

## Biology-Ecology

### Pollinators and seed dispersers of espinheira-santa (*Monteverdia ilicifolia* - Celastraceae), a Brazilian medicinal plant

Polinizadores e dispersores de sementes de espinheira-santa (*Monteverdia ilicifolia* - Celastraceae), uma planta medicinal Brasileira

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## ABSTRACT

*Espinheira-santa* (*Monteverdia ilicifolia*) is a native plant of Brazil widely used in folk medicine because it has several therapeutic properties. Regarding the pollination biology and seed dispersal of this species, there is no information available in the literature. In this study, pollinators and dispersers of *M. ilicifolia* were observed and identified, as well as the behavior of these biological agents during the period of flowering and fruiting. In addition, the frequency of pollinator visits and the efficiency of the seed disperser were recorded. It was verified that in *espinheira-santa*, pollination is carried out by diptera and by hymenoptera. Among the main groups of pollinators are the flies, being *Lucilia eximia*, *Limnophora* sp. and *Eristalis* sp. the most frequent, and the wasps, in which *Brachygastra mellifica*, *Polistes canadensis*, *Mischocyttarus* sp. and one species of the Tiphidae family were the most frequent. Eight species of birds consumed diaspores (arils with seeds), acting as dispersers of this species. However, the following birds were more efficient in the dispersion of seeds: *Tangara sayaca*, *Elaenia* sp. and *Pipraeidea bonariensis*. In this sense, *M. ilicifolia* is a species that has entomophilous pollination and ornithochoric dispersion.

**Keywords:** Active Germplasm Bank; Floral biology; Reproductive phenology

## RESUMO

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A espinheira-santa (*Monteverdia ilicifolia*) é uma planta nativa do Brasil amplamente utilizada na medicina popular por possuir várias propriedades terapêuticas. No que diz respeito à biologia da polinização e da dispersão de sementes dessa espécie não há informações disponíveis na literatura. Neste sentido foram observados e identificados polinizadores e dispersores de *M. ilicifolia*, bem como o comportamento desses agentes biológicos durante o período de floração e frutificação. Além disso, a frequência de visitas dos polinizadores e a eficiência do dispersor de sementes foram registradas. Verificou-se que na espinheira-santa, a polinização é realizada por dípteros e por himenópteros. Entre os principais grupos de polinizadores estão as moscas, sendo *Lucilia eximia*, *Limnophora* sp. e *Eristalis* sp. as mais frequentes e as vespas, nas quais *Brachygastra mellifica*, *Polistes canadensis*, *Mischocyttarus* sp. e uma espécie da família Tiphidae foram as mais frequentes. Oito espécies de aves consumiram diásporos (arilos com sementes), atuando como dispersores dessa espécie. No entanto, as seguintes aves foram mais eficientes na dispersão das sementes: *Tangara sayaca*, *Elaenia* sp. e *Pipraeidea bonariensis*. Nesse sentido, *M. ilicifolia* é uma espécie que possui polinização entomófila e dispersão ornitócorica.

**Palavras-chave:** Banco Ativo de Germoplasma; Biologia floral; Fenologia reprodutiva

## 1 INTRODUCTION

*Monteverdia ilicifolia* (Mart. ex Reissek) Biral (Celastraceae), known by the popular name of espinheira-santa, is a native species of Brazil that occurs predominantly in the states of Paraná, Santa Catarina and Rio Grande do Sul (GUARINO *et al.*, 2018). Espinheira-santa is widely used in Brazilian folk medicine (BARBIERI *et al.*, 2014; DUTRA *et al.*, 2016; PAULERT *et al.*, 2019) and it has several therapeutic indications (COPPEDE *et al.*, 2014; GROSS *et al.*, 2019).

Despite the existence of several recommendations for the conservation and sustainable use of medicinal plants, only a small portion of these genetic resources have been adequately protected in reserves or botanic gardens (CHEN *et al.*, 2016). The ex situ conservation by Active Germplasm Banks helps in this process, as it aims to avoid the loss of genetic resources, to conserve the gene sources and to promote the collection, identification and characterization of the genotypes for usage (BARBIERI, 2003). In order to contribute to its conservation and characterization, a Germplasm Bank of Espinheira-Santa is maintained since 2002 by Embrapa Temperate Agriculture in partnership with the Sul-rio-grandense Federal Institute.

It is known that studies related to reproductive biology, involving knowledge of the pollination ecology and seed dispersal in *M. ilicifolia*, are an efficient tool to contribute to the improvement of management and conservation strategies (STEENBOCK; REIS, 2004). However, there are still many gaps in the knowledge about the reproductive biology of this species, and studies on the biology of pollination and seed dispersal of *M. ilicifolia* were not found.

