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Studies on the Seeds of *Smilax goyazana* A.DC. (*Smilacaceae*)

By

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With 5 Figures

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Summary

PALHARES D., TINÉ M. A., VINHA D., SILVEIRA C. E. DOS S. & ZAIDAN L. B. P. 2009. Studies on the seeds of *Smilax goyazana* A.DC. (Smilacaceae). – Phyton (Horn, Austria) 49 (1): 117–130, with 5 figures.

Seed *Smilacaceae* – Although the genus Smilax presents a worldwide occurrence and occupies with a reasonable phytosociological dominance in a great variety of ecosystems, the seeds are very exigent: germination occurs only after many weeks to months under imbibition in a narrow range of optimal temperatures. In seeds of *Smilax goyazana*, the combined effect in the seed germination of four regimens of temperature, presence of photoperiod or constant darkness and previous exposition or not to gibberellin was verified. Also, the content of water, proteins, lipids and carbohydrates were measured. An anatomical study using standard technique was carried out. The embryo is dispersed at an immature stage with a torpedo format. The endosperm is made up of cells with thickened walls and with lipidic drops in the vacuoles. The seeds are aphotoblastic and indifferent to an exposition to gibberellin. The germination occurred only under constant temperatures between 25 °C and 30 °C. The germinability was inferior to 40% and the temperature of 25 °C was more fa-

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vourable to germination than the one of 30 °C. The seeds present low water content and are oleaginous. The polysaccharides are the second most abundant reservoir.

Zusammenfassung

PALHARES D., TINÉ M.A., VINHA D., SILVEIRA C.E. DOS S. & ZAIDAN L.B.P. 2009. Studies on the seeds of *Smilax goyazana* A.DC. (*Smilacaceae*). [Untersuchungen an Samen von *Smilax goyazana* A.DC. (*Smilacaceae*)]. – Phyton (Horn, Austria) 49 (1): 117–130, mit 5 Abbildungen.

Obwohl die Gattung *Smilax* ein Kosmopolit und in vielen unterschiedlichen Ökosystemen pflanzensoziologisch dominant ist, sind die Samen dieser Gattung relativ anspruchsvoll: Sie keimen nur nach einigen Monaten Imbibition und das innerhalb eines schmalen optimalen Temperaturbereichs. In dieser Arbeit werden die kombinierten Effekte von vier unterschiedlichen Ansätzen – unterschiedliche Temperaturen, Photoperiode oder Dauerdunkel, mit oder ohne Gibberellinapplikation – untersucht. Der Wasser-, Protein-, Lipid- und Carbohydratgehalt wurde bestimmt. Anatomische Untersuchungen zeigten, dass sich der Embryo zum unreifen Torpedo-Stadium entwickelt. Das Endosperm wird von Zellen mit verdickten Zellwänden mit Lipidtropfen in den Vakuolen gebildet. Die Samen reagierten weder auf unterschiedliche Beleuchtung, noch auf Gibberellinbehandlung. Sie keimten nur bei konstanten Temperaturen zwischen 25 °C und 30 °C. Die Keimfähigkeit war unter 40% und die Temperatur von 25 °C war wesentlich besser für die Keimung als 30 °C. Die Samen hatten einen geringen Wassergehalt und sind ölhaltig. Polysaccharide sind der zweitwichtigste Reservestoff.

Introduction

The plants of the genus Smilax are dioicous monocotyledons that occur in tropical and subtropical areas of all the continents (ANDREATA 1980), thus occupying a wide variety of ecosystems with a reasonable phytosociological dominance (MATALLANA & al. 2005). They accumulate steroidal saponins in the rhizome, which are substances of pharmacological interest, used in the production of medications and also as reagents in blood diagnostic tests (NAOUM 1990, BERNARDO & al. 1996, VERAS & al. 2005).

Paradoxically, in spite of the phytosociological dominance, the seeds of many species of *Smilax* are exigent: germination takes several weeks to months to occur, the optimal temperatures occur in narrow ranges and there is a quick loss of viability if the seeds are imbibed in suboptimal temperatures or exposed to formic acid, sulphuric acid or thiourea (POGGE & BEARCE 1989, ROSA & FERREIRA 1999, ANDREATA 1980, ANDREATA & PEREIRA 1990, ANTUONO & LOVATO 2003). However, contrasting to the other species, SANTOS & al. 2003 report, to *S. japecanga*, a germinability higher than 80% with imbibition in gibberellin solution or scarification with sulphuric acid before cultivation under constant 35 °C.

Notwithstanding, vegetative reproduction in plants of the genus *Smilax* is highly improbable to occur. POGGE & al. 1974 tried to obtain roots from cuttings of two common species in North America, *S. rotundifolia* and

S. glauca, but achieved a success rate of less than 30%. Moreover, in *S. goyazana*, the production of new plants from rhizome cuttings was not possible (PALHARES & SILVEIRA 2005, PALHARES & ZAIDAN, submitted). Micropropagation in vitro has been successful in only one species, *S. oldhami* from Japan (TAZAWA & SASAHARA 2003).

In the Cerrado of Central Brazil, a savannic vegetation, *S. goyazana* presents a marked phytosociological dominance in the herbaceous layer and is practically the only species of the genus *Smilax* that occurs in this environment (MUNHOZ & FELFILI 2005, 2006, 2007), since other species from the genus have only been cited occasionally in some floristic studies (ANDREATA 1997). Like the other species from the genus, *S. goyazana* accumulates saponins in the rhizome, but not in the leaves (PALHARES & al. 2009).

