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CROP PRODUCTION

Processing and physical and physiological quality of the native forest seeds of *Vernonanthura discolor*

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ABSTRACT. The objective was to study the processing of *Vernonanthura discolor* seeds, evaluating their physical and physiological qualities and defining parameters for purity analysis and determine the weight of one thousand seeds. The seeds extracted from the infructescences were processed in a blower, testing combinations of openings of the calibration valve and the side air passage. After processing, the seeds went through water content determination, germination tests, first count of the germination test, purity analysis, weight of one thousand seeds and percentage of full seeds. Compared to the control, seeds processed in a regulated blower with the calibrator valve in position 10 plus three 360° turns of side air passage obtained an approximately three-fold increase in germination and vigour and had 96% purity. We conclude that the processing of *V. discolor* seeds was best done by combining the rubbing of the seeds on circular sieves (1.8 and 1.6 mm in diameter) and then passage in an adjusted seed blower (calibration valve in position 10 plus three 360° turns of side air passage). The weight of one thousand seeds is 0.500 g, and the working sample for purity analysis must be 1.3 g.

Keywords: Vassourão-preto; post-harvest; purity analysis; weight of one thousand seeds; seeds blower; germination.

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Introduction

Vassourão-preto (*Vernonanthura discolor* (Spreng.) H. Rob.) is a native species in the mixed rain forest and is typical of secondary vegetation, which is common in clearings and young forests (Siminski & Fantini, 2011). It is considered a pioneer plant with fast growth and is tolerant to low temperatures; it is considered an option for mixed plantings in permanent preservation areas to prepare the environment for the development of climax species. The natural dispersion of the seeds occurs through the wind, also known as anemochory (Almeida, Watzlawick, Myszka, & Valerio, 2008), and with the help of *papus*, structures adhere to the seeds.

The disappearance of native species makes natural regeneration and survival of future generations impossible, causing environmental problems. However, it is necessary to rescue and spread species with the possibility of human applications; the introduction of a native plant can be an instrument for preserving the species (Chamas & Matthes, 2000; Heiden, Barbieri, & Stumpf, 2006; Masetto et al., 2012).

The development of commercial plantings of native species depends on the knowledge of their reproductive process, which includes the formation of seeds, determination of the harvest period, definition of extraction and processing methods, and determination of appropriate conditions for germination and storage (Alves, Sader, Bruno, & Alves, 2005; Pereira, Cuquel, & Panobianco, 2010; Maranho, Paiva, & Paula, 2013).

Post-harvest handling is highly important to ensure physical, physiological and sanitary quality of the seeds (Santos, 2015). Usually, after extracting the seeds, processing is required because, in general, there are remnants of materials that adhered to or mixed with the seeds, such as flight apparatuses; remnants of fruits, leaves, branches and dirt; and malformed seeds or seeds attacked by insects and fungi (Araujo, Viggiano, & Silva, 2009; Santos, 2015).

Processing may be classified as the set of operations that seek to improve physical purity, achieve a standard quality, preserve the germination percentage and avoid pests and diseases in the seeds (Santos

Neto, Carvalho, Oliveira, Fraga, & Souza, 2012). In cases of forest species seeds, processing is considered minimal because only impurities are eliminated, so knowledge and improvement of this operation is crucial to obtain pure seeds with great germinating potential, enabling the formation of seedlings.

The quality control of seeds, through purity evaluations, germination and sanity, is relevant both for private use and commercialization of the material (Flores, Ataíde, Borges, Silveira, & Pereira, 2011). The determination of the percentage of pure seeds in the batch is done through the physical purity analysis, whose general conduction protocol is described in the International Rules for Seed Testing (ISTA, 2015); however, for Vassourão-preto, there are no instructions for procedures and sizes of working samples.

The objective was to study the processing of *Vernonanthura discolor* seeds, evaluating their physical and physiological traits, and to define parameters for purity analysis and determine the weight of one thousand seeds.

