

Entomofauna associated with erva-de-touro (*Poiretia latifolia* Vogel) stands in the Santa Catarina plateau region

Entomofauna associada à erva-de-touro (Poiretia latifolia Vogel) em povoamentos na região do Planalto Catarinense

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ABSTRACT

Poiretia latifolia Vogel (Fabaceae), is a promising plant native of the altitude field ecosystem, inserted in the Atlantic Forest biome in the southern region of Brazil. It is valued for its monoterpene-rich essential oil and exhibits great potential in the pharmaceutical and cosmetic industries. This work aimed at studying the entomofauna associated with *P. latifolia*, as well as assessing the effects of these insects on this plant's reproductive success. The entomofauna were surveyed between December 2015 and January 2016, in two natural *P. latifolia* stands found in the Capão Alto and São José do Cerrito municipalities of Santa Catarina State. The entomofauna was monitored for 42 hours in each stand, being marked five plants in each stand that were accompanied hourly, from 9:00 am to 4:00 p.m. The effects of the climatic variables on the insect were analyzed using the Pearson correlation coefficient. To determine the damage due to herbivory, ten *P. latifolia* plants were marked at each stand, from which the flowers and seeds were collected and the insect damage estimated. The predominant insects in both stands were identified as belonging to the orders Coleoptera, Diptera and Hymenoptera. The Dipteran insects revealed a significant positive correlation with the mean daily temperature ($r = 0.84$, $p = 0.03442$) in the Capão Alto stand. Damages caused by insects to the *P. latifolia* flowers and seeds was higher in São José do Cerrito, corresponding to 54% and 88% of damaged structures, respectively. Environmental changes precipitated by the intense agricultural usage in the vicinity of the *P. latifolia* stands may have been the reason for these differences.

KEYWORDS: faunistic analysis, ecological interactions, florivory, seed predation, Fabaceae, altitude fields.

RESUMO

Poiretia latifolia Vogel (Fabaceae) é uma promissora planta nativa do ecossistema campos de altitude, inserido no bioma Mata Atlântica na região Sul do Brasil. Seu valor se encontra na composição do seu óleo essencial que é rico em monoterpenos com potencial de uso farmacêutico e cosmético. O objetivo deste trabalho foi estudar a entomofauna associada à *P. latifolia*, bem como avaliar a influência dos insetos no sucesso reprodutivo da planta. O levantamento da entomofauna foi realizado no período de dezembro de 2015 a janeiro de 2016, em dois povoamentos naturais de *P. latifolia* localizados nos municípios de Capão Alto e São José do Cerrito, Santa Catarina. A entomofauna foi monitorada por 42 horas em cada local, sendo marcadas cinco plantas em cada área que foram acompanhadas de hora em hora, das 9:00 às 16:00. A influência das variáveis meteorológicas sobre a presença de insetos foi analisada pelo coeficiente de correlação de Pearson. Para determinar os danos por herbivoria, foram marcadas dez plantas de *P. latifolia* em cada área, das quais foram coletadas flores e sementes para avaliar o dano por insetos. Insetos pertencentes às ordens Coleoptera, Diptera e Hymenoptera foram predominantes em ambos os povoamentos. A presença de insetos da ordem Diptera demonstrou correlação positiva significativa com a temperatura média diária ($r = 0,84$; $p = 0,03442$) no povoamento de Capão Alto. Os danos causados por insetos às flores e sementes de *P. latifolia* foram superiores em São José do Cerrito, correspondendo por 54% e 88% das estruturas danificadas, respectivamente. Alterações ambientais causadas pela intensidade de uso agrícola próximo aos povoamentos de *P. latifolia* podem ter causado estas diferenças.

PALAVRAS-CHAVE: análise faunística, interações ecológicas, florivoria, predação de sementes, Fabaceae, campos de altitude.