The conditions for the germination of seeds of *S. goyazana* are not known. Therefore, the objective of this study is to verify the conditions to obtain germination of the seeds, to characterize the main reservoir substances and to relate the anatomical structure to the physiological behaviour observed.

Material and Methods

Mature fruits of *Smilax goyazana* A.DC. were collected from plants growing in the Cerrado area of the Olimpic Center of University of Brasília, a place of 160 ha located between the co-ordinates of 15°46'S – 47°50'W e 15°45'S – 47°51'W, providing a representative flora of cerrado *strictu sensu*, according to the phytosociological study of ASSUNÇÃO & FELFILI 2004. Voucher specimens were deposited at the herbarium of the University of Brasília (UB) under the registration numbers of UB 15269 and UB 15270. The fruits were air dried at room temperature for 15 days. Following this, the seeds were removed, cleaned in absorbent paper towels, dried at room temperature overnight and stored for 2 to 12 months in dark and dry glasses at 8 °C before the studies.

Anatomy of the Seeds

Seeds were imbibed overnight in distilled water, transversely sectioned in the medium portion, fixed in FAA_{50} for 48 hours, dehydrated in ethanolic series and included in methacrylate resin (KRAUS & ARDUIN 1997). Sections up to 10 μm of thickness in transversal and longitudinal plans were made in a rotatory microtome. After adherence to slides, they were stained with methylene blue or neutral red, rinsed in tap water, dried at room temperature and mounted with acrylic resin Acrilex® (FANK-DE-CARVALHO & GRACIANO-RIBEIRO 2005, PAIVA & al. 2006).

Histochemical tests were then done in hand-free sections of imbibed seeds for the detection of starch (with Lugol's iodine), phenolic compounds (with ferric chloride) and lipids (with Sudan IV). To confirm the observation of lipids, a negative control was done, as follows: sections of seeds were immersed in ethanol-chloroform solution (1:1) for 10 minutes under smooth shaking (1 part of seeds to 5 parts of solvent). After filtration, the sectioned material was dried in an oven at 40 °C for about 10 minutes and then submitted to reaction with Sudan IV (JOHANSEN 1940, KRAUS & ARDUIN 1997).

Seed Viability

Tetrazolium solution at 0.5% was prepared and stored in dark flasks under 8 °C according DELOUCHE & al. 1962. For estimating seed viability, 30 seeds were put to imbibe in distilled water at room temperature for 48 hours. The seeds were hand-free sectioned close to the basis, showing the embryo and the endosperm. The sections were immersed in the tetrazolium solution and kept in darkness at room temperature for 6 hours. The seeds that presented with a red stained embryo and endosperm were considered as viable (Fig. 1).

Effect of Gibberellin, Light and Temperature on Germination

The experiment was a factorial of $4 \times 2 \times 2$ (thus, totaling 16 groups), each group being made up of three repetitions of 20 seeds, for cultivation in BOD chambers. The combined effect on the total germination of the seeds of four temperature regimens (constant 20, 25, 30 °C and alternating 20–30 °C), absence or presence of photoperiod (darkness or 12/12 hour photoperiod of white light at intensity of 100 µmol quanta.m^{-2}.s^{-1}) and exposure or not to gibberellin (gibberellic acid, GA_3) was tested. The seeds were sowed in Petri dishes covered by filter paper and kept humid with distilled water. The darkness treatments were obtained by wrapping the Petri dishes with aluminum paper.

For exposition to gibberellin, gibberellic acid (GA_3) 1.6 mM was prepared with dissolution in distilled water under vigorous shaking. The pH was adjusted to 6.8 to 7.0 with NaOH 0.01 N. The seeds were imbibed in the solution for 48 hours at room temperature before being sowed.

Each four days, the germination of the seeds, defined as the protrusion of the radicle, was observed. The darkness groups were observed in dark chambers illuminated by green light (LABOURIAU & COSTA 1976).

The seeds of the 25 °C group were observed until 60 days of cultivation. The seeds of the other groups that had not germinated after 45 days were put in BOD chambers under a constant 25 °C with a 12/12 hour photoperiod and the germinability was counted after another 60 days of cultivation.

Additionally, a 2×2 factorial experiment for cultivation in a glasshouse was drawn. Each one of the four groups contained three repetitions of 20 seeds. The combined effect of exposure or not to natural light and exposure or not to gibberellic acid was tested. The temperature varied, during the experimental period, from 19 to 28 °C. The darkness treatments and the exposure to gibberellin were carried out as described above. The seeds that had not germinated after 45 days were put in BOD chambers under a constant 25 °C with a 12/12 hour photoperiod and the germinability was counted after 60 days.

Statistical treatment was carried out after the transformation of the percentages of germination into arcsin (in degrees). The transformed data was submitted to the normality test of Shapiro-Wilk and then to ANOVA, being compared by Tukey's test at 0.05 (MOTULSKY 1995, SANTANA & RANAL 2004).

