

Pollination biology of *Syngonanthus elegans* (Eriocaulaceae – Poales)

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Abstract. Studies on the pollination biology of Eriocaulaceae are scarce although particularly interesting because of its inclusion in the Poales, a predominantly wind-pollinated order. The pollination biology of *Syngonanthus elegans* (Bong.) Ruhland was studied during two annual flowering periods to test the hypothesis that insect pollination was its primary pollination system. A field study was carried out, including observations of the morphology and biology of the flowers, insect visits and pollinator behaviour. We also evaluated seed set, seed germination and seedling development for different pollination modes. Although seeds were produced by self-pollination, pollination by small insects contributed most effectively to the reproductive success of *S. elegans*, resulting in the greatest seed set, with the highest germination percentage and optimum seedling vigour. The floral resources used by flower visitors were pollen and nectar that was produced by staminate and pistillate flowers. Self-pollination played a minor role and its consequence was inbreeding depression.

Introduction

Eriocaulaceae is included in the Poales, an order that comprises about one-third of the monocotyledons and, together with Zingiberales, Commelinales, Arecales and Dasypogonaceae, constitutes the Commelinid clade (APG 2003). Owing to the high representativeness of Poales within the monocotyledons, families in this order have been widely studied in recent years, to provide support information for phylogenetic analyses. Within this context, studies on pollination biology can be very informative, although they are scarce for the group and are predominantly restricted to Bromeliaceae (Sazima *et al.* 1989; Siqueira Filho and Machado 2001; Kahler *et al.* 2005; Vosgueritchian and Buzato 2006). As to the other families, information has been so far obtained for Cyperaceae (Leppik 1955), Poaceae (Soderstrom and Calderón 1971; Adams *et al.* 1981; Guilherme and Ressel 2001; Koshy *et al.* 2001) and Eriocaulaceae (Ramos *et al.* 2005; Sawyer *et al.* 2005).

Considering the floral morphology of the 17 families included in Poales (Chase *et al.* 2000; APG 2003; Soltis *et al.* 2005), it is believed that only Bromeliaceae and Rapateaceae (more basal families), Mayacaceae, Xyridaceae and Eriocaulaceae (xyrids) are pollinated predominantly by animals (Linder and Rudall 2005). The other families are considered characteristically anemophilous (Linder and Rudall 2005), particularly Cyperaceae and Poaceae, because of the reduction of their floral parts, even though some studies have reported the occurrence of pollination by insects in species of both families (Leppik 1955; Soderstrom and Calderón 1971; Adams *et al.* 1981; Koshy *et al.* 2001).

Anemophily has been suggested as the pollination syndrome in Eriocaulaceae as well (Kral 1966; Giulietti 1984). However, other authors, on the basis of morphological and anatomical characteristics of the flowers, have suggested that entomophily is the main syndrome (Hare 1950; Cronquist 1981, 1988; Dahlgren *et al.* 1985; Hensold 1988; Stützel 1998; Scatena and Bouman 2001; Rosa and Scatena 2003, 2007). According to Rosa and Scatena (2007), all Paepalanthoideae species have nectariferous structures, favouring entomophily.

The only experimental study about reproductive biology of Brazilian Eriocaulaceae species was conducted by Ramos *et al.* (2005), and supplied evidence for insect pollination in *Syngonanthus mucugensis* Giul. and *S. curralensis* Mouldenke. According to the authors, who investigated the floral biology, visitor behaviour and mating systems, this is the main pollination system of *S. mucugensis* and *S. curralensis*, although self-pollination also occurs. Effective pollinators included Diptera, Coleoptera and Hymenoptera species that use only the pollen as a floral resource (Ramos *et al.* 2005). Because the centre of genetic diversity of Eriocaulaceae is in Brazil, more specifically in the Espinhaço range, which extends through the states of Minas Gerais and Bahia (Giulietti and Hensold 1990; Giulietti *et al.* 1995), studies involving Brazilian species in their natural habitat are extremely important to understand the reproductive aspects of the family. Furthermore, studies of floral morphology and biology may contribute to the identification of characteristics that permit the exploration of the factors that have influenced evolution in Poales.

Syngonanthus species are found in the American and African continents, and most of them occur in South America (Parra 2000). *S. elegans* has a restricted distribution on some mountains in Minas Gerais State, Brazil (Parra 2000), and its populations have declined in the range of occurrence, because its long-lasting inflorescences are used for making crafts.

The objectives of the present work were to study the floral morphology and biology of *S. elegans*, including aspects such as the occurrence of dichogamy, nectar presence, stigma receptivity and pollen viability. We also identified the community of floral visitors of *S. elegans*, reporting on the activities they perform on the flowers, and we demonstrated the relative contribution of insects, wind, and self-pollination to the reproductive success of the species. We aimed to fill some gaps on the pollination biology of Eriocaulaceae, specifically addressing the following questions: do flower visitors use nectar as a food resource; how important are flower visitors in terms of seed set; and what are the consequences of self-pollination?

Materials and methods

Species and study area

Two populations of *S. elegans* in the Parque Nacional da Serra do Cipó (19°12'30"S–19°30'35"S and 43°20'25"W–43°40'00"W), Minas Gerais State, Brazil, were studied. Parque Nacional da Serra do Cipó (a national park) has an area of ~33 800 ha and a perimeter of 154 km, and is predominantly characterised by the 'campo rupestre' (rocky outcrops) vegetation, where a large number of Eriocaulaceae species are found. The local climate is upland tropical, with cool summers and a well-defined dry season. The mean annual temperature is ~21.2°C and the mean annual precipitation is 1622 mm.

The individuals of *S. elegans* are perennial, and flowering occurs from February to June, peaking in April, with seed dispersal from August to December (Scatena *et al.* 1997). Monthly scientific expeditions to the study area were conducted in the period from May to June 2005 and from February to June 2006, with a mean duration of 7 days per trip. The pollination experiments were carried out from March to June 2006. Voucher specimens of *S. elegans* were deposited in the Herbarium Rioclaurens (HRCB), at São Paulo State University – UNESP, in Rio Claro, São Paulo (HRCB 45959).

Floral morphology and biology

Flowers were fixed in formalin : acetic acid : alcohol (FAA 50% alcohol concentration) (Johansen 1940) and dissected under a stereomicroscope (MZ 125, Leica Heerbrugg, Switzerland) for analysis, description and illustration ($n = 30$ flowers). The floral structures were described by using the terminology adopted by Rosa and Scatena (2007) for Paepalanthoideae (Eriocaulaceae) species. Fixed flowers were dehydrated in an absolute ethanol series, critical-point dried, coated with gold and examined with a scanning electron microscope (JSM-5410, JEOL Tokyo, Japan).

The time and duration of anthesis, as well as the duration of the pistillate and staminate phases of capitula were determined by field observations of 30 capitula. Stigma receptivity was tested with hydrogen peroxide and benzidine (Dafni 1992) on five flowers from different plants per hour from 0900 to 1800 hours, during 2 days. Pollen viability was analysed on

pollen grains removed from the anthers of flowers from four individuals, stained with acetocarmine (Kearns and Inouye 1993). In addition, the presence of lipid substances in the pollen grains (pollenkitt) was tested with Sudan IV (Johansen 1940).