INTRODUCTION

The altitude fields reveal a rich diversity of plant species, with an enormous potential for several uses including food for human consumption, grazing, and the manufacture of medicines or cosmetics (AMORIM & BOFF 2009). The Santa Catarina plateau region is outstanding for its high levels of conservation of the native vegetation. This was possible because of the less intense anthropization of this region, coupled with the type of agricultural activities practiced here, mostly extensive cattle rearing (AMORIM 2010).

However, this type of rural exploration model has been drastically altered over the recent years, when agricultural crops such as soybean and corn were introduced, and reforestation was done with the *Pinus* sp. and *Eucalyptus* sp. (PEREIRA et al. 2006, POLÊSE 2014). These changes currently put the survival of many native plants species to great risk, several of which have been used in regional and traditional therapies.

In this context, the local populations have extensively used the erva-de-touro *Poiretia latifolia* Vogel (Fabaceae: Dalbergieae), to treat stomach disorders (JANKE et al. 1988). Besides being believed to have aphrodisiac properties, this plant is also used as a flavoring agent in the beverage chimarrão (MÜLLER 1984). Recent research gives evidence of its high potential in the manufacture of cosmetics and medicinal products, because of its uniquely aromatic essential oils, and abundance of monoterpenes like carvone, dihydrocarvone and limonene (PORTO et al. 2010).

This chemical compound class present in plants is also associated with the insects being attracted to them (CERUTI 2007). The monoterpenes contained in *P. latifolia* may indicate the presence of the entomophilic type of pollination, a fact reinforced by the flowers revealing several characteristics linked to entomophily. AMORIM (2010) reported the paucity of studies on the pollinating agents in *P. latifolia* as a limiting factor in domesticating this species.

The global decline in biodiversity noted is principally caused by the destruction of the habitat and its fragmentation (GANEM & DRUMMOND 2011). Biodiversity loss can produce a loss in the stable functioning of the ecosystem (LANARI & COUTINHO 2010). Man-made landscapes suffer species loss, mainly due to agricultural practices, as the natural habitats are destroyed and isolated (FERREIRA et al. 2012).

Habitat fragmentation may also result in a drop in the species richness and abundance of the pollinator guilds; these changes disrupt the foraging activities of the visiting floral insects, disturb the plant-pollinator relationships, and decrease the seed output and gene flow in the isolated plant populations (DAUBER et al. 2010). Therefore, the biotic interactions between the different organisms involved in this study were conducted in the Santa Catarina State University (Brazil).

The density and biodiversity of the hosts present in an environment determines the abundance of the entomofauna (SILVEIRA NETO et al. 1995). In their study on *Sophora tomentosa* L. and *Crotalaria vitellina* Ker Gawl, BRITO et al. (2010) reported that, apart from being important pollen vectors of these Fabaceae, the entomophilic pollinators also strongly affect the survival of these insects in the resting sites, thus indicating the interdependence of these players in this ecosystem.

Faunistic analysis is a tool, utilized over several years, to characterize and delimit communities, discover the predominant species, assess the environmental influences on a specific region, as well as compare the different locations based on the insect species identified (FRIZZAS et al. 2003), to bring clarity in terms of the insect community dynamics in certain ecosystems (BICELLI et al. 1989, DORVAL et al. 1995). In such a study, it is vital that the entomofauna be identified, as this will enable a solution to be found to make up the pollination deficit for many cultivable vegetable crops, and is also applicable for the erva-de-touro *P. latifolia*. Thus, the protection and restoration strategies for specific insect habitats can be developed so as to guarantee species pollination (SHULER et al. 2005).

Knowledge regarding the entomofauna associated with *P. latifolia* permits a clear understanding of the interactions occurring in the stands of this plant, including the protooperation relationship with the pollinating insects. Insects that feed on the flowers and seeds of *P. latifolia* and thus exert a negative influence on the reproductive success of the plant can be identified. Therefore, this work aimed at identifying the entomofauna associated with *P. latifolia*, as well as estimating the negative influence exerted by these predatory insects on the flowers and seeds, and on the reproduction of this plant species.

