



Insect galls in three species of *Copaifera* L. (Leguminosae, Caesalpinioideae) occurring sympatrically in a Cerrado area (Bahia, Brazil)

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Abstract: The aim of this study was to characterize galls of three species of the *Copaifera* genus (*C. sabulicola*, *C. luetzelburgii* and *C. depilis*), which occur sympatrically in a Cerrado area from Bahia state. A total of 23 gall morphotypes were found, 9 morphotypes on *C. sabulicola*, 7 on *C. luetzelburgii* and 7 on *C. depilis*. Galls predominated on leaves and stems. The most abundant colours found among galls were brown and green. Regarding the period of the year, from 35,511 leaflets analysed, 16,322 were collected in the dry season and 19,189 in the rainy season. In the dry season, 1,635 leaflets having galls were observed, and the highest proportion was observed on *C. luetzelburgii* (16.31%). In relation to the rainy season, there is statistical difference in the incidence of leaflets with galls between *C. sabulicola* and *C. depilis*, these two species are affected differently during the rainy season. In general, most of the gall morphotypes were more abundant in the dry season. The galls of 18 morphotypes cannot be determined, because the gall samples were collected occupied by parasitoids or without dwellers. Cecidomyiids induced galls in five morphotypes. Associated invertebrates belonging to the orders Hymenoptera, Diptera, Coleoptera and Acari were found among all gall morphotypes. The results obtained here indicate that the three species of *Copaifera* have an expressive number of galls, but lower than reported in the literature for *C. langsdorffii*, which is considered a super host.

Keywords: *Copaifera depilis*, *Copaifera luetzelburgii*, *Copaifera sabulicola*, *Cecidomyiidae*, insect-plant interaction

Galhas entomógenas em três espécies de *Copaifera* L. (Leguminosae, Caesalpinioideae) que ocorrem em simpatria em área de Cerrado (Bahia, Brasil)

Resumo: O objetivo deste trabalho foi caracterizar as galhas de três espécies do gênero *Copaifera* (*C. sabulicola*, *C. luetzelburgii* e *C. depilis*) que ocorrem em simpatria no Cerrado do Estado da Bahia. Um total de 23 morfotipos de galhas foi encontrado, sendo 9 morfotipos em *C. sabulicola*, 7 em *C. luetzelburgii* e 7 em *C. depilis*. As galhas predominaram nas folhas e caules e os morfotipos com coloração marrom e verde foram os mais abundantes. Em relação à época do ano, do total de 35.511 folíolos analisados, 16.322 foram coletados no período seco e 19.189 no chuvoso. Na estação seca foram observados 1.635 folíolos com galhas, sendo que a maior proporção de folíolos galhados foi observada em *C. luetzelburgii* (16.31%). Já em relação à estação chuvosa, há diferença estatística na incidência de folíolos com galhas entre *C. sabulicola* e *C. depilis*, estas duas espécies são afetadas diferentemente durante a estação chuvosa. De forma geral, a maioria dos morfotipos foi mais abundante na estação seca. Os galhadores de 18 morfotipos não foram determinados, porque as galhas coletadas apresentavam parasitóides ou estavam vazias. Os cecidomídeos foram os responsáveis pela indução de cinco morfotipos. Os invertebrados associados às galhas pertencentes às ordens Hymenoptera, Diptera, Coleoptera e Acari. Os resultados obtidos neste estudo apontam que as três espécies de *Copaifera* possuem um número expressivo de galhas, porém menor que o relatado na literatura para *C. langsdorffii*, a qual é considerada uma super hospedeira.

Palavras-chave: *Copaifera depilis*, *Copaifera luetzelburgii*, *Copaifera sabulicola*, *Cecidomyiidae*, interação inseto-planta

Introduction

The genus *Copaifera* L. (Leguminosa, Caesalpinioideae) has 38 species, distributed in Central America (4 spp.), South America (29 spp.), Africa (4 spp.) and Asia (1 sp.). In Brazil, 28 species of this genus have been recorded, with 22 species restricted to Brazilian territory (Costa & Queiroz 2007). *Copaifera* species are popularly known as “copaibeiras” or “pau d’óleo” and are mainly found in the Amazon region and in Central-western Brazil (Veiga Junior & Pinto 2002). Bahia is the Brazilian state with the largest number of *Copaifera* species (13 species). Furthermore, the species *C. langsdorffii* Desf., *C. depilis* Dwyer, *C. luetzelburgii* Harms and *C. sabulicola* Costa & Queiroz occur sympatrically in western region of this state (Santos et al. 1985a).

