

Evaluation of the Seed vigor of *Tournefortia paniculata* Cham. Post-Digestive Tract of the Ruby-Crowned Tanager *Tachyphonus Coronatus* (Vieillot, 1822)

Avaliação do Vigor de Sementes de *Tournefortia paniculata* Cham. Pós-Trato Digestório do Tiê-Preto *Tachyphonus coronatus* (Vieillot, 1822)

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Abstract

Endozoochory (dispersion with passage through the digestive system of animals) is one of the most important processes. It is included in the ecological process known as dispersive mutualism, benefit the species involved. The most used method to evaluate the physiological quality of seeds is carried out based on the traditional germination test. However, electrical conductivity tests with seeds of forest species are still scarce, especially those developed with the individual method, which provide greater fidelity to the results so that through this one can define the test by the mass methods. The current work aimed to investigate the vigor, from the electrical conductivity of the seeds of *Tournefortia paniculata* Cham. after passage through the digestive tract of a ruby-crowned tanager *Tachyphonus coronatus* (Vieillot, 1822). An expedition was conducted in a fragmented area of Atlantic Forest in process of reforestation in the campus of the IFRJ. After the capture of the passerine with mist nets, proceeded the identification of seeds shed in its feces. The experimental design was completely randomized with one treatments with five replicates. The results allowed concluded that the seed of the *T. paniculata* Cham. after digestive tract of the ruby-crowned tanager presented high electrical conductivity in three of its replicates at the individual level.

Keywords: Ornithochoric; Forest Seed; Seed deterioration

Endozoocoria (dispersão com passagem pelo sistema digestivo de animais) é um dos processos mais importantes. Está incluído no processo ecológico conhecido como mutualismo dispersivo, beneficiando as espécies envolvidas. O método mais utilizado para avaliar a qualidade fisiológica das sementes é realizado com base no tradicional teste de germinação. Entretanto, os testes de condutividade elétrica com sementes de espécies florestais ainda são escassos, especialmente aqueles desenvolvidos com o método individual, que proporcionam maior fidelidade aos resultados para que, através deste, seja possível definir o teste pelos métodos de massa. O presente trabalho teve como objetivo investigar o vigor, a partir da condutividade elétrica das sementes de *Tournefortia paniculata* Cham. após passagem pelo trato digestório de um tiê-preto *Tachyphonus coronatus* (Vieillot, 1822). Uma expedição foi realizada em uma área fragmentada da Mata Atlântica em processo de reflorestamento no campus do IFRJ. Após a captura da ave com redes de neblina, procedeu-se à identificação das sementes eliminadas em suas fezes. O delineamento experimental foi inteiramente casualizado com um tratamento com cinco repetições. Os resultados permitiram concluir que a semente de *T. paniculata* após trato digestivo do tiê-preto apresentou alta condutividade elétrica em três de suas réplicas no nível individual.

Palavras-chave: Ornithocoria; Semente Florestal; Deterioração de sementes

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1 Introduction

The process of reforestation can happen through the production of seedlings and, through seed dispersal, this can occur due to the action of the plant itself (autochory), water (hydrochory), wind (anemochory), animals (zoochory), etc. However, special attention should be given to the action of the birds, given their ample capacity of migration between the different environments. However there are few studies that relate the effect of zoochory, mainly ornithochory on the physiological quality of seeds (REIS, 2003; DARIO, 2004).

It is known that seed dispersal can be an important regulatory factor, promoting the coexistence of species in a complex ecosystem (JÁNOSI; SCHEURING, 1997), and this process depends on several aspects, such as behavior, physiology and other characteristics of the interaction between animals and plants, such as retention time, number of seeds released and the frequency at which they are distributed (BARNEA et al., 1992).

The dispersing animals act at different levels of the process and at different spatio-temporal scales. The primary dispersers are those that take the seeds directly from the fruits and have great influence on the initial pattern of seed rain (WANG; SMITH, 2002).

Endozoochory (dispersion with passage through the digestive system of animals) is one of the most important processes (75% occurrence). It is included in the ecological process known as dispersive mutualism, benefit the species involved. In this, the animals extract from the fruits the necessary nutrients to their diet and the vegetables have their material dispersed and deposited at great distances from the mother plant (VAN DER PIJL 1972; KUNZ, 1982; FENNER, 1985). The Birds are among the most important seed dispersers, not only because of their abundance, but also because of the frequency with which they feed on fruits and the great capacity to move and occupy different environments (JORDANO, 2006; BOCCHESE et al., 2008). They are still important for the maintenance of forests and the recovery of areas that suffered anthropic actions (GALINDO-GONZÁLEZ et al., 2000; GARCIA et al., 2000; MANHÃES et al., 2003).