MATERIAL AND METHODS

A survey was conducted from December 2015 to January 2016, of the *P. latifolia* associated entomofauna in two natural stands, in the Capão Alto and São José do Cerrito municipalities, Santa Catarina State, Brazil.

The Capão Alto experimental area is located at 27°58'33" S and 50°30'26" W, at 966 m altitude. The São José do Cerrito experimental area is separated into two sections, the first situated at 27°46'6.60" S and 50°30'2.50" W, at 906 m altitude, and the second at 27°46'8.50" S and 50°30'3.37" W, at 905 m altitude.

The region climate is subtropical with well-distributed rainfall during summer (Cfb-type according to Köppen and Geiger), with average annual precipitations between 1300 and 1700 mm and 15 to 16 °C average annual temperature, and 78 to 80% average annual relative humidity (PANDOLFO et al. 2002). Cambissolo is the predominant soil type in this region (SOUTO 2005).

All the data related to the meteorological variables [relative humidity (RH) and temperature (°C)] during the time of the survey were drawn from the INMET Meteorological Database for Teaching and Research (BDMEP) (INMET 2016), to the municipality of Lages, SC, from a station 25.6 km from the Capão Alto stand and 16.7 km from the São José do Cerrito stand.

At each stand, five plants were marked, and every 60 minutes the insects visiting each plant were counted for a 10-minute observation period per plant per hour, with a 2-minute interval between each plant. Using an entomological net, aspirator or tweezers in the case of caterpillars, the visiting insects were captured (ALMEIDA et al. 1998), sacrificed with ethyl ether in a lethal chamber and transported to the laboratory for classification up to the level of order, according to the methodology of FUJIHARA et al. (2016). Utilizing the data thus collected, the analysis of the fluctuation of the Orders to which the predominant insects belonged was performed, to estimate the period of high insect activity.

Every week, observations were made from 9:00 am to 4:00 p.m., for a total of 42 observation hours, of the entomofauna associated with this plant in each stand. Photographic records of the visiting insects were maintained and their behavior and interactions with the *P. latifolia* species were recorded.

To assess the extent of herbivory damage caused by the insects during the *P. latifolia* reproductive phase, the fully opened flowers (anthesis) 5 per plant from 10 plants per site were collected, apart from the weekly collection of all the fruits produced by the 10 plants specified, as the fruits reached maturation. After fixing the flowers in Carnoy's solution, they were left undisturbed for 24 hours. They were then transferred to labeled vials containing 70% alcohol. The fruits were oven-dried at 40 °C for 24 hours and refrigerated at 5 °C until the articles opened for seed evaluation. Dissection of the flowers was done under a stereo microscope, and the total and percentage of flowers having damaged reproductive organs were counted. Similarly, the fruits were also opened under a stereo microscope to count the total number of seeds the insects had damaged.

The correlation between the number of insects caught per order and the meteorological variables [mean daily temperature (°C) and relative humidity (RH)] were calculated using the Pearson correlation coefficient, with PAST v. 2.17c (HAMMER et al. 2001), and standardized data, according to FIGUEIREDO FILHO & SILVA JÚNIOR (2009).

RESULTS AND DISCUSSION

During the evaluations, 9,566 individuals belonging to eight different orders of insects were observed (Table 1). In both stands, the most predominant orders included Hymenoptera, Diptera and Coleoptera, with values of 62.1%, 33.6%, and 3.9% of the total number of individuals counted, respectively.

