Floresta e Ambiente 2020; 27(4): e20170960
https://doi.org/10.1590/2179-8087.096017
ISSN 2179-8087 (online)

SHORT COMMUNICATION – Silviculture

Causes of Low Seed Quality in *Ilex paraguariensis* A. St. Hil. Samples (aquifoliaceae)

Anieli Cioato de Souza[1] 0000-0001-5989-5740
Luciana Magda de Oliveira[1] 0000-0001-7362-1041
Gabriela Fernanda Souza[1] 0000-0002-1875-9950
Sharline Schossler Schmidt[1] 0000-0003-0606-5692
Patrícia Paloma Liesch[1] 0000-0003-4880-8677

Abstract

This study sought to determine the possible causes of the low seed quality of *I. paraguariensis* A. St. Hil. Seeds from six samples collected at different sites were classified as empty, decayed, herbivorous and full. Viability was assessed by tetrazolium test in seeds filled with a visualized embryo. High amounts of empty and deteriorated seeds (54% to 93%) were verified in four of the evaluated samples, and insect attack was observed in two samples. The viability of visualized embryo-filled seeds was 83% to 100%. Low quality generally results from the presence of empty and deteriorated seeds, as well as dormancy.

Keywords: empty seeds; herbivorous; yerba mate.

1. INTRODUCTION AND OBJECTIVES

Yerba mate (*Ilex paraguariensis* A. St. Hil.) plays an important socioeconomic role in the Southern region of brazil, where it is produced. The leaves are dried to prepare a traditional tea that is consumed by millions of people in South America (Oliveira & Waquil, 2015).

Seedling production of the species is commonly performed by pyrenees (hereafter called "seeds"), which have around 5% germinability (Cuquel et al., 1994). This low germinability may be associated with physiological deformations such as absence of embryo and deterioration, as well as herbivory. Therefore, the presence of insects and empty and deteriorated seeds has been observed in *Ilex* species such as *I. latifolia* (Takagi & Togashi, 2013) and *I. aquifolium* (Arrieta & Suarez, 2004; Garcia et al., 2005). Thus, this study sought to determine the possible causes of the low quality of *I. paraguariensis* seeds.

Six seed samples were obtained from at least five matrices with a minimum distance of five meters and a maximum of five hundred meters in native areas of different locations and/or years:

- **OC-15**: collected in 2015 in Otacílio Costa, SC. The average annual temperature is 16.3 °C, with an average annual rainfall of 1,519 mm and 871 m altitude (Climate Data, 2014) (27° 31' 41.82" S; 50° 8' 17.14" W – SIRGAS 2000).
- **UR-15**: collected in 2015 and **UR-16**: in 2016 in Urupema, SC. The average annual temperature is 14.1° C, with an average annual rainfall of 1,634 mm and 1,324 m altitude (Climate Data, 2014) (27° 57' 53.3" S; 49° 50' 20.3" W – SIRGAS 2000).
- **CE-16**: collected in 2016, in Campo Erê, SC. The average annual temperature is 16.7° C, with an average annual rainfall of 2,045 mm and 903 m altitude (Climate Data, 2014) (26° 24' 35.55" S; 53° 11' 46.53" W – SIRGAS 2000).
- **IJ-16**: collected in 2016 in Ijuí, RS. The average annual temperature is 19.9 °C, with an average annual rainfall of 1,774 mm and an altitude of 307 m (Climate Data, 2014) (28° 21' 34.7" S; 53° 55' 10.35" W – SIRGAS 2000).
- **PU-16**: collected in 2016 in Porto União, SC. The average annual temperature is 17.7 °C, with an average annual rainfall of 1,667 mm and 780 m altitude (Climate Data, 2014) (26° 15' 57.33" S; 51° 4' 50.78" W – SIRGAS 2000).

[1] Universidade do Estado de Santa Catarina (Udesc), Lages, SC, Brasil

The fruits were ripe and with dark purple coloration – 2.5/1 F: 5Y (Kollmorgen, 1975) (Figure 1). The seeds were extracted in a sieve and running water, dried on paper towels and used in the experiments.