Staminate and pistillate flowers were submerged in neutral red solution (1 : 10 000) to locate the nectariferous tissues (Kearns and Inouye 1993) ($n = 20$ flowers). Nectar production was analysed by chemical tests conducted in the field, with Diabur-test 5000 test strips (Roche Mannheim, Germany), which are sensitive to glucose ($n = 20$ flowers).

Floral visitors

Floral visitors were surveyed by collecting them between 0900 and 1800 hours (period during which the capitula were open). Each insect was anaesthetised with sulfuric ether, placed in a separate vial and the presence or absence of pollen grains adhered to their bodies was determined in the laboratory with a stereomicroscope. The pollen grains collected from visitors were compared with the pollen grains from *S. elegans* with a microscope. Insect species were identified by specialists. Voucher specimens were deposited in the Instituto de Biociências collection, at UNESP, in Rio Claro, São Paulo.

The relative abundance of visitor species was evaluated for each *S. elegans* population studied (Populations A and B) on two consecutive days, in different months. The populations were walked through in steady steps every 2 h, from 0900 to 1800 hours, and all floral visitors observed were recorded (Dafni 1992). Insect-visitation frequency was analysed on only 1 day for each population; the number of visits for each visiting species and the time they remained on the capitula were recorded by simultaneously observing three *S. elegans* individuals for 20 min, every hour, from 0900 to 1800 hours (Dafni 1992). The temperature and relative humidity were monitored with a thermohygrometer (Incoterm Porto Alegre, Brazil). Visitor behaviour was studied using a stereomicroscope, supplemented by an analysis of photographs made during the visits.

On the basis of their abundance, visitation frequency and behaviour on the capitula, the insects were classified as effective pollinators, occasional pollinators or merely visitors. Effective pollinators represented the abundant insects that had frequently visited capitula both in the staminate and in the pistillate phases, and that carried pollen grains from a capitulum to another. Occasional pollinators represented the insects that exhibited such behaviour, but with a low visitation frequency. The insects that were observed on only one occasion or that had only visited flowers of the same sex were taken as visitors.

Pollination systems

The occurrence of spontaneous self-pollination, wind pollination and insect pollination was examined by selective exposure experiments. To test spontaneous self-pollination, capitula at the flower-bud stage (in preanthesis) were marked and isolated in polyester bags (25 × 25 µm mesh), thus negating the passage of pollen grains and preventing cross-pollination (Treatment 1). To test wind pollination, other capitula (in preanthesis) were marked and isolated in jersey bags (500 × 500 µm mesh) that prevented the arrival of flower visitors but allowed pollen grains carried by

the wind (Treatment 2). Because scapes (inflorescence stalks) are fragile and break easily, the bags employed to isolate the inflorescences were attached to iron frames and were sewn on the bottom to avoid contact with the scape and the capitulum. To test insect pollination, capitula in pre-anthesis were marked and maintained under natural conditions (Treatment 3). For each treatment, 25 capitula were marked at random in Population A, whereas 35 capitula were marked in Population B. After 90 days, all marked capitula were collected and placed in separate plastic bags. The number of pistillate flowers and seeds formed in each capitulum were counted in the laboratory with a stereomicroscope. Reproductive success was calculated by the formula: number of seeds/(number of flowers \times 3), where 3 is the number of ovules per flower. The three treatments were compared by means of the Kruskal–Wallis test ($\alpha=0.05$). The Nemenyi test was the multiple-comparison procedure deployed ($\alpha=0.05$) (Zar 1999), and the reproductive success was the variable analysed.

Seed germination

Seed-germination percentages were compared among the three pollination treatments by selecting viable seeds of Population B (48 seeds in Treatment 1 and 100 seeds in Treatments 2 and 3). These seeds were distributed among four Petri dishes (10 cm diameter) per treatment, lined with filter paper moistened with distilled water. The dishes were placed in clear germination boxes and maintained in an incubator at 25°C, under continuous white light (15 W). Germination percentage was checked weekly for 3 months. The results were analysed statistically with the analysis of variance (ANOVA, $\alpha=0.05$), and the Tukey test was the multiple comparison method used ($\alpha=0.05$) (Zar 1999).

Results

Floral morphology and biology

Syngonanthus elegans has declinous, trimerous flowers, with a hyaline aspect (Fig. 1*a–d*). These flowers are grouped in bisexual capituliform inflorescences, 0.6–1.2 cm in diameter, enveloped in involucre bracts (Fig. 1*a*). The bracts are arranged in ~10 series. The outermost bracts are light brown, 2.4–3.4 mm long, whereas the innermost ones are snow-white and larger than the flowers, 4.5–6.5 mm long.

The staminate flowers (Fig. 1*b*) are isostemonous, with sepals connate at the base, 1.2–1.4 mm long, and petals connate from the base to the mid-region, 1.5–1.7 mm long. They have free nectariferous pistillodes developing from the receptacle (Fig. 1*b*, arrow), 0.5–0.6 mm long, stamens with free filaments, 1.6–1.8 mm long, and bithecate, dorsifixed anthers, with longitudinal dehiscence. The pistillate flowers (Fig. 1*c, d*) have free sepals, 0.8–0.9 mm long, petals fused in the mid-region, 1.9–2.1 mm long, and the ovary measuring ~0.5 mm in length, superior, trilocular, with one ovule per locule. The style (Fig. 1*d*) is terminal, hollow, branching out into more elongated stigmatic portions (1.5–1.7 mm long) and shorter nectariferous portions (0.8–1.0 mm long, arrow).

Pollen grains germinating on the stigmatic portion of the style were observed by scanning electron microscopy (Fig. 2*a*). The nectariferous portions of the style (Fig. 2*b*) and the nectariferous pistillodes (Fig. 2*c, d*) possess an epidermis with papillose cells.

These cells are short in flowers at the bud stage (Fig. 2*c*, arrow) and elongated in flowers at anthesis (Fig. 2*d*, arrow), owing to the production and secretion of nectar. When the flowers undergo anthesis, both the nectariferous pistillodes (staminate flowers) and the nectariferous portions of the styles (pistillate flowers) stand out in the capitula because of their yellow colour, in contrast to the other whitish flower parts (Fig. 3*b, c*, arrow). These structures were stained with neutral red, indicating their secretory function, and tested positive for glucose with test strips.

The capitula are protandrous and undergo centripetal maturation (Fig. 3*a–c*). The flowers are distributed on verticils in the capitula and verticils of staminate flowers at anthesis alternate with verticils of pistillate flowers at anthesis. Although the capitula present dichogamy (Fig. 3*a, b*), contact between the stigmas of pistillate flowers at anthesis and anthers of staminate flowers from the previous phase was observed in some capitula. Capitula in the pistillate phase and capitula in the staminate phase were observed simultaneously in the same individual.

