Abundance of gall-inducing insect species in sclerophyllous savanna: understanding the importance of soil fertility using an experimental approach

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Abstract: Although many studies have now demonstrated that both richness and abundance of gall-inducing insect species are directly and indirectly (via the host plant) influenced by soil quality, the empirical evaluation of it in the field remains anecdotal at best. The effects of soil fertility on richness and abundance of gall-inducing insects associated with a widespread savanna species, *Eremanthus glomerulatus*, were evaluated under experimental field conditions in Brasilia, central Brazil. The effect of soil fertility on gall-inducing insects species richness was evaluated using three treatments: (1) plots fertilized with nitrogen; (2) plots fertilized with phosphorus; and (3) control plots: soils without fertilization. Species richness of gall-inducing insects (six species of Cecidomyiidae) did not differ among the treatments. Leaves with galls had higher nitrogen concentrations (mean = 15.0 ± 0.5 mg g^{-1}), compared with leaves without galls (mean = 9.0 ± 0.7 mg g^{-1}) on plants that occurred in soils with addition of nitrogen. Similarly, leaves with galls had higher foliar phosphorus concentration (mean = 1.0 ± 0.04 mg g^{-1}) than leaves without galls (mean = 0.6 ± 0.05 mg g^{-1}) in plots with addition of phosphorus. In galled leaves, a negative relationship between gall density and nitrogen concentration was found for one gall-inducing insect species, while three species showed a positive relationship between gall density and leaf nitrogen concentration. A negative relationship between gall density and concentration of leaf phosphorus was observed for four of the six gall-inducing insect species studied. No relationship was found between gall density and leaf nitrogen and phosphorus concentration in ungalled leaves. We argue that foliar nitrogen and phosphorus concentration respond to gall density in galled leaves and therefore, gall-inducing insect species are capable of manipulating their host plant, modifying the foliar nutrients of *E. glomerulatus* in sclerophyllous savanna.

Key Words: *Eremanthus glomerulatus*, gall-inducing insects, plant fertilization, sclerophyllous savanna, soil fertility

INTRODUCTION

Of all plant–herbivore interactions, probably the most complex and poorly understood is that of gall-inducing insect species and their host plants (Cuevas-Reyes *et al.* 2006, Fernandes & Carneiro 2009). Both biotic (e.g. natural enemies, host-plant composition, plant structural complexity, host density and host resistance) and abiotic factors (e.g. hygrothermal stress, scleromorphic environments and soil fertility) can influence both richness and the abundance of gall-inducing insect species over both temporal and spatial scales (Cuevas-Reyes *et al.* 2004a, 2004b; Fernandes & Price 1988, 1991, 1992; Oyama *et al.* 2003). These factors are not mutually exclusive and can be formalized at least into three hypotheses.

The plant species richness hypothesis proposes that more plant species represent more potential sites to colonize by insect herbivores (Fernandes & Price 1988). In some cases the abundance of gall-inducing insect species at the level of the plant community is positively (Cuevas-Reyes *et al.* 2004a, Fernandes & Price 1988) while in others abundance is negatively associated with plant

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species richness (Oyama *et al.* 2003). Floristic diversity of habitats may be responsible for differences in local and regional patterns of richness and abundance of gall-inducing insects (Cuevas-Reyes *et al.* 2004a, Stone & Schönrogge 2003).

The hygrothermal stress hypothesis states that abundance of gall-inducing insects increases with the environment hygrothermal stress (Fernandes & Price 1988, 1991). Some studies show that gall-inducing insects are more common in scleromorphic than mesophytic vegetation, because sclerophyllous leaves are generally long-lived and hence present lower rates of abscission, while under ambient hygrothermal stress the probability of attack by natural enemies such as parasitoids and pathogens is diminished. The combination of plant traits and habitat stress provide favourable sites for colonization by gall-inducing insect species even under low resource availability (i.e. water, soil fertility) (Blanche & Westoby 1995). Finally, Fernandes & Price (1991) argued that soil fertility is an important factor that affects the abundance and species richness of gall-inducing insects in relation to latitudinal gradients. This hypothesis states that lower abundance of gall-inducing insect species should occur in fertile soils (Cuevas-Reyes *et al.* 2004b, Fernandes & Price 1991). Soil stress may induce nutrient deficiencies and favour the presence of toxic elements in the soil (Cuevas-Reyes *et al.* 2004b, Price *et al.* 1998). Plant species that grow under conditions of dystrophic soils tend to have lower rates of growth and accumulate higher concentrations of secondary metabolites such as phenols, alkaloids and terpenoids (Fernandes *et al.* 1994). Gall-inducing insects may incorporate or sequester the biologically active chemicals of their host plants during the formation of gall tissues, and thereby gain protection from their predators and parasites (Hartley & Lawton 1992, Pascual-Alvarado *et al.* 2008).