Subsequently, 200 seeds, divided into four repetitions, were immersed in water for 24 h for each batch to facilitate longitudinal cutting. The seeds were evaluated visually with the aid of a Stemi-305® stereo microscope and classified into: Empty – without endosperm and embryo; Deteriorated – with necrotic endosperm and embryo or coalescent tissues (Catapan, 1998); Filled with unseen embryo; Filled with visualized embryo; and Herbivorous – with the presence of insect or signs of herbivory (Figure 2).

For viability evaluation, seeds filled with a visualized embryo were immersed in 0.1% tetrazolium solution for 24 h at 35 °C (Catapan, 1998).

The experiments were installed in a completely randomized design. Data were tested for normality by the Shapiro-Wilk test and homogeneity by the Bartlett's test, and analysis of variance was performed. Data with heterogeneous variance were transformed into arc sine $\sqrt{x/100}$. Means were compared by the Tukey test at 5% probability. The Pearson's correlation was used to correlate environmental conditions and seed sample characterization.

The characteristics of *I. paraguariensis* seeds differed according to the sample; however, higher percentages of decayed and empty seeds were generally observed (Table 1).

The empty seeds (Figure 2a) were counted in greater number in the UR-16, UR-15, OC-16 and PU-16 samples with values between 27 and 44% (Table 1). These results may be related to pollination, distance between matrix trees, and the efficiency of natural pollinators (Ayub & Mariath, 1996 apud Carvalho, 2003), since this is a dioecious species.

Figure 1. Mature *Ilex paraguariensis* fruit with a dark purple coloration -2.5/1 F: 5Y (Kollmorgen, 1975). Scale bar: 10 mm.

For the removal of empty seeds from a sample, Zanon (1988) suggests to immerse them in water and discard the supernatants. Sousa et al. (2003) observed that controlled pollination for *I. paraguariensis* is efficient and indicated for most of fruits and full seeds.

The PU-16 sample had the highest number of deteriorated seeds (Figure 2b and Table 1), differing from the others. Although the presence of pathogens was not evaluated, Oliveira (2013) reports that seed deterioration may be fungi-related.

Regarding herbivory, micro-hymenopterans belonging to the family Torymidae were identified (Figure 2c) by isolating the insect in OC-15 and UR-15 samples. These microwaps feed on the seed, and then exit through a hole that could be visually identified in the seeds (Figure 3).

In *I. paraguariensis* seeds from vintage 1982, provenances São Mateus do Sul-PR, Catanduvas-SC and Centenario-RS, Zanon (1988) verified the presence of unidentified micro hymenopteran, which caused approximately 50% of damaged seeds.

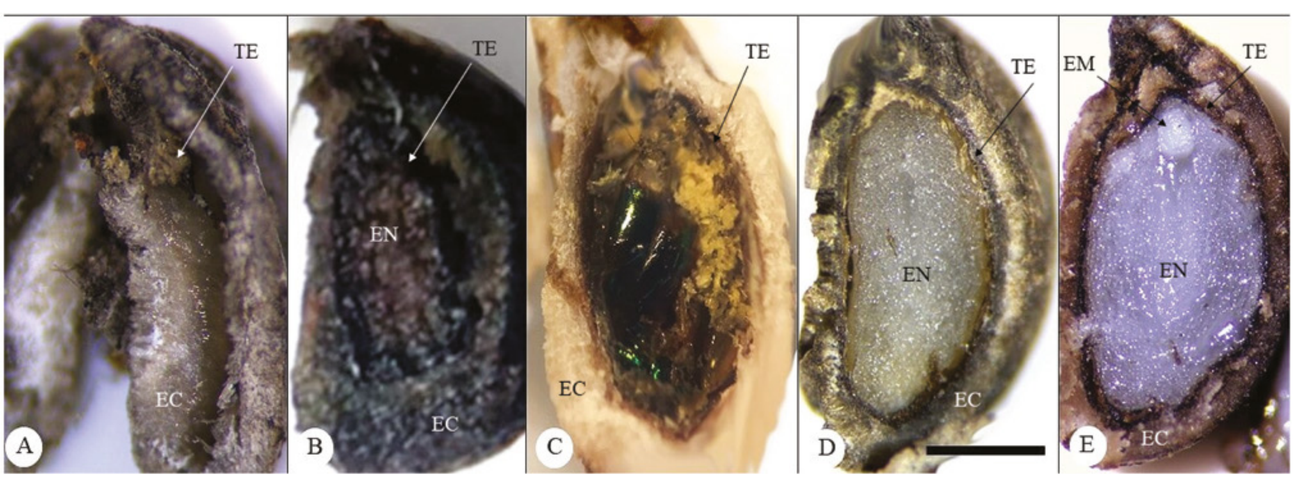

Figure 2. *Ilex paraguariensis* seeds classified as: empty (a); deteriorated (b); herbivorous (c); filled with unseen embryo (d); and filled with visualized embryo (e). Scale bar: 1 mm.