The most used method to evaluate the physiological quality of seeds is carried out based on the traditional germination test (PASSOS et al., 2008; BOCCHESE et al., 2008). However, such testing for seed of forest species may take weeks or even months to complete the results. Thus, the development and improvement of the methodology for rapid feasibility evaluation tests promises to make the production process more efficient and to aid decision making on seed lots. However, electrical conductivity tests with seeds of forest species are still scarce, especially those developed with the individual method, which provide greater fidelity to the results so that through this one can define the test by the mass methods. Stallbaun et al. (2015), using the individual electrical conductivity test with seeds of *Anadenanthera falcate* (Benth.) Speg. concluded that the applied method was compatible with the standard germination test. The current work aimed to investigate the vigor, from the electrical conductivity of

the seeds of *Tournefortia paniculata* Cham., after passage through the digestive tract of a ruby-crowned tanager *Tachyphonus coronatus* (Vieillot, 1822).

2 Materials and Methods

An expedition was conducted in a fragmented area of Atlantic Forest in process of reforestation in the campus of the Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (IFRJ) in the Municipality of Pinheiral, State of Rio de Janeiro (22°31'29.73"S; 43°59'37.08"W). One ruby-crowned tanager *T. coronatus* was captured with mist nets. After identification of the species (SIGRIST, 2014), the bird was photographed and measured (total length, length, width and height of the beak, wing, tail and tarsi lengths) (NASCIMENTO et al., 1994). The bird was kept in an individual box with clean ground paper until defecation. The seeds was taken from the fresh feces and identified according to the Manual of Identification and Cultivation of Native Arboreal Plants of Brazil (LORENZI, 2016). Seeds of *T. paniculata* was shed by the ruby-crowned tanager. After identification, asepsis was employed leaving the seeds in sodium hypochlorite solution (2%) for 5 min and then washing under running water. In the field, drying was carried out with filter paper with a temperature of approximately 30-35 °C for 30 min. For conduct the Electrical Conductivity (EC) test, the recommendations proposed by Krzyzanowski et al. (1999), Flavio and Paula (2010), Guollo et al. (2017) and Thode-Filho (2018) was followed with adaptations. The experimental design was completely randomized with one treatments with five replicates. The tests was performed by the individual method (by seed) for 24 hours. The seeds was placed in individual plastic containers containing 15 mL of distilled water. Additionally, the EC was evaluated in five soak periods: 3, 6, 9, 12 and 24 hours. The parameters of the study was evaluated using a multiparameter probe AKSO Combo 5 and the results was expressed in $\mu\text{S cm}^{-1}$.

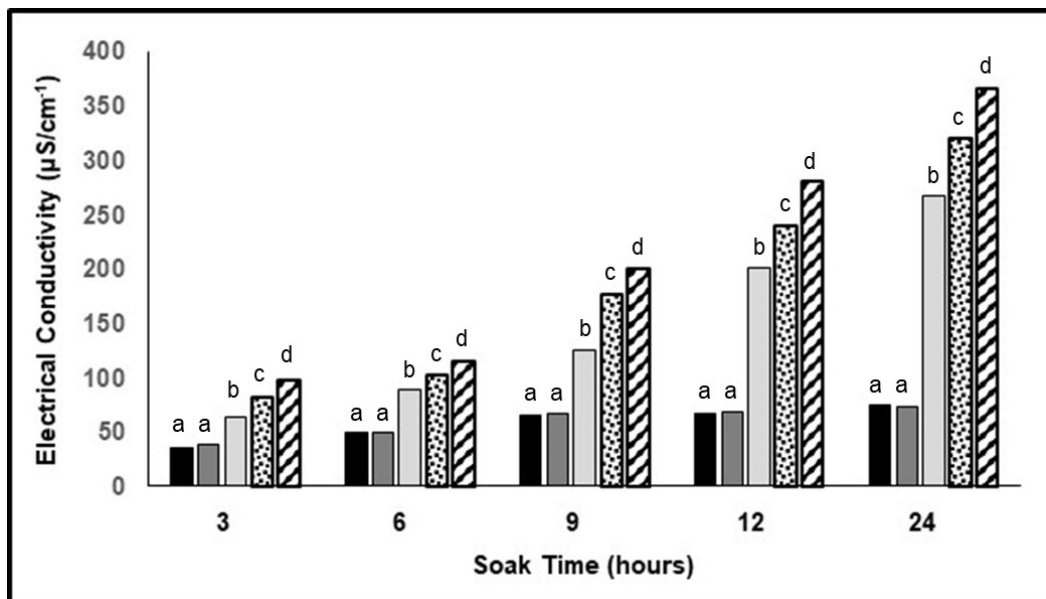
The data was submitted to analysis of variance (ANOVA) and the means was compared by the Tukey test $p \leq 0.05$ of probability between the same ones (COSTA NETO, 1977; MILLER; MILLER, 1993).