Anthesis of the flowers starts in the morning, before the opening of the capitula (Fig. 3*c*), which begins between 0900 and 0930 hours. Anthesis of the pistillate flowers lasts from 1 to 2 days, whereas the staminate flowers remain open for only a single day. Each pistillate phase of the capitulum lasts from 4 to 5 days, and each staminate phase lasts from 5 to 8 days. The staminate flowers detach from the capitula 1 or 2 days after anthesis (Fig. 3*d*, arrowhead), and only pistillate flowers can be seen towards the end of capitulum maturation. Total maturation of the capitulum lasts about 3 months.

Stigmas are receptive as soon as they are exposed and positioned above the extended perianth, in the morning. When receptive, the stigmas are shiny and papillose and remain receptive throughout the day. Anther dehiscence occurs mainly between 1130 and 1400 hours. Pollen viability was 98.4% (± 0.4 ; $n=4$ flowers). The pollen grains agglutinate owing to the presence of lipid substances (pollenkitt). Nectar is secreted in small quantities during the entire period of anthesis and accumulates in the central region of the flowers, on the nectariferous structures.

Floral visitors

In all, 29 floral-visitor species were recorded, distributed across 19 insect families belonging to the orders Diptera (10 species), Hymenoptera (9 species), Coleoptera (8 species), Hemiptera (1 species) and Psocoptera (1 species) (Table 1). Among these species, the following had the highest relative abundance: *Brachiacantha australe* Leng (36.81%), *Neotropicomiris longirostris* Carvalho & Fontes (15.28%), *Solenopsis* sp. (14.26%), *Poecilognathus* sp. nov. (11%), *Mordella* sp. (6.90%), *Stomopogon* sp. (4.75%), Eumolpini sp. 1 (2.98%) and *Dialictus* sp. (2.14%) (Fig. 4*a–h*).

The visitation frequency and time spent in the capitula for each floral-visitor species are presented in detail in Table 2. The visitation frequency for each species was variable throughout the day, being higher at higher temperatures with low relative humidity.

The floral visitors began their activities in the flowers between 0900 and 0930 hours, this coinciding with the capitulum opening

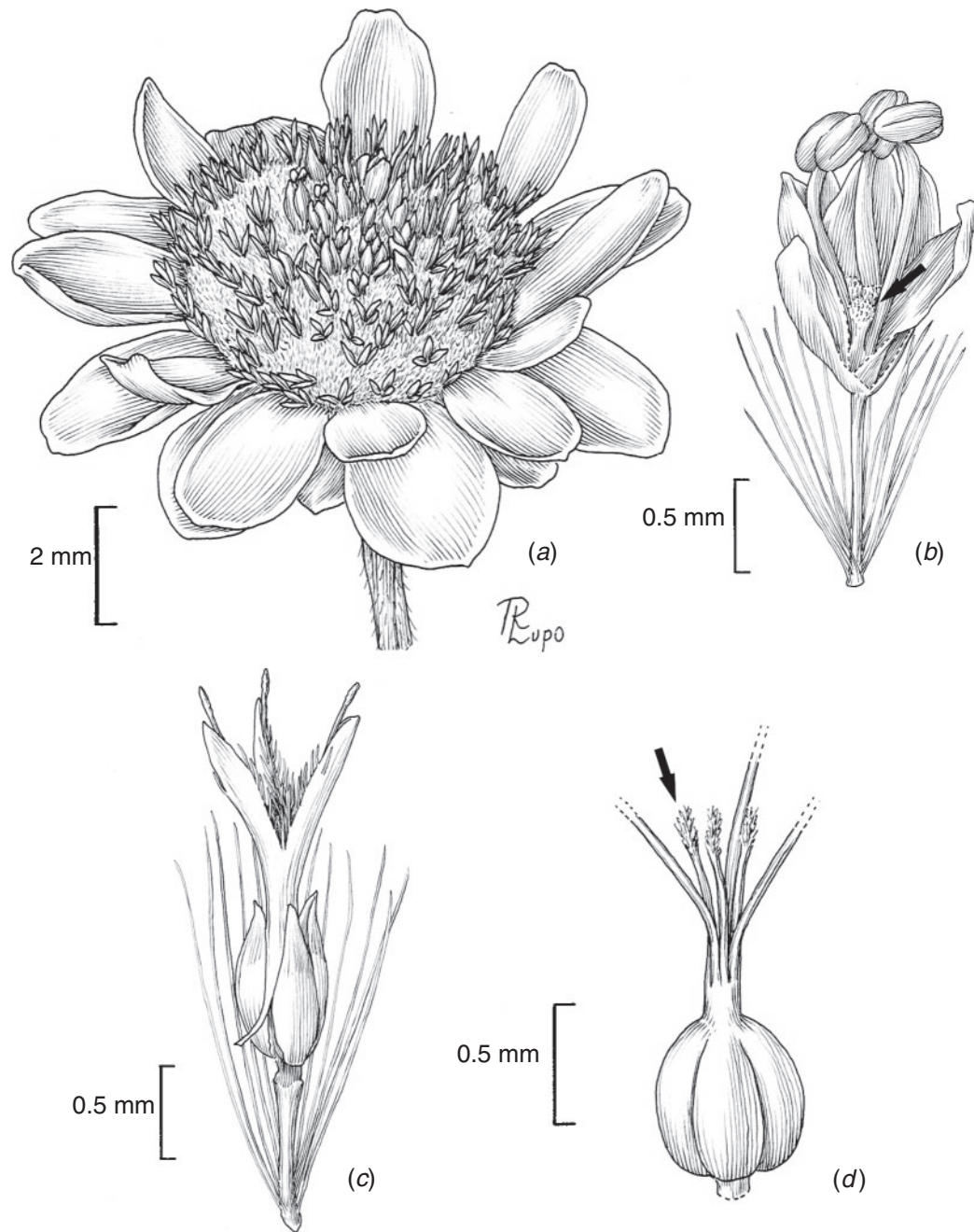


Fig. 1. Morphology of the capitulum and flowers of *Syngonanthus elegans*. (a) General aspect of the capitulum. (b) Staminate flower. The arrow indicates nectariferous pistillodes. (c) Pistillate flower. (d) Detail of the gynoecium. The arrow indicates the nectariferous portions of the style.

time, and extended these activities until ~1800 hours, when the capitula began to close. The highest frequency of visits occurred mainly between 1130 and 1400 hours, which is the same period as for anther dehiscence. When approaching the flowers of *S. elegans*, visitors used the capitula as a landing base and visited capitula in both the staminate and pistillate phases. The nectariferous pistillodes (staminate flowers) and nectariferous portions of the styles (pistillate flowers) were the structures searched by most visitors. While they collected nectar, the insects came into contact with pollen and stigmas, causing

pollination to occur. Most of the pollinators usually visited all the flowers of a capitulum (then moved to another).