The effect of soil properties on the abundance, survivorship and species richness of gall-inducing insects at local or regional scales has been evaluated in some studies (Blanche & Ludwig 2001, Blanche & Westoby 1995, Cuevas-Reyes *et al.* 2003, 2004b). Less understood are the effects of soil fertility via sclerophyllous hosts on gall-inducing insects. In this study, we evaluated the effects of soil fertilization in Brazilian savanna on gall-inducing insects' attack on *Eremanthus glomerulatus* (Asteraceae) under experimental field conditions. This is the first experimental study in fertilized fields of savanna that attempts to document the interaction between gall-inducing insects and soil fertility. In particular, our hypotheses were: (1) soil fertility positively affects the nutritional quality of tissues in *E. glomerulatus*, (2) soil fertility reduces the gall-inducing insect species richness in *E. glomerulatus*, (3) the abundance of galls by different gall-inducing insect species is negatively affected by soil fertility.

METHODS

Study area

The experiment was carried out at the Ecological Reserve of the Instituto Brasileiro de Geografia e Estatística covering 1300 ha, near Brasília ($15°56'$S, $47°53'$W) in central Brazil. Soil is characterized as acidic oxisols, with high Al levels and low cation exchange capacity. The study site elevation ranges between 900–1100 m with an average annual precipitation of 1453 mm (Pereira *et al.* 1993). The vegetation is characterized as savanna with trees and shrubs varying in cover from 10% to 60%. *Eremanthus glomerulatus* Less is a dominant native species with a density of 176 individuals ha^{-1} (Andrade *et al.* 2002, Pereira *et al.* 1993). We performed the experiment in permanent plots with additions of nitrogen and phosphorus to soils (for details see Kozovits *et al.* 2007).

Experimental design

To determine the effects of soil fertility on gall-inducing insect species richness, we used three treatments: (1) plots fertilized with nitrogen: addition of 100 kg^{-1} ha^{-1} y of $(NH_4)_2SO_4$; (2) plots fertilized with phosphorus: addition of 100 kg^{-1} ha^{-1} y of $Ca(H_2PO_4)_2 + CaSO_4$ $2H_2O$; and (3) Control plots: soils without fertilization. Treatments were randomly distributed in 15×15-m plots separated by 20 m with four replicates per treatment. The amount of fertilizer followed recommendations for removing nutrient limitations in *Eucalyptus* plantations in the savanna region. Nutrients were applied from 1998 to 2007 at the beginning and end of the wet season over the litter layer to avoid major disturbance in the plots and in granular form in order to release N and P more slowly. Three samples were collected in each plot using a 5-cm-diameter PVC coring device. The field-moist soil samples were extracted with 1 M KCl for 1 h, and the inorganic N concentrations were determined by colorimetry. N-NH_4 was analysed through reaction with Nessler reagent and N-NO_3 by UV absorption according to the method proposed by Meier (1991). We collected all samples of *E. glomerulatus* in 2007.

Soil fertility and nutritional quality of Eremanthus glomerulatus

To determine the effects of nitrogen and phosphorus on nutritional quality of *E. glomerulatus* tissues, we sampled 12 individual plants in each treatment plot (i.e. 36 plants overall) and collected 20 leaves with galls and 20 leaves without galls per individual (i.e. 480 leaves per plot).

We determined nitrogen concentration per leaf using a Kjeldahl distillation method (Pontes *et al.* 2009) and phosphorus concentration by nitroperchloric digestion (Campbell & Plank 1998).

Soil fertility and gall-inducing insect species richness

To evaluate the potential relationships between soil fertility and gall-inducing insect species richness, we randomly sampled 12 individuals of *E. glomerulatus* in each plot. Because it is generally assumed that gall morphology is unique to a gall-inducing insect and that each gall species is specific to a single plant species (Ananthakrishnan 1984, Cuevas-Reyes *et al.* 2003, Dreger-Jauffret & Shorthouse 1992, Weis *et al.* 1988), we counted and separated all gall-inducing insects on the basis of gall morphology and plant organ.

