

Germination and seedling morphology of four South American *Smilax* (Smilacaceae)

Aline Redondo Martins¹, Anielca Nascimento Soares², Aline Bertolosi Bombo², Alessandra Fidelis³, Ana Dionisia da Luz Coelho Novembre⁴ & Beatriz Appezzato da Glória²

1. Institute of Biology, Universidade Estadual de Campinas, 13083-970, Campinas, SP, Brazil; aline_martins_botanica@yahoo.com.br
2. Department of Biological Sciences, Escola Superior de Agricultura 'Luiz de Queiroz', Universidade de São Paulo, 13418-900, Piracicaba, SP, Brazil; anielcasoares@hotmail.com, aline_bbombo@hotmail.com, bagloria@esalq.usp.br
3. Department of Ecology, Universidade de São Paulo, 05508-900, São Paulo, Brazil; fidelis@usp.br
4. Department of Crop Science, Escola Superior de Agricultura 'Luiz de Queiroz', Universidade de São Paulo, 13418-900, Piracicaba, SP, Brazil; adlenove@esalq.usp.br

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Abstract: Species of *Smilax*, also known as greenbrier, are widely distributed in Brazil and their commercial trades are carried out by the extractivism of native species. We the aim to provide information about the germination and development of seedlings in four *Smilax* species, different experiments were developed under controlled conditions. We evaluated two germination treatments: temperature (30°C and 20-30°C) and light (presence/absence), and for few cases the tetrazolium treatment was applied. A different treatment response was observed among the studied species. Light had a significant influence in *S. brasiliensis*, with the highest germination rates at 20-30°C in dark conditions. *S. campestris* showed significant differences among temperature treatments, but not to light; while *S. cissoides* showed high germination rates (66-78%), independently of treatment. However, *S. polyantha* had low germination rates (19-24%). After one year, the expanded leaves showed different characteristics among the studied species. Leaves of *S. brasiliensis* were ovate, coriaceous, three main veins and prickle-like structures only on the midrib on abaxial face. *S. campestris* leaves were oblong, coriaceous and prickle-like structures were located at the leaf midrib and margin. *S. cissoides* had ovate-elliptic, membranaceous leaves, with three main veins with prickle-like structures on the abaxial face. *S. polyantha* leaves showed ovate-elliptic, coriaceous leaves, with three main veins, translucent secondary veins and no prickle-like structures. A seedling identification key was elaborated based on morphological characteristics. Rev. Biol. Trop. 60 (1): 495-504. Epub 2012 March 01.

Key words: greenbrier, leaf morphology, light, medicinal plant, temperature.

Smilax species, also known as “salsaparrilha” (Greenbrier), are usually used by people due to their anti-rheumatic properties attributed to infusions of their roots and rhizophores. These species are widely distributed in Brazil and their commercial trades are carried out by the extractivism of native species (Andreata 1997).

Studies about the germination of species are important tools to help solving taxonomical and phylogenetical questions and to help the

understanding of morphology using ontogeny (Andreata & Pereira 1990, Oliveira 2001). Additionally, the establishment of conditions for germination is of crucial importance for the production and trade of viable seeds.

Several authors carried out studies about germination of *Smilax* species (Holm 1890, Andreata 1980, Pogge & Bearce 1989, Andreata & Pereira 1990, Barnea *et al.* 1991, Andreata & Menezes 1999, Rosa & Ferreira 1999,

D'Antuono & Lovato 2003, Santos *et al.* 2003, Palhares *et al.* 2009, Martins *et al.* 2011). These studies tested different treatments and they found different results in relation to responses to light, dormancy break and germination rates. However, *Smilax* is a dioecious genus and therefore the plants are generally markedly present in the ecosystems, their seeds are difficult to germinate.

Seedlings of monocotyledons have a complex structure. Unfortunately, the literature contains many superficial seedling descriptions, together with an inexact and often incorrect or misleading terminology (Tillich 2007). There is a lack of studies about the morphology of seedlings of *Smilacaceae* family. One of the few studies described the morphology of seedlings of five different Brazilian species of *Smilax* considering the post-germination period and the appearance of the first eophyll (Andreatta & Pereira 1990). Martins *et al.* (2011) presented seed germination and seedling and young plant morphology of *Smilax polyantha* and discusses the terminology of the underground system.