As a functionally dioecious species (PERLEBERG, 2017), *M. ilicifolia* requires a pollinating agent for its reproduction. In general, flowers of species of the Celastraceae family are pollinated by bees, flies and beetles, offering nectar as a floral reward (JUDD *et al.*, 2009). However, *M. ilicifolia* pollinators are not known, although small insects such as bees, wasps and ants have been observed in their flowers (SCHEFFER *et al.*, 2009). The espinheira-santa flowers are inconspicuous, actinomorphic, have a yellowish-green coloration, diurnal anthesis, do not exude odor perceptible to human smell and produce nectar as a floral resource (MAZZA *et al.*, 2011; PERLEBERG, 2017). This floral morphology allows the nectar to be easily accessed by several groups of insects, including bees, flies and wasps that can act as pollinators (PINHEIRO *et al.*, 2014).

When ripe, the pericarp of the fruits of the espinheira-santa acquires an orange, reddish or brownish color and presents dehiscence, exposing the white and succulent aril that surrounds the seeds (CARVALHO-OKANO, 1992; PERLEBERG, 2017). The contrast between the color of the pericarp and the aril is likely to be attractive to birds (NEGRELLE *et al.*, 1999; JUDD *et al.*, 2009) however, there is no information in the literature about which species perform the dispersion of the seeds of *M. ilicifolia*.

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information in the literature about which species perform the dispersion of the seeds of *M. ilicifolia*.

In this sense, the objectives are to identify pollinators and seed dispersers of *M. ilicifolia* in the Active Germplasm Bank of Espinheira-Santa in Embrapa Temperate Agriculture/Sul-rio-grandense Federal Institute. As well as, to identify the most frequent pollinators and the dispersers with greater efficiency in the dispersion of the seeds.

## **2 MATERIAL AND METHODS**

### **2.1 Study area**

The work was carried out from 2015 to 2017 at the Active Germplasm Bank of Espinheira-Santa - Embrapa Temperate Agriculture/Sul-rio-grandense Federal Institute (31°42'S/52°18'W, Pelotas, Rio Grande do Sul state, southern Brazil), during the flowering and fruiting periods of *M. ilicifolia*. The area is located on the Coastal Plain and it presents anthropogenic vegetation with grazing fields, eucalyptus forestry (*Eucalyptus* spp., Myrtaceae) and experimental areas with annual and perennial crops. According to Köppen's classification, the climate of the region is the Cfa type, humid subtropical, with hot summers and without dry season (MORENO, 1961).

### **2.2 Floral visitors and pollinators**

Floral visitors were observed and collected in two plants with functionally female flowers and in two plants with functionally male flowers, totaling 120 hours. These observations were made during the flowering period of *M. ilicifolia*, from 8 am to 6 pm on alternate days from August to October, considering 60 hours in each floral morphotype. Individuals of each species of floral visitors were collected and

dry assembled for identification by specialists in the Insect Ecology Laboratory of the Federal University of Pelotas.

In order to identify the potential pollinators of espinheira-santa, observations were made of intrafloral behavior, analyzes of pollen deposition on the visitor's body and records of visits to floral morphotypes (functionally female flowers and functionally male flowers). For each species of floral visitor, direct observation and recording of photographs were performed to identify the floral resource collected (pollen or nectar), as well as their intrafloral behavior during the collection of this resource. It was observed if there was contact of any part of the floral visitor's body in the anthers of the functionally male flowers and in the stigma of the functionally female flowers. The deposition of pollen in parts of the body of the visiting insect was verified by a stereomicroscope.

The relative frequency of visits was obtained by calculating the total number of visits accomplished per species divided by the total number of visits of all species and multiplied by 100. A visit meant every act of searching for the floral resource by a given visitor when landing on the flowers.

The authorization for collecting and transporting specimens of wildlife in situ was granted by the Brazilian Chico Mendes Institute for Biodiversity Conservation (ICMBIO) and is registered in the Brazilian System of Authorization and Information on Biodiversity (SISBIO) under number 51282.

### **2.3 Seed Dispersers**

Thirty ripe fruits, orange, reddish or brown, with indehiscent capsules, were evaluated for length (distance between insertion of peduncle and apex) and diameter. Diameter and length of the aril with the seeds (diaspore) were also obtained after the removal of the pericarp. All measurements were performed using a digital caliper with a precision of 0.1mm (SPUJT, 1994).

The registration of the visiting birds was done by the method of focal observation, which consists in observing directly a group of plants with mature fruits, recording the frugivory events and behaviors of interest (JORDANO; SCHUPP, 2000). The observation period occurred from November to January, when the fruits were ripe, presenting an open capsule exposing the diaspore (white aril with seeds). Two plants, close to each other, were observed on non-consecutive days, totaling 60 hours from 6:30 a.m. to 7:30 p.m. The observations were made at a minimum distance of 5 m. Whenever possible the visiting birds were photographed.

