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ASSESSING THE PERFORMANCE OF TROPICAL FOREST SEEDS ENCAPSULATED WITH GELS FROM *VITIS VINIFERA* AND SODIUM ALGINATE FOR DIRECT SEEDING

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The objective of the study was to evaluate the encapsulation of seeds of tropical forest species with cellulose gel (CG) from *Vitis vinifera* and sodium alginate, with and without biostimulant, in order to optimise its size, shape and performance for direct seeding. Four species used in direct seeding were selected, i.e., *Bixa orellana L., Mimosa bimucronata (DC.) Kuntze, Trema micrantha (L.) Blume and Psidium guajava L.*. The experimental design was completely randomised with eight treatments and four repetitions of 100 seeds each. Encapsulation affected the physical quality of the seeds, however, it facilitated their handling and visualisation in germination tests, especially for *T. micrantha*, with a 4-fold increase in size with alginate gel and 2-fold with CG. Sodium alginate performed better than CG, synchronising the growth of *B. orellana* seedlings. The biostimulant showed potential to synchronise germination, vigor and seedling growth when associated with dormancy break for *M. bimucronata,* and promoted seedling growth for *P. guajava*. Sodium alginate gel has potential for seed enhancement, while CG encapsulation needs to be improved in relation to its physical characteristics, requiring further studies. The biostimulant can be recommended for enhancing uniformity of seed emergence in direct seeding.

Keywords: Seedling establishment, germination, small seeds, coating, seed enhancement

INTRODUCTION

Brazil proposed to recover 12.5 million hectares of tropical forests, assuming international restoration commitments (Guerra et al. 2020). This scenario highlights the need for large-scale efforts of ecosystem restoration requiring more than 3.6, and up to 15.6 thousand tons of native seeds (Bustamante et al. 2019, Urzedo et al. 2020). However, the production of native seeds is far from this goal and involves the adoption of a highly efficient restoration methodology to reduce seed waste and improve seedling establishment (Urzedo et al. 2019).

Direct seeding has been widely used in the restoration of degraded areas (Grossnickle & Ivetic 2017). It consists of using seeds with different life cycles and successional groups, which are sown directly in the soil (Campos-Filho et al. 2013). In a short time, sowed species cover the soil, decreasing the coverage of invasive exotic competitors and facilitating the establishment of seedlings of tree species (Silva et al. 2015). Besides, direct seeding is a technology applicable to rural producers that can contribute to reducing the restoration deficit (Ferreira et al. 2007).

Despite the low cost and facility of planting, direct seeding is limited due to the requirement of a large amount of seeds and low seedling establishment (Ceccon et al. 2016, Grossnickle & Ivetic 2017). The emergence fail due to species with small seeds, predation and unsuitable microsites for germination (Moles & Westoby 2004, Guarino & Sccariot 2014, Grossnickle & Ivetic 2017). Furthermore, seedling survival is affected by competition with grasses and low survival rates (Doust et al. 2006, Palma & Laurance 2015).

Factors such as sowing, planting practices, soil conditions, vegetative cover and seed predation are the main focus in direct seeding, nevertheless, seed quality and emergence are the initial drivers of the restoration trajectory (Grossnickle & Ivetic 2017, Pellizzaro et al. 2017). To overcome these problems, priming and seed coating can be used in large scale direct seeding of crops and horticultural species to uniformise seed size, to facilitate mechanised plantation and to accelerate and synchronise germination in the field by scaling-up seed use for global restoration (Madsen et al. 2016, Pedrini et al. 2020).

The diversity of shape, size and structures in seed coat can be standardised by seed coating, favoring their handle in ecological restoration projects, while uniformising seed size and providing nutrients and hormones to increase germination, as well as protectors to reduce predation losses (Madsen et al. 2016). Encapsulation is similar to seed coating, differing by the use of polymers with emulsifying properties to regulate water changes.

The encapsulating agent, alginate, has been the most used coating, due to its solubility at temperature environment, permeable gel ability, good gelling property, low cost, ease of use and absence of toxicity, and has proven to be proficient in developing artificial seeds (Guerra et al. 1999, Gantait & Sinniah, 2013). Cellulose gel (CG) or cellulose-based hydrogels can be obtained from a natural source and is considered a sustainable product (Kabir et al. 2018, Klemm et al. 2005). They can be prepared from pure and native cellulose by chemical dissolution with LiCl dimethylacetamide (DMAc), N-methylmorpholine-N-oxide (NMMO), ionic liquids (ILs), alkali / urea (or thiourea), or by manufacturing / designed with bacterial cellulose (Shen et al. 2016). This material has many applications and is used in different areas (Kabir et al. 2018). Seed enhancement by encapsulation with biodegradable and hydrophilic polymers associated with biostimulant enables the use of unmanned vehicles for direct aerial seeding in hard-to-reach forest areas, and can reduce the ecological load on the environment (Vovchenko et al. 2020).

In this study, it was hypothesised that assessing seed enhancers is an important contribution to direct seeding in general, as both seed size and germination will be uniformised and increased, and can contribute to reducing seed waste of tropical forest seeds. Thus, the objectives were to develop and evaluate the performance of tropical forest tree seeds encapsulated with biodegradable CG and sodium alginate.

MATERIAL AND METHODS

Target species

Four target species were selected among those previously tested in direct seeding: (a) smaller seeds (> 100,000 seeds kg^{-1}) with low establishment rate (< 20% of success), *Trema micrantha* (Cannabaceae), and high establishment rate (> 80% of success), *Mimosa bimucronata* (Fabaceae), (b) medium-sized seeds (20,000 to 100,000 seeds kg^{-1}) with low establishment rate, *Bixa orellana* (Bixaceae) and high establishment rate, *Psidium guajava* (Myrtaceae) (Table 1). Small seeds were selected because they generally show low survival and establishment rates in direct seeding, compared to medium to large species with greater seed weights and high establishment rates (Moles & Westoby 2004, Doust et al. 2008, Gonzalez-Rodriguez et al. 2011, Wang et al. 2011, Guarino & Scariot 2014, Hossain et al. 2014).