Scarification of Seeds

Experimental groups of one repetition of 20 seeds each was weighed and either mechanically (a small opening in the middle portion of the seed made with a needle) or chemically (for 30 seconds, 1 minute or 3 minutes of immersion in sulphuric acid

98%) scarified. The control group was made up of 20 intact seeds. The seeds were then vigorously washed in tap water for some minutes, imbibed in distilled water for 48 hours, slightly dried in paper towel and again weighed for verification of the gain of mass of water absorption. Then, the seeds were divided into two groups for verification of the tegument in the germination: one group was put in BOD chambers under a constant 25 °C with 12/12 hour photoperiod and the other in a glasshouse, whose temperature varied from 9 to 28 °C, under natural variation of light. Germination was observed each four days for a 45-day period.

Seed Viability after Exposure to high Temperatures

Exposure to high temperatures was done by immersion in boiling water for one minute or exposure to a dry 80 °C temperature for one or three minutes according to the experimental design of PALHARES 2004. Each group, consisting of 30 seeds, was evaluated by the tetrazolium test. Statistical analysis was done as described above.

Germination in Cerrado Soil

Three repetitions of 20 seeds each were put in 200 mL plastic cups containing sifted cerrado soil and kept moist in a glasshouse. After 45 days, the seeds were removed for counting germination.

Small nylon bags with 20 seeds each (two repetitions for each depth) were buried during the rainy season in the cerrado area of the Olimpic Center of University of Brasilia at depths of 5, 10, 20 and 50 cm. After 45 days, the seeds were removed for counting germination.

Water Content

The water content of 50 seeds was expressed in percentages of fresh mass and calculated through the difference of mass of seeds before and after drying in oven at 105 °C according to TARRÉ & al. 2007.

Protein Content

Three repetitions of 100 mg of triturated fresh seeds were put in 2 mL of phosphate buffer pH 7.0 at 1% (m/v) at room temperature under vigorous shaking for two hours. The material was centrifuged for one minute and the supernatant was added to Bradford's reagent (BRADFORD 1976). Absorbance was read at 595 nm. Protein content was calculated according to a calibration curve for a solution of bovine seroalbumin.

Lipid Content

Three repetitions of 1.0 g of triturated fresh seeds were put in a bag of filter paper in a Soxhlet's extractor for 5 hours using hexane:ethanol 2:1 (v/v) as eluent. After extraction of the lipids, the excess of eluent was removed in a dry oven under 40 °C for two hours. Lipid content was considered as the difference of mass measured before and after the extraction (SILVA 1981).

Extraction and Carbohydrate Analysis

Three repetitions of triturated fresh seeds were put in distilled water at 80 °C in the proportion of 1% (m/v) under vigorous shaking for 5 hours. For the precipitation

of the polysaccharides, ethanol 80 °GL was added in the proportion of 3 parts of ethanol to each part of extract. The material was decanted under 4 °C overnight and then centrifuged at 20 g for 20 minutes.

The supernatant, containing the soluble sugars was dried at 40 °C and rediluted in distilled water. The soluble sugars were quantified by the phenol-sulphuric method in spectrophotometer at 480 nm, having glucose as a pattern (DUBOIS & al. 1956).

After drying under 40 °C, the sediment was re-suspended in distilled water, frozen and lyophilized.

The polysaccharides were analyzed regarding the presence of arabinose, fructose, fucose, galactose, glucose, manose, saccharose, xylose and polyalcohols. According to CARVALHO & DIETRICH 1993 as follows: the lyophilized sediment was subjected to acid hydrolysis with sulphuric acid: 100 µL of sulphuric acid 72% (p/p) were added to the sediment under 30 °C for 45 minutes. Following this, 1.7 mL of distilled water was added and the material was autoclaved for 1 hour. After acid hydrolysis, the final volume was completed to 5.0 mL with distilled water and the pH corrected to 7.0 with sodium hydroxide. The extract was filtered in anionic and cationic Dowex resin and then analyzed regarding the released sugars.

The analysis of sugars was carried out by HPLC in a system SX-300, using a Carbo-Pac PA1 column eluted with 0.25 mL.min-1 of isocratic NaOH 16mM. The identification and measurement of sugars was made by comparison with the time of retention obtained for known commercially available patterns of arabinose, fructose, fucose, galactose, glucose, manose, saccharose, xylose and myoinositol.