Brachiacantha australe (Coccinellidae, Coleoptera) (Fig. 4a) was the most abundant visitor (Table 1). Although Coccinellidae are reported to be hunters, it was observed that these beetles fed on pollen and nectar. Moreover, the inflorescences of *S. elegans* were the main mating sites for these beetles. Individuals could be found on capitula throughout the day, walking over the flowers, feeding on pollen and nectar, and copulating. During these activities, pollen grains became attached to the ventral region

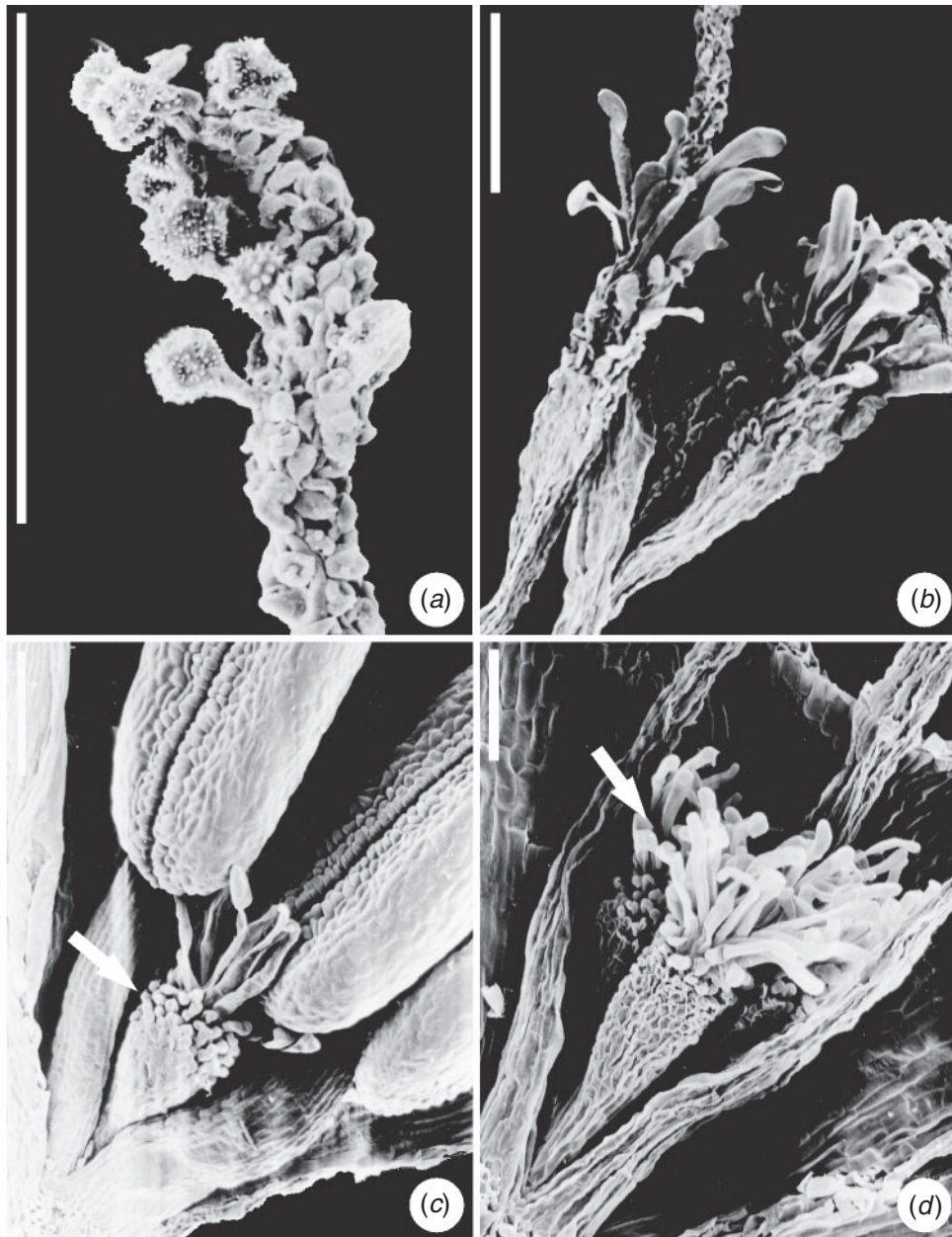


Fig. 2. Scanning electron micrographs of *Syngonanthus elegans* flowers. (a) Detail of the stigmatic portion of the style, with germinating pollen grains. (b) Detail of the nectariferous portions of the style. (c) Detail of the nectariferous pistillodes in a flower at the bud stage, showing the unelongated epidermal cells (indicated by the arrow). (d) Detail of nectariferous pistillodes in a flower at anthesis, showing the elongated epidermal cells (indicated by the arrow). Scale bars = 20 μm .

of their abdomen and to their legs (Fig. 4a, arrow), and were then removed on the stigmas when these beetles visited capitula in the pistillate phase. The peak activity in this species occurred from 1130 to 1400 hours. Although they were the most abundant insect species, their visiting frequency was lower than that for less abundant species such as *Poecilognathus* sp. nov. (Bombyliidae, Diptera) (Table 2), because they spent a longer period of time on the same capitulum.

Specimens of *Neotropicomiris longirostris* (Miridae, Hemiptera) (Fig. 4b) were observed on capitula throughout the day, and were more abundant in May (Table 1). While

retrieving nectar with their proboscis, the pollen grains adhered to the ventral and lateral regions of their abdomen, and to their legs and antennae. Specimens of *Solenopsis* sp. (Formicidae, Hymenoptera) (Fig. 4c), however, had their peak activity from 1100 to 1300 hours, and were more abundant in April (Table 1). A large number of pollen grains was observed on their entire body. They moved among different plants via the scapes (inflorescence stalks), which were close to each other because of the aggregated distribution of *S. elegans*.

Poecilognathus sp. nov. (Bombyliidae, Diptera) (Fig. 4d) individuals flew quickly from one capitulum to another