Table 1 Target species for encapsulation seed testing: seed size classification, number of seeds kg^{-1} and temperature (°C) of the germination test in controlled laboratory conditions

Species	Seed size classification	Number of seeds kg^{-1}	Type of dormancy	Seed testing temperature (°C)
Bixa orellana	Medium	31,000	None	30
Mimosa bimucronata	Small	88,500	Tegument impermeability	30
Psidium guajava	Small	96,900	Tegument impermeability	25
Trema micrantha	Extremely small	625,000	Tegument impermeability	30

Seed encapsulation and germination tests

Germination and encapsulation tests were carried out at the Forest Seedlings and Seedlings Laboratory (LASEM), Federal University of São Carlos, Sorocaba Campus, São Paulo, Brazil. The seeds were classified according to seed size and dormancy type (Table 1). The seeds were prepared before the treatments, T_1 and T_{6A} (Table 2). For *B. orellana*, the aril was removed and washed intensively with running water. The dormant seeds of *M. bimucronata* remained 24 hours in water immersion at temperature 80 °C. *Psidium guajava* seeds were scarified in sand by a magnetic stirrer for 15 minutes and *T. micrantha* was immersed in water at 50 °C for 5 minutes (Tavares et al. 1995, Kramer & Zonetti 2018).

Test was carried out on encapsulating seeds with CG, an agent prepared with cellulose fibre extracted from grape skins (*Vitis vinifera*). This material shows interesting properties related to water absorption. Cellulose was obtained by KRAFT process modification, as proposed by Lu and Hsiesh (2012). The grape skin was submitted to an alcoholic extraction using 92% ethanol solution under constant stirring for 60 minutes at 55 °C, using 0.07 g grape skin for each mL of solution. The extract was then filtered and the solid part dried at 37 °C for 12 hours. Then, a 5% sodium hydroxide solution was used to divide the lignin compounds from the cellulosic compounds, using a proportion of 0.09 g from biomass to each mL of alkali solution.

Evaluation was also carried out on sodium alginate, a gel applied for aerial seeding by seed balls (Mohamed 2020). A sodium alginate dispersion was prepared in a 2% m/v proportion, using distilled water as solvent. To obtain the capsules, aluminum chloride hexahydrate solution at 30% m/v was used as a complexing agent. The cellulose gel was obtained by dispersing the cellulose fibres extracted from grape skin in NaOH/urea solution at 12 °C under constant stirring. Then, the dispersion was heated up to 40 °C to increase the viscosity and improve the gel formation.

The encapsulation process was carried out with CG and sodium alginate dispersion individually (Figure 1). In both cases, the seeds were transferred to a beaker containing the gel and the dispersion, under manual stirring (Figure 1, 3rd step). To form the capsules, a Pasteur pipette with 5 mm diameter was used to transfer the seeds encapsulated by the gel into the complex solution (Figure 1, 3rd step). The seeds were dropped one-by-one into the complex solution and left for one hour. Then, the capsules were filtered and dried on filter paper for 24 hours at room temperature (Figure 1, 4th step).

Besides the polymers, evaluation was carried out on the presence or absence of the commercial biostimulant (Stimulate®), a growth regulator composed of 0.09 g L^{-1} of kinetin, 0.05 g L^{-1} of gibberellic acid and 0.05 g L^{-1} of 4-indole-3-butyric acid. As control (T_1 and T_{1A}), seeds of all species were used with and without breaking dormancy or pre-preparation (Table 2). For T_{1A} control, the species were not subjected to any treatment. In treatments with biostimulant, previous tests were carried out and the results were applied in this study. The product was manually mixed in order to incorporate all the seeds without leaving any residues. Thus, 0.3 ml was used for 100 seeds of *B. orellana*, *M. bimucronata*, *P. guajava* and 0.1 ml for *T. micrantha*.

Table 2 Treatments applied in the encapsulation essay of forest seeds used in direct seeding

Treatment code	Treatment description
T_1	Control with seed pre-preparation
T_{1A}	Control without pre-preparation**
T_2	Encapsulation with cellulose gel of *Vitis vinifera* (CG)
T_3	Encapsulation with cellulose gel of *Vitis vinifera* (CG) + germination biostimulant Stimulate®
T_4	Encapsulation with alginate gel
T_5	Encapsulation with alginate gel + germination biostimulant Stimulate®
T_6	Germination biostimulant Stimulate® without pre-prepare**
T_{6A}	Germination biostimulant Stimulate® with seeds pre-prepared