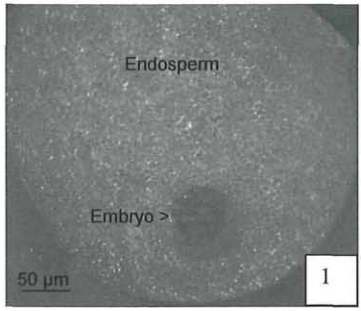
Results

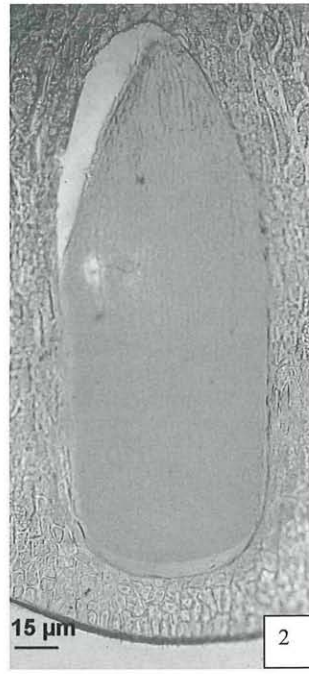
Anatomical Description

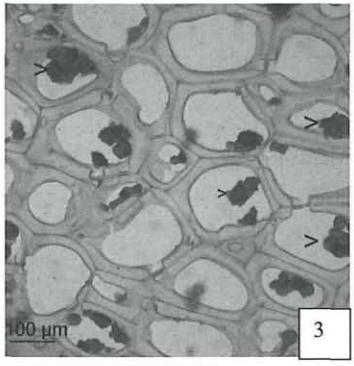
The seed is round-shaped (when only one seed is formed inside the fruit) or plan-convex (when two or three seeds are formed inside the fruit), with a diameter (or length) of about 50 mm. The embryo is dispersed in an immature stage, in the format of a torpedo (Fig. 2). It is placed in the opposite pole of the hilum, at about 50 µm distance from the basis and with about 120 µm of length and 45 µm of width being taken by a massive endosperm. The endosperm is made up by cells of thickened walls containing lipidic drops in the vacuole (Fig. 3). The seed coat is thin, presenting 3 to 5 µm of thickness and is composed by a reduced testa and 4 to 5 external layers of the endosperm, which present deposition of phenolic compounds (Fig. 4). Starch or amyloid substances were not seen.

Effects of Temperature, Light and Gibberellin in Germination

The stored seeds presented, by the tetrazolium test, a viability of 65%. The germination occurred only at constant 25 °C and 30 °C, but reached less than half of the value estimated by the tetrazolium test (Table 1). The temperature of 25 °C was more favourable than that of 30 °C. The exposure to light or darkness did not interfere with germination, characterizing an aphotoblastic response. In a same way, exposure or not to gibberellin did







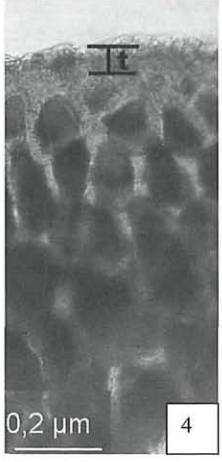
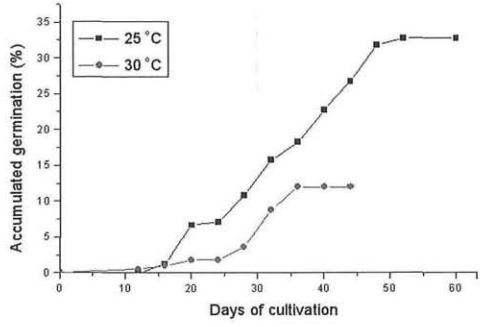


Fig. 5



Figs. 1–4. Seeds of *Smilax goyazana* A.DC. Fig. 1. Transversal section of viable seed as seen after the tetrazolium test: the embryo and the endosperm stained red. Fig. 2. Longitudinal section showing the immature embryo in a torpedo format. Fig. 3. Detail of the endosperm, after reaction with Sudan IV, showing the thickened cell walls. Arrow-heads point lipidic drops inside the vacuoles. Fig. 4. Reaction with ferric chloride showing the reduced testa (t) and an abundance of phenolic compounds in the seed coat. Fig. 5. Cumulative germination of seeds of *Smilax goyazana* under the temperatures of a constant 25 °C (n = 240) and 30 °C (n = 240).

not alter the germinability of the groups. Germination started after 12 days under temperature of 30 °C and after 16 days under that of 25 °C. After 44 days no more germination occurred (Fig. 5). The seeds of the groups of constant 20 °C and 30 °C that had not germinated after 45 days and were transferred to constant 25 °C presented a residual germinability: 3% in the ones coming from 20 °C and 8% in the ones from 30 °C. The seeds of the groups of alternate temperature and glasshouse did not germinate when transferred to a constant 25 °C.

Table 1. Germinability of seeds of *Smilax goyazana* under the constant temperatures of 20, 25 and 30 °C and alternate 20/30 °C, under light (12/12 hour photoperiod) or darkness, imbibed or not in gibberellic acid 1.6 mM pH 7.0.

	Germinability (%)			
	Photoperiod		Darkness	
Temperature	Control	Gibberellin	Control	Gibberellin
20 °C	0a	0a	0a	0a
25 °C	30b	32b	33b	36b
30 °C	12c	13c	10c	10c
20–30 °C	0a	0a	0a	0a

Effect of Scarification

All the seeds scarified with sulphuric acid were infested by fungus within 5 days and died. The mechanically scarified seeds presented a blackish colour surrounding the lesion, suggesting phenolic oxidation. The germinability was 8% when kept under a constant 25 °C. No scarified seeds germinated in the glasshouse. The seeds scarified with sulphuric acid tended to absorb more water than the control: after 48 hours of imbibition, the control seeds enhanced 19.4% in mass, while the seeds scarified with 3 minutes of immersion in sulphuric acid gained 31.4% in mass. The fresh mass of 1000 seeds was about 70 g.

Exposure to high Temperatures

All the seeds immersed in boiling water died. The viability reduced as the time of exposure to high dry temperatures increased (Table 2).

Composition of the Seed

Table 3 resumes the composition of the seeds. The seeds present low water content, low proteic content and are oleaginous. Polysaccharides are the second most abundant reservoir compounds. Among the soluble sugars, sucrose is the most abundant. For the proportion of sugars released, it is probable that the polysaccharides are a mix of arabinoxylans and galactans.

Table 2. Viability of seeds of *Smilax goyazana* after exposure to a dry 80 °C or boiling water, as estimated by the tetrazolium test.