Material and methods

The experiments were conducted from October 2014 to January 2015. Materials were collected from Vassourão-preto matrices, natural populations with distances between the trees ranging from 10 to 25 m, located in the city of Bocaiúva do Sul, state of Paraná, Brazil (25° 15.139' S e 49° 06.096' W).

Twenty matrices were selected randomly, identified, measured and mapped by a georeferencing system. The plants in the matrices had an average Diameter at Breast Height (DBH) of 19.94 cm, with a maximum of 37.50 cm and minimum of 9.50 cm; the average total height (basis of the trunk to the edge of the canopy) was 9.42 m, ranging from 6.92 to 13.50 m.

The collection of seeds (achene dry fruit) began on October 20, 2014, when the seeds had their structure formed and natural dispersion was initiated (Figure 1A and B). Collection lasted until to the end of the natural seed drop from the selected matrices.

Initially, the achenes (containing seeds or not) extracted from the infructescences (Figure 2A) were rubbed on circular 1.8 and 1.6 diameter sieves (Figure 2C and D) to remove the *papus* (structure that helps dispersion) and then processed in seed blowers (general model - Elo's) (Figure 2B) to remove impurities. They were homogenized afterwards through the manual method (ISTA, 2015).

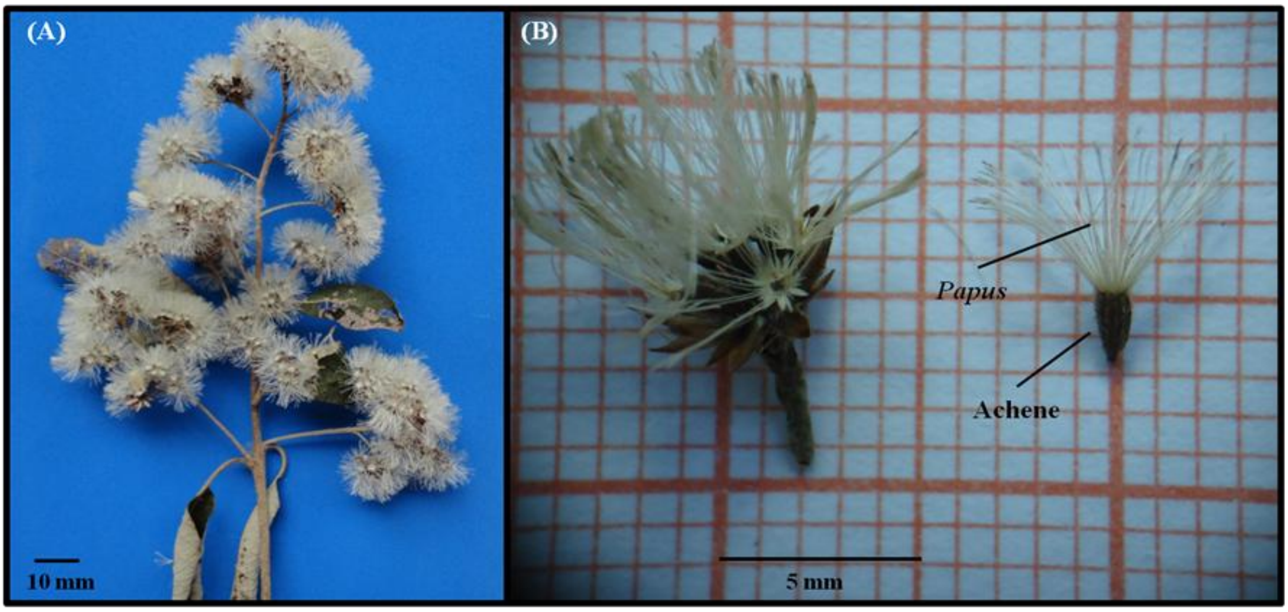

Figure 1. Characteristics of the branches and infructescence of Vassourão-preto at the moment of natural dispersion: (A) branch with 100% of the seeds ready for dispersion and (B) detail on the infructescence at the natural dispersion point and the achene + *papus*.