To assess the effects of soil fertility on density of stem galls, we used the same 12 individuals of the soil fertility and nutritional quality analyses, and recorded the total number of stem galls divided by the number of stems per individual. For leaf galls, on the same individual plants of each treatment (i.e. 36 plants overall), we sampled five branches and randomly collected 10 leaves, and on each leaf we estimated the gall density (i.e. number of galls divided by the total foliar area). We first obtained a digital image of each leaf and then estimated the total area of the leaf using Sigma Scan Pro software.

Statistical analyses

We used a two-way ANOVA to determine the effects of soil on the nutritional quality of tissues of *E. glomerulatus* (after Box–Cox transformation) (Stokes *et al.* 2000). We considered nitrogen and phosphorus addition as independent variables and foliar nitrogen concentration and foliar phosphorus as response variables. A LSMeans test was used for a posteriori comparisons (SAS 2000). To determine if gall density depends on soil fertility, for each gall-inducing insect species, we applied a one-way ANOVA (after Box–Cox transformation) (Stokes *et al.* 2000) and a posteriori LSMeans test for the comparison of means (SAS 2000). In addition, we used a t-test to determine the differences in gall density between insect guilds (foliar vs. stem galls). We considered insect guilds as independent variables and gall density as response variable. To evaluate the relationship among gall density and foliar nitrogen and foliar phosphorus concentration, in each fertilizer treatment and for each gall-inducing insect species, we applied a linear regression analysis. Finally we used a linear regression analysis to determine the relationship between gall density and foliar nitrogen and foliar phosphorus concentration in ungalled leaves for each gall-inducing species.

Table 1. Differences in foliar nitrogen and phosphorus concentration between treatments of plant fertilization (mg g^{-1}). Numbers indicate the mean $\pm$ SE. Statistical comparisons are shown in the text.

Treatments	Foliar nitrogen concentration	Foliar phosphorus concentration
Control	5.0 ± 0.6	0.5 ± 0.06
Nitrogen addition	13.0 ± 0.7	0.7 ± 0.05
Phosphorus addition	10.0 ± 0.4	1.2 ± 0.07

RESULTS

During the study period six species of Cecidomyiidae (Diptera) were found on *E. glomerulatus*. Four species attacked leaves (sp. 1 to sp. 4) while two induced galls on stems (sp. 5 and sp. 6). We did not find differences in gall-inducing insect species richness (F = 2.3; df = 2, P > 0.3) and plant density (F = 1.6; df = 2, P > 0.2) between the fertilization treatment plots.

The foliar nitrogen concentration of *E. glomerulatus* was significantly higher in plants from plots in which nitrogen was added (F = 7.2; df = 2; P < 0.004). Similarly, in plants from plots in which phosphorus was added, we found higher phosphorus concentration in leaves (F = 11.9; df = 2; P < 0.0003) (Table 1). Notably, leaves with galls had higher nitrogen concentration compared with leaves without galls in plants that occur in soils with addition of nitrogen (t = 2.5; df = 1; P < 0.01) (Figure 1a). In addition, leaves with galls had higher foliar phosphorus concentration than leaves without galls in plots with addition of phosphorus (t = 2.4; df = 1; P < 0.02) (Figure 1b).