	Viability (%)
Control	65a
1 minute – dry	45ab
3 minutes – dry	20b
Boiling water	0c

Table 3. Biochemical composition ($mg.g^{-1}$ of fresh mass) of seeds of *Smilax goyazana*.

Proteins	60.0
Lipids	250.0
Soluble carbohydrates	60.0
Sucrose	49.0
Polyol	4.8
Glucose	3.5
Fructose	2.6
Polysaccharydes	140.0
Xylose	52.0
Glucose	46.0
Arabinose	21.0
Galactose	21.0

Discussion

The anatomical data reflect the germination behavior observed. The long time required for the commencement of the germination is probably related to the immaturity of the dispersed embryo. In this case, a period of post-maturation is likely to occur. So, considering that the germination was less than half of what was predicted by the tetrazolium test, it is possible that adjustments in the protocol are necessary, but it can also indicate that the long period of post-maturation can somehow affect the viability of the seeds. Anyway, small immature embryos are also observed in other species from the genus *Smilax* (Guaglianone & Gattuso 1991, Andreata & Menezes 1999).

The thick wall cells and the lipidic drops of the endosperm are, respectively, the anatomical feature of the abundance of polysaccharides and of the oleaginous character of the seeds. The fact of not having reacted with Lugol's iodine also indicates that there are no xyloglucans (Buckeridge & al. 2000), as confirmed by other assays (data not shown).

In some seeds, the testa is a very well developed tissue (Rao 1979/80, 1986), but in *S. goyazana* it is reduced. In savannahs it is common plants with seeds with dormancy related to the coat (Morris & al. 2000). In Cer-

rado, plants with impermeable coat are common and in such cases, it is necessary to remove the coats for the seeds to germinate (Rede De Sementes Do Cerrado 2003). In the seeds of *Eugenia dysenterica*, a fructuous tree of the Cerrado, the seed coat is permeable to water, however, it presents inhibitory phenolic compounds and a small lesion in the seed coat is necessary for the oxidation of the phenolic compounds and the subsequent germination of the seeds (Rizzini 1970). Again, certain Cerrado species, such as *Melilotus alba* and *Ageratum conyzoides* present allelopathic phenolic compounds, that can eventually inhibit the germination of their own seeds (Zaidan & al. 1985, Ladeira & al. 1987).

Although the seeds with the coats removed by sulphuric acid had absorbed more water, the intact seed coat is a protection for the seed. Probably, the phenolic compounds of the coat are microbicidal, as when it is removed there is a quick deterioration of the seeds. The weakness of the coat may also explain why the seeds of the Mediterranean species *Smilax aspera*, although swallowed by many species of birds, survive in only a few of the species, thus making them the effective dispersers (Herrera 1981, Izhaki & Safridl 1990, Barnea & al. 1994).

The fruits of *Smilax goyazana* are edible and it is probable that the dispersers are birds, lizards and ants, since the fruits remain mature attached to the plant, which is typical for plants presenting such animals as dispersers (Herrera 1981). Myrmechory is observed in *S. aspera*, whose seeds are intensely collected by ants, insects with a predilection to oleaginous seeds (Aronne & Wilcock 1994). Also, there are reports of seeds of *S. brasiliensis* germinating in anthills (Andreata 1997), indicating that myrmechory is a common feature for the genus.

The soil is generally a very good thermal insulator (Gavande 1972). Under just a few centimeters of soil, the temperature is not altered when the vegetation is burned (Coutinho 1978). Also, at about 20 cm depth, the temperature is constant throughout all day long (Gavande 1972). So, the myrmechory of the seeds together with laboratory findings led us to expect that the seeds would germinate under the soil (Klein & al. 1996, Sassaki & al. 1999a, b, c, d, Rondon 2001): the ants would carry the seeds underground where they would germinate. However, it was not observed. So, the common presence of *S. goyazana* in the field together with the difficulty of observing germination under natural conditions can lead to the conclusion that a more complex ecological interaction is required for the germination of the seeds in the environment (Kahn & Ungar 1996). Not only *S. goyazana* but also many common species of the Cerrado present seeds with difficult germination, indicating that in this ecosystem, the generation of seedlings is the result of a complex ecological interaction (Ruggiero & Zaidan 1997, Cesarino & Zaidan 1998, Cesarino & al. 1998, Sassaki & al. 1999d, Rede De Sementes Do Cerrado 2003).

In the Cerrado, seeds that break the dormancy after exposure to high temperatures are very rare. In the opposite direction, in the Mediterranean vegetation and in the Australian savannahs, seeds that require previous exposure to high temperatures to germinate are very frequent (BORGHETTI 2000). The most common, in the Cerrado, is the presence of seeds that are just resistant to high temperatures. The cell wall composition confers some thermal insulation (PALHARES 2004), so that the thermo-resistant seeds represent an evolutive amelioration in this respect, with a biological limit around 120 °C (HANLEY & al. 2001). Anyway, the seeds of *S. goyazana* do not resist too long to high temperatures.

Just a few other species from the genus *Smilax* have been studied regarding the germination requirements. In five Brazilian species of *Smilax*, the germinability was also higher under a constant 25 °C and the germination takes 40 to 100 days to start. Alternate temperatures and scarification with sulphuric acid reduce seed viability. The seeds tend to be aphotoblastic, with the exception of *S. rufescens* that presents a partial positive photoblastism (ANDREATA 1980, ANDREATA & PEREIRA 1990).