**No break dormancy or no seed preparation of *Bixa orellana*

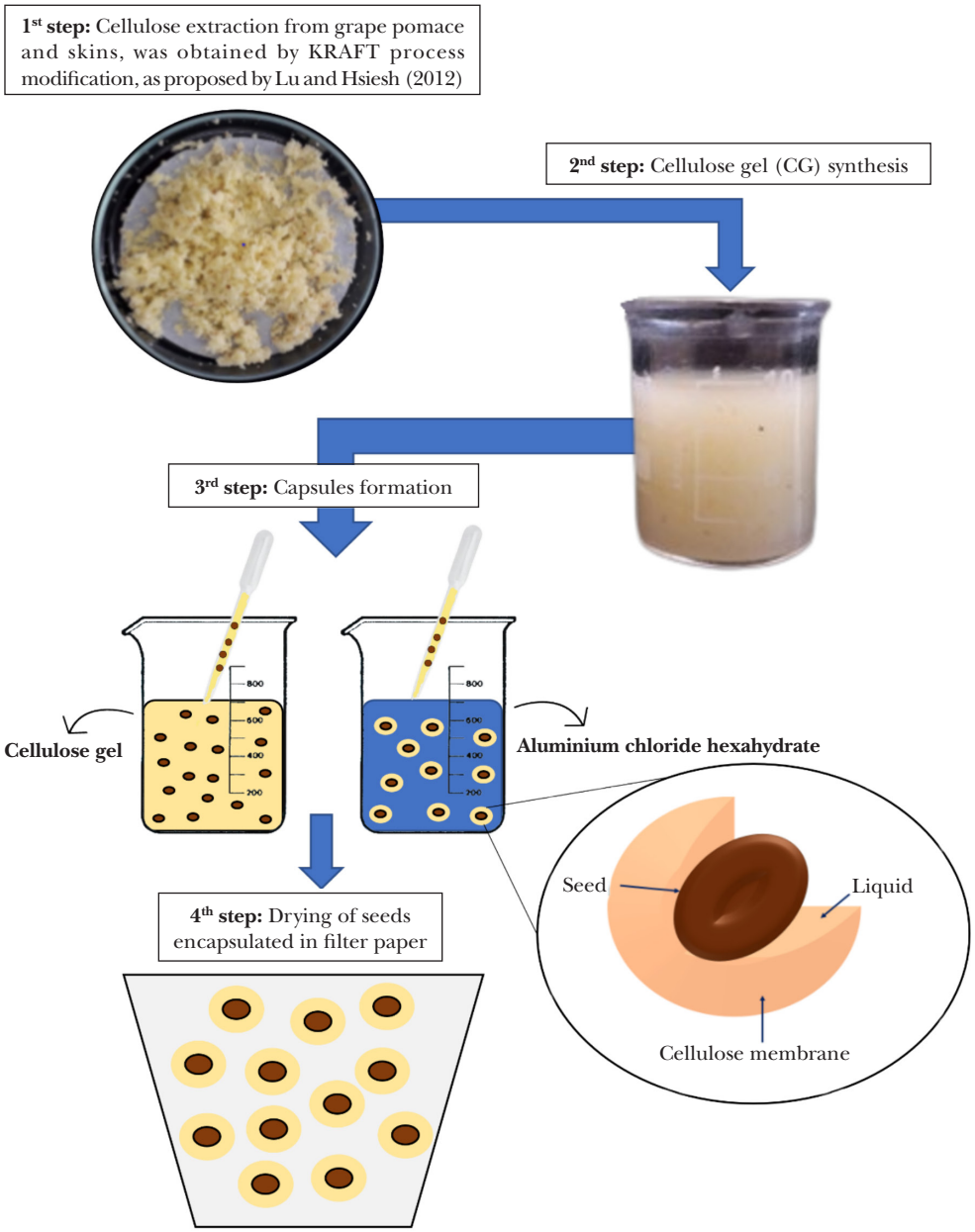

Figure 1 Flowchart of the encapsulation process of the four species of tropical forests

For T_4 and T_5, sodium alginate was applied, and the encapsulation process was the same as used for CG. The effect of Stimulate® was tested to improve seed germination of all species (T_6) and to evaluate seed pre-preparation and dormancy break for *M. bimucronata*e and *P. guajava* (T_{6A}). Changes in size and shape of 10 seeds/species were evaluated by measuring length (larger size) and width (smaller size) before and after the encapsulation treatments.

Germination and physical analysis was adapted from Brasil (2013) and Mori et al. (2012). The germination tests were carried out in the laboratory; four replicates of 25 seeds each was adopted for all treatments and species. Vermiculite was used as a substrate placed in Gerbox® for *B. orellana* and *M. bimucronata*, and in a petri dish for *T. micrantha*, moistened in a proportion of 2:1 (v:v) of substrate and water. For *P. guajava*, filter-paper was used as substrate in a petri dish. To evaluate seed germination, the radicle protrusion (> 1 mm) was used as a criterion.

After the emergence of the first pair of leaves, the seedlings were measured (aerial part and root) and the dry mass was evaluated for the aerial part (hypocotyl and epicotyl) and roots in an oven at 65 °C for 24 hours. The treatments with encapsulated seeds (T_2, T_3, T_4 and T_5) were not performed as a dormancy break test because a condition similar to soil seed bank, in direct seeding, was adopted.

Data analysis

The number of germinated seeds per treatment was counted every day, to calculate the percentage of seed germination and vigor by the speed of germination index (IVG) (Ranal et al. 2009). Seedling vigor was evaluated by the length and dry mass of the aerial part and roots of the seedlings (Ranal et al. 2009). To assess the normality of the data, the Shapiro-Wilk test and residual plotting were applied to verify the data distribution. In the germination analysis, a completely randomised experimental design with four replications was applied, and the Kruskal-Wallis test was performed, complemented by Dunn's post-test to verify the differences between the treatments at a probability level of 5%. Treatments, species and replications that did not germinate were excluded from the analysis of variance. Regarding the other parameters (number and percentage of germinated seeds, IVG, seed size, seedling length and dry mass), exploratory data analysis was performed with boxplots and average graphs. All analyses were realised with the R software (R Core Team 2021).

RESULTS

The two polymers increased the seed size (Figure 2). The alginate gel provided the greatest size increament in all the four species studied. *Trema micrantha* increased four times its dimension (5.8 ± 1.4 mm²), compared to the seeds without encapsulation (1.3 ± 0.2 mm²). For the same species, the CG represented an expansion of more than two times its size (2.9 ± 0.6 mm²) (Figure 3).