The mechanical processing study was conducted using one 20.0 g sample of achenes per treatment. Different combinations of blower calibrations were tested to remove most impurities from the samples, leaving only the achenes, or reproductive units, which are considered seeds. For this, combinations were tested for the opening of the calibration valve (positions 0, 5, 7.5, 10, and 12.5) with side air passage (0, 1, 2, 2.5, and 3 360° turns) (Figure 2B).

Figure 2. Steps of processing Vassourão-preto seeds: (A and C) rubbing on a circular sieve with 1.8 mm diameter, (D) rubbing on a circular sieve with 1.6 mm diameter, (B and E) passage through the blower with valve calibration and side air passage for the removal of impurities, and (F) detail of the seeds obtained after the entire process.

Seeds with no processing (achene + *papus*) and seeds at each processing treatment stage were submitted to the following evaluations:

Physical purity analysis: With the aid of a magnifying glass and transmitted light, samples of 3,000 g of seeds per treatment were separated into two components (pure seeds and inert material), and the amount of pure seeds per weight of working sample was expressed as a percentage. Achenes with or without *papus* (Figure 1B and Figure 3A) and pieces of achenes bigger than half their original size, except when evident that they did not have any seeds, were considered pure seeds, according to the International Rules for Seed Testing (ISTA, 2015).

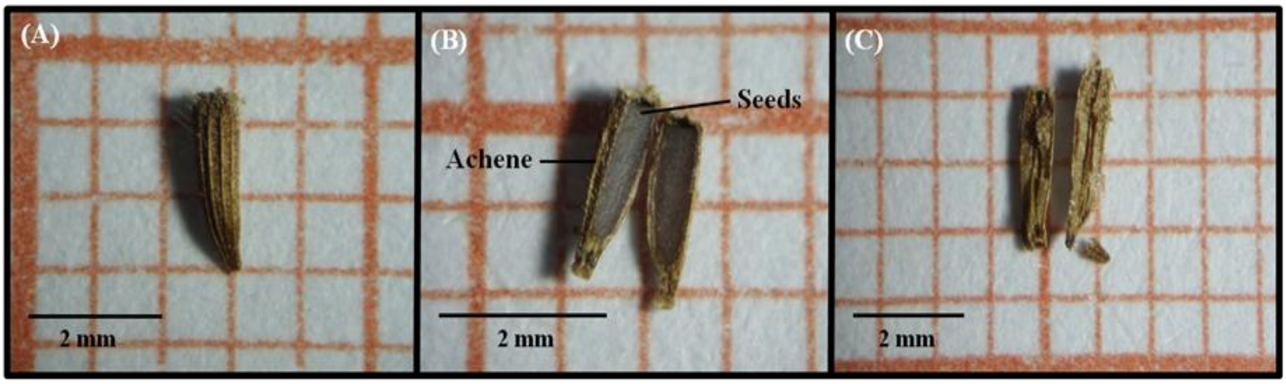

Figure 3. Characteristics of the Vassourão-preto seeds: (A) achene (seed), (B) achene with seed, and (C) empty achene.

Detection of full samples: Four repetitions of 25 pure seeds (achenes) per treatment were observed with a stereomicroscope with the help of a sectioned-in-half scalpel, and the presence or absence of seeds inside the achene was checked (Figure 3B and C). The percentage of full seeds per treatment was computed.

Weight of one thousand seeds: Eight subsamples containing 100 pure seeds each were used. They were weighed on a balance scale with four decimals, following the recommendations of the International Rules for Seed Testing (ISTA, 2015). The weight of one thousand seeds was calculated by multiplying the average mass obtained in the subsamples of 100 seeds by 10, and a variation coefficient (< 6%) was adopted (ISTA, 2015).

Determination of the seeds water content: Seed water content was determined before and after the purity analysis by the greenhouse method at 103 ± 2°C for 17 hours (ISTA, 2015). There were two replications of approximately 0.500 g per treatment.

Germination test: Germination tests were carried out before and after the purity analysis. There were four replications of 50 seeds sown in transparent plastic boxes (11.0 x 11.0 x 3.5 cm) on two blotter sheets of paper and humidified with 2.5 times the mass of the dry substrate using water. The seeds were germinated at 25°C with constant light, and the counts were done on the 13^{th} and 29^{th} days after sowing (Grzybowski, Silva, Vieira, & Panobianco, 2016).The percentage of normal seedlings was computed.

