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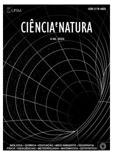

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Biology-Botany

Apomictic seed formation and viability in *Ilex paraguariensis* A.St.-Hil. (Aquifoliaceae)

Formação e viabilidade de sementes apomíticas de *Ilex paraguariensis* A.St.-Hil. (Aquifoliaceae)

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ABSTRACT

The objective of this study was to investigate fruit and seed formation in *Ilex paraguariensis* A.St.-Hil. without sexual reproduction, in addition to evaluating their viability. This study was based on the hypothesis that the formation of apomictic fruit and seeds occurs, but the seeds are non-viable, contributing to the low germination percentage of the species. Five branches of 20 pistillate mother plants were bagged before anthesis (T1) and five immediately after fruit formation (TC). Once they reached maturity, the fruit was collected and counted. Their seeds were cut lengthwise and classified as intact (with embryo and endosperm) or non-intact (empty and/or deteriorated). The viability of intact seeds was evaluated with the tetrazolium test when they were classified as viable or non-viable. The data were submitted to the Kruskal-Wallis Test. The formation of viable fruit and seeds occurred in smaller numbers in T1, all statistically different from TC (p<0.05). Although in smaller quantities and with a statistical difference, the formation of viable fruit and seeds occurs without sexual reproduction, suggesting that the process responsible for their formation is the facultative apomixis. The higher percentage of viable fruit and seeds in TC is probably a result of the occurrence of both apomixis and sexual reproduction. The percentage of non-viable seeds does not differ between the two treatments, suggesting that apomixis may not be the cause of the low percentage of viable seeds and germination, requiring further studies to understand its causes.

Keywords: Apomixis; Dioecious species; Tetrazolium test; Viable seeds; Yerba mate

RESUMO

O estudo objetivou investigar a formação de frutos e sementes em *Ilex paraguariensis* A.St.-Hil. sem a ocorrência de reprodução sexuada e avaliar a viabilidade destas. Partimos da hipótese de que ocorre a formação de frutos e sementes apomíticos, porém que as sementes sejam inviáveis, contribuindo para o baixo percentual de germinação da espécie. Cinco ramos de 20 matrizes pistiladas foram ensacados antes da antese (T1) e cinco imediatamente após a formação de frutos (TC). Quando maduros, os frutos foram colhidos e contabilizados. Suas sementes foram cortadas em sentido longitudinal e classificadas em íntegras (com embrião e endosperma) e não íntegras (vazias e/ou deterioradas). A viabilidade das sementes íntegras foi avaliada pelo teste de tetrazólio, quando foram classificadas em viáveis e não viáveis. Os dados foram submetidos ao Teste de Kruskal-Wallis. Houve a formação de frutos e sementes viáveis em menor número em T1, todos diferindo estatisticamente de TC ($p<0.05$). Embora em menor quantidade e com diferença estatística, ocorre a formação de frutos e de sementes viáveis sem reprodução sexuada, sugerindo que o processo responsável pela sua formação seja a apomixia facultativa. O maior percentual de frutos e sementes viáveis em TC é, provavelmente, resultado da ocorrência de apomixia e reprodução sexuada. O percentual de sementes inviáveis não difere entre os dois tratamentos, sugerindo que a apomixia pode não ser a causa do baixo percentual de sementes viáveis e de germinação, exigindo a continuidade dos estudos para compreender suas causas.

Palavras-chave: Apomixia; Espécie dioica; Teste de tetrazólio; Sementes viáveis; Erva-mate

1 INTRODUCTION

Ilex paraguariensis A.St.-Hil. (Aquifoliaceae), popularly known as *yerba mate*, is a tree species native to the Atlantic Forest (Flora e Funga do Brasil, 2023) with distribution in Argentina, Paraguay, and Brazil (Bruxel et al., 2018). In these countries and Brazil, especially in the state of Rio Grande do Sul, the species has cultural and economic importance, where the consumption habit of “chimarrão” promotes the market (Dalmoro, 2018).