The seeds of *S. campestris*, a common species in Argentina, present a partial negative photoblastism and germinability higher than 80%, under temperatures varying from 25 to 35 °C (ROSA & FERREIRA 1999). *S. glauca* and *S. rotundifolia*, two common species of North America, are negative photoblastic and present germinabilty higher than 80% when kept under room temperature. Germination takes 30 to 180 days to occur and the seeds do not tolerate exposure to sulphuric acid, thiourea, formic acid nor imbibition under suboptimal temperatures (POGGE & BEARCE 1989). *S. aspera* presents a post-maturation period of more than one year under laboratory conditions and the germinability is less than 50%. Gibberellin did not alter the germinability, but the seeds that were ingested by dispersing birds showed a higher germinability (IZHAKI & SAFRIDL 1990, ANTUONO & LOVATO 2003).

In contrast to all these species, SANTOS & al. 2003 described, for *S. japecanga*, a higher germinability after mechanical or chemical scarification and/or after imbibition in gibberellic acid. It is possible that the authors in fact studied another plant, since they do not mention the deposition of voucher specimens and *S. japecanga* is not considered a valid species in the taxonomic review of ANDREATA 1997.

Regarding the biochemical composition, in the Cerrado, there seem to be two great groups of seeds: one that accumulates proteins and lipids and the other that accumulates carbohydrates: among the studied species, protein content varied from 4.4 to 152 $mg.g^{-1}$, the lipidic content, from 10 to 400 $mg.g^{-1}$, and the polysaccharid content reached up to 850 $mg.g^{-1}$ (CARAMORI & al. 2004, MAYWORM & al. 1998, SILVA & al. 1998). Among the polysaccharides, galactomannans are very common, while xyloglucans appear only in some species (PANEGASSI & al. 2000, MATUDA & MARIA-NETTO 2005). By the proportion of sugars that compose the polysaccharides

of the seeds of *S. goyazana*, it is possible that the xylose is linked to arabinose, forming an arabinoxylan. In such way, the species is in an intermediary position, predominantly accumulating lipids and polysaccharides, and with a lower content of proteins and soluble sugars.

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References

ANDREATA R. H. P. 1997. Revisão das espécies brasileiras do gênero *Smilax* Linnaeus (*Smilacaceae*). – Pesquisas Botânica 47: 7–244.

ANDREATA R. H. P. 1980. *Smilax* LINNAEUS (*Smilacaceae*): ensaio para uma revisão das espécies brasileiras. – Arquivos do Jardim Botânico do Rio de Janeiro 24: 179–301.

ANDREATA R. H. P. & PEREIRA T. S. 1990. Morfologia das plântulas de algumas espécies de *Smilax* L. – Pesquisas-Botânica 41: 7–24.

ANDREATA R. H. P. & MENEZES N. L. 1999. Morfoanatomia do embrião, desenvolvimento pós-seminal e origem do rizóforo de *Smilax quinquenervia* VELL. (*Smilacaceae*). – Boletim de Botânica da Universidade de São Paulo 18: 39–51.

ANTUONO L. F. & LOVATO A. 2003. Germination trials and domestication potential of three native species with edible sprouts: *Ruscus aculeatus* L., *Taunus communis* L., *Smilax aspera* L. – ISHS Acta Horticulturae 598: 211–218.

ARONNE G. & WILCOCK C. C. 1994. First evidence of myrmecochory in fleshy-fruited shrubs of the Mediterranean region. – New Phytologist 127: 781–788.

ASSUNÇÃO S. L. & FELFILI J. M. 2004. Phytosociology of a cerrado *strictu sensu* fragment at the Paranoá Environmental Protect Área, DF,Brazil. – Acta Botanica Brasilica 18: 903–909.

BARNEA A., YOM-TOV Y. & FRIEDMAN J. 1994. Does ingestion by birds affect seed germination? – Functional Ecology 5: 394–402.

BERNARDO R., PINTO A. & PARENTE J. 1996. Steroidal saponins from *Smilax officinalis*. – Phytochemistry 43: 465–469.

BORGHETTI F. 2000. Ecofisiologia da germinação das sementes. – Universa 8(1): 149–180.

BRADFORD M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. – Analytical Biochemistry 72: 248–254.

BUCKERIDGE M. S., SANTOS H. P. & TINÉ M. A. 2000. Mobilisation of storage cell wall polysachharides in seeds. – Plant Physiology and Biochemistry 38: 141–156.

CARAMORI S. S., LIMA C. S. & FERNANDES K. F. 2004. Biochemical characterization of selected plant species from Brazilian savannas. – Brazilian Archives of Biology and Technology 47: 253–259.

CARVALHO M. A. M. & DIETRICH S. M. 1993. Variation in fructan content in the underground organs of *Vernonia herbaceae* (VELL) RUSBY at different phenological phases. – New Phytologist 123: 735–740.

CESARINO F. & ZAIDAN L. B. P. 1998. *Vernonia cognata* LESS. (*Asteraceae*): armazenamento e viabilidade dos aquênios. – Hoehnea 25(1): 59–70.

CESARINO F., ARAÚJO J. E. & ZAIDAN L. B. P. 1998. Germinação de sementes e crescimento de plantas de *Diplusodon virgatus* POHL, *Lythraceae*. – Acta Botanica Brasílica 12(suppl.3): 349–356.

COUTINHO L. M. 1978. Aspectos ecológicos do fogo no cerrado. – Revista Brasileira de Botânica 1: 93–96.