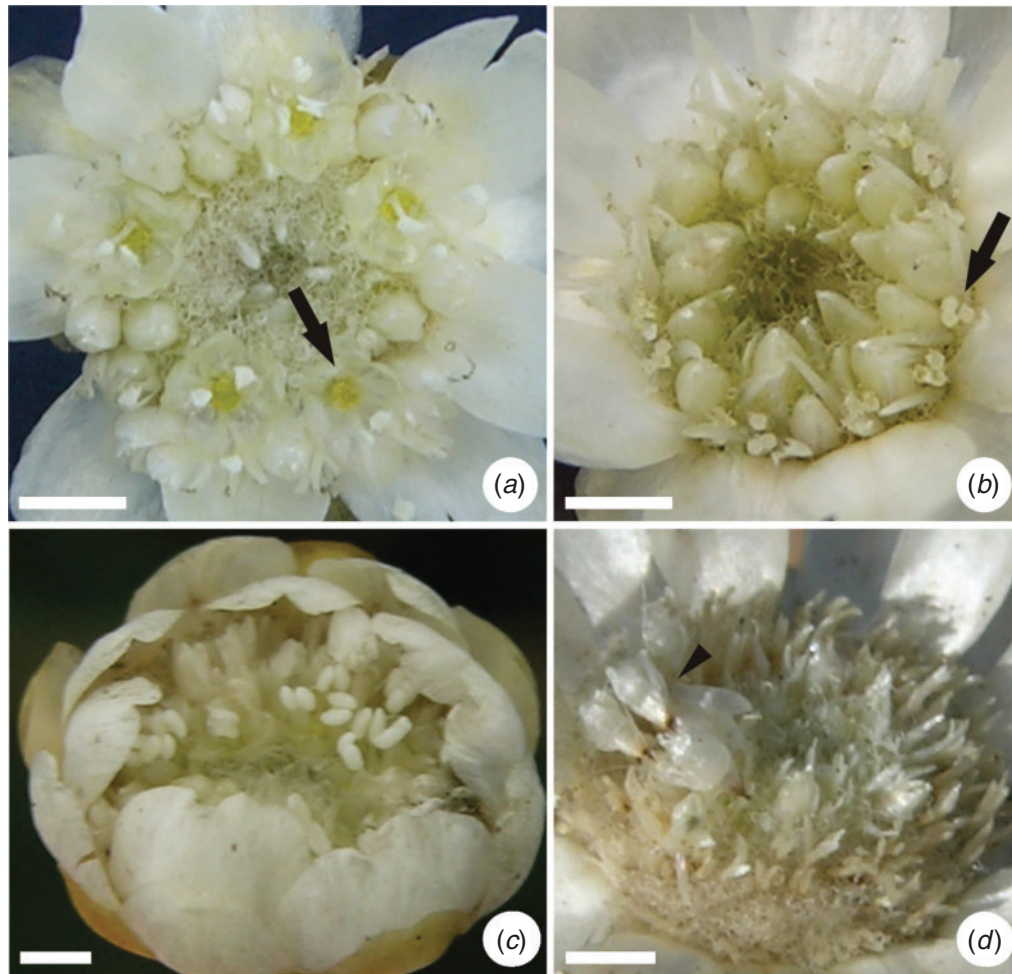


Fig. 3. The dichogamic capitula of *Syngonanthus elegans*. (a) Open capitulum with staminate flowers at anthesis. The arrow indicates the nectariferous pistillodes in the central region of the flowers. (b) Open capitulum with pistillate flowers at anthesis. The arrow indicates the nectariferous portions of the style. (c) Opening capitulum, showing staminate flowers at anthesis. (d) Detail of the capitulum, showing staminate flowers detaching (indicated by the arrowhead). Scale bars = 0.1 cm.

(Table 2) and visited all flowers in the capitulum, searching for nectar. They extracted the nectar with their proboscis, and the pollen grains became attached to the ventral region of their abdomen and legs. Peak activity in this species occurred from 1200 to 1500 hours, with greater abundance in April (Table 1).

Stomopogon sp. (Muscidae, Diptera) (Fig. 4f) and *Dialictus* sp. (Halictidae, Hymenoptera) (Fig. 4g) presented a visiting behaviour similar to the one observed for *Poecilognathus* sp. nov. The peak activity in these species was from 1200 to 1400 hours, and they were more abundant in April (Table 1). A large number of pollen grains was observed attached to the body of these insects.

Although abundant, *Mordella* sp. (Mordellidae, Coleoptera) (Fig. 4e) and *Eumolpini* sp. 1 (Chrysomelidae, Coleoptera) (Fig. 4h) remained for a long time on a single inflorescence (Table 2), on the flowers or on the involucre bracts, and only a few pollen grains were observed adhered to their bodies. *Eumolpini* sp. 1 individuals were observed only in May (Table 1). Its specimens, as well as *Brachiacantha australe* (Coccinellidae, Coleoptera) specimens, spent the night inside the capitula, which closed daily.

Brachiacantha australe, *Stomopogon* sp., and *Eumolpini* sp. 1 individuals were also observed in species of other genera of Eriocaulaceae, which grew during the same period as *S. elegans*.

In addition to the listed floral visitors, the occurrence of mites of the order Oribatida (*Humerobates flechtmanni* Pérez-Iñigo & Pérez-Iñigo Jr. *Hemileius* sp.) was recorded in 92% of the capitula ($n=60$). More than one mite occurred in each capitulum, and one specimen was observed being transported by a *Stomopogon* sp. individual.

Pollination systems

The formation of seeds in the polyester-bagged capitula (Treatment 1) demonstrated the occurrence of spontaneous self-pollination. The mean numbers of pistillate flowers and seeds formed per capitulum in each treatment are shown in Table 3.

The reproductive success among the three treatments was similar for both populations (Fig. 5). Reproductive success in Treatment 3 was greater and significantly different from that in Treatments 1 and 2 (Nemenyi test, $P<0.01$), indicating that

Table 1. Survey of floral visitors of *Syngonanthus elegans*, performed during two consecutive days for each studied population in May 2005, April 2006 and May 2006 at Serra do Cipó (Minas Gerais)

Classification of the floral visitors was based on their behaviour and visitation frequency: EF, effective pollinator; OC, occasional pollinator; V, visitor

Floral visitor	No. of observed individuals					Total	Relative abundance (%)	Classification
	May 2005 Pop. A	April 2006 Pop. A	Pop. B	May 2006 Pop. A	Pop. B			
Coleoptera								
Coccinellidae								
<i>Brachiacantha australe</i>	58	88	116	37	96	395	36.81	EF
Mordellidae								
<i>Mordella</i> sp.	33	15	15	2	9	74	6.90	OC
Chrysomelidae								
Eumolpini sp. 1	2			23	7	32	2.98	OC
Alticini sp. 1	1		1		2	4	0.37	OC
<i>Paranapiacaba</i> sp.	1					1	0.09	V
<i>Parasyphraea</i> sp.					1	1	0.09	V
Curculionidae								
<i>Pandeleiteius</i> sp.	1					1	0.09	V
<i>Geraeus</i> sp.		1				1	0.09	V
Hemiptera								
Miridae								
<i>Neotropicomiris longirostris</i>	15	11	13	40	85	164	15.28	EF
Hymenoptera								
Formicidae								
<i>Solenopsis</i> sp.	5	58	74	2	14	153	14.26	EF
<i>Azteca</i> sp.	1	1	3			5	0.47	OC
<i>Camponotus</i> sp.		2	6			8	0.75	OC
Halictidae								
<i>Dialictus</i> sp.		11	6	1	5	23	2.14	EF
Cynipidae sp. 1					3	3	0.28	OC
Colletidae								
<i>Chilicola</i> sp.					2	2	0.19	V
Andrenidae								
<i>Psaenythia</i> sp.		1				1	0.09	V
Braconidae								
<i>Apanteles</i> sp.					1	1	0.09	V
Pteromalidae								
<i>Lyrcus</i> sp.					1	1	0.09	V
Diptera								
Bombyliidae								
<i>Poecilognathus</i> sp. nov.	14	72	17	1	14	118	11.0	EF
Muscidae								
<i>Stomopogon</i> sp.	1	26	18		6	51	4.75	EF
Empididae								
<i>Empis</i> sp. 1	1	3			3	7	0.65	OC
<i>Empis</i> sp. 2	2					2	0.19	V
<i>Empis</i> sp. 3	1					1	0.09	V
<i>Hilarempis</i> sp.	1					1	0.09	V
<i>Lamprempis</i> sp.	1					1	0.09	V
Syrphidae sp. 1		6	3			9	0.84	OC
Dolichopodidae sp. 1		2	2	1	3	8	0.75	OC
Agromyzidae sp. 1	1	1	2			4	0.37	OC
Psocoptera								
Pseudocaeciliidae sp. 1	1					1	0.09	V

pollination by insects was the most effective pollination system in *S. elegans*. Treatments 1 and 2 were not statistically different from each other concerning the reproductive success (Nemenyi test, $P > 0.05$), indicating that wind did not effectively contribute towards pollination in this species. The Nemenyi test results are shown in Table 4.