The species is considered cryptic dioecious, because despite having flowers with stamens and pistil indicating perfect hermaphroditism, only the structures of one of the sexes is active, making it difficult to detect dioecy (Mayer & Charlesworth, 1991; Bruxel et al., 2018). These two types of flowers occur in separate individuals of the species (De Resende et al., 1995). In female or pistillate flowers, the gynoecium is well-developed with four ovules, while its anthers are smaller, modified, and sterile, called staminodes (Carvalho, 2003; Pires et al., 2014). In male or staminate flowers, the androecium has larger, perfect, and fertile anthers while the ovary is sterile, aborted, with a rudimentary appearance (pistillode) (Carvalho, 2003; Pires et al., 2014).

The fruit is of the spherical berry type with a red to black hue (Backes & Irgang, 2002), containing, on average, four pyrenes each with a seed formed by embryo, endosperm, and envelopes (Heuser et al., 1993). *I. paraguariensis* pyrenes have combined dormancy, including physical (preventing water absorption), morphological (due to the naturally underdeveloped embryo), and possibly physiological dormancy (with the presence of inhibitors) (Souza et al., 2022).

A work by Pires et al., (2014) in Urupema, Santa Catarina, indicates that the reproductive cycle of *I. paraguariensis* takes place between September and May, flowering between September and November and fruiting between January and May, with the influence of high temperatures in this cycle, a common characteristic in its family (Pires et al., 2014). Both pistillate and staminate flowers open throughout the day, without a defined time, with receptive stigma throughout the anthesis period on pistillate flowers, remaining open for at least three days, oxidizing after pollinator visits (Pires et al., 2014). The pollination of *I. paraguariensis* occurs in an entomophilous way, discarding the possibility of anemophily (pollination by wind) (Carvalho, 2003).

Due to its economic importance, the species is a constant target of research aimed at expanding its uses as raw material for food, beverages, cosmetics, textile, hygiene and cleaning, and health promotion industries (Dallabrida, 2016). The leaves are also widely used for medicinal purposes, due to their stimulating (Bortoli et al., 2018), anti-obesity, antioxidant, antimicrobial, and anti-ulcerogenic properties (Alkhatib & Atcheson, 2017; Santos et al., 2022), in addition to various potentials that still need studies. However, in addition to slow germination, its percentage is low, which makes it difficult to produce seedlings (Brasil, 2009; Winhelmann et al., 2022).

This low germination percentage may be related to dormancy and seed viability, as some works show high empty or deteriorated seed percentage (Galíndez et al., 2018; Winhelmann et al., 2022). De Sousa et al. (2015), in their experiment to define the methodology for controlled pollination of *I. paraguariensis* aiming at maximizing seed production, suggest the formation of fruit without sexual reproduction and consider the possibility of formation by apomixis.

The term apomixis (*stricto sensu*), used synonymously with agamospermy, is defined as the formation of seeds from the ovule tissues, without meiosis and fertilization for embryo development (De Resende et al., 1995; Dall'Agnol & Schifino-Wittmann, 2005). Lima (2005) states that in apomixis, the embryo formation is autonomous, from a reduced diploid nucleus arising from the embryo sac or ovary. The apomictic process replaces meiosis (by apomeiosis) and fertilization, occurring completely independently (Lima, 2005). This author also states that these seeds, through successive mitoses and processes of growth and development, form a new sporophyte identical to the mother plant, undergoing alternation of generations, where, unlike the sexual reproduction process, it is only morphological, without chromosomal reduction (diploid to haploid). It is noteworthy that in some species, seed formation can occur both asexually (by apomixis) and sexually (by fertilization), therefore named as facultative apomicts (Cardoso, et al., 2018; Albertini et al., 2019).

Thus, in *I. paraguariensis*, the apomictic seed formation, if non-viable, could contribute to the low germination rate of the species. For species with these characteristics, the tetrazolium test is indicated to evaluate seed viability (Brasil, 2009). Therefore, the objective of this work was to investigate the formation of fruit and seeds in *I. paraguariensis* without sexual reproduction and to evaluate their viability. We start from the hypothesis that the fruit and seed formation in the species occurs by fertilization and apomixis, and that the seeds formed without sexual reproduction are non-viable, contributing to the low germination percentage registered for the species.