The experimental design was entirely randomized with four replications, and the data was processed in a variance analysis and the averages were compared by the Scott-Knott test ($p \leq 0.01$). The water content data and the purity analysis were not analysed statistically.

Results and discussion

The processing of seeds is a crucial step in the production chain because it aims to improve batch characteristics, improve the physical attributes, preserve the germination percent and avoid pests and seed diseases (Araujo, Araujo, Zonta, Vieira, & Donzeles, 2011; Santos Neto et al., 2012; Santos, 2015).

For Vassourão-preto seeds, it is very important to remove the *papus* when handling the harvested seeds, that is, in the evaluation of the physiological quality, determining water content, during storage and when using to produce seedlings. This goes beyond eliminating possible pests and fungi that might have adhered to the structure.

The water content, germination and first count data from the germination test on Vassourão-preto seeds without processing and after processing was carried out on seedlots from different calibrations of the seed blower that consisted of combinations of openings of the calibration valve (CV) and the side air passage (SAP). These are presented in Table 1. One can observe that the water content values in the tested processing treatments were close, ranging from 8.3 to 10.7%, and the non-processed seeds had a higher water content (11.2%) but were not expressly different from some treatments, which was an important fact for the execution of the other evaluations of physiological quality. The high water content of the ones without processing is justified, given they absorb extra water in the natural dispersion structures (*papus*).

Regarding feasibility of the processed seeds (Table 1), it was seen that seven combinations of the blower's adjustments enabled a high germination percentage (approximately 90%), an important fact because usually the seeds of native forest species present a low germination percentage, with few species reaching 80% (Wielewicki, Leonhardt, Schlindwein, & Medeiros, 2006). In a study on the maturation process of Vassourão-preto, Grzybowski et al. (2016) obtained 57% germination at physiological maturity, which is theoretically the species' maximum germination. It was observed that the non-processed seeds and some tested treatments were negatively different and were classified as worse quality (Table 1).

Table 1. Average water content, germination and first count of the germination test of Vassourão-preto seeds without processing and after processing. Processing consisted of combinations of calibration valve openings - CV (position 0, 5, 7.5, 10 and 12.5) and the side air passage – SAP (0, 1, 2, 2.5, and 3 360° turns) of the blower.

Processing methods	Water content	Germination	First count
	....	%	....
No processing	11.2	42 d	30 b
CV 0 + SAP 2 360° turns	10.7	38 d	24 b
CV 0 + SAP 2.5 360° turns	9.2	42 d	27 b
CV 0 + SAP 3 360° turns	9.4	82 b	50 a
CV 5 + SAP closed	9.5	38 d	17 b
CV 5 + SAP 1 360° turns	10.4	45 d	26 b
CV 5 + SAP 2 360° turns	9.0	64 c	34 b
CV 5 + SAP 2.5 360° turns	9.1	82 b	49 a
CV 5 + SAP 3 360° turns	8.8	92 a	50 a
CV 7.5 + SAP closed	9.2	54 c	36 b
CV 7.5 + SAP 1 360° turns	8.3	77 b	36 b
CV 7.5 + SAP 2 360° turns	9.1	90 a	55 a
CV 7.5 + SAP 2.5 360° turns	9.1	79 b	43 a
CV 7.5 + SAP 3 360° turns	8.3	93 a	45 a
CV 10 + SAP closed	8.7	91 a	54 a
CV 10 + SAP 1 360° turns	8.4	81 b	42 a
CV 10 + SAP 2 360° turns	8.6	89 a	47 a
CV 10 + SAP 2.5 360° turns	8.6	85 b	51 a
CV 10 + SAP 3 360° turns	8.4	89 a	49 a
CV 12.5 + SAP closed	8.6	94 a	58 a
C.V. (%)	-	11.78	36.85

Means followed by the same letter in the column do not differ statistically according to the Scott-Knott test ($p \leqslant 0.01$).