Therefore, the aim of this study was to provide information about the germination and seedling morphology of four species of *Smilax* known as "salsaparrilha": *Smilax brasiliensis* Sprengel, *S. campestris* Grisebach, *S. cissoides* Martius ex Grisebach and *S. polyantha* Grisebach. Moreover, a seedling identification key was elaborated based on morphological traits, which can help further researches in identifying *Smilax* seedlings.

MATERIALS AND METHODS

Fruit sampling: Fruits were sampled randomly from individuals in different plant

populations and occurrence locations (Table 1). The plant samples were registered and added to the plant collection of the Herbarium (ESA) of the Escola Superior de Agricultura "Luiz de Queiroz" of the Universidade de São Paulo.

Germination experiments: We tested two different factors affecting germination rates: two temperature conditions (30°C and 20-30°C) and two light regimes (eight hours of light, and total absence of light). For each treatment, we used four replicates, with 25 seeds each. The seeds were previously selected in order to eliminate grossly damaged or malformed seeds. All seeds were transferred to germination box (11x3cm), with 292g of sterilized sand and 45mL of water. Observations were carried out weekly during 30 weeks. Germination was confirmed by the emission of aboveground parts, since seeds were buried (1.0cm deep) in sand.

We performed analysis of variance using randomization tests to test differences between treatments for each species. We chose this method, since there is no assumption of normal distribution of the data (Manly 2007). We also used Euclidean distance as resemblance measure (10 000 iterations). The statistical analyses were performed by the software MULTIV (Pillar 2005).

At the end of the experiments, the SGI (Speed Germination Index) was calculated according to Maguire (1962). The seeds that did not germinate had their viability tested by performing a modified tetrazolium test according to the methodology adopted by Cury *et al.* (2010). Seeds were also considered not viable when the tissue was damaged and/or altered by fungi, compromising the seedling development.

TABLE 1
Brazil location of sampling of *Smilax* seeds

Species/Voucher	Location State/City
<i>S. brasiliensis</i> (ESA 107638)	Minas Gerais/Itapagipe (19°32'39.6" S - 49°26'36.2" W)
<i>S. campestris</i> (ESA 107657)	Rio Grande do Sul/Caçapava do Sul (30°03'34.2" S - 51°07'31.8" W)
<i>S. cissoides</i> (ESA 107659)	Bahia/Feira de Santana (12°12'07" S - 38°57'57.4" W)
<i>S. polyantha</i> (ESA 107649)	São Paulo/Pratânia (22°48'54 5" S - 48°44'33.2" W)

Seedling morphology: Ten seedlings of each study species were analysed concerning their morphology. They were photographed with a digital camera during the following development stages (Andreata & Pereira 1990): early seedling development, seedlings with the first expanded eophyll, young plants with five eophylls and one-year old plants. The following traits were observed (Andreata & Pereira 1990): number of hyaline cataphylls formed in the primary axis, characteristics of the cotyledonary sheath, number and shape of young leaves, aerial stem ramification, formation of underground stems and distinction among primary and adventitious roots, as well as their establishment.

RESULTS

Germination experiments: The results showed that seeds of *S. brasiliensis* (Fig. 1A) showed an interaction between light and temperature ($p=0.0002$). The highest values of germination were reached under conditions of 20-30°C with the absence of light (55%,

$p\leq 0.05$). On the other hand, significant differences between treatments of temperature could be found for *S. campestris* ($p=0.04$, Fig. 1B), but not for light conditions ($p=0.81$). The 79% of seeds germinated in the treatment of 20-30°C.

No significant differences between treatments for both light and temperature conditions were found for *S. cissoides* and *S. polyantha* ($p=0.89$ and $p=0.06$ for *S. cissoides*, and $p=0.99$ and $p=0.44$ for *S. polyantha* Fig. 2C, D). The first species showed high germination rates (between 66 and 78%), whilst the second one had the lowest germination rates (19-24%) among all study species.

The first species to germinate was *S. campestris* (third week). This species also showed the highest values of SGI (2.45 to 4.22, Table 2). *S. cissoides* began to germinate at the fourth week and it had SGI values from 1.63 to 1.86. Finally, seeds of *S. brasiliensis* and *S. polyantha* had the lowest SGI values (0.09 to 1.57 and 0.5, respectively).