Seed germination

The total number of germinated seeds in each treatment and their corresponding percentages were as follows: Treatment 1 (seeds resulting from spontaneous self-pollination), 9 (18.75%); Treatment 2 (seeds resulting from spontaneous self-pollination + wind pollination), 44 (44%); Treatment 3 (seeds resulting

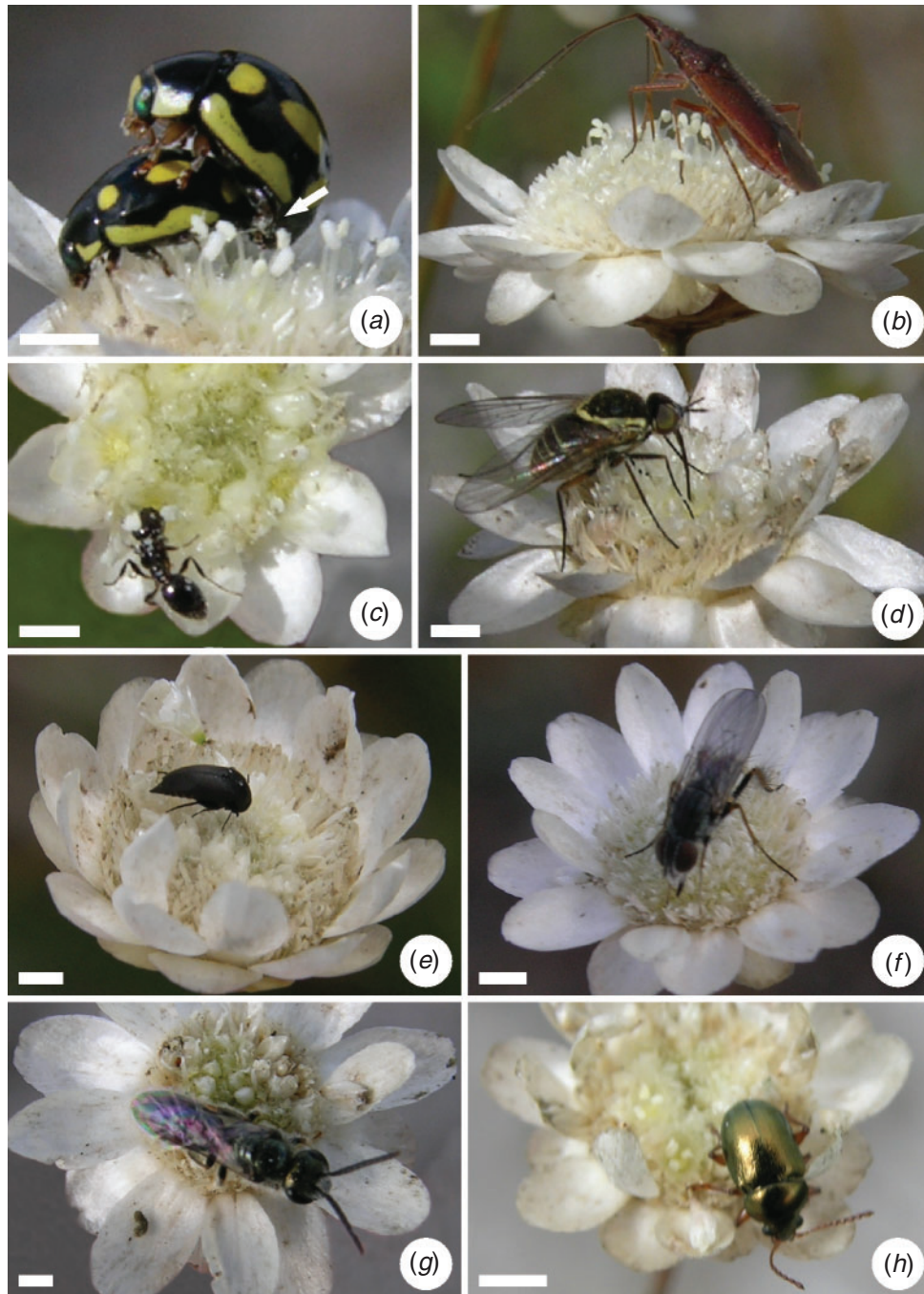


Fig. 4. The pollinators of *Syngonanthus elegans*. (a) *Brachiacantha australe* (Coccinellidae, Coleoptera), copulating on the capitulum. The arrow indicates the pollen grains attached to their legs. (b) *Neotropicomiris longirostris* (Miridae, Hemiptera), extracting nectar from staminate flower. (c) *Solenopsis* sp. (Formicidae, Hymenoptera), visiting a capitulum in the staminate phase; observe the insect extracting nectar from nectariferous pistillodes. (d) *Poecilognathus* sp. nov. (Bombyliidae, Diptera), extending its proboscis to extract nectar from a pistillate flower. (e) *Mordella* sp. (Mordellidae, Coleoptera), standing on capitulum. (f) *Stomopogon* sp. (Muscidae, Diptera), extracting nectar from a pistillate flower. (g) *Dialictus* sp. (Halictidae, Hymenoptera), standing on capitulum. (h) Eumolpini sp. 1 (Chrysomelidae, Coleoptera), standing on capitulum. Scale bars = 0.1 cm.

from spontaneous self-pollination + wind pollination + insect pollination), 67 (67%) (Fig. 6). Germination percentage was lower in Treatment 1 and significantly different from Treatment 3

(Tukey test, $P < 0.01$). Seedlings in Treatment 1 were also less vigorous than those in Treatment 3 because of their smaller length and yellowish leaves.