This study reveals that defining processing methods for native forest seeds enables the improvement of the harvested batch, which helps in commercial production because it reduces costs during the handling of seeds, avoids the loss of input and produces seedlings with better potential and good answers in the field (Santos, 2015).

The evaluation of seed vigour, carried out on the first count of the germination test (Table 1), confirmed the results found for viability, which showed that the non-processed seeds were classified as lower quality, and the best performing treatments in the germination test also had high vigour.

To confirm the procedures carried out in processing, a purity analysis of the tested treatments was performed (Table 2) with the objective of determining a percentage composition of the mass of the sample components, the identification of seeds, inert material and, inferentially, the composition of seed batches (ISTA, 2015).

It can be seen that the purities of the samples were close, with 93.9 to 97.0% purity. Only the combination of the calibration valve closed with two 360° turns of the side air passage had lower purity, approximately 75% (Table 2). The physiological quality of pure seeds, obtained from each processing treatment, was evaluated to define the processing parameters.

When evaluating the percentage of full seeds, some treatments had higher percentages compared to the control (non-processed) and treatments with smaller air passages (Table 2).

Regarding the weight of one thousand seeds, the treatments with adjustments CV 7.5 + SAP 3 360° turns, CV 10 + SAP 2.5 360° turns, CV 10 + SAP 3 360° turns and CV 12.5 + SAP closed were classified with higher masses. The average weight of one thousand seeds of Vassourão-preto ranged from 0.491 g to 0.513 g, with the highest weight obtained in the combination CV 10 + SAP 3 360° turns treatment (Table 2).

Based on the weight of one thousand seeds, we can suggest the working size sample. The working sample for the purity analysis must have at least 2,500 seeds (ISTA, 2015). Therefore, the working sample mass for Vassourão-preto was calculated by multiplying the average weight of one thousand seeds (0.5003 g) by 2.5, suggesting a working sample of Vassourão-preto seeds to be approximately 1.3 g. This ensures that the sample does not have a number of seeds less than 2,500 since the values used were the ones from treatments with a higher mass.

Table 2. Purity percentage, full seeds and weight of one thousand seeds (WOTS) of non-processed Vassourão-preto seeds and seeds after processing, using combinations of calibration valve openings - CV (position 0, 5, 7.5, 10, and 12.5) and the side air passage – SAP (0, 1, 2, 2.5, and 3 360° turns) of the blower.

Processing methods	Purity (%)	Full seeds (%)	WOTS (g)
No processing	-	54 e	0.429 f
CV 0 + SAP 2 360° turns	75.4	53 e	0.316 i
CV 0 + SAP 2.5 360° turns	93.9	51 e	0.333 h
CV 0 + SAP 3 360° turns	94.7	75 c	0.426 f
CV 5 + SAP closed	94.4	38 f	0.316 i
CV 5 + SAP 1 360° turns	95.1	51 e	0.333 h
CV 5 + SAP 2 360° turns	95.6	66 d	0.412 g
CV 5 + SAP 2.5 360° turns	94.2	89 b	0.447 e
CV 5 + SAP 3 360° turns	95.2	99 a	0.472 c
CV 7.5 + SAP closed	95.1	75 c	0.411 g
CV 7.5 + SAP 1 360° turns	95.0	90 b	0.432 f
CV 7.5 + SAP 2 360° turns	95.1	95 a	0.463 d
CV 7.5 + SAP 2.5 360° turns	95.0	96 a	0.464 d
CV 7.5 + SAP 3 360° turns	94.9	100 a	0.491 b
CV 10 + SAP closed	95.5	98 a	0.478 c
CV 10 + SAP 1 360° turns	96.0	99 a	0.473 c
CV 10 + SAP 2 360° turns	95.3	100 a	0.479 c
CV 10 + SAP 2.5 360° turns	95.9	100 a	0.499 b
CV 10 + SAP 3 360° turns	96.0	100 a	0.513 a
CV 12.5 + SAP closed	97.0	100 a	0.498 b
C.V. (%)	-	7.31	2.93

Means followed by the same letter in the column do not differ statistically according to the Scott-Knott test ($p \leq 0.01$).