2 MATERIALS AND METHODS

2.1 Preparation and collection of fruit and seeds

A total of 20 pistillate *I. paraguariensis* mother plants were selected in natural management areas in Ilópolis (28°55’57.5”S and 52°07’52.8”W) and Putinga (28°57’10”S and 52°10’33”W), state of Rio Grande do Sul, Brazil. In September 2021, the flower buds of 10 branches of each mother plant (totaling 200 branches) were quantified and properly identified (by the mother plant, branch, and treatment).

Figure 1 – Mother plant (a) of Ilex paraguariensis A.St.-Hil. (Aquifoliaceae) with bagged branches (b)

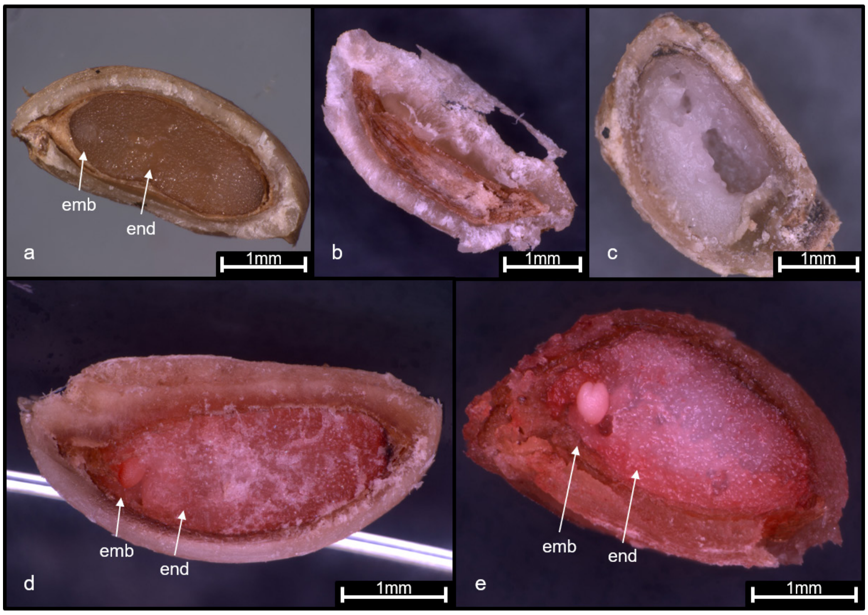
Source: Authors (2022)

Then, five branches of each mother plant (totaling 100 branches) were bagged and sealed (Fig. 1), constituting the apomixis treatment (T1). For bagging, a 120 g m²-1 Voil fine mesh fabric was used to prevent the entrance of pollen grains and pollinators from the outside (Lenza & Oliveira, 2005; Amorim & Oliveira, 2006; Fonseca, 2019). In November 2021, shortly after fruit formation, the other branches (five) of each of the mother plants (totaling 100 branches) had their fruit quantified. Then, the branches were also bagged and sealed (Fig. 1) to avoid fruit loss, constituting the control treatment (TC). After the bagged fruit ripening of both treatments (TC and T1), in February 2022, the branches were collected and transported to the Botany Laboratory of the Universidade do Vale do Taquari – Univates. The branches whose bags were damaged when collected were discarded (two bags). Each branch fruit was counted and opened for seed count. Aborted fruit was quantified, but not analyzed. The seeds were stored in falcon tubes, identified according to the mother plant, branch and treatment, without prior seed conditioning, under refrigeration (5°C) until evaluation.

2.2.1 Seed viability evaluation

The collected seeds were immersed in 10 mL of ultrapure water and kept in a DeLeo® TLK48 sterilization and drying oven (30°C) for 24 h to facilitate cutting (Catapan, 1998). Subsequently, a lengthwise cut was performed for integrity verification using a Leica® EZ4 HD stereoscopic microscope, with 20-30x magnification. The seeds were then classified as intact (Fig. 2a) (with embryo and endosperm) or non-intact (Fig. 2b-c) (Brasil, 2009). Data were tabulated according to the mother plant, branch, and treatment.