A significant difference was observed between the assessment dates for order Hymenoptera, in both stands, with the third week of December registering the highest number of insects sighted (Table 2). During the first four weeks, a substantial difference was visible between the stands, with the greatest number of insects being recorded in São José do Cerrito. The high number of visiting hymenoptera was chiefly the result of the presence of the ants visiting the *P. latifolia* inflorescences, most likely seeking aphids or plant exudates. However, neither the *Acromyrmex* nor *Atta* genera were observed during the study period, possibly indicating that the leaf-cutting ants, common in this region, are not attracted to this plant (GIESEL 2014, BACCARO et al. 2015). During the first assessment week, no flowering plants in anthesis were evident, which limited the hymenopteran visitors to only ants and some wasps that were seen. The *P. latifolia* flowering was visible in abundance between Dec 17 and Dec 29, when greater numbers of visiting hymenoptera were seen (Table 2). In both stands, the presence of bees visiting the *P. latifolia* flowers were noted, but the wasps were noticed only in Capão Alto. Normally, Hymenopterans are considered economically significant as they are the main pollinating agents (KLEIN et al. 2006, LOSEY & VAUGHAN 2006), parasitoids (FUJIHARA et al. 2016) and predators of pests (ELISEI et al. 2010) of various cultures. MELO et al. (2012) reported that bees have the greatest potential as pollinators as they possess posterior collecting legs that facilitate pollen transport unlike the other hymenopterans which have ambulatory legs.

Table 1. Total number and percentage of insects from the different orders observed in the *P. latifolia* (N = 5) plants, located in two different stands, from six weekly collections between 12/03/15 and 01/08/16.

Order	Capão Alto		S. J. do Cerrito		Total	
	No.	%	No.	%	No.	%
Hymenoptera	2728	60.92	3210	63.09	5938	62.07
Diptera	1522	33.99	1695	33.31	3217	33.63
Coleoptera	201	4.49	168	3.30	369	3.86
Blattodea	15	0.33	3	0.06	18	0.19
Lepidoptera	4	0.09	7	0.14	11	0.11
Hemiptera	2	0.04	3	0.06	5	0.05
Mantodea	3	0.07	2	0.04	5	0.05
Neuroptera	3	0.07	0	0.00	3	0.03
Total	4478	100.00	5088	100.00	9566	100.00

In both the stands, order Diptera revealed significant variations in the number of insects counted during the time of study (Table 2). The third and fourth weeks registered a significant difference, with a greater number of Diptera in the São José do Cerrito stand ($p < 0.05$). This finding was possibly because the *P. latifolia* stands were in the vicinity of a cattle raising region, which produces substances that are attractive to these insects, with the Calliphoridae (PINTO et al. 2010), Muscidae (SOUZA et al. 2005) and Sarcophagidae (MARCHIORI & LINHARES 1999) visiting these areas, seeking oviposition and feeding sites, such as in the feces or on cattle.

Table 2. Mean number of insects belonging to the orders Coleoptera, Diptera and Hymenoptera, present in the *P. latifolia* (N = 5) plants, observed by date, between 12/03/15 and 01/08/16, in the Capão Alto and São José do Cerro, SC stands.

Order	Data	Average number of insects per plant	
		Capão Alto	S.J. do Cerrito
Coleoptera	12/03-04/2015	9.8±10.6 aA	8.0±4.8 aA
	12/10-11/2016	12.2±8.4 aA	2.2±2.5 aB
	12/17-18/2015	6.6±4.7 aA	10.0±6.7 aA
	12/21-22/2015	3.2±3.6 aA	4.2±4.0 aA
	12/28-29/2015	4.0±2.4 aA	5.6±3.8 aA
	01/07-08/2016	4.4±3.3 aA	3.6±3.8 aA
Diptera	12/03-04/2015	34.0±11.3 cA	42.2±13.5 bA
	12/10-11/2016	48.0±7.8 bcA	53.6±11.7 bA
	12/17-18/2015	55.2±6.6 abB	90.0±26.0 aA
	12/21-22/2015	42.2±5.9 bcB	54.0±7.2 bA
	12/28-29/2015	58.0±12.0 abA	47.2±6.1 bA
	01/07-08/2016	67.0±5.0 aA	52.0±17.0 bA
Hymenoptera	12/03-04/2015	59.6±7.3 cB	78.6±10.7 cA
	12/10-11/2016	75.2±8.5 bcB	105.4±13.4 bcA
	12/17-18/2015	112.6±14.4 aB	149.6±19.1 aA
	12/21-22/2015	92.8±12.5 abB	117.2±19.5 bA
	12/28-29/2015	112.0±14.3 aA	103.8±6.3 bcA
	01/07-08/2016	93.4±10.4 abA	87.4±17.9 bcA

* Capital letters compare the stands within each date, while the lower case letters compare the evaluation dates within each stand. Means followed by the same letter do not differ by Tukey test at the 5% level of significance.