Despite the wide distribution of *Copaifera* in Brazilian territory, studies on the relationships between galling insects and species of this genus are mainly related to *Copaifera langsdorffii* Desf. This species is considered by Costa et al. (2010), as the tropical arboreal species with the richest galling insects ever described (23 morphotypes) and can be considered a super host (Gonçalves-Alvim & Fernandes 2001). The aims of this study were to characterize galls of three species on this genus, *Copaifera depilis* Dwyer, *Copaifera luetzelburgii* Harms and *Copaifera sabulicola* Costa & Queiroz, which occur sympatrically in Western Bahia and compare their abundance.

Materials and Methods

This study was carried out from samples of three species of *Copaifera* collected in Serra da Bandeira, Barreiras, Bahia (11° 37' and 12° 25'S and 44° 34' and 46° 23'W). This municipality is located in the Cerrado Biome and presents two well defined seasons, a dry and a rainy. The rainy season comprises the months from October to April, while the dry season corresponds to the months from May to September. The climatic type of the municipality, according to Köppen classification is Aw type, typical of savannah, presenting dry winter and average air temperature of the coldest month above 22°C. The mean annual rainfall is 1500 ± 500 mm (Neto et al. 2013).

The gall characterization of *C. sabulicola*, *C. luetzelburgii* and *C. depilis* was performed by sampling 20 individuals of each species randomly selected. The collections were carried out from December 2012 to January 2013 (rainy season) and from June to July 2013 (dry season). The plants used had the following averages for height: 2.19 m (± 0.35) in *C. sabulicola*; 2.27 m (± 0.29) in *C. luetzelburgii* and 1.96 m (± 0.49) in *C. depilis*.

The richness and abundance of galls per plant were determined by collecting four branches with 50 cm length of each host plant. To avoid the effect of the position of the branches on the occurrence of the galls, each plant was divided into quadrants following the north (N), south (S), east (E) and west (W) directions, and one branch of each of these quadrants was collected. The number and size of the branches as well as the number of collections were determined in order not to damage the host plants by excessive pruning. A total of 80 branches of each species were analysed in each season and the same individuals were investigated during the dry and rainy seasons. The galls were photographed and morphologically characterized according to the shape, colour, presence of trichomes and distribution in the host plant as reported by Maia & Fernandes (2004). The occurrence of leaf galls on adaxial and/or abaxial was verified.

In order to compare the proportion of leaves with galls between seasons and species, we counted the total number of leaflets and the number of leaflets with galls, in 20 individuals of each of the three species. We applied the test of comparison between two proportions with correction of continuity, at a significance level of 5%, with decision based on p-value. Firstly, we performed the comparison test between dry and rainy seasons considering the three species together. Secondly we compare the dry and rainy seasons considering the three separate species. Finally, we compare the species to each other, both for the dry season and for the rainy season. These analyses were conducted in the software R-Gui, 3.4.0 version.

Results and Discussion

In the three *Copaifera* species, a total of 23 gall morphotypes were obtained, 9 morphotypes found on *C. sabulicola*, 7 on *C. luetzelburgii* and 7 on *C. depilis*. The characterization of the galls is presented in Table 1 and Figure 1. Galls were found in leaves and stems, being foliar the most abundant in the three *Copaifera* species. Although galls were not observed in flowers and fruits, these organs were also inspected.