Fig 2 – Lengthwise cut seeds. (a) Intact seed with embryo (emb) and endosperm (end). (b-c) Non-intact seeds. (d) Viable seed with embryo (emb) and endosperm (end) within coloration and firmness pattern. (e) Non-viable seed with embryo (emb) and endosperm (end) outside coloration and firmness pattern



Source: Authors (2022)

2.2.2 Tetrazolium test

Seeds classified as intact were immersed in a 0.1% tetrazolium solution and kept in a DeLeo® TLK48 sterilization and drying oven (35°C) for 24 h, after which they were triple-washed in ultrapure water. Next, the seeds were observed using a Leica® EZ4 HD stereoscopic microscope, with 20-30x magnification. When seeds had a reddish color pattern indicating live tissue, with a firm embryo and intact endosperm, they were classified as viable (Catapan, 1998) (Fig. 2d). Seeds that did not have the characteristics described above were considered non-viable (Brasil, 2009) (Fig. 2e). Non-intact and non-viable seeds were grouped and considered non-viable. Data were tabulated according to the mother plant, branch, and treatment.

2.3 Data analysis

The percentage of formed fruit was defined as concerning to the number of flower buds counted per branch. The percentage of viable and non-viable seeds was defined in relation to the total number of seeds formed per branch. As they did not meet the assumptions of the ANOVA, and could not be transformed, they were analyzed using the non-parametric Kruskal-Wallis test with InfoStat® 2020 statistical software (Di Rienzo et al., 2023).

3 RESULTS AND DISCUSSION

The average fruit percentage, average number of formed seeds, average viable and non-viable seeds percentage failed the normality of the Shapiro-Wilk test and could not be transformed, being analyzed through the non-parametric Kruskal-Wallis test (Table 1). Fruit formation occurred both in TC and T1, however, the higher average percentage in relation to the number of flower buds per branch occurred in TC (39.07%), differing statistically from T1 (10.37%) (p<0.05). The fruit had seeds, however, the average formed seed number per branch in T1 was lower (69.68) and statistically different from TC (140.71) (p<0.05). The average viable seed percentage per branch also differed statistically between treatments (p<0.05). In TC 10.83% of seeds were viable, while in T1 the percentage was only 6.38%. There was no statistical difference regarding the average non-viable seed percentage between the two treatments (TC= 65.21% and T1= 51.19%).

Table 1 – Average fruit percentage and average formed seed number, average viable and non-viable seed percentage per branch of Ilex paraguariensis A.St.-Hil., analyzed by the non-parametric Kruskal-Wallis test

		Seeds		
Treatment	**Formed Fruit**	**Formed**	**Viable**	**Non-viable**
TC	39.07%*	140.71*	10.83%*	65.21%
T1	10.37%*	69.68*	6.38%*	51.19%

*Statistically different averages (p<0.05)

The fruit and seed formation in both treatments (TC = branches bagged after fruit formation; T1 = branches bagged and sealed with flower buds), even with smaller numbers in T1, when compared to the control treatment, indicates the occurrence of apomixis. This is because the fabric used in our experiment to isolate the branches before anthesis prevented the passage of pollinating organisms and pollen. Thus, the occurrence of sexual reproduction was prevented, which is reinforced by the fact that in *I. paraguariensis* pollination occurs exclusively by entomophily, having, as main floral visitors, insects of the Coleoptera, Diptera, and Hymenoptera orders (Carvalho, 2003; Pires et al., 2014). The size of these insects makes it impossible to pass through the fabric used in our experiment. The possibility of apomixis is reinforced by the dioecy registered for the species with one of the reproductive structures being stunted (Bruxel et al., 2018). Conversely, the branches bagged soon after the fruit formation in TC allowed pollinator visits. Thus, it is understood that in TC both sexual reproduction, through fertilization, and asexual reproduction through apomixis occurred in conjunction, which could explain the higher formed fruit percentage.

The formed fruit and seeds in TC, possibly due to the combination of apomixis and sexual reproduction, characterizes apomixis as facultative (Cardoso et al., 2018; Albertini et al., 2019). The availability of both apomixis and sexual reproduction guarantees greater reproductive success, dispersing a greater number of clone individuals originated by apomixis, and maintaining population heterogeneity with individuals generated by sexual reproduction (Mangla et al., 2015). In species with a wider distribution, such as *I. paraguariensis*, apomixis may occur more frequently in populations from less favorable condition environments, such as deserts or recently deglaciated areas (Mráz et al., 2019). Apomixis is also recorded in restricted plant species, being important due to the smaller number of individuals per population and fewer pollinator visits (Fonseca, 2019).