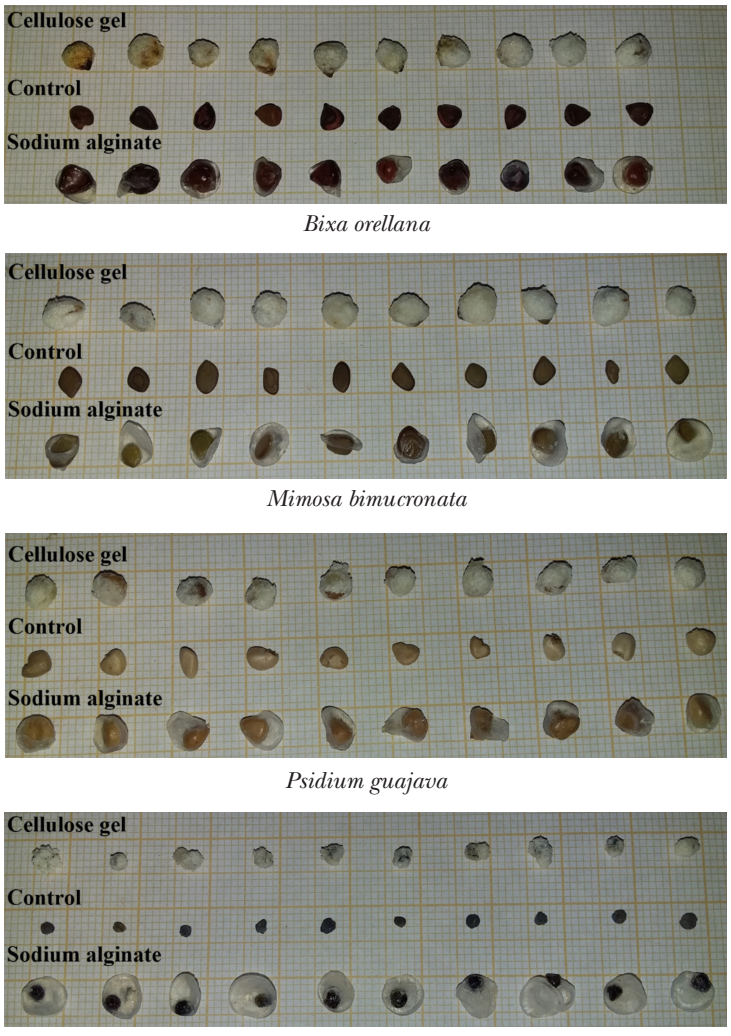

Figure 2 Visual comparison of seeds encapsulated with cellulose gel (CG) and sodium alginate with non-encapsulated seeds (control)

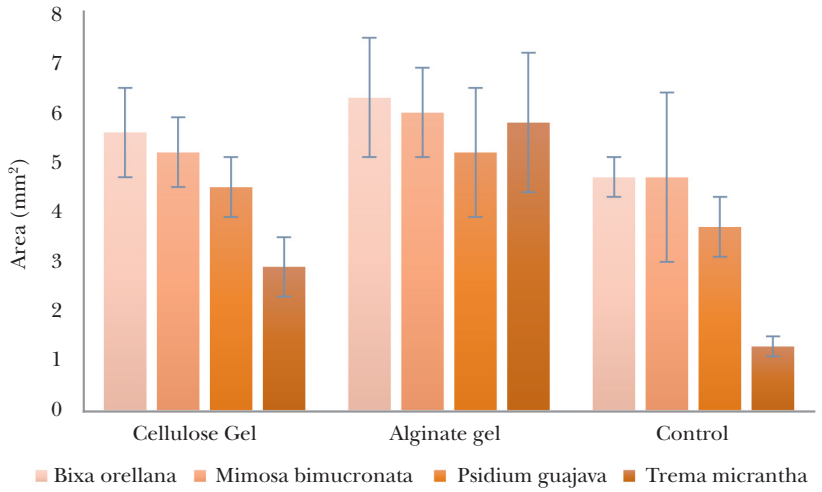

Figure 3 Area (mm²) and error bar of encapsulated seeds of the species with *Vitis vinifera* cellulose gel (CG) and alginate gel compared to seeds without encapsulation

Due to the low germination rate, *T. micrantha* was excluded from the analysis. Although no significant difference was reported between the treatments for germination of *B. orellana* ($X^2 = 8.69$, $p = 0.1915$), higher germination was observed in T_1 and T_4 (Figure 4). There was a high data dispersion between the replicates, mainly in T_1, which may have influenced the residue, reducing the effect of treatments on the analysis of variance. For vigour, there was a significant difference of IVG ($X^2 = 13.75$, $p = 0.032$) between controls (T_1 and T_{1A}) and encapsulated seeds. The encapsulation with biostimulant and alginate (T_5) affected germination similar to CG (T_2 and T_3) (Figure 4).

For *M. bimucronata*, breaking dormancy was more significant on seed germination ($X^2 = 19.30$, $p = 0.007$) than the encapsulation, mainly when associated with biostimulant (T_{6A}). The dormancy break showed less data dispersion (T_1 and T_{6A}) due to germination uniformity among replications. The encapsulation of *M. bimucronata* reduced seed vigour significantly ($X^2_{IVG} = 21.26$, $p = 0.003$). There was a positive association between the biostimulant and dormancy breaking, increasing and synchronising seed germination and vigour (T_{6A}).

Encapsulation inhibited seed germination of *P. guajava*. The CG (T2, T3) allowed slight seed germination compared to alginate gel (T_4 and T_5) (Figure 4 and Table 3). Despite the variations between the replications of the same treatment (Figure 4), there was a significant difference ($X^2 = 29.82$, $p = 0.0001$). The biostimulant significantly favored vigour, mostly when compared to control with dormancy break (T_1). The CG and alginate gel, and the biostimulant, reduced seed germination compared to controls with (T_1) and without dormancy break (T_{1A}). The result points out a potential effect of encapsulation and biostimulant inhibiting germination, but not the vigor of non-scarified seeds of *P. guajava*.

Regarding seedling vigour, there was a significant difference between *B. orellana* seedlings in aerial part length ($X^2 = 12.9$, $p = 0.04$) and roots ($X^2 = 25.9$, $p = 0.002$). There was no effect of the treatments on shoot dry mass ($X^2 = 9.03$, $p = 0.171$), but on root dry mass ($X^2 = 24.32$, $p = 0.004$) (Figure 3). The CG induced an increasing non-uniformity of length of the aerial part and a reduction in the root length, mainly associated with the biostimulant (Figure 5).

For *M. bimucronata*, the T_5 was excluded from the analysis because only one seedling survived (Figure 6). An effect was observed only on the length of the aerial part ($X^2 = 29.02$, $p = 0.0001$) and roots ($X^2 = 24.96$, $p = 0.0007$) with no significant effect in mass accumulation in aerial part ($X^2 = 13.48$, $p = 0.06$) and roots ($X^2 = 13.09$, $p = 0.051$). The growing of aerial part of CG-encapsulated seeds (T_2 and T_3) differed and were non-uniform compared to controls (T_1 and T_{1A}), and did not differ from the other treatments with alginate (T_4) and biostimulant, without breaking dormancy (T_6) (Figure 6). The use of biostimulant with dormancy break was similar to controls and uniformised the growing of aerial part, more than roots.