The density of galls differed significantly between the fertilizer treatments, but the response varied according to the galling species. For example, in one species, we found that gall density was lower in plots with addition of nitrogen (sp. 2: F = 5.4; df = 2; P < 0.02) and in three species we found lower density of galls in plots with addition of phosphorus in the soil (sp. 1: F = 5.2; df = 2; P < 0.03); (sp. 4: F = 7.2; df = 2; P < 0.01); (sp. 5: F = 7.9; df = 2; P < 0.01). In contrast, in one species, we found that gall density was higher in treatments with addition of nitrogen (sp. 3: F = 5.9; df = 2; P < 0.02). Similarly, in one species, gall density was higher in treatments with addition of phosphorus (sp. 6: F = 22.7; df = 2; P < 0.0003) (Figure 2). We found differences in gall density between insect guilds (foliar galls vs. stem galls). The density of foliar galls was higher than stem galls (F = 9.7, df = 1; P < 0.002). In galled leaves, we found a negative relationship between gall density and concentration of leaf nitrogen for only one leaf gall-inducing insect species (sp. 2: F = 12.0; R^2 = 0.6; P < 0.006). Conversely, for three leaf gall-inducing insect species, we found a positive relationship between gall density and concentration of leaf nitrogen
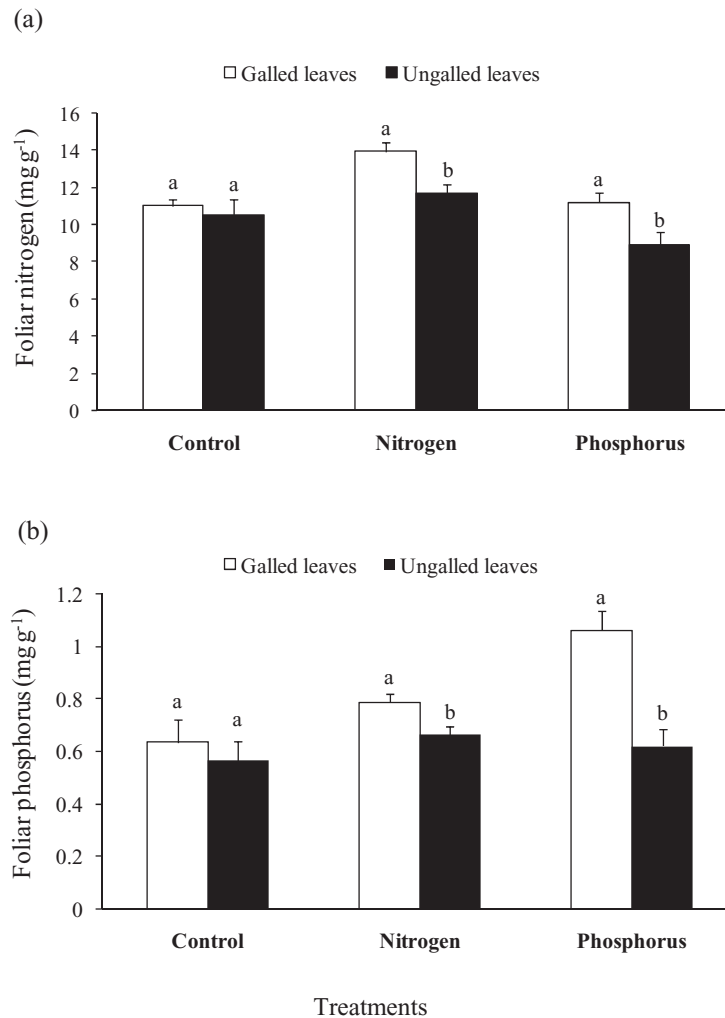
(a)

(b)

Treatments

Figure 1. Effects of soil fertilization on galled and ungalled leaves of *Eremanthus glomerulatus*. Nitrogen addition in the soil on foliar nitrogen concentration (a); phosphorus addition on foliar phosphorus concentration (b). Common letters identify means within treatments that were not significantly different according to LSMeans test ($P > 0.001$) following two-way ANOVA analysis.

(sp. 1: $F = 9.7$; $R^2 = 0.5$; $P < 0.01$); (sp. 3: $F = 7.4$; $R^2 = 0.42$; $P < 0.02$); (sp. 4: $F = 10.0$; $R^2 = 0.5$; $P < 0.01$) (Figure 3). No relationship between gall density and concentration of leaf nitrogen was observed in two gall-inducing insect species (sp. 5: $F = 0.2$; $R^2 = 0.01$; $P > 0.05$); (sp. 6: $F = 2.2$; $R^2 = 0.2$; $P > 0.05$).

Negative relationships between gall density and concentration of leaf phosphorus were observed in galled leaves for four gall-inducing insect species (sp. 1: $F = 11.0$; $R^2 = 0.52$; $P < 0.007$); (sp. 2: $F = 7.1$; $R^2 = 0.41$; $P < 0.01$); (sp. 4: $F = 15.5$; $R^2 = 0.6$; $P < 0.002$); (sp. 5: $F = 7.3$; $R^2 = 0.42$; $P < 0.01$) (Figure 3). In contrast, only two gall-inducing insect species presented a positive relationships between both variables (sp. 3: $F = 6.0$; $R^2 = 0.37$; $P < 0.03$); (sp. 6: $F = 13.9$; $R^2 = 0.58$; $P < 0.003$).

In only one species did we find a negative relationship among gall density and leaf nitrogen concentration in ungalled leaves (sp. 6: $F = 9.9$; $R^2 = 0.46$;

$P < 0.005$). In no gall-inducing insect species was a significant relationship found between gall density and concentration of leaf phosphorus in ungalled leaves.