3 Results and Discussion

The mean values of EC as a function of the different soak periods are shown in figure 1. Significant differences was observed in all the soak periods. It was verified that of the five replicates analyzed, only two presented EC low all the time. At the end of the soak time the EC of the two replicates was below $76\mu\text{S cm}^{-1}$. On the other hand, the other three replicates presented a considerable increase at the end of each period. At the end of 24 hours the EC presented a variation between 267 and $36576\mu\text{S cm}^{-1}$. Thus, of the five seeds analyzed, three was showed great permeability.

As the seed grows older and your bark is broken, its

Figure 1 – Evaluation of the electrical conductivity in different times of seed soak of the *Tournefortia paniculata* Cham. Means followed by the same lowercase letter do not differ statistically from each other by Tukey test $p \leq 0.05$ probability.



deterioration occurs, consequently, the loss of the integrity of the cell membrane systems, causing an increase in its permeability, which results in leaching of electrolytes and higher electrical conductivity (OLIVEIRA, 2012).

Frugivorous birds after fruit consumption and after regurgitation or excretion of the seeds have been considered extremely important to favor seed germination and recovery of impacted fields (BARNEA et al., 1992; WENNY; LEVEY, 1998; YAGIHASHI et al., 1999). They act as pulp and seed separat agents, besides modifications in the external structure of the seeds as a consequence of their ingestion, such as: thermal action, chemical and mechanical. Also considered to be seed dormancy removers (IZHAKI; SAFRIEL, 1990; BARNEA et al., 1991; FIGUEROA; CASTRO, 2002).

The passage of some types of seeds through the digestive system allows a chemical scarification without damage to the seed, causing gas exchange with the environment and/or the elimination of germination inhibitors present, besides facilitating water penetration and reactivation of the metabolic processes (TRAVESET; VERDÚ 2002).

In anatomical comparative study of digestive tracts of birds Santos et al. (2016) report that the digestive tube has, on average, 13.38% of the total length of the birds. Following this proportion, the ruby-crowned tanager of this study that measured the total length of 17.4 cm has a digestive tract estimated to be approximately 2.3 cm. This information becomes relevant considering that the retention time of the seeds is an important factor in the dispersion process. Small seeds are retained longer in the digestive tract of the disperser and can be carried away from the parent plant, although a very long retention decreases its viability (MURRAY et al., 1994).

Barnea et al. (1992) have suggested that the removal of the outer layer of the seed (shell) occurs only in seeds

that remain for longer in the animal's digestive system, increasing the permeability and, consequently, the chances of germination in the environment. Birds have the ability to give different seed treatments, which depends partly on seed size, and when they are very small, this mechanism does not work. In addition, many studies show results in which the ingestion of seeds by birds interferes positively or negatively (BARNEA et al., 1991; CASTIGLIONI et al., 1995).

Thode-Filho et al. (2018) when study the feasibility of using the individual electrical conductivity test to evaluate the physiological quality of seeds of forest species, *Ficus calyptroceras* (Miq.) Miq. (Moraceae), *Ficus enormis* (Mart. ex Miq.) Mart., *Ficus obtusiuscula* (Miq.) Miq., *Annona emarginata* (Schltdl.) H. Rainer, *Annona neosalicifolia* H. Rainer and *Xylopia brasiliensis* Spreng., after passage through the digestive tract of wild birds. They identified low electrical conductivity at the individual level in such a way that the seeds showed no loss of vigor. Still, according to the authors, the birds only played the role of individual disperser, failing to cause injury or physical damage to the shell.