The Capão Alto stand showed a significant positive correlation for order Diptera with temperature ($r = 0.84$, $p = 0.03442$) (Table 3). PINTO et al. (2010) reported that temperature induced an increase in the numbers of Calliphoridae; however, a decrease in the population of this family was recorded during the hot months, when the relative humidity was very low. ZIMMER et al. (2010) in their studies on family Muscidae, reported a population spurt between November and April, but with nil insect catches during the months when

the average temperatures dropped to below 12.7 °C or rose above 22.5 °C. *Haematobia irritans* L. (Diptera: Muscidae) showed strong correlation only with precipitation (BIANCHIN et al. 2006). This implies that the climatic factors which affect the main families of Diptera associated with the cattle were variable, although there were many examples of correlation with temperature.

Table 3. Pearson correlation between the number of insects observed from each order and the meteorological variables for the Capão Alto and São José do Cerrito, SC stands.

Order	Capão Alto		São José do Cerrito	
	Temp. (°C)	RH	Temp. (°C)	RH
Blattodea	0.08 ^{ns}	0.51 ^{ns}	-0.19 ^{ns}	0.13 ^{ns}
Coleoptera	-0.46 ^{ns}	-0.44 ^{ns}	-0.30 ^{ns}	0.40 ^{ns}
Diptera	0.84 [*]	-0.14 ^{ns}	-0.16 ^{ns}	0.03 ^{ns}
Hemiptera	0.48 ^{ns}	-0.39 ^{ns}	0.34 ^{ns}	-0.18 ^{ns}
Hymenoptera	0.71 ^{ns}	0.18 ^{ns}	-0.09 ^{ns}	-0.13 ^{ns}
Lepidoptera	0.35 ^{ns}	-0.21 ^{ns}	-0.28 ^{ns}	0.01 ^{ns}
Mantodea	-0.13 ^{ns}	-0.48 ^{ns}	-0.05 ^{ns}	-0.12 ^{ns}
Neuroptera	-0.04 ^{ns}	0.12 ^{ns}	x	x

*Significant at 5% probability.

^{ns} Not significant.

x Not found.

The predatory insects from the orders Mantodea and Neuroptera found the presence of the dipterans attractive, which is the reason they utilized the *P. latifolia* inflorescences as a hunting site, capturing the flies that came their way. These insect orders include the generalist predators (CHIARADIA et al. 2011), which facilitate biological pest control, and at times inflict some degree of indirect crop damage, by their predation on the pollinators. The complete lack of insects belonging to order Neuroptera in São José do Cerrito could indicate some environmental alterations. Insects from this order are hailed as bioindicators (MANSELL 2002), as they are susceptible to pesticide contamination (MAIA et al. 2016), and although they do not react to habitat fragmentation, they can be influenced by their heterogeneity, availability and microclimate within the fragments (SOUZA et al. 2008).

Both the stands showed the presence of caterpillars (Lepidoptera) feeding on the *P. latifolia* leaves, but avoiding the leaf margins which contain the oil glands (AMORIM 2010). To prevent such damage caused by herbivory, the plants exhibit several defense mechanisms, like the release of toxins, repellents, thorns, as well as physical and chemical defense mechanisms (AOYAMA & LABINAS 2012). The monoterpenes constituting the *P. latifolia* essential oil is capable of exhibiting a repellent effect against herbivores (KOUL et al. 2008) and an attractive effect on its natural enemies, which is released when the plant is experiencing damage (CERUTI 2007).