In control plots, we found a negative relationship among gall density and concentration of leaf nitrogen in two species (sp. 2: $F = 66.6$; $R^2 = 0.86$; $P < 0.0001$); (sp. 6: $F = 54.1$; $R^2 = 0.84$; $P < 0.0001$). No relationship between gall density and concentration of leaf nitrogen was observed in four species (sp. 1: $F = 0.6$; $R^2 = 0.05$; $P > 0.05$); (sp. 3: $F = 1.1$; $R^2 = 0.09$; $P > 0.05$); (sp. 4: $F = 0.7$; $R^2 = 0.05$; $P > 0.05$); (sp. 5: $F = 0.3$; $R^2 = 0.02$; $P > 0.05$). Finally, five gall-inducing insect species presented a negative relationship between gall density and concentration of leaf phosphorus (sp. 1: $F = 45.1$; $R^2 = 81.8$; $P > 0.0001$); (sp. 3: $F = 56.6$; $R^2 = 0.84$; $P < 0.0001$); (sp. 4: $F = 24.8$; $R^2 = 0.7$; $P < 0.0001$); (sp. 5: $F = 19.6$; $R^2 = 0.6$; $P < 0.001$); (sp. 6: $F = 14.9$; $R^2 = 0.6$; $P < 0.003$).
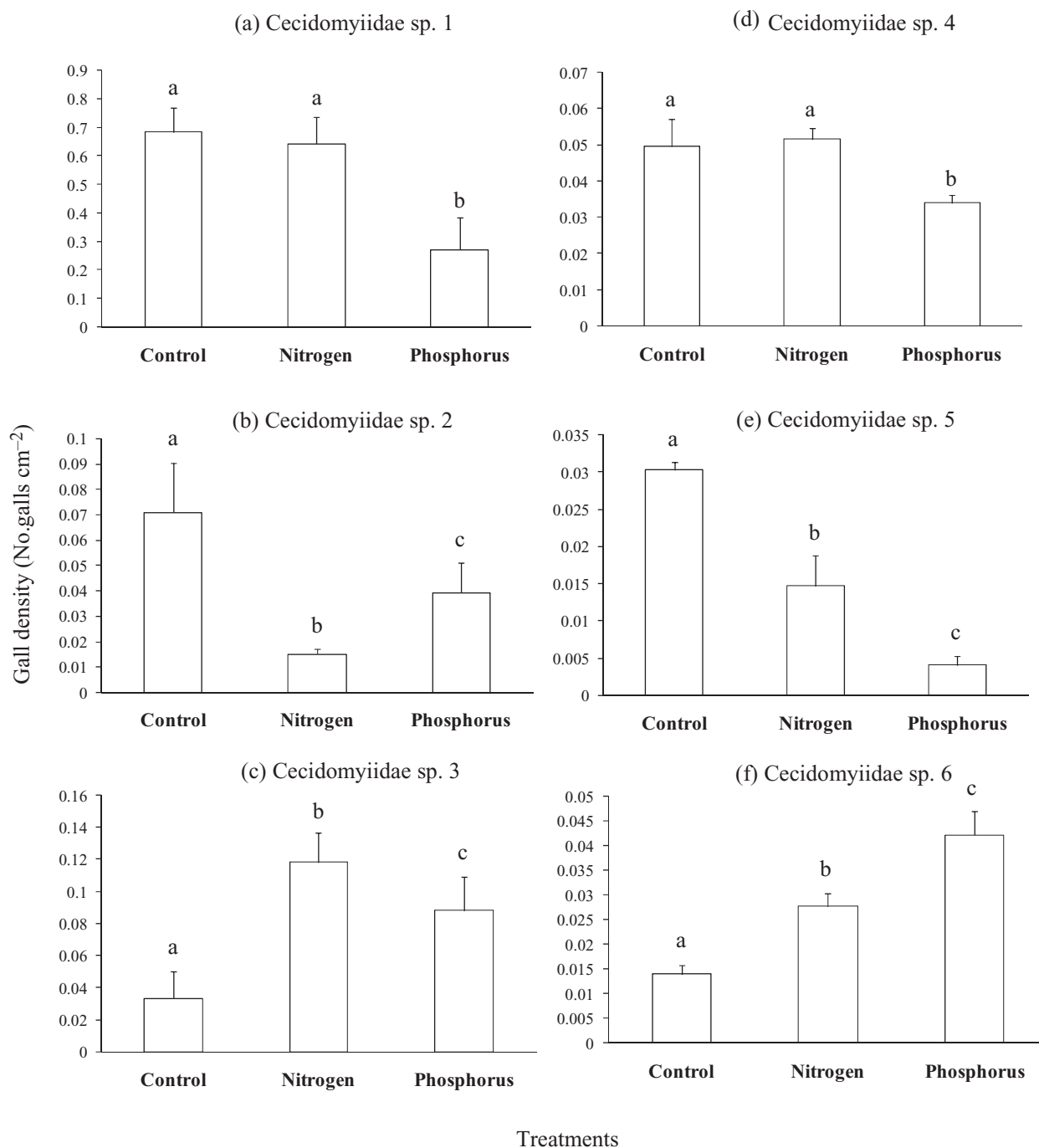
Figure 2. Comparison of gall density between different treatments of soil fertility in six galling species associated to *Eremanthus glomerulatus*. Untransformed data are shown. Values with the same letter did not differ significantly after a LSMeans multiple comparison test (P > 0.001).