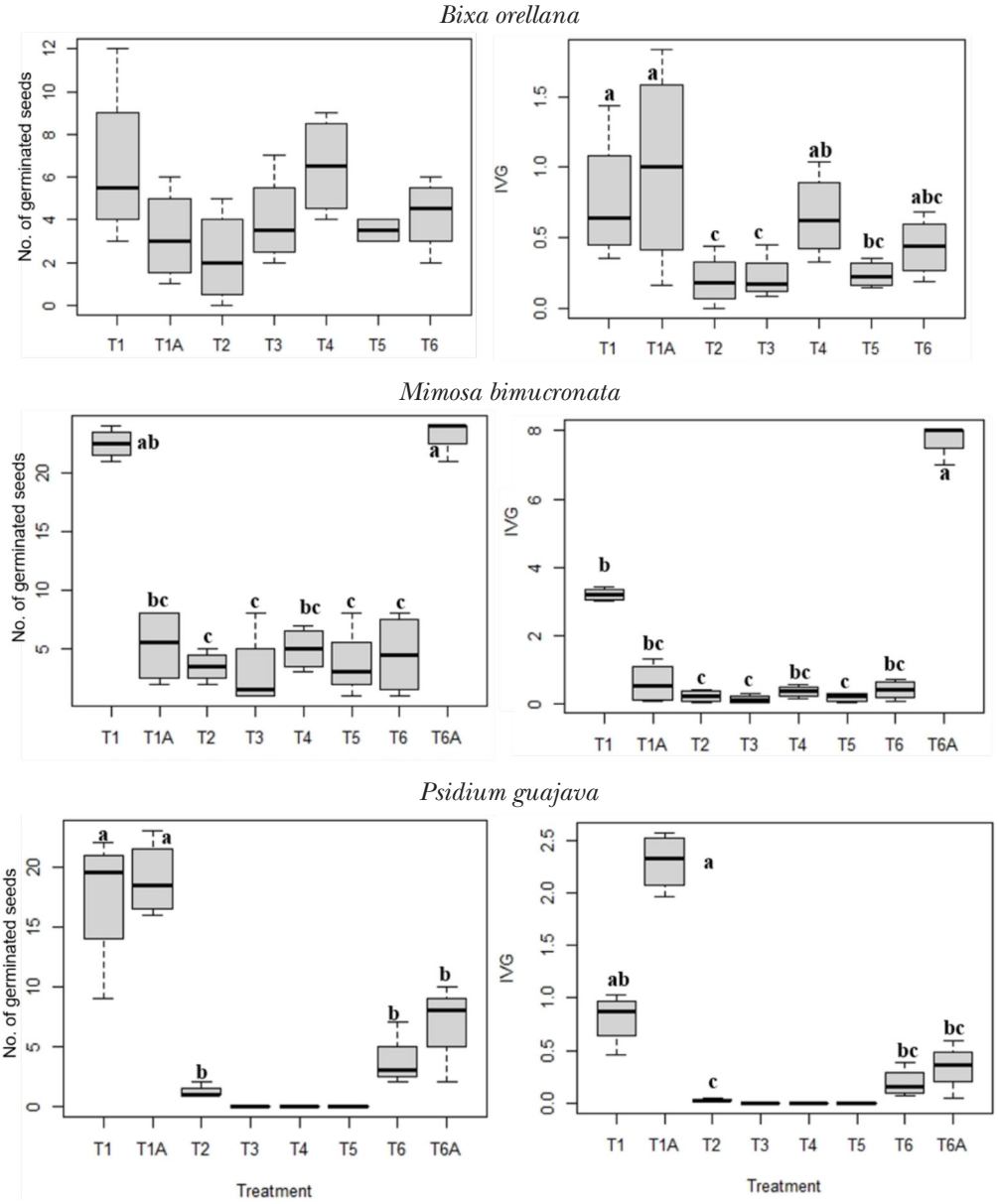

Figure 4 Boxplot of the median and quartiles of the number of germinated seeds and the speed of germination index (IVG) for each species in the different treatments; same letters do not have significant differences by Dunn's post-hoc test ($p < 0.05$) for each of the studied variables

For *M. bimucronata*, T_5 was also excluded from the analysis due to the survival of only one seedling (Figure 4). An effect was observed only on the length of the aerial part ($X^2 = 29.02$, $p = 0.0001$) and roots ($X^2 = 24.96$, $p = 0.0007$). Both the treatment with cellulose gel (T_2) and biostimulant (T_3) differed from the control without dormancy break, and did not differ from the other treatments with encapsulation, in the length of the aerial part (Figure 6). There were no significant differences for aerial dry mass ($X^2 = 13.48$, $p = 0.06$) and roots ($X^2 = 13.09$, $p = 0.051$).

In the case of *P. guajava*, there were no healthy seedlings to carry out measurements in the treatments of CG and alginate gels, therefore, they were excluded from the analysis (Figure 7). There was a significant difference in the length ($X^2 = 56.49$, $p < 0.0001$) and uniformity of roots elongation promoted by biostimulant and dormancy break (T_{6A}), which did not interfere in root mass accumulation. For the dry mass of the aerial part, the significant difference was obtained only between T_1 and T_6 ($X^2 = 56.71$, $p < 0.0001$).

Table 3 Mean germination in percentage and speed of germination index (IVG) for each species in the different treatments

Treatments	*Bixa orellana*		*Mimosa bimucronata*		*Psidium guajava*		*Trema micrantha*	
	%G	IVG	%G	IVG	%G	IVG	%G	IVG
T_1	24 ± 13.4	0.76 ± 0.47	90 ± 5.2	3.21 ± 0.18	75 ± 23.2	0.8 ± 0.25	0	0
T_{1A}	13 ± 8.8	1.00 ± 0.73	21 ± 12.8	0.61 ± 0.59	72 ± 12.6	2.3 ± 0.27	1 ± 2	0.004 ± 0.009
T_2	10 ± 8.8	0.20 ± 0.18	14 ± 5.2	0.64 ± 0.17	5 ± 2.0	0.03 ± 0.01	1 ± 2	0.01 ± 0.011
T_3	13 ± 8.6	0.21 ± 0.15	12 ± 13.4	0.14 ± 0.11	0	0	0	0
T_4	25 ± 9.5	0.65 ± 0.30	20 ± 7.3	0.38 ± 0.17	0	0	0	0
T_5	13 ± 2.3	0.23 ± 0.09	17 ± 11.9	0.20 ± 0.12	0	0	0	0
T_6	16 ± 6.8	0.63 ± 0.21	18 ± 14.0	0.41 ± 0.28	15 ± 8.8	0.2 ± 0.13	1 ± 2	0.01 ± 0.02
T_{6A}	-	-	93 ± 6.0	7.50 ± 0.50	28 ± 13.8	0.3 ± 0.20	-	-