During each observation session, we recorded the visiting bird species, the time of each visit and the total number of visits of each species (with or without seed consumption). When it was possible to carry out visual observation of the bird during the entire period of stay in the plant, this visit was called a complete visit. In the complete visits, the time of the visit, the total number of diaspores removed from the fruits (including those not ingested and those in which there was only the consumption of aryl) and the number of diaspores ingested were recorded. The manipulation of fruits during the foraging of birds was classified as swallowing the entire diaspore (aryl with the seeds), removing the seeds and swallowing only the aryl and pecking the aryls (PIZO, 1997). The classification of bird species related to their diet follows SICK (1997).

The behavior that visiting birds use during foraging is called by Moermond and Denslow (1985) "foraging tactics" and is classified as follows:

- picking, when the bird collects the diaspora, without extending the body or assuming special positions;
- reaching, when the bird extends the body below or above the perch to reach the diaspore, including the behavior of extending the body to the sides in order to reach the diaspore;
- hanging, when the bird is with the whole body below the perch, with the ventral region facing upwards;
- stalling, when the bird in flight carries out a direct attack on the diaspora.

The relative percentage of consumption (RPC) for each species was calculated by multiplying the mean number of whole swallowed diaspores in the total visits by the total number of complete visits, divided by the sum of the values obtained for all species and multiplied by 100 (FRANCISCO; GALETTI, 2001). The dispersion potential (DP), which measures the efficiency of a species as seed disperser from the number of whole swallowed diaspores and the time spent on the complete visits, was also estimated for each species of bird using the calculation  $DP = [f/t.(n)]$ . Given *f* the mean number of intact swallowed diaspores from fruits; *t* the average time the bird stayed on the plant in complete visits; *n* the number of complete visits per hour (GONÇALVES *et al.*, 2015).

The taxonomic nomenclature follows the Brazilian Convention of Ornithological Registries (PIACENTINI *et al.*, 2015).

## 3 RESULTS

### 3.1. Floral visitors and pollinators

There were 35 species of floral visitors, belonging to 4 orders and 18 families, all of Insecta class. The orders with the greatest number of species (88.6%) were Diptera, represented by flies, and Hymenoptera, represented by wasps and bees. The order Diptera was the one that presented the largest number of families (9), emphasizing the Syrphidae family, with 8 species. Following this is the order Hymenoptera, with 5 families, in which the Vespidae family stood out, with 5 species (Table 1). The activity of the floral visitors began at 9 a.m. and lasted throughout the daytime period, with greater intensity between 10 a.m. and 3 p.m.

Most species (65.7%), belonging to the orders Diptera and Hymenoptera, were considered potential pollinators of the espinheira-santa, because they visited both the floral morphotypes, touched the reproductive organs of the flower (anther and stigma) with some part of the body (head, thorax, paws, eyes, wings and

mouthparts) and presented pollen adhered to some part of their body (Table 1). These insects made quick visits to several flowers of the same plant, remaining a few seconds in each flower and later moved to other plants of the germplasm bank. Twelve species were observed only once or twice in a single floral morphotype or had no pollen adhered to parts of the body. These species were classified as nectar pickers (Table 1). The asian lady beetle, *Harmonia axyridis* (Pallas, 1773), was considered a nectar picker, although pollen was adhered to the body and collected nectar in functionally female and male flowers, because all the individuals observed remained in the same flower and in the same plant throughout the observation period. The only floral resource collected by floral visitors was nectar.

The flies (Diptera) were the most frequent pollinators, contributing with 55.5% of the visits. The Syrphidae family (flower flies) was the most diversified, with eight species, highlighting two species of *Eristalis* that together represented 11% of visits (Table 1, Fig. 1A). The blow fly *Lucilia eximia* (Wiedemann, 1819), Calliphoridae family, was the most frequent pollinator (Fig. 1B), contributing with 11.9% of the visits. *Limnophora* sp. was the second most frequent pollinating fly and a species of *Eristalis* sp.2 the third (Fig. 1C). These two species were observed repeatedly in plants with functionally female flowers and plants with functionally male flowers and they visited the flowers during the whole observation period, with greater activity between 11 a.m. and 5 p.m. All species of flies presented similar behavior, using the proboscis to suck or lick the nectar throughout the nectariferous disk, making circular movements around the stylet, touching the head and other parts of the body in the anthers and stigma (Table 1).

The group of wasps (Argidae, Tiphiidae and Vespidae) presented frequency of visits of 44.5%. *Brachygastra mellifica* Say, 1837 (Fig. 1D) was the most remarkable. Considering all the insects, this species was the second most frequent pollinating species, behind only *L. eximia* (blow fly). Among the most common wasps are also: *Polistes canadensis* (Linnaeus, 1758) (Fig. 1E), a species of family Tiphiidae (Fig. 1F) and one species of *Mischocyttarus* (Table 1). Wasps used the



mouthpiece to collect nectar, chewing the nectariferous disk at the base of the stamens, touching the head and other parts of the body on the anthers and stigma.

An undetermined species of the family Tiphidae presented a curious behavior: male and female always visit the flowers in copulation (Figure 1f). The winged male, when moving among the flowers to collect nectar, carries the aptera female, which also feeds on nectar. In this process, the pollen covers completely the female body.