DISCUSSION

The patterns of herbivore attack on host plants have been associated with nutrient availability and chemical defences as a consequence of physiological stress (Cobb *et al.* 1997, Pires & Price 2000). Nitrogen and phosphorus are the two primary limiting resources for plant growth in many terrestrial ecosystems (Bobbink *et al.* 2010, Perring *et al.* 2008). Therefore, an increment of nutrient availability may modify plant–herbivore interactions as a result of uptake and use efficiency of nutrients by plants (Crawley *et al.* 2005, Gough *et al.* 2000). Nutrient concentration, primarily nitrogen and phosphorus, is crucial to organ/module production
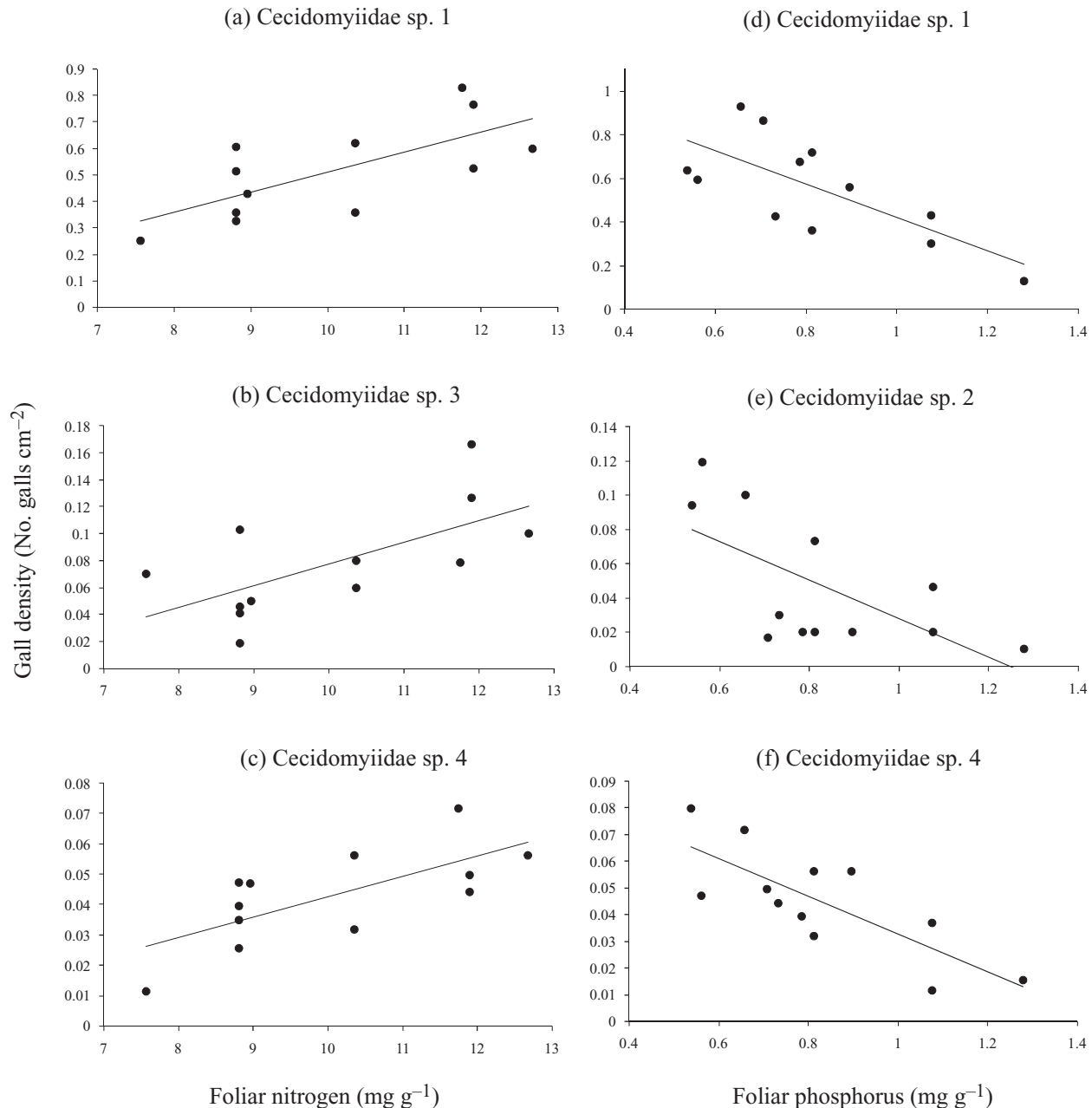
Figure 3. Relationships between foliar nitrogen of galled leaves and gall density in plots with addition of nitrogen in three galling species associated with *Eremanthus glomerulatus*. Cecidomyiidae sp. 1 (a); Cecidomyiidae sp. 3 (b); Cecidomyiidae sp. 4 (c); and relationships between foliar phosphorus of galled leaves and gall density in plots with addition of phosphorus in three galling species. Cecidomyiidae sp. 1 (d); Cecidomyiidae sp. 2 (e); Cecidomyiidae sp. 4 (f).