EM: embryo; EN: endosperm; TE: coat; EC: endocarp.

Table 1. Percentage of empty seeds (V), deteriorated (D), herbivorous (H), with visualized embryo (EV) and with unseen embryo (ENV), and viability (Viab) observed in samples of *Ilex paraguariensis* seeds.

				Full		
SAMPLES	V	D	H	EV	ENV	Viab
OC-15	30.41 ab	24.37 c	0.99 a	6.22 b	34.82 b	100 a
UR-15	27.76 abc	42.23 ab	0.24 ab	28.62 a	0.70 c	100 a
UR-16	44.64 a	44.00 ab	0 b	3.94 b	7.42 c	88 c
CE-16	2.12 c	27.30 bc	0 b	33.16 a	37.42 b	83 d
IJ-16	4.44 bc	3.53 d	0 b	26.50 a	65.53 a	96 b
PU-16	36.18 a	57.71 a	0 b	2.00 b	4.11 c	100 a
CV %	48.37	23.19	92.01	63.19	33.69	12.48

Means followed by the same letter comparing samples do not differ from each other by the 5% Tukey test.

The presence of full seeds with or without visualized embryos (Figures 2d and 2e) ranged from 6.11 to 92.03%, according to the sample (Table 1).

Correlation between climatic factors and seed quality was observed (Table 2). There was a strong linear correlation between empty seeds and altitude, showing that the higher the altitude, the greater the number of empty seeds. The relationship between temperature and rainfall was inverse, that is, the lower the temperature and rainfall, the higher the number of empty seeds.

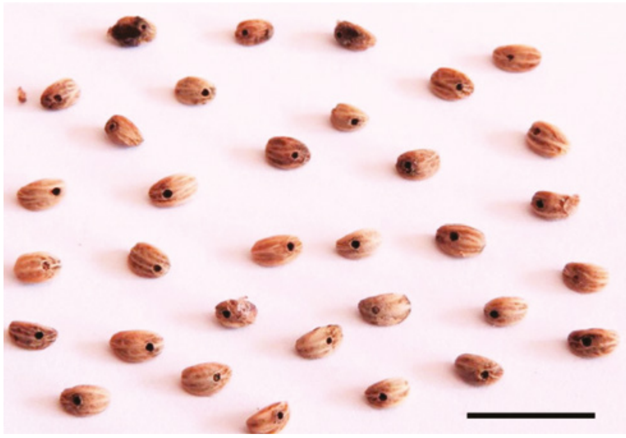

Figure 3. *Ilex paraguariensis* seeds with signs of herbivory. Scale bar: 1 cm.

The correlation between temperature and empty seeds can be explained by pollination as it is entomophilous, and low temperatures may decrease insect visits. When evaluating pollination in *I. paraguariensis*, Pires et al. (2014) and Liebsch & Mikich (2009) observed there was greater visitation of flower pollinators in the periods with higher temperatures during the day. *I. paraguariensis* flowering occurs in the warmer months after winter, with average temperatures above 13 °C (Pires et al., 2014).

Table 2. Pearson correlation between *Ilex paraguariensis* seeds classes and climatic variables.

	Altitude	Temperature	Rainfall
Empty	0.81	−0.84	−0.74
Died	0.63	−0.59	0.46
Herbivory	0.33	−0.39	0.46
Full	−0.95	0.94	0.08

Most of the seeds filled with visualized embryo were considered viable by the tetrazolium test (Table 1). Although viable, *I. paraguariensis* seeds are classified as dormant (Niklas, 1987; Heuser, 1990; Galíndez et al., 2018).

Based on the results, the low germination reported in *I. paraguariensis* seeds is not only related to dormancy, but also to factors that influence seed formation, considering the high number of empty/deteriorated seeds observed, in addition to the low temperature and higher altitude observed at the collection sites correlated with the high number of empty seeds.