The tetrazolium tests showed that ca. 36-58% of the total number of seeds of

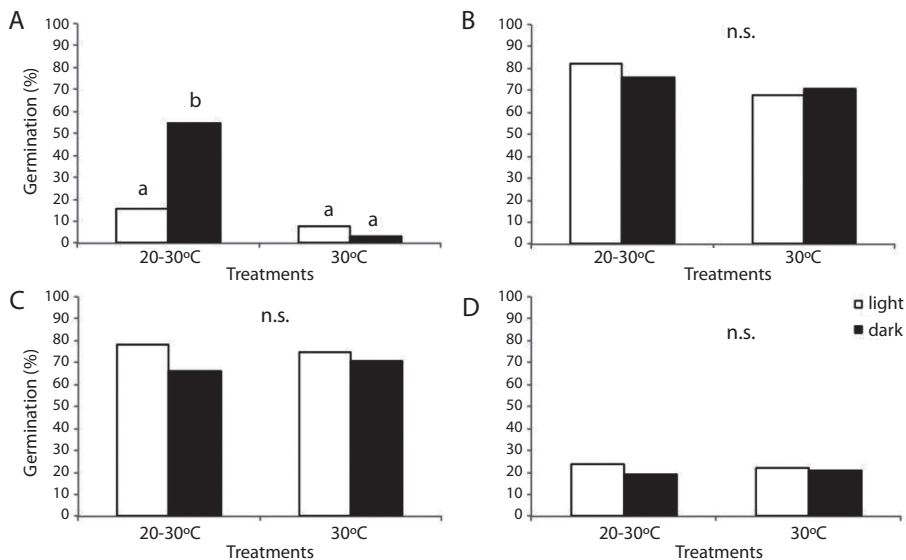


Fig. 1. Germination of *Smilax brasiliensis* (A), *Smilax campestris* (B), *Smilax cissoides* (C) and *Smilax polyantha* (D) under two different temperature conditions (20°-30°C and 30°C) and light regimes (light/dark). Different letters mean significant differences among treatments.