such as leaves, roots and inflorescences (Abrahamson & McCrea 1985, Aerts & Chapin 2000). Plant species adapted to nutrient-poor environments are well-known to retranslocate nitrogen and phosphorus out of above-ground organs, especially leaves (Chapin 1991, Chapin et al. 1980). Variation in the quality of foliage, particularly nitrogen, may influence the incidence of herbivores by changes in survival, fecundity and mortality (Helms & Hunter 2005). In our study, a significantly higher

nitrogen and phosphorus concentration was observed in galled compared with ungalled leaves when these nutrients were added experimentally to soil. These results support the findings of other experiments that suggest that galls act as nutrient sinks (Blanche & Westoby 1995). Limiting macronutrients in the soils such as nitrogen and phosphorus, affected the density of gall-inducing insect species on *E. glomerulatus* in the savanna study area. It has been suggested that

species from low-nutrient (nitrogen and phosphorus) habitats have higher nutrient resorption efficiencies (percentage of a nutrient withdrawn from mature leaves before leaf abscission) (Van Heerwaarden *et al.* 2003). Nitrogen and phosphorus fertilization result in higher concentration in the litter (Van Heerwaarden *et al.* 2003, Vitousek 1998) indicating lower nitrogen resorption efficiency of most species (Eckstein *et al.* 1999). However, Resende (2001) show that savanna plant species have a highly efficient and complete phosphorus resorption. In contrast, nitrogen concentrations in senesced leaves show intermediate or incomplete resorption efficiencies suggesting that savanna plants occur in conditions with low nutrient availability, especially nitrogen and phosphorus (Haridasan 2001, Kozovits *et al.* 2007). As result of conditions of low nutrient availability, these plant species have developed efficient systems to minimize nutrient losses such as higher resorption of nutrients (Kozovits *et al.* 2007, Nardoto *et al.* 2006) and scleromorphic leaves that in turn, reduce the probability of abscission and facilitate high concentration of chemical defences (Fernandes & Price 1988, Price *et al.* 1998). We argue that gall-inducing insect species are capable of manipulating their host plants extending to control above chemical composition of gall tissues, that in turn, usually have elevated concentrations of nutrients and low concentrations of secondary chemical compounds (Hartley & Lawton 1992, Pascual-Alvarado *et al.* 2008). These results corroborate the higher incidence of gall-inducing insect species found in scleromorphic vegetation (Fernandes & Price 1988, Fernandes *et al.* 1994) and suggest that scleromorphic plant species represent favourable sites to colonize by gall-inducing insect species (Price *et al.* 1998).