SUBMISSION STATUS

Received: 19 Sept. 2017
Accepted: 3 Nov. 2019
Associate editor: Juliana Müller Freire
0000-0002-4758-2533

CORRESPONDENCE TO

Anieli Cioato de Souza
Universidade do Estado de Santa Catarina (Udesc), Av. Luís de Camões, 2.090, CEP 88520-000, Lages, SC, Brasil
e-mail: anielicioato@hotmail.com

ACKNOWLEDGEMENTS

The authors thank Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (Fapesc) (2017TR639) for the financial support, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) for awarding doctoral scholarship to the first author, and Ph.D. Tiago Geog Pikart for insect identification.

REFERENCES

Arrieta S, Suárez F. Germination and seed bank depletion of holly (*Ilex aquifolium* L.) in four microhabitat types. *Seed Science Research* 2004; 14: 305-313. 10.1079/SSR2004180

Catapan MIS. *Influência da temperatura, substrato e luz na germinação de sementes de Ilex paraguariensis St. Hil.* [thesis]. Curitiba: Universidade Federal do Paraná, 1998.

Carvalho PER. *Espécies arbóreas brasileiras*. Colombo: Embrapa Florestas; 2003.

Climate Data. *Dados climáticos para cidades mundiais* [Internet]. 2014 [cited 2016 Sept. 9]. Available from: http://bit.ly/2EGIkV2

Cuquel FL, Carvalho MLM, Chama HMCP. Avaliação de métodos de estratificação para a quebra de dormência em sementes de erva-mate. *Scientia agricola* 1994; 51(3): 415-221. 10.1590/S0103-90161994000300006

Galíndez G, Ceccato D, Bubillo R, Lindow-López L, Malagrina G, Ortega-Baes P, Baskin CC. Three levels of simple morphophysiological dormancy in seeds of Ilex (Aquifoliaceae) species from Argentina. *Seed Science Research* 2018; 28(2): 1-9. 10.1017/S0960258518000132

Garcia D, Obeso JR, Martinez I. Rodent seed predation promotes differential recruitment among bird-dispersed trees in temperate secondary forests. *Oecologia* 2005; 144: 435-446. 10.1007/s00442-005-0103-7

Heuser ED. Ilex paraguariensis *St. Hill: endosperma e embrião durante a embriogênese tardia* [thesis]. Porto Alegre: Universidade Federal do Rio Grande do Sul, 1990.

Liebsch D, Mikich BOS. Fenologia reprodutiva de espécies vegetais da Floresta Ombrófila Mista do Paraná, Brasil. *Revista Brasileira de Botânica* 2009; 32: 375-391. 10.1590/S0100-84042009000200016

Kollmorgen. *Munsell soil color charts*. Baltimore; 1975.

Niklas CO. Estudios Embriologicos y citologicos en la yerba mate *Ilex paraguariensis* (Aquifoliaceae). *Instituto de Botánica del Nordeste* 1987; 6: 45-56. 10.30972/bon.611504

Oliveira ME. *Patologia de sementes*. Palmas: Universidade Federal do Tocantins; 2013.

Oliveira SV, Waquil PB. Dinâmica de produção e comercialização da erva-mate no Rio Grande do Sul, Brasil. *Ciência Rural* 2015; 45: 750-756. 10.1590/0103-8478cr20140276

Pires EZ, Stedille LIB, Machado S, Mantovani A, Bortoluzzi RLC. Biologia reprodutiva de erva-mate (*Ilex paraguariensis* A. St. Hil) em remanescente de Floresta Ombrófila Mista Altomontana. *Revista de Ciências Agroveterinárias* 2014; 13(2): 171-180.

Sousa VA, Daros TL, Sturion JA. *Fenologia reprodutiva de erva-mate (Ilex paraguariensis St. Hil)*. Brasília, DF: Embrapa Florestas-CNPF, 2003.

Takagi E, Togashi K. Oviposition of the seed parasitoid wasp *Macrodasyceras hirsutum* (Hymenoptera: Torymidae) into seeds of nonhost tree *Ilex latifolia*. *Journal of the Entomological Research Society* 2013; 15(2): 17-20.

Zanon A. *Circular técnica nº 16: produção de sementes de erva mate*. Brasília, DF: Embrapa; 1988.