Evaluating the occurrence of apomixis in a rare experiment on dioecious species populations with *Baccharis dracunculifolia* DC. and *Baccharis elliptica* Gardner, Fonseca (2019) isolated flower buds from pistillate plants and showed seed formation by facultative apomixis in both species. The formation of apomictic seeds in these species is consistent with their reproductive strategies. The results with *B. dracunculifolia* and *B. elliptica* corroborate with those obtained by other authors who used similar methods (Fonseca, 2019). Amorim & Oliveira (2006), for example, when characterizing the sexual structure and reproductive ecology of *Amaioua guianensis* Aubl., isolated flower buds from pistillate plants in pre-anthesis and observed the formation of apomictic fruit. The authors mention that many of these fruit were aborted after two months of development and some that reached maturation were smaller and apparently stunted. In our study, stunted fruit were found in small amounts for both treatments.

Another study by Lenza & Oliveira (2005) on the reproductive biology of *Tapirira guianensis* Aubl. also confirmed the formation of fruit through apomixis when the authors isolated pistillate inflorescences. The authors state that it is an insignificant process in the species, but its occurrence indicates that sexual reproduction is not exclusive in *T. guianensis*. It is the same case observed in the present work with *I.*

paraguariensis, nevertheless, the occurrence of apomixis in taxonomically closer species is suggested only for *I. aquifolium* L. by Obeso (1996) who analyzed its fruit and seed production, but without studying the viability of apomictic seeds.

The observation of viable seeds in T1, although in smaller quantities compared to TC and with the statistical difference between both ($p<0.05$), indicates that apomixis in *I. paraguariensis* promotes its propagation. The greater amount of viable seeds in TC is likely to be the result of the occurrence of apomixis and sexual reproduction. Conversely, the experiment by Campacci et al. (2017), with a population of *Zygopetalum mackayi* Hook., indicated that there was no statistical difference in the seed viability between apomixis and sexual reproduction. Based on the results, the authors also state that the amount of viable apomictic seeds varies according to the species. Thus, it would be appropriate to evaluate the variation of viable apomictic seeds in taxonomically closer species. However, considering *I. paraguariensis*, no experiment evaluating apomictic seed viability of species from the same genus or family was found. Neverthless, when compared to studies without differentiation between apomixis and sexual reproduction, our study indicated lower viability (10.83%). Winhelmann et al. (2022) analyzed the viability of seeds from 10 mother plants from three municipalities in Vale do Taquari, Rio Grande do Sul, and discarded supernatant seeds when they were immersed in a water container (approximately 10%). Thus, the remaining seed viability was 26.2%. The high non-viable seed percentage, recorded in TC and T1, did not differ statistically between treatments, which seems to be typical of the species.

In dioecious populations, such as *I. paraguariensis*, pistillate plants can avoid biparental inbreeding through post-pollination processes such as the selection of pollen tubes before fertilization and selective abortion of seeds, preventing complete development or germination. (Austerlitz et al., 2012). These processes may have occurred in TC, increasing the amount of non-viable seeds. Normally, apomictic species have low genetic variability in their individuals, and the combination with sexual reproduction and diverse environmental pressures allows population genetic diversity

(Cardoso et al., 2018). For *I. paraguariensis*, Wendt et al. (2007) indicates greater genetic variability within populations (88.3%) when compared to variation between populations (11.37%). These authors also say that genetic variability between progenies of the same population is greater (55.5%) than between individuals of the same progeny (32.8%).

Seeds from asexual reproduction (such as apomixis) can be used as a propagation method of the species for seedling production, in which, according to Hand & Koltunow (2014), the production of genetically identical individuals from seeds is of interest in agriculture, due to the possibility of maintaining desirable genes. Further works are still needed to understand facultative apomixis in *I. paraguariensis*, as well as its occurrence, viability and influence on the genetic variability of different populations from the species.

4 CONCLUSIONS

Fruit and seed formation occurs in *I. paraguariensis* through sexual and asexual reproduction, the latter possibly through facultative apomixis. In addition, contrary to our initial hypothesis, the seeds formed without sexual reproduction are viable. Also, these occurred in greater quantities in the control treatment, probably due to the occurrence of both facultative apomixis and sexual reproduction. The percentage of non-viable seeds did not differ between treatments, which suggests that apomixis is not the cause of the low viable seed and germination percentages in the species, requiring further studies to understand its causes.