We used nitrogen and phosphorus as indicators of soil fertility in our study and we found two different patterns: the first indicated that in treatments with addition of nitrogen and phosphorus, the density of galls decreased in four gall-inducing insect species (Cecidomyiidae sp. 1, sp. 2, sp. 4 and sp. 5). Gall density was higher on hosts with lower foliar phosphorus concentration in four gall-inducing insect species (Cecidomyiidae sp. 1, sp. 2, sp. 4 and sp. 5), while in control plots, we found a similar pattern that shows a negative relationship between gall density and concentration of leaf phosphorus in five gall-inducing insect species. In addition, no relationship was found among gall density and foliar nitrogen and phosphorus concentration in ungalled leaves. The relative nutrient status of plants is likely to be reflected in the foliar nutrient concentration of ungalled leaves. Therefore, these results suggest that foliar nitrogen and phosphorus concentration respond to gall density in galled leaves and corroborate a chemical manipulation of the host plant by gall-inducing insects (Hartley & Lawton 1992). In contrast, the second trend

shows that gall density presented positive relationships with foliar nitrogen concentration in three gall-inducing insect species (Cecidomyiidae sp. 1, sp. 3 and sp. 4). Herbivore responses to host plant stress (i.e. low nitrogen, phosphorus and water availability) may be negative, positive or in some cases show no response to plant stress (Larsson 1989, Waring & Cobb 1992). Blanche & Westoby (1995) showed that abundance and richness of gall-inducing insects were not directly linked to soil fertility but, instead, via host plant taxon in a community dominated by *Eucalyptus* spp. The mechanism that explained this result is that eucalypts are adapted to infertile soils and may indirectly affect the incidence of gall-inducing insects that were already in association with the *Eucalyptus* community. However, it has been suggested that plants that occur in low soil fertility, especially low soil phosphorus have high abundance of gall-inducing insect species (Blanche & Ludwig 2001, Cuevas-Reyes *et al.* 2004b, Fernandes & Price 1991). These plants usually have high concentrations of secondary chemical compounds such as oils and phenols and each gall-inducing insect species has the ability to manipulate the growth and development of plant tissue (Weis *et al.* 1988) and may also be capable of modifying host nutritional quality and plant secondary metabolites for protection against natural enemies (Fernandes & Price 1992, Hartley & Lawton 1992, Pascual-Alvarado *et al.* 2008).

We conclude that soil fertility affected in different ways the density of gall-inducing insect species on *E. glomerulatus* under experimental soil conditions. In the first case, a given host that occurred in different conditions with low levels of soil phosphorus and nitrogen had greater density of galls than the same host species that occurred in fertile plots. Therefore, direct effects of soil fertility may also explain the reduction in the density of galls on *E. glomerulatus* that are present in different soil fertility conditions. Adaptive phenotypic plasticity within each individual may explain differences in the incidence of gall-inducing insects for *E. glomerulatus* analysed in our study (Cuevas-Reyes *et al.* 2004b, Schlichting & Pigliucci 1998). Host plants of the same species adapt to variable soil fertility and gall-inducing insects negatively respond to the quality of the environment experienced by their hosts. Because some plant species respond to low soil fertility by having long-lived parts with a high concentration of chemical secondary compounds (Coley *et al.* 1985) and gall-inducing insects may sequester these chemicals in the gall chamber for protection against natural enemies, we expect galling insects to select individuals of a given host species with greater chemical defences under low nutrient availability. In the second case, our study shows that gall density presented positive relationships with foliar nitrogen concentration in three gall-inducing insect species. The herbivore responses to plant quality may be a continuum that included herbivore

species that feed preferentially on stressed plants to herbivores that feed on vigorous plants or plant modules (Price 1991). The evidence indicates that the patterns of herbivory are higher in plants that grow in rich environments, compared with plant species adapted to low resources (Coley *et al.* 1985, Price 1991). Therefore, plants with more nitrogen and phosphorus availability represent sites more vigorous to colonize by gall-inducing insect species because the larval performance may be highest in these sites (Fritz *et al.* 2000, Price *et al.* 1990). This suggests an oviposition preference for more vigorous plant modules and potential relationship among oviposition site selection and larval survival (Craig *et al.* 1989, Price *et al.* 1990). In our study the gall density was different between insect guilds (foliar vs. stem galls). Because stem galls were larger than foliar galls, it is probable that gall-inducing insect species with large galls produce many insects and occur on their host plants at low densities. This idea is in accord with some studies that indicated that the number of galling insects present within galls is positively related to gall size for many insect species (Freeman & Geoghagen 1987, Honek 1993). Because gall size is a good indicator of insect fitness (Sopow & Quiring 2001), the differences observed in gall density between insect guilds may reflect different strategies of uptake and use efficiency of nutrients by foliar and stem gall-inducing insect species. Finally, this study provides the first experimental evidence for the incidence of gall-inducing insects on the sclerophyllous savanna. These results show the ecological importance of abiotic factors in structuring plant–insect interactions.

ACKNOWLEDGEMENTS

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