In *C. sabulicola*, the most frequent morphotype found was the globoid one, which was found in 14 and 13 of the 20 plants analysed in the dry and rainy seasons, respectively (Figure 1h). Regarding the foliar surface, it was verified that four morphotypes occurred in the adaxial and abaxial surfaces. In *C. luetzelburgii* 7 morphotypes were found, however only 1 (7.42%) occurred in the stem. In this species, the parenchymal was the most frequent (Figure 1p). In *C. depilis*, 7 morphotypes were also identified, the stem types being less frequent than the foliar ones. In *C. depilis* and *C. sabulicola*, galls with trichomes were not found, whereas in *C. luetzelburgii* two morphotypes presented these structures (Table 1).

The results indicate that the pattern of occurrence of galls in the three *Copaifera* species studied is similar to that observed by Almeida et al. (2006) in *C. langsdorffii*, in which the greatest diversity and abundance of galling insects occurs in leaves, followed by branches. The occurrence of a greater number of leaf galls is corroborated by several studies in different Brazilian biomes and for several plant species (Gonçalves-Alvim & Fernandes 2001, Araújo et al. 2011; Santos et al. 2011b; Silva & Almeida-Cortez 2006), and this may be justified by the greater abundance of nutrients in this organ (Mani 1964).

According to the colour, brown and green morphotypes were the most frequent in galls on three species of *Copaifera*, however, differences were found in colour and it may be related to gall senescence. Santos et al. (2010c), in a study carried out in a Cerrado area, also verified in 20 species of plants that galls might have a wide variety of colours, especially during the maturation period. Another difference between the three species of *Copaifera* refers to the presence or absence of pubescence. In *C. sabulicola* and *C. depilis* were not observed the presence of trichomes while in *C. luetzelburgii* was observed. This result can be associated with the characteristics of the host species, due to *C. luetzelburgii* present a large number of trichomes on leaves, while the other two species are glabrous. Other possibility is that trichomes on leaves conferred some protection to the galler by killing parasitoids of the larva as pointed by Fernandes et al. (1987). More detailed studies should be performed to explain this finding.

In this analysis of abundance of gall morphotypes and the relationship with leaflet numbers and period of the year, it was observed that although the collected branches had the same length (50 cm), the number of leaflets verified in the three *Copaifera* species was different depending on the species and according to the period of year. A total of 35,511 leaflets were analysed, being 16,322 collected in the dry season and 19,189 in the rainy season.

We observed that the proportion of leaves with galls during the dry season is 0.1002 (percentage approximately equal to 10%), while in the rainy season this proportion is 0.0459 (percentage approximately equal to 5%) (Table 2). The test for comparison of two proportions indicated a statistical difference between the two periods of the year (p-value approximately equal to zero), in other words, the period of year interferes with the incidence of leaves with galls in such a way that, in this study, the proportion of leaves with galls in the dry season is double the proportion in the rainy season.

The results presented in Table 3 demonstrated that *C. sabulicola* is the only species which did not present statistical difference between dry and rainy seasons, in other words the period of year did not interfere

Table 1. Gall characterization (organ, shape, colour, trichomes, foliar surface and plant frequency) in species of *Copaifera* (Serra da Bandeira, Barreiras, Bahia, Brazil). Abbreviations: Ga: galler, Pa: parasitoid, Su: successor