T_1 = control with preparation to uniformise germination, T_{1A} = control without preparation to uniformise germination, T_2 = encapsulation with *Vitis vinifera* cellulose gel (CG), T_3= encapsulation with *Vitis vinifera* cellulose gel (CG) + germination biostimulant (Stimulate®), T_4 = encapsulation with alginate gel, T_5 = encapsulation with alginate gel + germination biostimulant (Stimulate®), T_6 = germination biostimulant (Stimulate®), T_{6A} = germination biostimulant (Stimulate®) + preparation to uniformise germination, - = treatment not applied

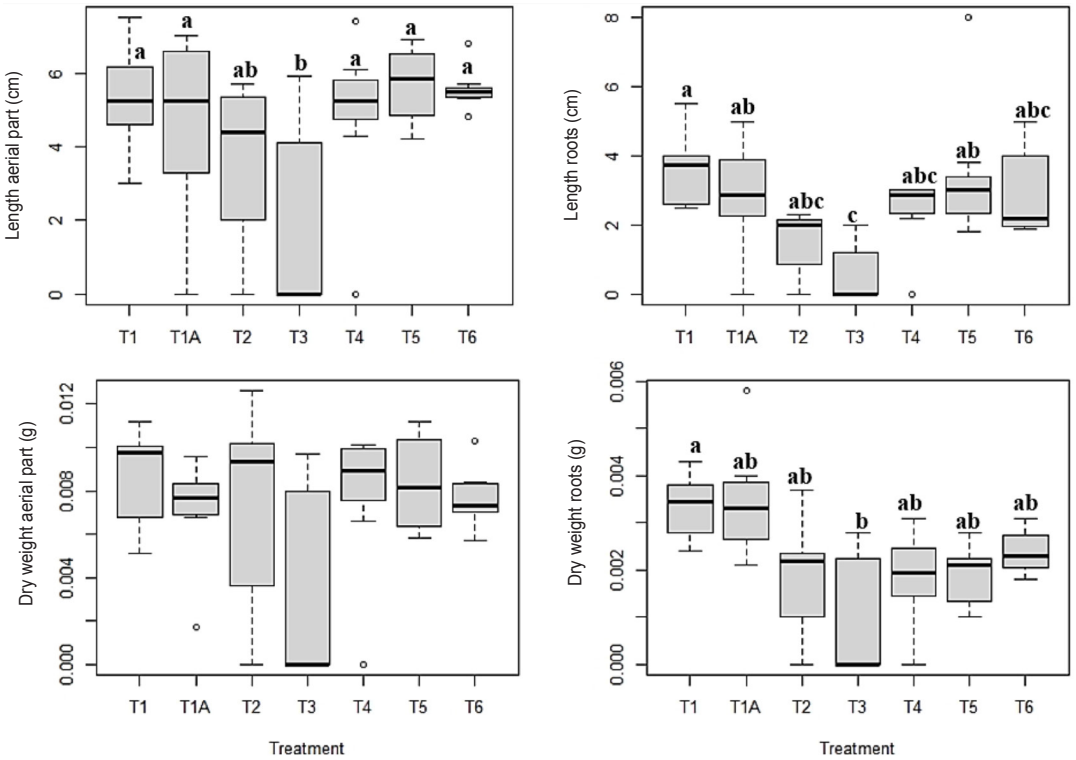

Figure 5 Boxplots of the median and quartiles of the length (cm) and dry mass (g) of aerial part and roots of seedlings of *Bixa orellana*; same letters do not have significant differences by Dunn's post-hoc test ($p < 0.05$) for each of the studied variables

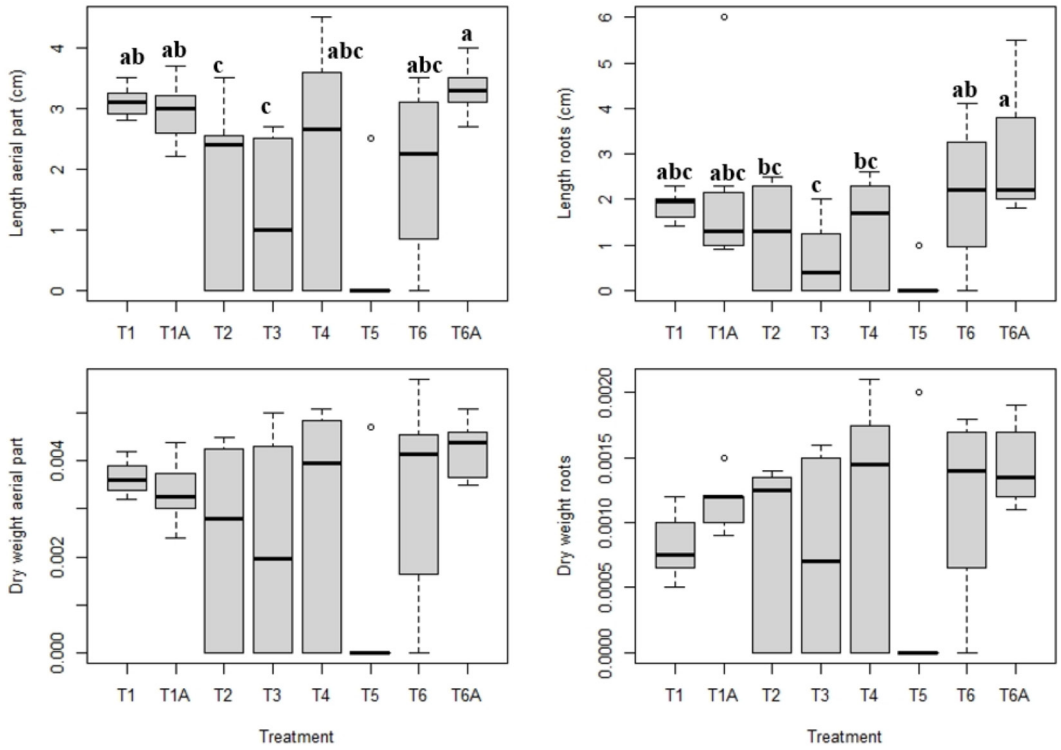

Figure 6 Boxplots of the median and quartiles of the length (cm) and dry mass (grams) of aerial part and roots of seedlings of *Mimosa bimucronata*; same letters do not have significant differences by Dunn's post-hoc test ($p < 0.05$) for each of the studied variables