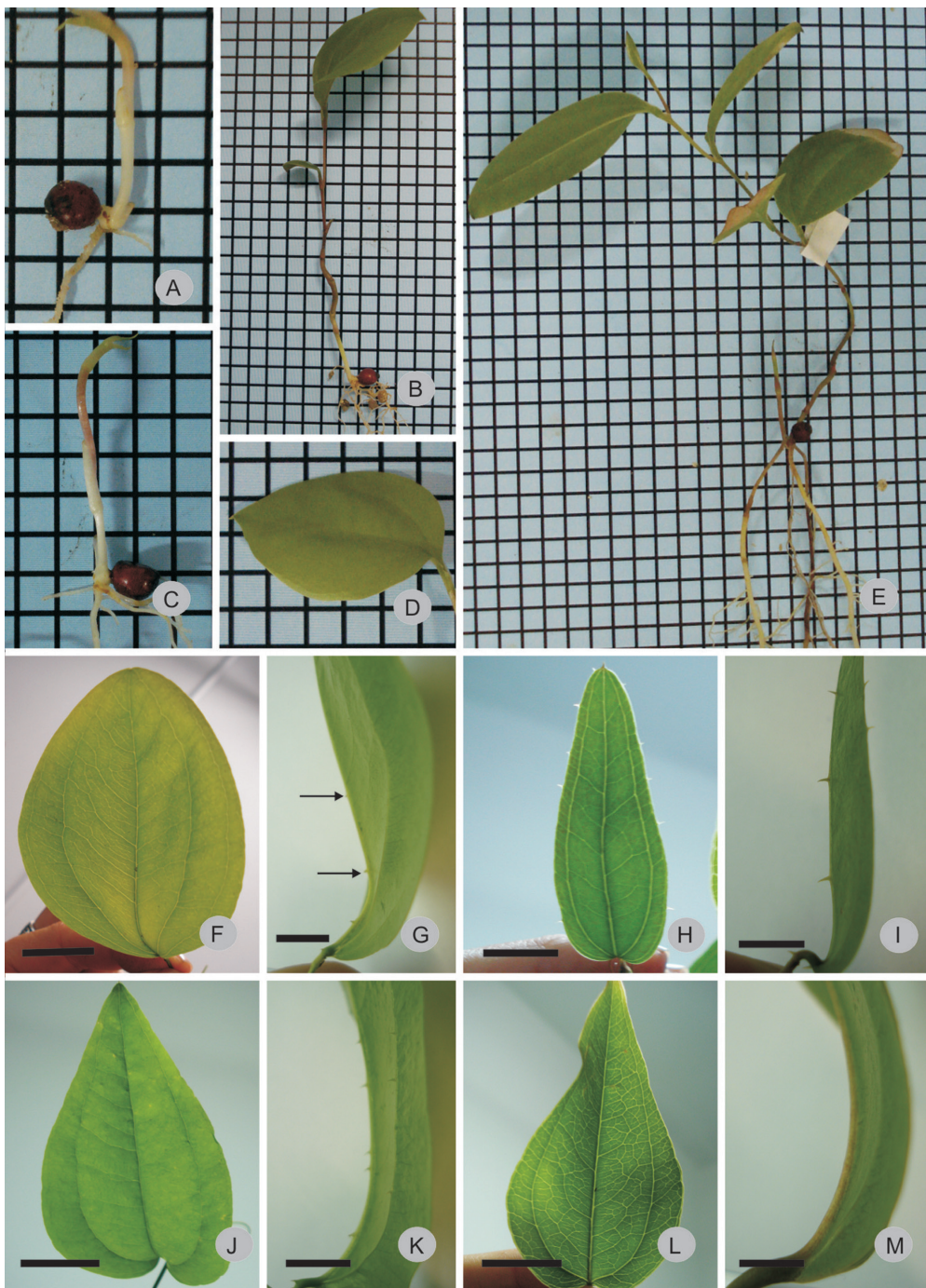


Fig. 2. A.-B., H.-I. *Smilax campestris*. C.-D., J.-K. *Smilax cissoides*. E., L.-M. *Smilax polyantha*. F.-G. *Smilax brasiliensis*, arrow = prickles on the midrib. A, C. Early seedling development. B., D. Seedling with eophyll. E. Seedling with the fourth protophyll. F.-M. Leaves of one-year old plants of *Smilax*. Scale: A.-E. Side of the square is 0.5cm, F., J., L. 2cm. G., H., K., M. 1cm, I. 0.5cm.

TABLE 2
Speed Germination Index (SGI) for *Smilax* species under two different temperature conditions and light regimes

Species	Light			Dark	
	30°C	20-30°C	30°C	20-30°C	
<i>S. brasiliensis</i>	0.23	0.4	0.09	1.57	
<i>S. campestris</i>	3.5	2.47	4.22	2.45	
<i>S. cissoides</i>	1.86	1.8	2.11	1.63	
<i>S. polyantha</i>	0.55	0.5	0.52	0.48	

S. polyantha had some kind of tissue alteration due to the presence of fungi at the end of the experiments (Table 3).

The other species showed lower percentages of fungi infection. *S. campestris* and *S. cissoides* had about 20% of infected seeds. Seeds of *S. brasiliensis* showed variation according to the treatment: 5% of all seeds were infected by fungi for the treatment 20-30°C (dark), whilst this percentage increased to 50% for 20-30°C (light). *S. campestris* and *S. cissoides* seeds had the lowest percentage of viable seeds at the end of the experiments (2-10%). *S. brasiliensis* had 23-44% of viable seeds and *S. polyantha* between 23-34% (Table 3).

Morphological analysis: The primary axis was formed by three (rarely four) translucent cataphylls before the formation of the first eophyll, which occurred 15-30 days after the germination. The cotyledonary sheath had at first a yellowish colour and a soft consistency (Fig. 2 A, C), surrounding completely the reduced epicotyl. Further at the development

phase, the sheath became brownish and chaffy. The morphology of the first eophyll did not differ among the study species, showing ovate-shape membranaceous leaves with convex apex, margin entire, rounded base, acrodrome leaf venation base and three main veins (Fig. 2 B, D). This first eophyll dried out when the plant had five leaves and thus, the eophyll was not visible in one-year old plants (Fig. 2 E, 3).

The completely expanded leaves in one-year old plants had distinct characteristics among the study species. The largest leaf of *S. brasiliensis* was 8x7cm, ovate-shaped, with a coriaceous consistency and three main veins. The midrib had prickle-like structures on the abaxial face (Fig. 2 F, G). Leaves of *S. campestris* had 4.5x2cm, oblongate-elliptic shape, coriaceous consistency and only one midrib, also with prickle-like structures on the abaxial face. However, prickle-like structures were also observed on the leaf margins (Fig. 2 H, I).