Gall	Organ	Shape	Colour	Trichomes	Foliar surface	Plant frequency		Associated fauna	Figure
						Dry	Rainy		
<i>Copaifera sabulicola</i>									
1	Stem	Discoid	Brown	Absent	-	1 (5%)	6 (30%)	Hymenoptera (Pa) Acari (Su)	1a
2	Stem	Fusiform	Brown	Absent	-	1 (5%)	0 (0%)	-	1b
3	Stem	Globoid	Brown	Absent	-	1 (5%)	0 (0%)	Formicidae (Su)	1c
4	Leaf	Parenchymatical	Green/ Brown	Absent	Adaxial	9 (45%)	10 (50%)	Cecidomyiidae (Ga)	1d
5	Leaf	Discoid	Green / Red	Absent	Both	2 (10%)	10 (50%)	Cecidomyiidae (Ga)	1e
6	Leaf	Discoid	Green / Red	Absent	Abaxial	2 (10%)	1 (5%)	-	1f
7	Leaf	Discoid	Green	Absent	Both	7 (35%)	3 (15%)	Cecidomyiidae (Ga)	1g
8	Leaf	Globoid	Green / Brown	Absent	Both	14 (70%)	13 (65%)	-	1h
9	Leaf	Cylindrical	Green/Red / Brown	Absent	Both	4 (20%)	1 (5%)	Coleoptera (Su)	1i
<i>Copaifera luetzelburgii</i>									
1	Leaf	Cylindrical	Green /Red/ Brown	Present	Abaxial	17 (85%)	8 (4%)	Hymenoptera (Pa)	1j
2	Leaf	Globoid	Green /Red/ Brown	Absent	Both	15 (75%)	18 (90%)	Hymenoptera (Pa)	1k
3	Leaf	Discoid	Green	Absent	Adaxial	3 (15%)	0 (0%)	Hymenoptera (Pa)	1l
4	Leaf	Discoid	Brown	Present	Abaxial	13 (65%)	5 (25%)	Hymenoptera (Pa)	1m
5	Stem	Discoid	Brown	Absent	-	12 (60%)	2 (10%)	Hymenoptera (Pa)	1n
6	Leaf	Discoid	Brown	Absent	Abaxial	4 (20%)	3 (15%)	-	1o
7	Leaf	Parenchymatical	Green / Brown	Absent	Both	18 (90%)	15 (75%)	-	1p
<i>Copaifera depilis</i>									
1	Stem	Discoid	Brown	Absent	-	0 (0%)	2 (10%)	Hymenoptera (Pa)	1q
2	Stem	Globoid	Brown	Absent	-	2 (10%)	1 (5%)	-	1r
3	Leaf	Globoid	Green / Brown	Absent	Both	19 (95%)	14 (70%)	-	1s
4	Leaf	Cylindrical	Green/Red/ Brown	Absent	Both	17 (85%)	13 (65%)	Cecidomyiidae (Ga)	1t
5	Leaf	Discoid	Green	Absent	Adaxial	3 (15%)	2 (10%)	-	1u
6	Leaf	Discoid	Brown	Absent	Both	15 (75%)	15 (75%)	-	1v
7	Leaf	Parenchymatical	Green / Brown	Absent	Abaxial	13 (65%)	10 (50%)	Cecidomyiidae (Ga) Hymenoptera (Pa)	1x

with the incidence of leaves with galls (p -value = 0.05417). The species *C. luetzelburgii* and *C. depilis* seemed to be sensitive to the seasons as dry and rainy seasons significantly interfered with the incidence of leaves with gall (p -value close to zero). In the *C. luetzelburgii* species, this proportion in the dry season is more than triple the rainy season, suggesting that this species is the most sensitive season of the year. In the species *C. depilis* the proportion in the dry season is double of the rainy season.

Regarding to dry season, all three species presented differences between them in relation to the incidence of leaves with gall. Specifically, during the dry season each of the three species is affected differently ($p < 0.0001$) such that the most sensitive species during the dry season is *C. luetzelburgii* (16.31%), followed by *C. depilis* (8.04%). The least sensitive species among the three was *C. sabulicola* (4.36%). Referring to rainy season, there is no statistical difference in the incidence of leaves with galls between *C. sabulicola* and *C. luetzelburgii* (p -value = 0.1073, $\chi^2 = 2.5932$) and between *C. luetzelburgii* and *C. depilis* (p -value = 0.1480, $\chi^2 = 2.0931$). However, there are differences between *C. sabulicola* and *C. depilis* (p -value = 0.002230, $\chi^2 = 9.3503$), that is, these two species are affected differently during the rainy season.

Several hypotheses search for ecological-evolutionary evidences in order to explain the association between galling insects and their host plants. Some authors point out that species richness for galling insects is different from others insects, because they require xeric environments or dry seasons instead of mesic environments and rainy seasons (Fleck & Fonseca 2007).

Although the largest number of leaflets in *Copaifera* was observed in the rainy season, the highest number of galling leaflets occurred in the dry season.