Table 1 – Floral visitors of *Monteverdia ilicifolia* (Celastraceae) in the Active Germplasm Bank of Espinheira-Santa - Embrapa Temperate Agriculture/Sul-rio-grandense Federal Institute, Pelotas, Rio Grande do Sul state, Brazil

Order/Family	Species	Flowers		Absolute frequency (n°)	Relative frequency (%)	Place of deposition of pollen	Classification
		FM	FF				
Hymenoptera							
Apidae	<i>Apis mellifera</i> <i>Linnaeus, 1758</i>	X	X	11	3,4	B	PO
Argidae	<i>Arge</i> sp.	X	no	1	0,3	WP	NP
Ichneumonidae	<i>Mesostenus</i> sp.	X	X	7	2,2	P	PO
	<i>Theronia</i> sp.	no	X	2	0,6	WP	NP
Tiphidae	<i>Indeterminate</i> <i>species</i>	X	X	23	7,2	B	PO
Vespidae	<i>Agelaia</i> sp.	X	X	2	0,6	H	PO
	<i>Brachygastra mellifica</i> <i>Say, 1837</i>	X	X	37	11,6	M, H	PO
	<i>Mischocyttarus</i> <i>sp.</i>	X	X	18	5,6	H, M, P	PO
	<i>Polybia scutellaris</i> <i>(Write, 1841)</i>	X	X	5	1,6	H	PO
	<i>Agelaia</i> sp.	X	X	2	0,6	H	PO

FM = functionally male; FF = functionally female; B = in the whole body; WP = without pollen; P = paws; H = head; M = mouthparts; W = wings; T = thorax; E = eyes; PO = pollinator; NP = nectar picker; no = not observed

Continued...

Table 1 – Continuation

Order/Family	Species	Flowers		Absolute frequency (n°)	Relative frequency (%)	Place of deposition of pollen	Classification
		FM	FF				
Hymenoptera							
Vespidae	<i>Brachygastra mellifica</i> Say, 1837	X	X	37	11,6	M, H	PO
	<i>Mischocyttarus</i> sp.	X	X	18	5,6	H, M, P	PO
	<i>Polybia scutellaris</i> (Write, 1841)	X	X	5	1,6	H	PO
	<i>Polistes canadensis</i> (Linnaeus, 1758)	X	X	23	7,2	H, M	PO
Diptera							
Anthomyiidae	Indeterminate species	X	X	12	3,8	B	PO
Bibionidae	<i>Biblio johannis</i> (Linnaeus, 1767)	X	X	7	2,2	B	PO
Calliphoridae	<i>Lucilia eximia</i> (Wiedemann, 1819)	X	X	38	11,9	H, W, P	PO
	<i>Hemilucilia segmentaria</i> (Fabricius, 1805)	X	X	5	1,6	T	PO
Muscidae	<i>Coenisia</i> sp.	X	no	1	0,3	T	NP
	<i>Neodexiopsis</i> sp.	no	X	2	0,6	T	NP
	<i>Limnophora</i> sp.	X	X	26	8,1	B	PO
Tachinidae	Indeterminate species	no	X	1	0,3	WP	NP
Sarcophagidae	<i>Oxysarcodexia riograndensis</i> (Lopes, 1946)	X	X	2	0,6	T	PO

FM = functionally male; FF = functionally female; B = in the whole body; WP = without pollen; P = paws; H = head; M = mouthparts; W = wings; T = thorax; E = eyes; PO = pollinator; NP = nectar picker; no = not observed

Continued...

Table 1 – Conclusion

Order/Family	Species	Flowers		Absolute frequency (n°)	Relative frequency (%)	Place of deposition of pollen	Classification
		FM	FF				
Diptera							
Sarcophagidae	<i>Peckia ingens</i> (Walker, 1849)	X	no	1	0,3	T	NP
	Indeterminate species	X	X	12	3,8	P	PO
Stratiomyidae	<i>Odontomya</i> sp.	no	X	1	0,3	WP	NP
Syrphidae	<i>Eristalis</i> sp. 1	X	X	15	4,7	T	PO
	<i>Eristalis</i> sp. 2	X	X	20	6,3	T, P	PO
	<i>Temnostoma</i> sp. 1	X	X	7	2,2	B	PO
	<i>Temnostoma</i> sp. 2	X	X	2	0,6	T	PO
	Indeterminate species 1	X	X	2	0,6	W	PO
	Indeterminate species 2	X	X	2	0,6	E, P	PO
	Indeterminate species 3	X	X	2	0,6	T	PO
	Indeterminate species 4	X	X	5	1,6	T, P	PO
Tabanidae	Indeterminate species 5	no	X	2	0,6	H, W	NP
Hemiptera							
Coreidae	<i>Acantocephala</i> sp.	X	no	5	1,6	P	NP
Pyrrhocoridae	<i>Dysdercus</i> sp.	X	no	1	0,3	WP	NP
Coleoptera							
Coccinellidae	<i>Harmonia axyridis</i> (Pallas, 1773)	X	X	19	5,9	T, H	NP
Lampyridae	Indeterminate species 6	no	X	1	0,3	W	NP

