genetic variability in *Q. parviflora*

Sixty-nine polymorphic RAPD markers were detected using five selected primers, and 41 polymorphic bands (6-8 bands per primer) were used to test which factors are significant in influencing variation in attacks by free-living herbivores and gall-formation on the host-plant (Zar, 1996). Herbivorous attack was estimated by the number of attacked leaves and percentage of leaf area damaged, while gall-forming insect attacks were estimated from the number of leaves with galls and number of galls per individual plant. Afterwards, simple and multiple linear regressions were used to verify the relationship between number of species and attack by free-living herbivores and gall-forming insects and the new axis obtained from PCA (see Marquis *et al*., 2001).

RESULTS

Genetic variability in *Q. parviflora*

Sixty-nine polymorphic RAPD markers were detected using five selected primers, and 41 polymorphic bands (6-8 bands per primer) with better resolution were used in subsequent analyses (Table 1). From these, 80.5% were found in plants of all three sampling sites, 14.6% were present at two sites, and 4.9% were only found in one type of vegetation.

The results showed large genetic similarity among plants from different populations (Fig. 1). The highest similarity \( (J = 0.82) \) occurred between two individuals from different types of vegetation, the cerrado *sensu stricto* and campo sujo (individuals 17 and 21; Fig. 1). Seven clusters could be identified: I—with plants from the three populations;
characteristics of the plants (Mantel test: $r$ (Fig. 2). Individuals from cerradão were plotted on the left side of axis 1, while contents of N, P, K and Na correlated positively with this axis, whereas Si and N-nitrate were negatively correlated. Individual plants from cerrado sensu stricto and cerradão could be separate by axis 1, while axis 2 separated the individuals from campo sujo from the other physiognomies (Fig. 3). There was no significant correlation between phenotypic traits and habitat characteristics ($r = 0.012; P = 0.594$).

A significant correlation obtained between axis 1 from phenotype PCA and axis 2 from habitat PCA ($r = 0.70; P < 0.0001$) indicated that some traits of $Q$. parviflora trees, such as sclerophyll, $C : N$ ratio, and N, P, K and Na concentrations in leaves, are related to features of the habitat. The other axis from phenotype, genotype and habitat PCA did not show any significant relationships (in all $P > 0.05$).

**Herbivory and effects of the genetic variability, phenotypic and habitat traits**

Adult insects of 79 species, in six orders, were found on leaves of $Q$. parviflora. Phytophagy was the most common diet among insects in the three types of vegetation ($n = 64$ species).

Variation in number of herbivorous insect species and attack by insects on leaves and shoots of $Q$. parviflora (Table 5) could be partially explained by some of the axes obtained by PCA based on genetic variability, phenotype and habitat characteristics (Table 6). Number of insect species was best explained by habitat characteristics (partial $r^2$ with HabAxis 2 = 0.24; $P = 0.006$), such as concentration of silica, nitrogen and water in the soil (Table 6). In addition, variation in percentage of damaged leaves (free-living herbivore attack) was better explained by genetic variability (PCA axes 2 and 4) and habitat (PCA axis 2). On the other hand, phenotypic traits were the most important factor affecting both variations in percentage of leaves with galls (partial $r^2$ with PhenAxis 1 = 0.14; $P = 0.04$), and number of galls per leaf (partial $r^2$ with PhenAxis 2 = 0.18; $P = 0.019$) (Table 6). Variation in the total number of galls per plant could not be explained by any of the axes obtained in PCA.

**DISCUSSION**

For RAPD markers, there is a high genetic diversity and low genetic divergence among $Q$. parviflora individuals from the three types of vegetation of the cerrado at the Ecological Station of Pirapitinga. A large percentage of variation accounting for differences among individuals within populations is commonly found in outcrossing plant species (e.g. Dawson and Powell, 1999; Gurgerli et al., 1999; Hsiao and Lee, 1999; Jordano and Godoy, 2000; Gomes, 2001). Thus, these results were expected due to the exclusive sexual reproduction, and the alogamous and self-incompatible reproductive system, in $Q$. parviflora (Barbosa, 1983).

Although PCA analysis based on phenotype and habitat characteristics could separate most of the individuals from

**TABLE 2. Analysis of molecular variance based on 41 RAPD markers from 30 individuals of $Q$. parviflora from three types of vegetation of the cerrado in the Ecological Station of Pirapitinga**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>SS</th>
<th>VC</th>
<th>% Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among populations</td>
<td>2</td>
<td>16-733</td>
<td>0-16222</td>
<td>2-35°*</td>
</tr>
<tr>
<td>Within populations</td>
<td>27</td>
<td>182-100</td>
<td>6-7444</td>
<td>97-65**</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>198-833</td>
<td>6-90667</td>
<td></td>
</tr>
</tbody>
</table>

d.f., degrees of freedom; SS, sum of squares; VC, variance component.
* $P > 0.05$.
** $P < 0.05$.