DELOUCHE J. C., STILL T. W., RASPET M. & LIENHARD M. 1962. The tetrazolium test for seed viability. Technical bulletin 51. – Mississippi State University, Mississippi.

DUBOIS N., GILLES K. A., HAMILTON J. K., KEBERS P. A. & SMITH F. 1956. Colorimetric method for determination of sugars and related substances. – Analytical Chemistry 28: 350–356.

FANK-DE-CARVALHO S. M. & GRACIANO-RIBEIRO D. 2005. Architecture, anatomy and histochemistry of the leaves of *Gomphrena arborescens* L.f. (Amaranthaceae). – Acta Botanica Brasilica 19: 377–390.

GAVANDE S. A. 1972. Fisica de suelos: Principios y aplicaciones. – Centro Regionale de Ayuda Tecnológica, Cidade do México.

GUAGLIANONE E. R. & GATTUSO S. 1991. Estudios taxonomicos sobre el genero *Smilax* (*Smilacaceae*). – Boletin de la Sociedad Argentina de Botanica 27(1–2): 105–129.

HANLEY M., FENNER M. & NE'EMAN G. 2001. Pregermination heat shock and seedling growth of fire following *Fabaceae* from four Mediterranean climate regions. – Acta Oecologica 22: 315–320.

HERRERA C. M. 1981. Fruit variation and competition for dispersers in natural populations of *Smilax aspera*. – Oikos 36: 51–58.

IZHAKI I. & SAFRIDL U. N. 1990. The effect of some Mediterranean scrubland frugivores upon germination patterns. – Journal of Ecology 78: 56–65.

JOHANSEN D. 1940. Plant Microtechnique. – McGraw-Hill Book Company, New York.

KHAN M. A. & UNGAR I. A. 1996. Influence of salinity and temperature on the germination of *Haloxylon recurvum* BUNGE ex. BOISS. – Annals of Botany 78: 547–551.

KLEIN A. L., ZAIDAN L. B. P. & FELIPPE G. M. 1996. Interaction between soil and photoperiod on development of *Bidens gardneri* BAKER (*Asteraceae*), a herbaceous species from the Brazilian cerrado. – Revista Brasileira de Botânica 19: 1–5.

KRAUS J. & ARDUIN M. 1997. Manual Básico de Métodos em Morfologia Vegetal. – Seropédica, Editora Universidade Rural.

LABOURIAU L. G. & COSTA J. A. F. 1976. Objetivos e instalações básicas de um laboratório de fisiologia vegetal. – Academia Brasileira de Ciências, Rio de Janeiro.

LADEIRA A. M., ZAIDAN L. B. P. & FIGUEIREDO-RIBEIRO R. C. L. 1987. *Ageratum conyzoides* L. (*Compositae*): germinação, floração e ocorrência de derivados fenólicos em diferentes estádios de desenvolvimento. – Hoehnea 14: 53–62.

MATALLANA G., WENDT T., ARAUJO D. & SCARANO F. 2005. High abundance of dioecious plants in a tropical coastal vegetation. – American Journal of Botany 92: 1513–1519.

MATUDA T. G. & MARIA-NETTO F. 2005. Caracterização química parcial do jatobá-do-cerrado (*Hymeneae stigonocarpa* MART.). – Ciência e Tecnologia de Alimentos 25: 353–357.

MAYWORM M. A. S., NASCIMENTO A. S. & SALATINO A. 1998. Seeds of species from the caatinga: proteins, oils and fatty acids contents. – Revista Brasileira de Botânica 21: 299–303.

MORRIS E. C., TIEU A. & DIXON K. 2000. Seed coat dormancy in two species of *Grevillea* (Proteaceae). – Annals of Botany 86: 771–775.

MOTULSKY H. 1995. Intuitive Biostatistics. – Oxford University Press, Oxford.

MUNHOZ C. B. R. & FELFILI J. M. 2005. Phenology of the herbaceous layer in a campo sujo community in the Fazenda Água Limpa, Federal District, Brazil. – Acta Botanica Brasilica 19: 979–988.

MUNHOZ C. B. R. & FELFILI J. M. 2006. Phytosociology of the herb-subshrub layer in an area of campo sujo, Distrito Federal, Brazil. – Acta Botanica Brasílica 20: 671–686.

MUNHOZ C. B. R. & FELFILI J. M. 2007. Reproductive phenology of an herbaceous-subshrub layer of a savannah (campo sujo) in the Cerrado Biosphere Reserve I, Brazil. – Brazilian Journal of Biology 67: 299–307.

NAOUM PC. 1990. Eletroforese: Técnicas e Diagnóstico. – Livraria Editora Santos, São Paulo.

PAIVA J. G. A., FANK-DE-CARVALHO S. M., MAGALHÃES M. P. & GRACIANO-RIBEIRO D. 2006. Verniz vitral incolor 500: a mounting medium alternative and economically viable. – Acta Botanica Brasilica 20: 257–264.

PALHARES D. 2004. Proposta de aula prática sobre a germinação de *Vellozia flavicans* MART ex. SCHULTZ (*Velloziaceae*). – Lecta 22: 7–9.

PALHARES D. & SILVEIRA C. E. S. 2005. Anatomy of rhizome of *Smilax goyazana* A. DC. (Smilacaceae). – Brazilian Journal of Medicinal Plants 8: 52–62.