S. cissoides showed ovate-elliptical shaped leaves of 7x4cm, with a membranaceous consistency and the presence of three main veins with

TABLE 3
Seed viability of *Smilax brasiliensis*, *S. campestris*, *S. cissoides* and *S. polyantha*

Species	Light						Dark					
	30°C		20-30°C		30°C		20-30°C		30°C		20-30°C	
	V	S/E	FU	V	S/E	FU	V	S/E	FU	V	S/E	FU
<i>S. brasiliensis</i>	23	29	40	30	0	54	44	26	27	34	11	0
<i>S. campestris</i>	10	1	21	8	0	10	4	4	21	3	0	21
<i>S. cissoides</i>	6	0	19	2	0	20	10	0	19	7	0	27
<i>S. polyantha</i>	33	1	42	34	6	36	23	0	58	27	4	48

(V) Number of viable seed, (S/E), number of seeds without embryo or with uncoloured embryo, (FU) number of seeds altered by fungi.

prickle-like structures on the abaxial face (Fig. 2 J, K). Finally, *S. polyantha* had coriaceous leaves, 7x4.5cm, with ovate-elliptical shape, three main veins and the absence of prickle-like structures. On the margin of the leaves, the vein was translucent, as well as the secondary veins. This characteristic was not observed by the other study species (Fig. 2 L, M).

The aerial caulinar ramification began after the formation of the five first leaves (five months after germination).

The one-year old individuals of *S. polyantha* and *S. cissooides* showed ca. five aerial stems, whilst *S. campestris* and *S. brasiliensis* had ca. seven stems (Fig. 3). The length of the stems by *S. cissooides* and *S. campestris* could be up to 1m, whilst stems of *S. polyantha* and *S. brasiliensis* were not larger than 0.5m (0.3 and 0.4, respectively). The average width of stems did not vary among study species (1-2mm, Fig. 3). All study species showed prickle-like structures at the

internode. However, they were more abundant by *S. campestris* and *S. cissooides*.

Each aerial branch was originated from an axillary bud present on the base of the early aerial stem. Therefore, the development of the underground system began from the fifth eophyll, which can be verified by the presence of reduced knotty from the early development phases of the underground systems on one-year old plants (Fig. 3).

The primary roots of *S. cissooides* and *S. brasiliensis* could not be differentiated from the adventitious roots in the early stages of seedling development (Fig. 2 C). The primary root was differentiated in *S. polyantha* until the formation of the first eophyll. In *S. campestris*, the primary root remained differentiated until the formation of the fifth eophyll. After one year, all study species showed well-developed adventitious radicular system, composed by white and brownish roots (Fig. 3).

**Identification key for one-year old plants of *Smilax brasiliensis*,
S. campestris, *S. cissooides* and *S. polyantha***

- 1. One main vein 2
- 1. Three main veins 3
- 2. Leaf oblong-shaped and prickle-like structures on the main vein and on the leaf margin *S. campestris*
- 3. Leaf ovate-shaped 4
- 3. Leaf elliptical-shaped 5
- 4. Prickle-like structures only on the main central vein *S. brasiliensis*
- 5. Prickle-like structures on the three main veins *S. cissooides*
- 5. Prickle-like structures absent and leaf translucent margin *S. polyantha*

DISCUSSION

The germination dawn, as well as the percentage of germination are factors that can be related to the time needed by the seeds for embryo maturation, absence and presence of light and to scarification (Andreato & Pereira 1990). According to Rosa & Ferreira (1999), the highest germination rates and more rapid germination were verified in dark conditions.

Pogge & Bearce (1989) indicated that the increase in light intensity as a factor can both enhance germination rates and reduce the time needed for germination to begin.

Light was an important factor affecting the germination of only one of the study species, *S. brasiliensis*, which usually occurs in open areas. On the other hand, the remaining study species, that occurs on forest edges, were not affected by this factor. Pogge & Bearce (1989)



Fig. 3. A.-C. *Smilax brasiliensis*. D.-F. *Smilax campestris*. G.-I. *Smilax cissooides*. J.-L. *Smilax polyantha*. A., D., G., J. One-year old plant. B.-C., E.-F., H.-I., K.-L. Detail of the new stems development from underground axillary buds. Scale: side of the square is 0.5cm.

tested the different light conditions for the germination of two *Smilax* species. They observed that the species that occurred in open areas (*S. glauca*) needed more light to germinate than *S. rotundifolia*, which could be found in shaded areas. According to the authors, light seemed to be a limiting factor for the germination of *S. glauca*.