II—with plants from cerrado sensu stricto and cerradão; III—with plants from cerradão; IV—with a plant from cerradão and others from the cerrado sensu stricto; V—with a plant from cerradão and others from the cerrado sensu stricto; VI—with a plant from cerradão and others from campo sujo; and VII—with plants from the campo sujo. Individuals 2, 13, 25 and 26 had similarity coefficients lower than 0.50 with other clusters (Fig. 1).

The analysis of molecular variance indicated that there was no significant difference between the ten trees selected in each of the three populations in different habitats (Table 2). Most of the genetic variation (97-65 %) of $Q$. parviflora is distributed among individuals of the same population (Table 2).

**Relationship among genetic variability, phenotypic and habitat traits**

A PCA based on presence and absence of RAPD markers on plants from the three types of vegetation showed that 56-6 % of the variation could be explained by four axes, and only 18 RAPD markers had a correlation above 0.50 with one of the new PCA axes. Furthermore, there was no significant correlation between the genetic similarity of individual $Q$. parviflora trees and the chemical and physical characteristics of the plants (Mantel test: $r = -0.026; P = 0.395$) or between genetic similarity and habitat characteristics ($r = 0.019; P = 0.356$).

Most of the variation (51-9 %) in phenotypic traits (Table 3) of $Q$. parviflora could be explained by two axes in the PCA. Sclerophyll, $C : N$ ratio, concentration of tannins and sulphur in leaves showed a direct relationship with axis 1, whereas Si and N-nitrate were negatively correlated. Individual plants from cerrado sensu stricto and cerradão could be separate by axis 1, while axis 2 separated the individuals from campo sujo from the other physiognomies (Fig. 3). There was no significant correlation between phenotypic traits and habitat characteristics ($r = 0.012; P = 0.594$).

A significant correlation obtained between axis 1 from phenotype PCA and axis 2 from habitat PCA ($r = 0.70; P < 0.0001$) indicated that some traits of $Q$. parviflora trees, such as sclerophyll, $C : N$ ratio, and N, P, K and Na concentrations in leaves, are related to features of the habitat. The other axis from phenotype, genotype and habitat PCA did not show any significant relationships (in all $P > 0.05$).
In fact, genetic similarity among individuals from the three populations of Q. parviflora is high and they probably belong to the same population. Variation in habitat quality among physiognomies may not be sufficient to cause genetic divergence due to differential selective pressure when there is no barrier to gene flow between sites.

There were no significant relationships between genetic similarity and the chemical and physical characteristics of plants, and between genetic similarity and the habitat traits of Q. parviflora. No significant correlation was found between phenotype traits and habitat characteristics. Telles et al. (2001) found a significant relationship between plant and soil characteristics for Eugenia dysenterica (Myrtaceae). They pointed out that the geographical distribution would be the main factor affecting the genetic divergence among local populations of this species. In addition, they did not find any significant correlation between genetic distance and plant phenotype, similar to the results presented here.

Habitat traits explained the variation in the number of herbivorous insect species on Q. parviflora better. The availability of water and nutrients in the soil and weather conditions are important factors that affect the growth and quality of plants (White, 1969, 1984), and indirectly influence the diet, and thus type, abundance and growth of herbivorous insects. The increase in soil nutrient concentration influences the plant’s physiology and changes the nutrient composition, such as protein (Schoonhoven et al., 1998), and secondary metabolite concentrations (Gershenzon, 1984). Differences in the number of insect herbivores...
Gonçalves-Alvim et al. — Insect Herbivory, and Genotype and Habitat of Q. parviflora

Variation in percent of damaged leaves was best explained by genetic variability and habitat. Similarly, Ribeiro and Brown (1999) observed a significant relationship between leaf damage by herbivorous insects and the genetic dissimilarity in two species of Tabebuia (Bignoniaceae). Also, an association was found between increasing concentrations of tannins and sclerophily, and the increase in percentage of leaf area lost by herbivore attack.

Contrary to the results of some authors (e.g. Coley, 1983; Salatino, 1993; Turner, 1994; Ribeiro et al., 1998), sclerophily may not play an import role in protecting Q. parviflora against herbivores, indicating that the insects could be adapted to the sclerophyllous characteristics of this species. Ribeiro and Brown (1999) did not observe any correlation between severity of herbivory and toughness of leaves in T. aurea and T. ochracea (Bignoniaceae). The results presented here also differ from those obtained by Marquis et al. (2001), who found a negative correlation of the concentrations of secondary compounds and their capacity to condense proteins.