PALHARES D., SILVEIRA C. E. S., ZAIDAN L. B. P.& PEREIRA L. A. R. 2009. Leaf anatomy of *Smilax goyazana* (*Smilacaceae*). – Acta Botanica Hungarica 51(1–2): 115–127.

PANEGASSI V. R., SERRA G. E. & BUCKERIDGE M. S. 2000. Potencial tecnológico do galactomanano de sementes de faveiro (*Dimorphandra mollis*) para uso na indústria de alimentos. – Ciência e Tecnologia de Alimentos 20: 406–415.

POGGE F. L. & BEARCE B. C. 1989. Germinating common and cat greenbrier. – Tree Planters'Notes 37: 34–37.

POGGE F. L., GILL J. D. & BEARCE B. C. 1974. Rooting common and cat greenbrier. – USDA Forest Service Research Note NE-189: 1–6.

RAO P. R. M. 1979/80. Seed and fruit anatomy of *Cassytha filiformis* L. with comments on its systematic position. – Israel Journal of Botany 28: 44–50.

RAO P. R. M. 1986. Seed and fruit anatomy in *Gyrocarpus americanus* with a discussion on the affinities of Hernandiaceae. – Israel Journal of Botany 35: 133–152.

REDE DE SEMENTES DO CERRADO 2003. Germinação de Sementes e Produção de Mudas de Plantas do Cerrado. – Rede de Sementes do Cerrado, Brasília.

RIZZINI C. T. 1970. Efeito tegumentar na germinação de *Eugenia dysenterica* DC. (Myrtaceae). – Revista Brasileira de Biologia 30: 381–402.

RONDON J. N., SASSAKI R. M., ZAIDAN L. B. P. & FELIPPE G. M. 2001. Effects of moisture content and temperature during storage on germination of the achenes of *Bidens gardneri* BAKER. – Revista Brasileira de Botânica 24: 35–41.

ROSA S. G. T. & FERREIRA A. G. 1999. Germination of medicinal plant: *Smilax campestris* GRISEB. – ISHS Acta Horticulturae 502: 105–111.

RUGGIERO P. G. C. & ZAINDAN L. B. P. 1997. Estudos de desenvolvimento de *Viguiera robusta* GARDN., uma *Asteraceae* do cerrado. – Revista Brasileira de Botânica 20: 1–9.

SANTANA D. G. & RANAL M. A. 2004. Análise da germinação: um enfoque estatístico. – Editora Universidade de Brasília, Brasília.

SANTOS M. R. A., PAIVA R., GOMES G. A. C., PAIVA P. D. O. & PAIVA L. V. 2003. Estudos sobre superação de dormência em *Smilax japecanga* GRISEBACH. – Ciência e Agrotecnologia 27: 319–324.

SASSAKI R. M., RONDON J. N., ZAIDAN L. B. P. & FELIPPE G. M. 1999a. Longevity of seeds from legume tree species artificially stored in cerrado and forest soils. – Hoehnea 26: 29–45.

SASSAKI R. M., RONDON J. N., ZAIDAN L. B. P. & FELIPPE G. M. 1999b. Number of buried seeds and seedlings emergence in cerradao, cerrado and gallery forest soils at Pedregulho, Itirapina (SP), Brazil. – Revista Brasileira de Botânica 22: 147–152.

SASSAKI R. M., RONDON J. N., ZAIDAN L. B. P. & FELIPPE G. M. 1999c. Germination of seeds from herbaceous plants artificially stored in cerrado soil. – Revista Brasileira de Biologia 59: 271–279.

SASSAKI R. M., RONDON J. N., ZAIDAN L. B. P. & FELIPPE G. M. 1999d. Effect of storage of achenes of *Bidens gardneri* BAKER on light sensitivity during germination. – Revista Brasileira de Botânica 22: 75–81.

SILVA D. J. 1981. Análise de alimentos – métodos químicos e biológicos. – Imprensa Universitária, Viçosa.

SILVA T. R. G., CORTELAZZO A. R. & DIETRICH S. M. 1998. Variations in storage compounds during germination and early plantlet growth of *Dalbergia miscolobium*. – Revista Brasileira de Fisiologia Vegetal 10: 119–124.

TARRÉ E., PIRES B. B. M., GUIMARÃES A. P. M., CARNEIRO L. A., FORZZA R. C. & MANSUR E. 2007. Germinability after desiccation, storage and cryopreservation of seeds from endemic Encholirium MART. ex SCHULT. & SCHULT. f. and *Dyckia* SCHULT. & SCHULT. f. species (*Bromeliaceae*). – Acta Botanica Brasilica 21: 777–783.

TAZAWA K. & SASAHARA T. 2003. Multiple bud formation and plant regeneration in another culture of shiode (*Smilax oldhami* MIQ.). – Breeding Science 53: 183–185.

VERAS T. N., LANNES G. M., PIVA J. P., STEIN R. T., JONES M. H., SILVA V. D., GARCIA P. C. R. & PITREZ P. M. C. 2005. Plastic bronchitis in a child with thalassemia alfa. – Journal de Pediatria 81: 499–502.

ZAIDAN L. B. P., BUCKERIDGE M. S. & FIGUEIREDO-RIBEIRO R. C. L. 1985. Composição do exsudato de sementes de *Melilotus alba* DESR. e seu efeito na germinação de sementes. – Hoehnea 12: 21–30.