In the present study, seed shell rupture was observed from EC increase. It is understood that the passage through the tract of the bird acted as a facilitator of the germination process eliminating the physical interferences. It can be affirmed that the process of digestion of the food by the bird (digestive physiology) acted as a mechanism of dormancy breaking for the seed, facilitating the shell rupture, this is due to variations in pH (2.5 to 6.8), the presence of organic acids and microorganisms throughout the entire digestive process (LONG, 1967; HULAN; BIRD, 1972; BIRD; MOREAU, 1978; DENBOW, 2000; BELL, 2002).

4 Conclusions

The results allowed concluded that the seed of the *T. paniculata* after digestive tract of *T. coronatus* presented high electrical conductivity in three of its replicates at the individual level. Thus, it is evident that the seed vigor of present study underwent post-tract alteration of *T. coronatus* according the EC elevation. It is observed that the bird has a good potential of dispersion of the seeds, besides contribution with the germinative process and recovery of anthropic areas.

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References

- BARNEA A, YOM-TOV Y, FRIEDMAN J. Does ingestion by birds affect seed germination? *Functional Ecology* 5: 1991;394-402.
- BARNEA A, YOM-TOV Y, FRIEDMAN J. Effect of frugivorous birds on seed dispersal and germination of multi-seeded fruits. *Acta oecologica* 13. 1992;(2): 209-219.
- BELL DD. Anatomy of the Chicken. In: Commercial chicken meat and egg production. 5a edição. Edited by Donald D. Bell e William D. Weaver, Jr.. Springer. 2002;p 41-58.
- BIRD FH, MOREAU GE. The effect of dietary protein levels in isocaloric diets on the composition of avian pancreatic juice. *Poult. Sci.* 1978;57: 1622-1628.
- BOCCHESI RA, OLIVEIRA AKM, LAURA VA. Germinação de sementes de *Cecropia pachystachya* Trécul (Cecropiaceae) em padrões anteriores e posteriores à passagem pelo trato digestório de aves dispersoras de sementes. *Revista de Biologia e Ciências da Terra*. 2008;8(2): 19-26.
- CASTIGLIONI GDA, CUNHA LST, GONZAGA LP. *Ramphocelus bresilius* como dispersor das sementes de plantas da restinga de Barra de Maricá, Estado do Rio de Janeiro (Passeriformes: Emberizidae). *Ararajuba*. 1995;3: 94-99.
- COSTA NETO PLO. Estatística. São Paulo: Edgard Blücher, 1977;264p.
- DARIO FR. A importância da fauna na dinâmica da floresta. 2004. Disponível em: <http://port.pravda.ru/brasil>. Acesso em: 16/05/2005.
- DENBOW M. Gastrointestinal anatomy and physiology. In: Sturkies avian physiology. 5a edição. Academic press, Londres. 2000; p:299-325.
- FIGUEROA JA, CASTRO SA. Effects of bird ingestion on seed germination of four woody species of the temperate rainforest of Chiloe Island, Chile. *Plant Ecology*. 2002;160, 17 -23.
- FLAVIO JJP, PAULA RC. Testes de envelhecimento acelerado e de condutividade elétrica em sementes de *Dictyoloma vandellianum* A. Juss. *Scientia Forestales*, 2010;v.38, n.87, p.391-399.
- GALINDO-GONZÁLEZ J, GUEVARA S, SOSA VJ. Bat and bird generated seed rains at isolate trees in pastures in a tropical rainforest. *Conservation Biology*. 2000;14(6): 1693-1703.
- GARCIA QS, REZENDE, JLP, AGUIAR LMS. Seed dispersal by bats in a disturbed area of southeastern Brazil. *Revista de Biologia Tropical*. 2000;48(1): 125-128.
- GUOLLO K, POSSENTI JC, FELIPPI M, DEL QUIQUI EM, DEBASTIANI AB. Avaliação da Qualidade Fisiológica de Sementes Florestais através do Teste de Condutividade Elétrica. *Sci. Agrar. Parana. Marechal Cândido Rondon*. 2017;v.16, n. 3, jul./set., p. 374-382.
- HULAN HW, BIRD FH. Effect of fat level in isonitrogenous diets on composition of avian pancreatic juice. *J. Nutr.* 1972;102: 459-468.
- IZHAKI I, SAFRIEL UN. The effect of some Mediterranean frugivores upon germination patterns. *Journal of Ecology*. 1990;78: 56-65.
- JÁNOSI IM, SCHEURING I. On the evolution of density dependent dispersal in a spatially structured population model. *Journal of Theoretical Biology*. 1997;187(3): 397-408.
- JORDANO P, GALETTI M, PIZO MA, SILVA WR. Ligando frugivoria e dispersão de sementes à biologia da conservação. - In: Rocha, C. D. F., Bergallo, H. D., Van Sluys, M. and Alves, M. A. S. (eds.), *Biologia da Conservação: Essências*. Rima Editora. 2006;p. 411-436.
- KRZYŻANOWSKI FC, VIEIRA RD, FRANCA NETO J de B. Vigor de sementes: conceitos e testes. Londrina: ABRATES, 1999. 218p.