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REFERENCES

Albertini, E., Barcaccia, G., Carman, J. G., & Pupilli, F. (2019). Did apomixis evolve from sex or was it the other way around?. *Journal of experimental botany*, *70*(11), 2951-2964.

Alkhatib, A., & Atcheson, R. (2017). Yerba Maté (*Ilex paraguariensis*) metabolic, satiety, and mood state effects at rest and during prolonged exercise. *Nutrients*, *9*(8), 882.

Amorim, F. W., & Oliveira, P. E. (2006). Sexual structure and reproductive ecology of *Amaioua guianensis* Aubl.(Rubiaceae), a dioecious species of" cerrado" woody plant formations. *Brazilian Journal of Botany*, *29*, 353-362.

Austerlitz, F., Gleiser, G., Teixeira, S., & Bernasconi, G. (2012). The effects of inbreeding, genetic dissimilarity and phenotype on male reproductive success in a dioecious plant. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1726), 91-100.

Backes, P., & Irgang, B. (2002). Árvores do Sul: guia de identificação & interesse ecológico; as principais espécies nativas sul-brasileiras. Inst. Souza Cruz.

Bortoli, P. M., Alves, C., Costa, E., Vanin, A. P., Sofiatti, J. R., Siqueira, D. P., ... & Kaizer, R. R. (2018). *Ilex paraguariensis*: Potential antioxidant on aluminium toxicity, in an experimental model of Alzheimer's disease. *Journal of inorganic biochemistry*, *181*, 104-110.

Brasil. (2009). Regras para análise de sementes. Ministério da Agricultura, Pecuária e Abastecimento, Brasília.

Bruxel, F., Avrella, E. D., Teixeira, M., Fior, C. S., & Freitas, E. M. (2018). Taxonomia, descrição e distribuição geográfica de *Ilex paraguariensis* A.St-Hil. *A cultura da erva mate e os ácaros: situação atual e perspectiva, 1st edn. Porto Alegre*.

Campacci, T. V. S., Castanho, C. T., Oliveira, R. L. F., Suzuki, R. M., Catharino, E. L. M., & Koehler, S. (2017). Effects of pollen origin on apomixis in *Zygopetalum mackayi* orchids. *Flora*, *226*, 96-103.

Cardoso, J. C. F., Viana, M. L., Matias, R., Furtado, M. T., Caetano, A. P. D. S., Consolaro, H., & Brito, V. L. G. D. (2018). Towards a unified terminology for angiosperm reproductive systems. *Acta Botanica Brasilica, 32*, 329-348.

Carvalho, P. E. R. (2003). Espécies Arbóreas Brasileiras. Embrapa Informação Tecnológica, Brasília.

Catapan, E. A. (1998). Influência da temperatura, substrato e luz na germinação de sementes de *Ilex paraguariensis* St. Hil.

Dallabrida, V. R., Dumke, C. I., Molz, S., Furini, V., & Giacomelli, M. B. O. (2016). Com erva-mate não se faz só chimarrão! Situação atual e perspectivas de inovação no setor ervateiro do Planalto Norte Catarinense. *DRd-Desenvolvimento Regional em debate*, *6*(2), 247-273.

Dall'Agnol, M., & Schifino-Wittmann, M. (2005). Apomixia, genética e melhoramento de plantas. *Current Agricultural Science and Technology*, *11*(2).

Dalmoro, M. (2018). A construção sociocultural e econômica da cadeia de produção e o consumo da erva-mate. *A cultura da erva mate e os ácaros: situação atual e perspectiva, 1st edn. Porto Alegre*.

Daniel, O. (2009). Erva-mate: sistema de produção e processamento industrial.

Sousa, V. A. D., Aguiar, A. V. D., & Spoladore, J. (2015). METHODOLOGY FOR THE CONTROLLED POLLINATION OF *Ilex paraguariensis* A. St. Hil.-Aquifoliaceae. *Revista Árvore*, *39*, 315-323.