Table 2. Number of visits (*n*) and total time spent (*t*) on *Syngonanthus elegans* capitula by the visiting species, recorded during one day in May 2006 at Serra do Cipó (Minas Gerais)

Floral visitor	Population A		Population B		Total	
	<i>n</i>	<i>t</i>	<i>n</i>	<i>t</i>	<i>n</i>	<i>t</i>
<i>Poecilognathus</i> sp. nov.	36	40 min 24 s	14	22 min 43 s	50	1 h 3 min 7 s
<i>Solenopsis</i> sp.	17	1 h 33 min 59 s	29	3 h 45 min 47 s	46	5 h 19 min 46 s
<i>Brachiacantha australe</i>	7	22 min 41 s	36	4 h 47 min 43 s	44	5 h 10 min 24 s
<i>Stomopogon</i> sp.	4	3 min 59 s	17	1 h 51 s	21	1 h 4 min 50 s
Eumolpini sp. 1	8	1 h 24 min 18 s	6	1 h 08 min 36 s	14	2 h 32 min 54 s
<i>Mordella</i> sp.	6	45 min 42 s	6	59 min 52 s	12	1 h 45 min 34 s
<i>Dialictus</i> sp.	1	23 s	6	57 min	7	57 min 23 s
<i>Neotropicomiris longirostris</i>	1	27 min	3	28 min 20 s	4	55 min 20 s
<i>Azteca</i> sp.	—	—	2	4 min 12 s	2	4 min 12 s
Dolichopodidae sp. 1	—	—	2	6 min 58 s	2	6 min 58 s
<i>Empis</i> sp. 1	1	7 min 30 s	—	—	1	7 min 30 s
Syrphidae sp. 1	1	57 s	—	—	1	57 s

Table 3. Mean and standard error for the number of flowers and seeds formed per *Syngonanthus elegans* capitulum, in each pollination treatment

Treatment 1 = spontaneous self-pollination; Treatment 2 = spontaneous self-pollination + wind pollination; Treatment 3 = spontaneous self-pollination + wind pollination + insect pollination. *n* = number of capitula

Population	Treatment	Pistillate flowers	Seeds
Population A	1	117.1 ± 8.6	12.8 ± 4.5; <i>n</i> = 15
	2	118.5 ± 9.5	30.5 ± 8.9; <i>n</i> = 15
	3	94.4 ± 6.4	82.3 ± 10.3; <i>n</i> = 20
Population B	1	145.2 ± 6.8	1.3 ± 0.5; <i>n</i> = 32
	2	130.2 ± 7.5	8.5 ± 2.9; <i>n</i> = 26
	3	124.2 ± 6.7	184.8 ± 13.5; <i>n</i> = 28

Discussion

Although anemophily is the main pollination syndrome of Poales, it was verified in this work that wind plays an insignificant role in the pollination of *Syngonanthus elegans*, and that the presence of easily accessible pollen and nectar attracts species of several insect orders.

Ramos *et al.* (2005) also considered entomophily to be the main pollination mode in *S. mucugensis* and *S. curralensis*, on the basis of floral-visitor survey and activities of insects on flowers. This hypothesis was confirmed in *S. elegans* by selective-exposure experiments that demonstrated that pollination by insects contributed more effectively to the reproductive success of the species.

Nectar is the primary resource searched for by insects in *S. elegans*. The nectar-producing structures correspond to the

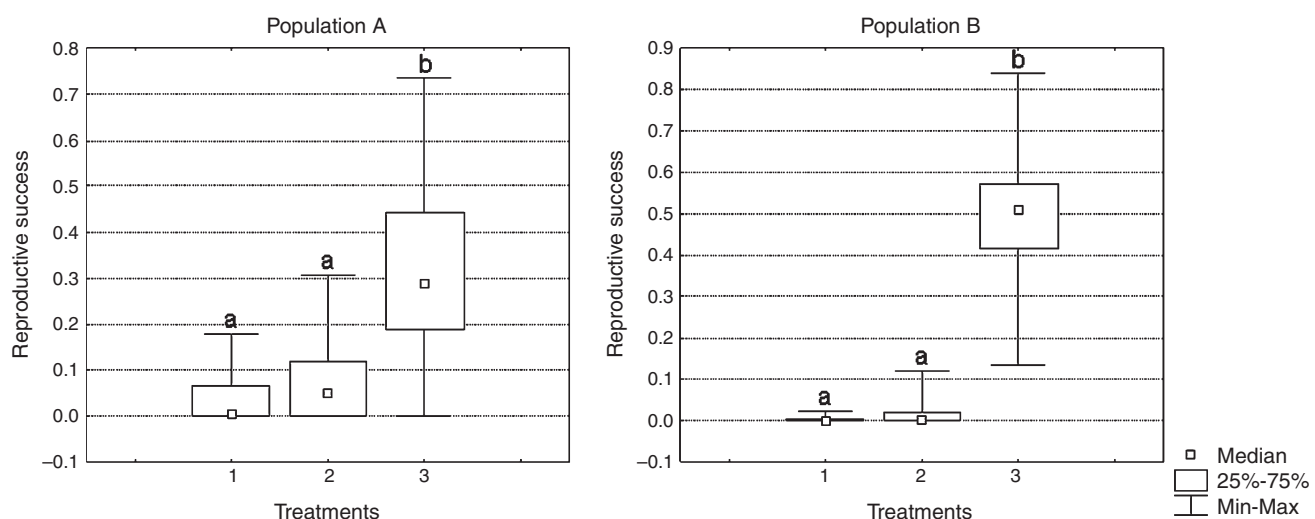


Fig. 5. Reproductive success of *Syngonanthus elegans* individuals submitted to the following three pollination treatments: Treatment 1 = spontaneous self-pollination; Treatment 2 = spontaneous self-pollination + wind pollination; and Treatment 3 = spontaneous self-pollination + wind pollination + insect pollination. Different letters on the graph represent a significant ($P < 0.05$) difference between treatments according to Nemenyi test. Reproductive success was calculated for each capitulum by the number of seeds/(number of flowers × 3).

Table 4. Results of the Nemenyi test, comparing the reproductive success among the three pollination treatments

Treatment 1 = spontaneous self-pollination; Treatment 2 = spontaneous self-pollination + wind pollination; Treatment 3 = spontaneous self-pollination + wind pollination + insect pollination. Reproductive success was calculated for each capitulum by the number of seeds/(number of flowers \times 3)

Treatments compared	Population A	Population B
1 v. 2	$P=0.9221$	$P=0.5829$
1 v. 3	$P<0.0001$	$P<0.0001$
2 v. 3	$P=0.0019$	$P<0.0001$

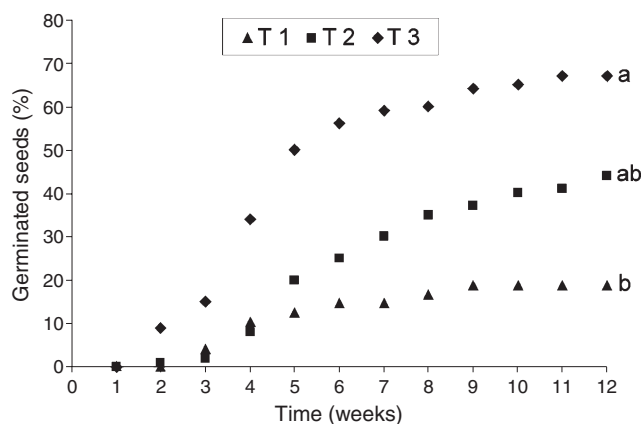


Fig. 6. Germination of *Syngonanthus elegans* seeds in the following three pollination treatments: Treatment 1 = seeds resulting from spontaneous self-pollination; Treatment 2 = seeds resulting from spontaneous self-pollination + wind pollination; Treatment 3 = seeds resulting from spontaneous self-pollination + wind pollination + insect pollination. Different letters on the graph represent a significant ($P < 0.05$) difference between treatments according to the Tukey test.

pistillodes (staminate flower) and to the nectariferous portions of the style (pistillate flower). Production of nectar by these structures has also been observed in other species of Eriocaulaceae, and was considered evidence of entomophily (Rosa and Scatena 2003, 2007). The fact that the nectariferous pistillodes and the nectariferous portions of the style are located below the anthers and the stigmas, respectively, causes the floral visitors in search of nectar to touch these structures, thus pollinating the flowers.