FM = functionally male; FF = functionally female; B = in the whole body; WP = without pollen; P = paws; H = head; M = mouthparts; W = wings; T = thorax; E = eyes; PO = pollinator; NP = nectar picker; no = not observed

Figure 1 – Pollinators of *Monteverdia ilicifolia* (Celastraceae) observed in the Active Germplasm Bank of Espinheira-Santa – Embrapa Temperate Agriculture/Sul-riograndense Federal Institute, Rio Grande do Sul state, Brazil



A. *Eristalis* sp. (Syrphidae, Diptera); B. *Lucilia eximia* (Calliphoridae, Diptera); C. *Limnophora* sp. (Muscidae, Diptera); D. *Brachygastra mellifica* (Vespidae, Hymenoptera); E. *Polistes canadensis* (Vespidae, Hymenoptera); F. Undetermined species – family Tiphidae (Hymenoptera)

### 3.2 Seed dispersers

Nine bird species of the Order Passeriformes visited the plants observed (Table 2). From these, only the bananaquit [*Coereba flaveola* (Linnaeus, 1758)] was not considered a seed disperser of the espinheira-santa. A species of *Elaenia* has not been determined because the representatives of this genus are difficult to identify: they are very similar and the differentiation is only by singing (SICK, 1997).

Table 2 – Visiting birds of *Monteverdia ilicifolia* (Celastraceae) in the Active Germplasm Bank of Espinheira-Santa – Embrapa Temperate Agriculture/Sul-rio-grandense Federal Institute, Pelotas, Rio Grande do Sul state, Brazil

Family/Species	PN	D	NV	NFV	TFV	DRF	WSD	RPC	DP
Tyrannidae									
<i>Elaenia</i> sp.	-	OM	106	23	45±61*	55	55	28,5	0,46
<i>Empidonomus varius</i> (Vieillot, 1818)	variegated flycatcher	IN	2	1	72	5	5	1,1	0,001
<i>Pitangus sulphuratus</i> (Linnaeus, 1766)	great kiskadee	OM	16	7	76,7±81,9	38	34	8,7	0,05
<i>Tyrannus melancholicus</i> Vieillot, 1819	tropical kingbird	IN	4	3	96±67,3	7	7	1,1	0,004
Turdidae									
<i>Turdus amaurochalinus</i> Cabanis, 1850	creamy-bellied thrush	OM	4	2	30	7	7	1,6	0,007
<i>Turdus rufiventris</i> Vieillot, 1818	rufous-bellied thrush	OM	17	2	480±594	8	8	7,7	0,0005
Thraupidae									
<i>Coereba flaveola</i> (Linnaeus, 1758)	bananaquit	NEC	4	4	50±6,7	2	0	0	0
<i>Pipraeidea bonariensis</i> (Gmelin, 1789)	blue-and-yellow tanager	FRU	28	17	65,9±39,9	81	79	14,6	0,34
<i>Tangara sayaca</i> (Linnaeus, 1766)	sayaca tanager	OM	124	51	61,4±42,2	21	134	36,7	1,9

PN = popular name; D = diet; NV = number of visits; NFV = number of full visits; TFV = time of full visits (seconds); DRF = diaspores removed from fruits; WSD = whole swallowed diaspores; RPC = relative percentage consumption; DP = dispersion potential; OM = omnivorous; IN = insectivorous; NEC = nectarivorous; FRU = frugivore; \* mean ± standard deviation

The visitation of the birds to the plants of the active germplasm bank began at the end of November and lasted until January, the fruit maturation stage when the opening of the capsule and the exposure of the white aril that surrounds the seeds occurs (Fig. 2A). Frugivory activity occurred from 6:45 a.m. to 6:30 p.m., totaling 305 visits in 60 observation hours, with an average of five visits per hour. The fruits presented, on average, a length of  $9.7 \pm 0.9$  mm and a diameter of  $8.5 \pm$

0.9 mm, while the diaspore (aril with seeds) presented, on average, a length of  $7.1 \pm 1.2$  mm and diameter of  $5.8 \pm 1.0$  mm.