Di Rienzo, J. A. (2022, April 04). *InfoStat versão 2014*. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. http://www.infostat.com.ar/

Flora e Funga do Brasil. (2023, April 04). *Aquifoliaceae.* Jardim Botânico do Rio de Janeiro. https://floradobrasil.jbrj.gov.br/FB4904

Fonseca, D. D. C. (2019). O conceito de raridade em populações dioicas: uma abordagem ecológica.

Galíndez, G., Ceccato, D., Bubillo, R., Lindow-López, L., Malagrina, G., Ortega-Baes, P., & Baskin, C. C. (2018). Three levels of simple morphophysiological dormancy in seeds of *Ilex* (Aquifoliaceae) species from Argentina. *Seed Science Research*, *28*(2), 131-139.

Heuser, E. D., Ferreira, A. G., & MARIATH, J. D. A. (1993). *Ilex paraguariensis* (Aquifoliaceae) Endosperma e embrião durante a embriogênese tardia. *Boletin de la Sociedad Argentina de Botanica, La Plata*, *29*(1/2), 39-48.

Lenza, E., & Oliveira, P. E. (2005). Reproductive biology of *Tapirira guianensis* Aubl.(Anacardiaceae), a dioecious species, in a gallery forest of Triângulo Mineiro, Brazil. *Brazilian Journal of Botany*, *28*, 179-190.

Lima, M. (2005). Melhoramento genético florestal.

Mangla, Y., Chaudhary, M., Gupta, H., Thakur, R., Goel, S., Raina, S. N., & Tandon, R. (2015). Facultative apomixis and development of fruit in a deciduous shrub with medicinal and nutritional uses. *AoB Plants*, *7*, plv098.

Mayer, S. S., & Charlesworth, D. (1991). Cryptic dioecy in flowering plants. *Trends in Ecology & Evolution*, *6*(10), 320-325.

Mráz, P., Zdvořák, P., Hartmann, M., Štefánek, M., & Chrtek, J. (2019). Can obligate apomixis and more stable reproductive assurance explain the distributional successes of asexual triploids in *Hieracium alpinum* (Asteraceae)?. *Plant Biology, 21*(2), 227-236.

Obeso, J. R. (1996). Fruit and seed production in European holly, *Ilex aquifolium* L.(Aquifoliaceae). In *ANALES-JARDIN BOTANICO DE MADRID* (Vol. 54, pp. 533-539). CSIC.

Pires, E. Z., Stedille, L. I. B., Machado, S., Mantovani, A., & Bortoluzzi, R. D. C. (2014). Reproductive biology of erva-mate (*Ilex paraguariensis* A. St. Hil.) in a remnant of High Montane Mixed Ombrophilous Forest.

Brown, B., & Aaron, M. (2001). The politics of nature. *The rise of modern genomics, 3rd edn. Wiley, New York*, *5*.

De Resende, M. D. V., Sturion, J. A., & Mendes, S. (1995). Genética e melhoramento da Erva-mate (*Ilex paraguariensis* St. Hil.). *EMBRAPA-CNPF. Documentos*, *25*.

Santos, A. F., Schiefer, E. M., Surek, M., Martins, C. A. F., Worfel, P. R., Sassaki, G. L., ... & de Souza, W. M. (2022). Chemical, biological, and pharmacological evaluation of the aqueous extract of *Ilex paraguariensis*, St. Hill.(Aquifoliaceae). *Research, Society and Development*, *11*(2), e301.122.5335-e301.122.5335.

Souza, A. C. D., Oliveira, L. M. D., Bagatini, K. P., Souza, G. F., Liesch, P. P., Schmidt, S. S., & Puchale, L. Z. (2022). Causes of dormancy in *Ilex paraguariensis* pyrenes. *Rodriguésia*, *73*, e01942020.

Wendt, S. N., Sousa, V. D., Quoirin, M., Sebbenn, A. M., Mazza, M. C., & Sturion, J. A. (2007). Genetic characterization of provenances and progenies of *Ilex paraguariensis* St. Hil. by using RAPD markers.

Winhelmann, M. C., Vargas, L. J. D., Gastmann, J., Jaeger, A. P., Freitas, E. M. D., & Fior, C. S. (2022). Evaluation of the quality of seeds of yerba mate (*Ilex paraguariensis* A. St.-Hil.) from different mother-plants. *Ciência Florestal*, *32*, 247-265.

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