The high germination rates of treatments with temperatures 20-30° and 30°C for *S. campestris* and *S. cissoides*, reinforce the idea that the low germination rates found for *Smilax polyantha* were not only related to temperature variation, substrate or light, but to an intrinsic factor of its metabolism and it thus can be an adaptation strategy of this species to the environment.

The germination rates of *Smilax campestris*, *S. cissoides* and *S. brasiliensis* found in this study are close to the ones obtained by Rosa & Ferreira (1999). Their results showed that 71% of total seeds of *Smilax campestris* germinated in the presence of light and temperatures of 30°C, whilst 51% germinated using temperatures of 25-35°C. Pogge & Bearce (1989) found similar results for germination under constant temperatures (22°C) for *Smilax rotundifolia* (95%) and *S. glauca* (82%).

Rosa & Ferreira (1999) showed that germination began 30 days after experiments, in the presence of light, and after 15 days in dark conditions. Seeds of *S. rotundifolia* germinated after two months, whilst seeds of *S. glauca* began to germinate only after ten months (Pogge & Bearce 1989). The process of germination can vary between 39 and 93 days for seeds of *Smilax rufescens*, *S. elastica*, *S. syphyllitica*, *S. quinquenervia* and *S. syringoides* (Andreata 1980). Our study showed the begin of germination after 22 days for *S. campestris* and 29 days for *S. cissoides*, confirming thus, the highest Speed Germination Indices for both species. Nevertheless, *S. brasiliensis* and *S. polyantha* showed the lowest values of Speed Germination Indices, beginning to germinate only after 36 days.

We conducted preliminary tests to break the dormancy of seeds of *Smilax polyantha*

collected in 2004, as described by several authors (Andreata 1980, Pogge & Bearce 1989, D'Antuono & Lovato 2003, Santos *et al.* 2003, Shao *et al.* 2006). We used chemical scarification (sulfuric acid) and mechanical (sandpaper), heat shock and gibberillic acid (GA₃), following the methodology described by Santos *et al.* (2003). However, no seeds germinated after the treatments described above. According to Shao *et al.* (2006) the main cause of dormancy of *Smilax riparia* was post-maturation of seed embryo. Additionally, we observed that seeds put to germinate using filter paper were more contaminated by fungi than the ones in vermiculite. These preliminary tests contributed to the further performance of experiments, because we did not scarified seeds before experiments and was used sand as substrate.

Seeds of study species showed hypogeal germination, the same one found in other species of Smilacaceae (Tillich 2007), and cryptocotylar, with the formation of hyaline cataphylls, as described by Duke (1965).

According to Andreata & Pereira (1990), the main root is difficult to be identified since the beginning of the development for *Smilax elastica*, *S. quinquenervia*, *S. rufescens*, *S. syphyllitica* and *S. syringoides* because the adventitious roots can be confused with the primary ones. The presence of the membranaceous cotyledonar sheath and differentiated leaf traits among the species observed in the present study were also verified by these authors. However, they stated that the first eophyll can differentiate the seedlings and this fact was not observed in our study, since the eophyll of the study species did not show significant differences.

Guaglianone & Gattuso (1991) and Andreata (1997) discuss that the detailed characterization of the leaves showed great taxonomic value for distinguishing species of the genus *Smilax*. Martins *et al.* (2010) observing the anatomy of the vegetative underground organs of six species of *Smilax* L. concluded that the morphology of this organs do not allow to distinguish among the studied species. The authors suggested that species still need to be

identified by leaf morphology as demonstrated in the present study.

Martins *et al.* (2011) showed that the horizontal growth and subsequent thickening of the underground organ of *S. polyantha* depend on the axillary buds development from the basal nodes of previous branches. According to them all adventitious roots originate from the underground stem called rhizophore. The same development of underground stem and root formation were observed in the present study.

Holm (1890) observed that the number of ramifications of *Smilax rotundifolia* and *S. glauca* could determine the age of plants, since the formation of a new stem could be observed every year. We did not find this relation for the study species, since after one year of development, individuals of *S. polyantha* and *S. cisoides* showed five aerial stems, whilst plants of *S. campestris* and *S. brasiliensis* had ca. seven stems.

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