This is the first study in Eriocaulaceae that shows that nectar, produced by the pistillodes and nectariferous portions of style, is used as a floral reward by pollinators. The production of nectar has not been reported in *S. mucugensis* and *S. curralensis* (Ramos *et al.* 2005), although their flowers also possess nectariferous structures.

In Eriocaulaceae, the branching of the styles into stigmatic and nectariferous portions in pistillate flowers does not occur in species of *Eriocaulon* L. and *Mesanthemum* Körn, which were identified by Ruhland (1903) as 'primitive' genera. According to Rosa and Scatena (2003, 2007), this morphological and functional modification of the style throughout the group's evolution is related to the attraction of pollinating agents. Analyses of breeding systems of *Eriocaulon parkeri*

B.L. Robinson demonstrated that this species reproduces by self-pollination (Sawyer *et al.* 2005).

Within the Poales, insect pollination, which is considered an ancestral pollination mode of the clade, has been reported only in Bromeliaceae, Rapateaceae, Mayacaceae, Xyridaceae and Eriocaulaceae (Linder and Rudall 2005). Regarding Eriocaulaceae, data produced in the current study are conclusive and strongly support insect pollination. With the exception of Mayacaceae, these are also the only families in Poales that possess floral nectaries (Givnish *et al.* 1999; Linder and Rudall 2005), demonstrating a strong association between these floral structures and the pollination system, since nectar is an important floral reward for pollinators. However, further studies in these families, as well as in other groups, are necessary to the better understanding of the evolution of reproductive systems in Poales.

On the basis of visitation frequency, behaviour on the capitula and the presence of pollen grains adhered to the body, the following were considered effective pollinators of *S. elegans*: *Brachiacantha australe* (Coccinellidae, Coleoptera), *Neotropicomiris longirostris* (Miridae, Hemiptera), *Solenopsis* sp. (Formicidae, Hymenoptera), *Poecilognathus* sp. nov. (Bombyliidae, Diptera), *Stomopogon* sp. (Muscidae, Diptera) and *Dialictus* sp. (Halictidae, Hymenoptera). The other floral visitors were considered occasional pollinators or, when observed on a single occasion or on flowers of the same sex, they were deemed to be visitors.

Species of Tenebrionidae (Coleoptera), Bombyliidae, Sarcophagidae and Syrphidae (Diptera) have been noted as effective pollinators of *S. mucugensis* and *S. curralensis*. In addition, species of Sarcophagidae, Syrphidae (Diptera) and Halictidae (Hymenoptera) are regarded as occasional pollinators (Ramos *et al.* 2005). Although most pollinators of *S. elegans* belong to different families than the pollinators of *S. mucugensis* and *S. curralensis*, these three species of *Syngonanthus* are generalist and belong to the category of plants pollinated by small diverse insects, which is common in 'campo rupestre' vegetation and could be a pattern for the family. It is also possible that most Eriocaulaceae species share pollinators, because some pollinators species of *S. elegans* from 'campo rupestre' of Minas Gerais State are also related to *S. mucugensis* and *S. curralensis* from 'campo rupestre' of Bahia State (Ramos *et al.* 2005), and were observed visiting other species in the family.

In *S. elegans*, most floral-visitor species are more abundant in April, possibly owing to a greater availability of resources, since blooming in this species peaks during that month (Scatena *et al.* 1997). Seasonal flowering, e.g. in *S. elegans* (Scatena *et al.* 1997), makes resources predictable in time, increasing the number of visitors that search for pollen and nectar. In addition, species with a clustered distribution, forming homogeneous populations with a large number of inflorescences per individual, such as in *S. elegans*, also favour the attraction of other groups of visitors that may act as occasional pollinators (Schmitt 1983; Klinkhamer and de Jong 1993) and may benefit from cross-pollination, because the pollinators transport the pollen from one plant to another (Rathcke and Lacey 1985; Taylor and Inouye 1985).

The variation observed in the visiting frequency throughout the day could be associated with abiotic factors, because different

species of insects require distinct optimal temperature and luminosity conditions for foraging (Antonini *et al.* 2005). Although the floral visitors of *S. elegans* showed differences in their activity times, their visitation peaks were similar, coinciding with high temperature and light intensity and low relative humidity. Because involucral bract movement is also influenced by air humidity (Oriani and Scatena 2009), visitation peaks coincide with the complete opening of the capitula (low relative humidity and high temperature conditions), favouring pollination by insects.

The results obtained in the present study demonstrated that seed formation also occurs by spontaneous self-pollination and, therefore, *S. elegans* is self-compatible. Although dichogamy occurs, self-pollination may occur within the same capitulum during the short period when the change from the staminate phase to the pistillate phase occurs, as suggested for *Paepalanthus tatei* (Stützel 1981). The consequence of self-pollination in *S. elegans*, however, was inbreeding depression, because seeds resulting from self-pollination showed lower germination percentage and seedlings were less vigorous. This could be due to the harmful effects of homozygous genes resulting from inbreeding, and such a fact also emphasises the importance of insect pollination to promote outcrossing.

The hypothesis that insect pollination is the primary pollination system of *S. elegans* is reinforced by the high pollen viability and by the fact that anther dehiscence coincides with the period of complete opening of the capitula and the highest visitation frequency. The presence of lipid substances (pollenkitt) in the pollen grains facilitates their adhesion to the insect bodies and also optimises the efficiency of the insect pollination. Consequently, as demonstrated in the present work, pollination by small diverse insects is the main system responsible for the reproductive success in this species, resulting in the greatest seed set, with the highest germination percentage and optimum seedling vigour.

Acknowledgements

We thank CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico (grants no. 130690/2005–8 and no. 301404/2004–6) and FAPESP – Fundação de Amparo à Pesquisa do Estado de São Paulo (grant no. 2005/02141–4), for financial support, and Fazenda Monjolos Pousada for logistical support. Special thanks go to E. Giannotti, C. J. E. Lamas, F. J. Zara, L. M. de Almeida, L. A. A. Costa, C. J. B. de Carvalho, B. W. T. Coelho, L. A. Moura, C. Campaner, R. Ale-Rocha, S. A. Vanin, D. S. Amorim and A. R. Oliveira, for identification of the floral visitors, and E. Simão for assistance with the germination and statistical tests. A. Bianconi is acknowledged not only for help with the English version, but also for helpful suggestions. We also thank L. P. C. Morellato, P. E. A. M. Oliveira and the anonymous reviewers, for critical comments that improved the manuscript.

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Manuscript received 3 July 2008, accepted 3 March 2009