In the present study, we observed that five morphotypes were induced by Cecidomyiids and the gallers of 18 morphotypes cannot be determined, because the gall samples were collected occupied by parasitoids or without dwellers (Table 1). According to Maia (2011), Cecidomyiidae are the most important galler in all zoogeographic regions. Micro-hymenopteras identified represents possibly parasitoids, natural enemy of Cecidomyiidae, with the exception of ants (Formicidae), which were found on abandoned galls, being considered in this study as a successor for *Copaifera langsdorffii* Desf. (Fabaceae), Oliveira et al. (2013) pointed that the level of parasitoidism is very high, and sampling for cecidomyiids adults has been unsuccessful. Coleoptera and Acari were considered successors.

The results obtained in this study indicate that the three species of *Copaifera* have an expressive number of galls, but lower than reported in the literature for *C. langsdorffii*, which is considered a super host (Costa et al. 2010). Among the morphotypes found in the *Copaifera* genus, some are morphologically similar among species, such as discoid occurring in *C. depilis* (Figure 1q) and *C. luetzelburgii* (Figure 1n), which were also observed in *C. langsdorffii* by Fernandes et al. (1988). These similarities can be better understood from anatomical gall studies, identification of inducers and parasitoids, which may clarify important aspects about the co-evolution between galling species and *Copaifera* genus.



Figura 1. Galls on *C. sabulicola* (a-i), *C. luetzelburgii* (j-p), and *C. depilis* (q-x) in Cerrado area (Serra da Bandeira, Barreiras, Bahia, Brazil).

Table 2. Comparison between dry and rainy seasons considering the three *Copaifera* species together (*Copaifera sabulicola*, *Copaifera luetzelburgii* and *Copaifera depilis*).

Seasons	Leaflets with galls	Total leaflets	Proportion	Confidence Interval (95%)	Test result
Dry	1,635	16,322	0.1002	[0.0956; 0.1048]	p -value < 0.0001
Rainy	881	19,189	0.0459	[0.0430; 0.0489]	$\chi^2=393,6214$

Table 3. Number of leaflets with galls and leaflets analyzed in *Copaifera sabulicola*, *Copaifera luetzelburgii* and *Copaifera depilis* in dry and rainy seasons in Cerrado area (Barreiras, Bahia, Brazil).

Species	Seasons	Leaflets with galls	Total leaflets (MD±SD)	Proportion	Confidence Interval (95%)	Test result
<i>Copaifera sabulicola</i>	Dry	193 (9.65±8.25)	4,426 (421.52±88.47)	0.0436	[0.0376; 0.0496]	$p\text{-value}=0,05417 \chi^2= 3,7073$
	Rainy	321 (30.57±13.56)	6,180 (588.57±106.97)	0.0519	[0.0464; 0.0575]	
<i>Copaifera luetzelburgii</i>	Dry	958 (47.90±8.89)	5,874 (559.43±84.84)	0.1631	[0.1536; 0.1725]	$p\text{-value} < 0,0001 \chi^2= 471,7436$
	Rainy	302 (28.76±8.90)	6,615 (630.00±127.24)	0.0457	[0.0406; 0.0507]	
<i>Copaifera depilis</i>	Dry	484 (24.20±24.21)	6,022 (573.52±81.95)	0.0804	[0.0735; 0.0872]	$p\text{-value} < 0,0001 \chi^2= 87,6910$
	Rainy	258 (24.57±9.04)	6,394 (608.95±97.95)	0.0404	[0.0355; 0.0452]	

Author Contributions

Isana Martins dos Santos: contributed to the study conception and design, to data analysis and interpretation, to the drafting and revision of the manuscript's intellectual content.

Valdeir Pereira Lima: contributed to the data analysis and interpretation, to the drafting and revision of the manuscript's intellectual content.

Edyany Kellen Souza Soares: substantial contribution to data collection and data analysis.

Marcelo de Paula: contribution to data analysis and interpretation, and revision of the manuscript's intellectual content.

Daniela Cristina Calado: contributed to the study conception and design, to data analysis and interpretation, to the drafting and revision of the manuscript's intellectual content.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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