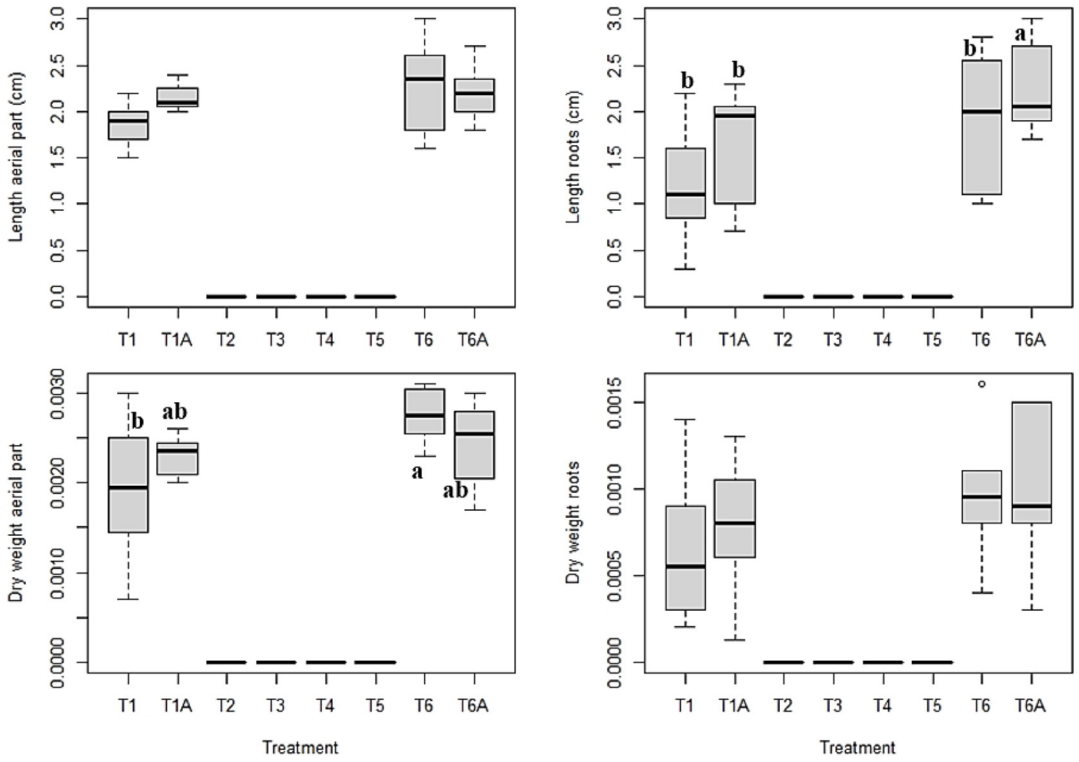

Figure 7 Boxplots of the median and quartiles of the length (cm) and dry mass (g) of aerial part and roots of seedlings of *Psidium guajava* in the different treatments; same letters do not have significant differences by Dunn's post-hoc test ($p < 0.05$) for each of the studied variables

DISCUSSION

In forest restoration, encapsulation is a technique with high potential for aerial seeding with capsules containing seeds mixed to repellents, fungicides, organic substrate, agglutinating substances and fertilisers to improve seed viability (Mohamed 2020, Vovchenko et al. 2020). Furthermore, it facilitates seed management and permits mechanisation of seed sowing. In the present study, encapsulation favored seed management and visualisation in germination tests, *Trema micrantha* had the highest increase in seed size, mostly with alginate. The CG also promoted an increase in seed size, but inferior to the alginate. Seed size is essential for direct seeding since small seeds are those that present more problems in field emergence (Palma & Laurance 2015, Ceccon et al. 2016). The expansion of the size of small seeds can facilitate and allow the use of species that have this characteristic, mainly in restoration by direct seeding (Pedrini et al. 2020).

The germination of *B. orellana* seeds was indifferent to treatments. Encapsulation with alginate and isolated biostimulants increased germination speed (IVG), similar to control, while CG reduced germination speed. In the analysis, *B. orellana* seeds showed low viability, but the same vigor (Table 3) of the highly viable *P. guajava*, and the encapsulation with alginate gel favored the performance of seed vigor and synchronised *B. orellana* germination. Since, in the direct seeding, field emergence is more related to seed vigour than germination and low vigour can prevent seedling emergence, encapsulation with alginate is an alternative (Madsen et al. 2016, Wendt et al. 2017).

Many native forest species show high variability in seed quality with values below 45–50% (Schmidt et al. 2018, Urzedo et al. 2020). At the same time, variation between replicates is a common problem in forest seed analysis (Piña-Rodrigues et al. 2015). The sample variability occurred due to matrix mixture and high natural genetic diversity of natural populations. Besides, the large seed size of many species and their irregular seed production require sample-size reduction in germination tests, and is frequent in the four repetitions of 25 seeds (Piña-Rodrigues & Freire 2010). This issue highlights the complexity of developing technologies to reduce seed waste and to enable large scale restoration, regardless of seed initial viability.