The definition of the parameters for the conduction of the purity analysis is important because it is usually the first analysis performed with the sample of seeds received by the laboratory. Parameter definitions are also important for enabling the evaluation of the procedures and harvest methods, assessing the efficiency of the handling and processing process, and establishing values for payment in commercialization and information for research (Lima Junior, Martins, Groth, & Lopes, 2015).

The pure seeds from each processing treatment were also submitted to water content, germination and vigour tests (Table 3). We see that, once again, the humidity values were close, and this is very important for the tests. The uniformity of water content is crucial for the standardization of the evaluations and to obtain consistent results (Marcos-Filho, 2015).

Table 3. Average water content, germination and first count of the germination test of pure seeds of Vassourão-preto without processing and after processing, using combinations of calibration valve openings - CV (position 0, 5, 7.5, 10, and 12.5) and the side air passage – SAP (0, 1, 2, 2.5, and 3 360° turns) of the blower.

Processing methods	Water content	Germination	First count
	....	%	....
No processing	9.4	35 e	26 d
CV 0 + SAP 2 360° turns	9.7	33 e	21 d
CV 0 + SAP 2.5 360° turns	9.3	46 d	36 d
CV 0 + SAP 3 360° turns	8.8	76 b	63 c
CV 5 + SAP closed	9.6	42 d	30 d
CV 5 + SAP 1 360° turns	9.9	41 d	29 d
CV 5 + SAP 2 360° turns	9.2	65 c	56 c
CV 5 + SAP 2.5 360° turns	8.3	79 b	60 c
CV 5 + SAP 3 360° turns	8.3	96 a	90 a
CV 7.5 + SAP closed	8.6	64 c	40 d
CV 7.5 + SAP 1 360° turns	8.7	81 b	57 c
CV 7.5 + SAP 2 360° turns	8.3	87 a	64 c
CV 7.5 + SAP 2.5 360° turns	8.5	92 a	74 b
CV 7.5 + SAP 3 360° turns	8.2	92 a	86 a
CV 10 + SAP closed	8.3	95 a	68 b
CV 10 + SAP 1 360° turns	8.1	88 a	54 c
CV 10 + SAP 2 360° turns	7.4	93 a	72 b
CV 10 + SAP 2.5 360° turns	8.2	95 a	91 a
CV 10 + SAP 3 360° turns	8.5	97 a	88 a
CV 12.5 + SAP closed	8.2	96 a	75 b
C.V. (%)	-	6.82	18.2

Means followed by the same letter in the column do not differ statistically according to the Scott-Knott test ($p \leq 0.01$).

The viability and vigour evaluation of the pure seeds (Table 3) enabled the restriction of adjustments possibilities for the seed blower for mechanical processing. The following combinations stand out: CV 5 + SAP 3 360° turns, CV 7.5 + SAP 3 360° turns, CV 10 + SAP 2.5 360° turns and CV 10 + SAP 3 360° turns.

Based on all procedures and analyses carried out in this experiment, the combination of blower adjustments using the calibration valve on position 10 plus three 360° turns of side air passage was the most efficient at seed processing, allowing the maximum number of pure seeds with high physiological quality.

However, it is important to highlight that, depending on the harvest year, the seeds can present a variable specific weight due to the non-uniformity in maturation (Gadotti, Villela, & Baudet, 2011) because the formation of seeds depends on external and internal factors. Thus, according to the harvest year, the settings of the blower opening can be adjusted for processing Vassourão-preto seeds to get better results.

Conclusion

We conclude that the processing of *Vernonanthura discolor* seeds must be done with a combination of manual processing and mechanical processing, namely, rubbing the seeds on circular sieves of 1.8 and 1.6 mm diameter and then passing them through a seed blower adjusted with a calibration valve in the 10 position plus three 360° turns of side air passage.

The weight of one thousand seeds of *Vernonanthura discolor* is 0.500 g, and the working sample for a purity analysis must be 1.3 g.

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