The results given here suggest that gall-forming insects respond to the nutritional quality of Q. parviflora because leaves richer in nutrients and poorer in tannins had more galls. Also, these results do not corroborate the plant stress hypothesis (White, 1969, 1976, 1984; Rhoades and Cates, 1976; Rhoades, 1979; Mattson and Haack, 1987), which predicts that nutritionally stressed plants should have smaller concentrations of chemical defences, so becoming more susceptible to attack by herbivores. In addition,
Gonçalves-Alvim et al. — Insect Herbivory, and Genotype and Habitat of Q. parviflora

Table 5. Number of species of free-living herbivores, and attack of herbivorous and gall-forming insects of 30 individuals from three populations of Q. parviflora

<table>
<thead>
<tr>
<th>Plant no.</th>
<th>TNISSP</th>
<th>PDLEAV</th>
<th>PGLEAV</th>
<th>LEAFAD</th>
<th>TNGALL</th>
<th>NLEAF</th>
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<tbody>
<tr>
<td>1</td>
<td>08</td>
<td>73-48</td>
<td>1-73</td>
<td>4-13</td>
<td>11</td>
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</tr>
<tr>
<td>2</td>
<td>07</td>
<td>77-99</td>
<td>2-51</td>
<td>4-81</td>
<td>10</td>
<td>2.0</td>
</tr>
<tr>
<td>3</td>
<td>03</td>
<td>80-87</td>
<td>0-41</td>
<td>5-28</td>
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<td>0.3</td>
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<td>4</td>
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<td>86-99</td>
<td>1-99</td>
<td>7-06</td>
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</table>

TNISSP, total number of herbivorous insect species; PDLEAV, percentage of damaged leaves; PGLEAV, percentage of galled leaves; LEAFAD, leaf area damaged; TNGALL, total number of galls; NLEAF, number of galls per leaf.

Table 6. Results of stepwise regression analyses between number of species of free-living herbivores, attack of herbivorous and gall-forming insects and the axis obtained by principal component analysis (PCA) of 30 individuals of Q. parviflora, in cerrado in the Ecological Station of Pirapitinga

<table>
<thead>
<tr>
<th>Number of species and attack</th>
<th>Regression models</th>
<th>R²</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of insect species</td>
<td>Log y = 0.761 - 0.0412*HabAxis2</td>
<td>24.0 %</td>
<td>8.69</td>
<td>0.006</td>
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<tr>
<td>% Damaged leaves</td>
<td>y = 78.3 + 2.49<em>GenAxis 2 + 3.15</em>GenAxis 4 - 6.53*HabAxis 2</td>
<td>64.6 %</td>
<td>17.46</td>
<td>0.000</td>
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<td>% Galled leaves</td>
<td>y = 0.841 - 0.0938* PhenAxis 1 + 0.181* PhenAxis 2 + 0.0899*HabAxis 1</td>
<td>21.7 %</td>
<td>3.68</td>
<td>0.025</td>
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<td>Leaf area damaged (-%)</td>
<td>y = 605 + 0.308<em>GenAxis 1 + 0.446</em>PhenAxis 1 - 0.973*HabAxis 2</td>
<td>34.1 %</td>
<td>5.99</td>
<td>0.03</td>
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<tr>
<td>Number of galls per leaf</td>
<td>Log y = 0.219 - 0.0486<em>PhenAxis 1 + 0.0589</em>PhenAxis 2 + 0.0416*HabAxis2</td>
<td>26.2 %</td>
<td>4.42</td>
<td>0.012</td>
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</table>

GenAxis 1 and GenAxis 2, Axis 1 and 2 from PCA based on the RAPD markers; PhenAxis 1 and PhenAxis 2, Axis 1 and 2 from PCA based on plant phenotypic traits; HabAxis 1 and HabAxis 2, Axis 1 and 2 from PCA based on habitat characteristics.

All parameters included in the models are statistically significant (P < 0.05). R², coefficient of determination; F, critical value for ANOVA; P, probability value.

Gall-forming insects were more abundant in plants on more fertile soils and with less sclerophyllous leaves, again not corroborating the soil fertility hypothesis (Fernandes and Price, 1988, 1991; Blanche and Westoby, 1995) that predicts higher richness and abundance of gall-forming insects in environments with smaller concentrations of nutrients in the soil.

In conclusion, the increase of Si and N and decrease of K and water in soil were positively related with the percentage of damaged leaves, leaf area lost and number of insect species on Q. parviflora. Additionally, the increasing concentrations of tannins, S, C : N ratio and sclerophyll were associated with increased percentage of leaf area lost by herbivore attack. A decrease in sclerophyll, concentration of tannins and C : N ratio, together with increasing concentrations of nutrients, such as N, P, K, Ca and Na, increase attack by gall-forming insects. Thus, in Q. parviflora the indirect effects of nutrient concentrations in soil (i.e.
nutrients available to the plant) and the direct effects of defensive compounds (i.e. tannins) could influence the behaviour of herbivorous insects. However, free-feeding herbivorous insects may avoid the negative effects of tannins in their feeding and increase consumption of leaves to satisfy their nutritional requirements by moving from one host plant to another. Gall-forming insects, on the other hand, are more affected by the quality of leaves of Q. parviflora, with fewer sclerophyllous leaves that have a larger concentration of nutrients and a smaller concentration of tannins being attacked more by gall-forming insects.

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