- LEVEY DJ. 1990. Habitat-dependent fruiting behaviour of an understory tree *Miconia centrodesma*, and tropical treefall gaps as keystone habitats for frugivores in Costa Rica. *Journal of Tropical Ecology*. 1990;6: 409-420.
- LONG JF. Gastric secretion in anesthetized chickens. *Am. J. Physiol*. 1967;212:1303-1307
- LORENZI H. Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas do Brasil, vol. 1/Harri Lorenzi. Nova Odessa, SP: Instituto Plantarum, 2016.
- MANHÃES MA. Dieta de traupíneos (Passeriformes, Emberezidae) no Parque Estadual do Ibitipoca, Minas Gerais, Brasil. *Iheringia, Sér. Zool*. 2003;93(1): 59-73.
- MILLER JC, MILLER JN. *Statistics for analytical chemistry*. 3. ed. Chichester: Ellis Horwood, 1993;233p.
- MURRAY KG, RUSSEL S, PICONE CM, WINNETT-MURRAY K, SHERWOOD W, KUHLMANN ML. Fruit laxatives and seed passage rates in frugivores: Consequences for plant reproductive success. *Ecology*. 1994;75(4):989-994.
- NASCIMENTO ILS, NASCIMENTO JLX, ANTAS PTZ. *Manual de Anilhamento de Aves no Brasil*. Brasília: Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis; 1994.
- OLIVEIRA S. *Tecnologia de sementes florestais: espécies nativas*. Curitiba: UFPR; 2012.
- PASSOS MAA, SILVA FJBC, SILVA ECA, PESSOA RC, SANTOS RC. Luz, substrato e temperatura na germinação de sementes de cedro-vermelho. *Pesquisa Agropecuária Brasileira*. 2008;v.43,n.2,p.281-284.
- REIS A. *Fundação o Boticário de Proteção a Natureza. Natureza et Conservação*. Curitiba-PR. v.1. n°1, 2003.
- SANTOS ALQ, ANDRADE MB, MENEZES LT, de SOUZA RR, FERREIRA CH, KAMINISHI ÁPS, de MORAES FM. Estudo anatômico comparativo do tubo digestório de aves da ordem Columbiformes. *PUBVET*, 6, 2016;Art-1319.
- SIGRIST T. *Guia de Campo: Avifauna Brasileira*. São Paulo: Avis Brasilis; 2014.
- STALLBAUN PH, SOUZA PA, MARTINS RCC, MATOS JMM, MOURA TM. Testes rápidos de vigor para avaliação da viabilidade de sementes de *Anadenanthera falcata*. *Enciclopédia Biosfera*. 2015;11(21), 1834-1842.
- THODE FILHO S, FRANCO HA, de LIMA FIGUEIREDO ENN, DA SILVA FERNANDES M, FERREIRA I, BERTO BP. (2018). Evaluation of the electrical conductivity of forest seeds after digestive tract of wild birds. *Ciência e Natura*, 2018;40, 79.
- TRAVESET A, VERDÚ M. A meta-analysis of the effect of gut treatment on seed germination. In: LEVELY, D. J. & GALETTI, M. (Eds.). *Seed dispersal and frugivory: ecology, evolution and conservation*. Wallingford: CABI Publishing. 2002;p.339-350.
- VAN DER PIJL L. *Principles of dispersal in higher plants*. Berlin, New York: Springer-Verlag. 1972;161 p.
- WANG BC, SMITH TB. Closing the seed dispersal loop. *Trends in Ecology and Evolution*. 2002;17(8): 379-385.
- WENNY DG, LEVEY DJ. Directed seed dispersal by bell-birds in a tropical cloud forest. *Proc. Natl. Acad. Sci*. 1998;95: 6204-6207.
- YAGIHASHI T, HAYASHIDA M, MIYAMOTO T. Effects of bird ingestion on seed germination of two *Prunus* species with different fruit-ripening seasons. *Ecological Research*. 1999;14: 71-76.

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