When fruiting started (Dec 18), and flowering declined, an apparent drop in the average number of beetles sighted was noted; however, no significant difference was observed between the assessment dates in either stand. Between the stands, a significant difference was recorded only during the second week of December (12/11/2015, $p < 0.01$), with the greatest number of insects being sighted in Capão Alto (Table 2).

When the flowers were collected, larvae were found in their interior regions. In São José do Cerrito the floral damage was estimated at 54%, whereas at Capão Alto it was 38% (Table 4). All the damaged flowers or those in the process of damage revealed an identical pattern, with the destruction of only the androecium and gynoecium, while the rest of the floral parts remained almost intact, barring the presence of a minute lateral opening in the keel, the possible entryway for the caterpillars during the bud stage. Florivory can exert a direct negative influence on the reproductive success of the plants, altering the floral aspects and restricting the pollinators from visiting (LEAVITT & ROBERTSON 2006), and destroying the reproductive flower parts (POHL et al. 2006).

The percentage of the damaged flowers in the São José do Cerrito stand (54%) has a direct bearing on the decrease in the seed output (Table 5) because the seed production in this stand was less, and lasted only for a two-week period, whereas in the Capão Alto the fruit ripening lasted seven weeks.

The seeds collected in São José do Cerrito had a higher percentage of damage, above 87% in the two collection dates (Table 5). The Larvae which occurred within the seeds, probably belonged to genus *Apion*

(Coleoptera: Brentidae), the only one of this family registered as a seed pest in *P. latifolia* (AMORIM 2010, OLIVEIRA et al. 2013).

Table 4. Percentage of damage caused to the *P. latifolia* flowers by the insects in both the stands evaluated at different collection dates, with 10 collected flowers / stands (5 flowers / plant).

Local	Flowers collected (No.)	Insect attack		
		Flowers damaged (No.)	Androecium damaged (%)	Gynoecium damaged (%)
S. J. do Cerrito	50	26	54	54
Capão Alto	50	19	38	38

Table 5. Percentage of insect damage to the *P. latifolia* seeds in both the stands evaluated at different collection dates, in the seeds harvested from 10 plants / stand.

Local	Date	Seeds collected (No.)	Insect attack	
			Seeds damaged (No.)	%
S. J. do Cerrito	01/07/16	214	189	88.32
	01/14/16	327	287	87.77
	Total	541	476	87.99
Capão Alto	01/08/16	249	88	35.34
	01/15/16	348	211	60.63
	01/22/16	497	317	63.78
	01/29/16	381	259	67.98
	02/05/16	311	241	77.49
	01/07/16	223	141	63.23
	01/14/16	71	53	74.65
Total	2080	1310	62.98	

The high prevalence of insects which damage the flowers and, particularly, the seeds of *P. latifolia*, may be the result of some ecological alterations in the São José do Cerrito stand. This can possibly be confirmed by the lack of insects from the family Vespidae, the significant natural enemies, through visual observation. Insects of this family are often used as environmental indicators because of their sensitivity to changes in their habitats, resulting from chemical pollution (ROCHA et al. 2010). The vespidae population may have been affected by the agricultural activities, like fruit growing and cattle breeding in the vicinity of the São José do Cerrito stand. According to VÁZQUEZ et al. (2008) cattle breeding supposedly influenced the hymenopteran abundance; however, as the present study extended for only one year, this finding may not be representative in the long term. As similar considerations can be applied to the current study, a more detailed study extending over a longer time period is required to ascertain the particular agricultural practices that could have induced this imbalance (for instance, the application of agrochemicals); this will facilitate the development of suitable management techniques with the aim of recovering this agroecosystem, as well as maintaining the *P. latifolia* populations, which may prove to be a good alternative source of income in the future, in the Santa Catarina plateau region.

CONCLUSION

The *P. latifolia* stands revealed a predominance of the insects belonging to the orders Hymenoptera, Diptera and Coleoptera.

The *P. latifolia* seed production was found to be affected by the insects which destroyed the flowers and seeds.

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