Figure 2 – Seed dispersal in *Monteverdia ilicifolia* (Celastraceae) – Active Germplasm Bank of Espinheira-Santa – Embrapa Temperate Agriculture/Sul-rio-grandense Federal Institute, Pelotas, Rio Grande do Sul state, Brazil



A. mature fruits exposing the aril just after opening of the pericarp; B. sayaca tanager (*Tangara sayaca*) removing the seed to consume only the aril; C. foraging tactic picking (sayaca tanager); D. foraging tactic reaching (sayaca tanager); E. foraging tactic hanging (blue-and-yellow tanager, *Pipraeidea bonariensis*)

In 110 complete visits, the birds consumed 329 diaspores, with an average consumption of 5.5 units per hour and 3 diaspores per visit (Table 2). Complete visits lasted on average 115.9 seconds and most of the birds remained on the plants only for the necessary time for foraging. *Elaenia* sp. was the bird that stayed

less time in the plant, while the rufous-bellied-thrush was observed in the soil or in basal branches of the plants of espinheira-santa, for a long period, more than 20 minutes, before or after feeding. During this period, in 65% of the visits, this bird regurgitated seeds under the plants of *M. ilicifolia* after feeding.

The birds removed the diaspore from the dehiscent fruits and swallowed it whole in 80% of the time (Table 2). The sayaca tanager [*Tangara sayaca* (Linnaeus, 1766)] mandibulated 36.5% of the diaspores, removing the seeds, which fell under the plant, feeding only on the aril. In some occasions, this species was observed rubbing the diaspore in the branches of *M. ilicifolia* or in nearby plant branches, in order to remove the seed and swallow only the aril (Fig. 2B). The great kiskadee [*Pitangus sulphuratus* (Linnaeus, 1766)] and the blue-and-yellow tanager [*Pipraeidea bonariensis* (Gmelin, 1789)] dropped respectively 10.5% and 2.5% of the diaspores without swallowing them. The other species swallowed the whole diaspores, except for the bananaquit that ate only the aril, pecking the diaspore, exposing the seeds or knocking them under the plant (Fig 2C). The birds did not consume fruits with the bivalve capsule.

The omnivorous birds represented 55.5% of the species and were responsible for the consumption of 83.2% of the diaspores. Blue-and-yellow tanager was the only registered frugivorous species, accounting for 14.6% of the total consumption of diaspores. The two insectivorous species consumed only 2.2% of the diaspores (Table 2).

The most used foraging tactics for the removal of diaspores were the picking type (Fig. 2C), wherewith the birds consumed 60.2% of the diaspores, and the reaching tactic (Fig. 2D) whereby 26% of the diaspores were consumed (Table 3). Besides these two tactics, used by all species, *Elaenia* sp. used the stalling tactic, and the rufous-bellied-thrush and the blue-and-yellow tanager used the hanging tactic (Fig. 2E). As most of the fruiting branches were flexible and ripening of the fruits occurred randomly, rarely forming aggregates of mature fruits, some birds were more efficient in collecting diaspores. Sayaca tanager, *Elaenia* sp., and blue-

and-yellow tanager used different foraging tactics to reach the diaspores, demonstrating agility and dexterity in fruit harvesting. Sayaca tanager and blue-and-yellow tanager, on several occasions, hung, suspending the body, or landed on vertical branches to reach the diaspores. In turn the *Elaenia* sp. made short flights from a nearby perch (branches of the plant itself) to catch the diaspores and, after landing again, swallowed them. The three species moved quickly and with agility between the branches of the plant in search of dehiscent fruits. In contrast, larger species, such as great kiskadee and rufous-bellied thrush, perched on a branch, usually horizontal, and remained in it trying to reach the diaspores.

Sayaca tanager (Fig. 3A) and *Elaenia* sp. (Fig. 3B) performed the highest number of visits and presented the highest relative percentages of consumption (RPC), consuming 65.2% of the diaspores. They were recorded at all times and on all observation days. Blue-and-yellow tanager (Fig. 3C-D), great kiskadee and rufous-bellied thrush (*Turdus rufiventris* Vieillot, 1818) represented 31% of the relative percentage of consumption. These five species were responsible for the consumption of 96.2% of the diaspores. Tropical kingbird (*Tyrannus melancholicus* Vieillot, 1819), creamy-bellied thrush (*Turdus amaurochalinus* Cabanis, 1850) and variegated flycatcher [*Empidonomus varius* (Vieillot, 1818)] performed the lowest number of visits and the lowest relative percentage of consumption (Table 2). In most of the visits, the rufous-bellied thrush tree fed inside the canopy and it was not possible to count the number of diaspores consumed. The relative percentage of consumption of this species may therefore be underestimated, since it remains for a long time foraging.

According to the dispersion potential, sayaca tanager was the most efficient species in the dispersion of espinheira-santa seeds (Table 2). *Elaenia* sp. and blue-and-yellow tanager also showed good efficiency in seed dispersal. Tropical kingbird, creamy-bellied thrush, variegated flycatcher, great kiskadee and rufous-bellied thrush presented low potential for dispersion (Table 2). Although rufous-bellied thrush was ranked as fourth in the relative percentage of diaspore consumption,



the long period of time it remains in the plant decreases its dispersal potential, since it increases the chances of defecating or regurgitating the seeds under the espinheira-santa plants.