Among the studied species, *T. micranta* had the lowest seed germination rate, despite the dormancy break. This genus presents species with low and asynchronous germination and field emergence with notorious prolonged dormancy, caused by the presence of tegument with a strong physical barrier (Daws et al. 2002, Rodrigues & Rodrigues 2014). Seed dormancies affect seedling establishment rate, and some species can remain in the seed soil bank for 2 to 4 years after direct seeding (Löf et al. 2004). Species with weak seed dormancy can emerge after a few months, while those with deep seed dormancy emerge and spread out over time, and need to overcome seed dormancy to avoid seed loss by predation (Grossnickle & Ivetić 2017). The small size and deep dormancy indicated that more than increasing seed size, the species requires specific procedures for dormancy break before seed encapsulation.

Without dormancy overcoming, *M. bimucronata* had a reduction in viability and vigor similar to encapsulated seeds, therefore, encapsulation would not be the unique cause of the low performance. When overcoming dormancy was associated with biostimulant, the number of germinated seeds increased, similar to control with dormancy break, while germination speed was significantly accelerated. Thus, overcoming dormancy and biostimulant can provide a faster and more synchronised emergence, also favoring a uniform growth of seedling (Figure 6). The application of the biostimulant favored *M. bimucronata*, promoting uniformity and increasing germination speed. The same was observed for agricultural legume seeds, such as beans and soybeans, which increased vigor in response to the biostimulant Stimulate® (Abrantes et al. 2011, Oliveira et al. 2020).

Although encapsulation affected *M. bimucronata* and *P. guajava* by reducing germination speed and the number of germinated seeds in relation to control (Figure 4), for *P. guajava*, both gels lowered seed germination and vigour, while the biostimulant synchronised seed germination and velocity. The encapsulation may be a physical barrier preventing water and oxygen uptake, a determining condition for the beginning of seed germination (Bewley 1997). As observed in *P. guajava*, the encapsulation inhibited seed germination, and the biostimulant allowed it and uniformised germination and vigour. Many polymers applied in encapsulation can modify

the environment around the seed and affect seed emergence and seedling establishment (Grossnickle & Iveti 2017).

Seed enhancement using film coating, encrusting and pellets, besides facilitating physical manipulation, also boost seed emergence in the field and seedling growth (Madsen et al. 2016). There was an elongation effect of the hypocotyl of *B. arborea* seedlings, increasing the shoot length with no difference in mass (Figure 5). On the other hand, the alginate gel synchronised root growth, independent of the biostimulant, while the biostimulant alone uniformised the hypocotyl and root growth (length and mass) of the seedling. Thus, for *B. orellana*, the encapsulation showed potential to improve the field emergence of vigorous seeds, and favored and standardised the growth of seedlings in direct seeding, mainly the alginate gel. However, due to costs, the application of the biostimulant alone is recommended to improve seed field emergence and boost the establishment of seedlings.

The elongation effect also occurred for seedling growth (aerial part and root length) of *M. bimucronata* (Figure 6). Nevertheless, alginate with biostimulant (T_5) caused mortality of all seedlings. Cellulose and alginate gels provoked asynchronous growth in hypocotyl length, and the biostimulant uniformised such process (Figure 4). More than encapsulation, the biostimulant showed capacity to synchronise and increase seed germination and vigour in *M. bimucronata*, mostly when associated with dormancy break, and revealed a potential to uniformise and promote seedling growth. On the other hand, for *P. guajava*, the absence of seed germination caused by CG + biostimulant (T_3) and by alginate, without (T_4) or with biostimulant (T_5) (Table 3), did not allow seedling emergence (Figure 7). The isolated biostimulant favored the uniformity of seedling mass accumulation, similar to seeds with overcoming dormancy. The association between dormancy break and biostimulant produced longer roots and uniformised root growth (length) and dry mass accumulation.

In temperate and Mediterranean zones, many studies have reported the increase of seed viability and seedling establishment using seed shelters and encapsulation in direct seeding, although this did not guarantee the same performance under all field conditions (Pausas et al. 2004, Madsen et al. 2016, Grossnickle & Ivetić 2017, Vovchenko et al. 2020). Seed protectors studied in tropical zones increased the probability of success (seed emergence and seedling survival), but not seedling growth. Some types of seed shelters, such as capsules and seed bombs, not only increased the success of direct seeding, but also expanded planting costs (Ivetić & Stanković 2015, Ceccon et al. 2016, Grossnickle & Ivetić 2017).

Encapsulation has reduced germination in some species. Some materials incorporated to seeds can affect seed germination, delaying growth and causing unsynchronised seedling emergence (Podlaski et al. 2003, Govinden-Soulange & Levantard 2008, Podlaski et al. 2019). The CG is hydro soluble and hydrophilic and created a thin layer around the seed. This affected seed germination, vigour and seedling development more than alginate. The encapsulation of tropical native seeds using CG is little studied, and no studies have been reported on CG of *V. vinifera*, applied to direct seeding. Positive effects of the native seed encapsulation with sodium alginate were observed by Pradella et al. (1989), providing favorable conditions for germination and development of plants in the soil. In agriculture, it is applied to soybean seeds and does not cause negative effects on plant germination (Duarte et al. 2018).

The biostimulant (Stimulate®) promoted greater seed vigour and, in general, demonstrated potential to improve seedling performance in the field. Likewise, it was found that, regardless of species, it reduced variability, synchronised germination, uniformised vigour, root growth and dry root mass accumulation. The effects provided can increase the viability of direct seeding, since one of the disadvantages of this technique is the great variability in the onset and duration of seed emergence in the field (Ceccon et al. 2016). Thus, the use of shelters or protective biological and chemical assets can play an important role in the success of direct seeding restoration in the tropics, targeting specific challenges that limit plant recruitment in a location, such as variable soil moisture, low soil nutrients, pests and diseases (Gornish et al. 2019).

CONCLUSION

The encapsulation with CG from *V. vinifera* and sodium alginate gels provided an increase in seed size. However, it affected seed germination and vigour, and the seedling development

of *M. bimucronata* and *P. guajava*. The biostimulant (Stimulate®) showed potential to synchronise seed germination and vigor, and to uniformise seedling growth in direct seeding. Encapsulation of *B. arborea* seeds with sodium alginate uniformised emergence and favored seedling performance, indicating a potential application for seed enhancement in direct seeding. Seed encapsulation with CG of *V. vinifera* requires further studies, especially on seedling establishment in field level, to develope a viable technique for ecological restoration through direct seeding in tropical regions.

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