Sayaca tanager (n = 3), great kiskadee (n = 2) and *Elaenia* sp. (n = 2) were observed feeding nestlings with diaspores of *M. ilicifolia*. These species and the blue-and-yellow tanager female also collected diaspores and carried them in the beak until their nestlings, which were on branches of trees near the Active Germplasm Bank. Sayaca tanager usually foraged in pairs or in groups of three individuals, probably the parents and the nestling. Due to the sexual dimorphism of blue-and-yellow tanager (Fig. 3C-D) it was possible to observe that 85% of the visits were performed by the female and, only in one occasion, the couple was seen foraging together.

Figure 3 – *Monteverdia ilicifolia* seed dispersers (Celastraceae) – Active Germplasm Bank of Espinheira-Santa – Embrapa Temperate Agriculture/Sul-rio-grandense Federal Institute, Pelotas, Rio Grande do Sul state, Brazil



A. sayaca tanager (*Tangara sayaca*). B. *Elaenia* sp. C. blue-and-yellow tanager female (*Pipraeidea bonariensis*). D. blue-and-yellow tanager male (*Pipraeidea bonariensis*).

Table 3 – Foraging tactics and number of diaspores consumed by seed dispersal birds of *Monteverdia ilicifolia* (Celastraceae) in the Active Germplasm Bank of Espinheira-Santa – Embrapa Temperate Agriculture/Sul-rio-grandense Federal Institute, Pelotas, Rio Grande do Sul state, Brazil

Family	Species	Foraging tactics and number of diaspores consumed			
		Picking	Reaching	Hanging	Stalling
Tyrannidae	<i>Elaenia</i> sp.	30	10	0	15
	<i>Empidonomus varius</i>	3	2	0	0
	<i>Pitangus sulphuratus</i>	26	8	0	0
	<i>Tyrannus melancholicus</i>	3	4	0	0
Turdidae	<i>Turdus amaurochalinus</i>	6	1	0	0
	<i>Turdus rufiventris</i>	6	1	0	1
Thraupidae	<i>Pipraeidea bonariensis</i>	41	26	12	0
	<i>Tangara sayaca</i>	83	35	16	0

## 4 DISCUSSION

### 4.1 Floral visitors and pollinators

*M. ilicifolia* presented a generalist and entomophile pollination system, since its flowers attracted a diversity of insects with different trophic niches, belonging to two orders, Diptera and Hymenoptera, and to different families. These two orders are among the most representative in the pollination of several dioecious species (LENZA; OLIVEIRA, 2005; SOMAVILLA *et al.*, 2010; FERNANDES *et al.*, 2012).

The dipterans, considered pollinators of small size, showed more frequent visits to the flowers of the espinheira-santa. This fact is common for dioecious species, where pollinators of small size (smaller than 12 mm) are adapted to small flowers of white, yellow or pale green coloration (BAWA; OPLER, 1975; LENZA; OLIVEIRA, 2005; NADIA *et al.*, 2007; FERNANDES *et al.*, 2012). In addition, flowers pollinated by small animals usually produce nectar in small quantities (FAEGRI; VAN

DER PIJL, 1979), which leads to the search for a greater number of flowers, including in different plants, increasing the percentage of cross-pollination (CRUDEN *et al.* al., 1983; PEREIRA, 2014). We observed this in the fast visits to several flowers of the same plant and in the subsequent movement of the pollinating insects to other plants of the Active Germplasm Bank.

In *M. ilicifolia*, flies use the nectar of the flowers as a food resource, carrying pollen from the male flowers to the female flowers, and they are therefore considered important pollinators of the species. Syrphidae, the most diverse family recorded, is considered the most important family among pollinator dipterans. It consists of flies specialized in feeding on pollen and nectar, depending, in adulthood, almost exclusively on flowers for feeding (WILLMER, 2011). The movement of these flies between different flowers is more systematic and regular than that of other species of flies and it is therefore recognized as important and efficient pollen vectors (WILLMER, 2011).

The visit of flies of larval saphagic habit, such a *L. eximia*, to the flowers of *M. ilicifolia* can be related to the periurban location of the study area, where there are chickens, cattle and horses. The existence of such animals and manure attracts flies of habit saphagus and necrophage, belonging to the families Anthomyiidae, Calliphoridae, Muscidae and Sarcophagidae (CARVALHO *et al.*, 2002; MACEDO *et al.*, 2011). These flies also use the nectar of the espinheira-santa flowers as a food resource, carrying pollen from the functionally male flowers to the functionally female flowers, and they are considered important pollinators of this species in this location.

Wasps, an equally important group in pollination of the espinheira-santa, usually visit flowers with nectar for their own feeding or maintenance of the colony and, in this process, they carry the pollen on the body (PEREIRA, 2014). Social wasps belonging to the genus *Brachygastra*, *Mischocyttarus*, *Polistes* and *Polybia* were cited as pollinator for Brazilian pepper (*Schinus terebinthifolius* Raddi, Anacardiaceae),

a functionally dioecious native plant of South America (SÜHS *et al.*, 2009; SOMAVILLA *et al.*, 2010) and quite common in the study area.

Only one species of bee was recorded in espinheira-santa: *Apis mellifera* Linnaeus, 1758. The flowers of *M. ilicifolia* are not very attractive to bees, they do not present sweet odor, as observed in other dioic species of melitophilic pollination (FERNANDES *et al.*, 2012; LENZI; ORTH, 2004), what may explain the low frequency of bee visits and the visit of only one species of bee.

#### 4.2 Seed dispersers

At the study site *M. ilicifolia* presented ornithochoric dispersion of its seeds, attracting birds from different trophic niches. We observed that the contrast between the coloration of the pericarp (orange, reddish or brownish) with the aril (white) during fruit maturation promoted the beginning of frugivory, since the visitation and consumption of the diaspores occurred only after dehiscence of the fruits. Bicoloured fruits (presenting a combination of two colors) are associated with the dispersion carried out by birds (GALETTI *et al.*, 2011). GALETTI *et al.* (2011) recorded five species of *Maytenus* sensu lato (genus to which the espinheira-santa belonged), which showed bicolour fruits with colored pericarp and white aril dispersed by birds in an Atlantic rain forest in the São Paulo state, in the Southeast of Brazil. NEGRELLE (1999) had already drawn attention to the question that the contrast between the pericarp and the aril was an attraction to the birds in espinheira-santa.

The consumption of diaspores only in dehiscent fruits may have contributed to the optimization of the frugivory and reduction of the stay time of the birds on the plants of espinheira-santa, which was less than 2 minutes. This time is considered short in terms of the probability that the seeds ingested are regurgitated or defecated under the mother plant (JORDANO; SCHUPP, 2000), increasing the efficiency of the birds as dispersers (SCHUPP, 1993). In species with

capsulated fruits, in which the birds do not expect the fruit to open and remove the capsule with the beak to feed on the aril, there was a greater demand in the feeding time and, consequently, a low consumption (PASCOTTO, 2006).

In addition to this, the reduced size of the diaspores, smaller than 1 cm, allowed the majority to be swallowed whole without the need of mandibulation, increasing the area of dispersion by the possibility of the seeds being regurgitated or defecated in places far from the Active Germplasm Bank. The cambacica was the only bird that did not swallow the diaspores, only pecked them, which may be related to the fact that this bird feeds on nectar (SICK, 1997) and, probably, on the arils because they are sweet.

Omnivorous and insectivorous birds act as seed dispersers of plant species (FRANCISCO; GALETTI, 2001, 2002; ATHIÊ; DIAS, 2011). The fact that these birds do not feed exclusively on fruits does not prevent them from being good seed dispersers. Sayaca tanager is an omnivorous species that consumes fruits of several plant species (CAZETTA *et al.*, 2002; FADINI; MARCO JÚNIOR, 2004; MACHADO; ROSA, 2005), however, in some cases, it is not considered disperser because it consumes only the mesocarp and leaves the seeds attached to the fruit, not swallowing them (FADINI; MARCO JÚNIOR, 2004; MACHADO; ROSA, 2005). We observed, sometimes, sayaca tanager mandibulating the diaspore, in order to swallow only the aril, allowing the seeds to fall under the plant. On the other hand, it is an important seed disperser of *M. ilicifolia* seeds because it had the highest number of visits, the highest percentage of diasporic consumption and the greatest dispersion potential.

In the Active Germplasm Bank the fruit maturation period is between November and January (PERLEBERG, 2017), corresponding to the breeding season of several bird species (SICK, 1997) and, therefore, it is a time of great demand for resources for feeding nestlings. The aril gives the fruit greater nutritional value, due to the high content of lipids in its composition, making it very attractive to birds (PIZO, 1997). Our record of these birds feeding their nestlings demonstrates the

importance of the diaspores of espinheira-santa in the diet of these species from an early age.

Considering the potential for dispersion, the relative percentage of consumption and the use of different foraging tactics, sayaca tanager (*T. sayaca*), *Elaenia* sp. and blue-and-yellow tanager (*P. bonariensis*) are the main seed dispersers of *M. ilicifolia* in the study area.

## 5 CONCLUSIONS

The evaluations carried out at the Active Germplasm Bank of Espinheira-Santa - Embrapa Temperate Agriculture/Sul-rio-grandense Federal Institute provided the following conclusions:

Flies (Order Diptera) and wasps (Order Hymenoptera) are the main pollinators of *M. ilicifolia*. The most frequent species in pollination of *M. ilicifolia* are, in order of importance, *L. eximia*, *B. mellifica*, *Limnophora* sp., a species of the family Tiphiidae, *P. canadenses*, *Eristalis* sp.2 and *Mischocyttarus* sp.

Birds are the seed dispersing agents of *M. ilicifolia*, being *T. sayaca*, *Elaenia* sp. and *P. bonariensis* the species with the greatest potential for dispersion.

*M. ilicifolia* presents entomophilic pollination and ornithochoric dispersion.

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