Assessing seed desiccation responses of native trees in the Caribbean

Efisio Mattana1 · Brígido Peguero2 · Alice Di Sacco3 · Wilvin Agramonte2 · Wilkin Rafael Encarnación Castillo2 · Francisco Jiménez2 · Teodoro Clase2 · Hugh W. Pritchard4 · Pablo Gómez-Barreiro5 · Elena Castillo-Lorenzo1 · Marianny Terrero Encarnación2 · Michael J. Way3 · Ricardo García2 · Tiziana Ulian1

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Abstract
Native trees from the Caribbean were tested for seed desiccation responses, by adapting the “100-seed test” protocol. Ninety-seven seed lots of 91 species were collected in the Dominican Republic and tested for germination immediately after collecting, and after drying and moist storage. Seed desiccation sensitivity was assessed as a continuous variable (Viability Loss Index; VLI), based on seed germination values before and after drying. The results were compared with predictions of seed desiccation responses based on seed lot traits (initial moisture content and thousand-seed weight) and with those of published predictive models based on plant and seed traits. VLI could be calculated for seed lots of 40 species. 80% of these seed lots showed consistent results among experiments and predictive models. Issues on the set up of the experiments were discussed, as well as the species for which experimental results and predictions led to contrasting results. Overall, the “100-seed test” confirmed to be an effective tool for assessing seed desiccation responses of a diverse under-investigated woody flora, guiding the seed conservation of trees and their use in reforestation programmes. In addition, by providing new data, it might improve the performance of available predictive models.

Keywords Ex situ conservation · Orthodox seeds · Recalcitrant seeds · Seed collections management · Seed mass · Seed moisture content

Introduction
Trees have a high socio-economic and cultural value (Dawson et al. 2014) and provide a variety of ecosystem services at the species and agroforest levels (Thompson et al. 2011). Despite their importance, it is estimated that over 8000 tree taxa, 10% of the world’s total,
are globally threatened with extinction (https://globaltrees.org/threatened-trees/red-list/).
At the same time, although international conventions and national policies for biodiversity
conservation and climate change mitigation call for increased forest protection, globally
forest cover continues to decline (Löf et al. 2019). In Central and South America, agri-
culture is the main cause of deforestation (FAO 2011), with rates above the world average
(Ceddia 2019). In addition, the Caribbean Islands are small and densely populated, resulting
in intense competition between biodiversity conservation and economic activities such
as agriculture (Lugo et al. 1981).

Although, theoretically, there is no technological reason why a plant species should
become extinct (Smith et al. 2011), challenges should still be addressed to guarantee the
effective conservation of trees (Pritchard et al. 2014). Ex situ conservation through stand-
ard seed banking (i.e. seed storage at—18 °C after drying to ca. 5% of moisture content;
FAO 2014; FAO/IPGRI 1994; Linnington 2003) is considered the best option for long term
preservation of plants, at least for those with desiccation tolerant seeds (Iriondo and Pérez
1999). “Orthodox” (desiccation tolerant) seeds can tolerate drying to a moisture content
(mc%) of ca. 7%, with little to no negative effect on viability, as opposite to “recalcitrant”
(desiccation sensitive) seeds (Roberts 1973), whereas “intermediate” seeds are those that
can tolerate drying to ca. 10% (Hong and Ellis 1996). However, species response to seed
desiccation should be considered as a continuum through the whole spectrum between
the two extremes (i.e. fully desiccation tolerant or sensitive species) and evaluated quan-
titatively (Walters 2015). In addition, intraspecific variability has been reported for this
seed trait, with environmental conditions during seed development affecting the level of
seed desiccation tolerance at dispersal (Daws et al. 2004). Knowledge of seed desiccation
responses is vital in reforestation, as seed storage can be useful in streamlining tree produc-
tion, sharing seeds among nurseries and accumulating seeds for direct seeding (Elliott et al.
2013).

According to recent estimates, 33% of the tree species worldwide are likely to produce
desiccation sensitive seeds (Wyse and Dickie 2018), with the highest percentages recorded
in tropical and subtropical habitats (Pritchard et al. 2014; Tweedle et al. 2003; Wyse and
Dickie 2017). However, little is known on the seed biology of many tropical trees, par-
ticularly regarding their seed storage behaviour (Sacandé et al. 2005). Such issues can be
addressed through the screening of tree species for their seed desiccation physiology and
the development of models to predict their responses (Pritchard et al. 2014).

Seed traits may be used to predict seed desiccation tolerance. As a rule of thumb, des-
iccation sensitive seeds are larger and have a higher moisture content at dispersal. Hong
and Ellis (1996) found that in 94 contrasting species, seeds with a thousand seeds weight
(TSW) lower than 2500 g and a moisture content up to 23% showed orthodox behaviour.
According to Chin et al. (1984), desiccation sensitive seeds have usually a TSW higher
than 500 g and a moisture content ranging from 30 to 70%. These criteria have been used
by the Xishuangbanna Germplasm Bank in China as a decision-making tool in the han-
dleng of species with unknown desiccation sensitivity (TSW–MC model; Lan et al. 2014).

Daws et al. (2006) developed a predictive model for 104 Panamanian tree species in
which seeds with a relatively thin seed coat (measured as seed-coat ratio, SCR), combined
with a large seed mass are likely to be desiccation sensitive. This model was then validated
for 38 African and European woody species (see Pritchard et al. 2014), 71 Australian rain-
forest species (Hamilton et al. 2013), and 101 woody species of tropical southern China
(Lan et al. 2014). Wyse and Dickie (2018) developed a set of models based on boosted
regression trees analysis (BRT) to predict the likelihood of seed desiccation tolerance/sen-
sitivity. These BRT models are informed by available data on seed and plant traits (i.e.
seed mass and plant woodiness), the climate(s) of the species’ distribution and the known seed storage behaviour of the species’ relatives (Wyse and Dickie 2018). As stated by the authors, these BRT models do not intend to replace the seed physiology/biology models (e.g., Daws et al. 2006; Lan et al. 2014), but should rather be used in conjunction with them (Wyse and Dickie 2018).

The most widely used protocol to assess seed responses to multiple desiccation states and subsequent storage at a range of temperatures was developed by Hong and Ellis (1996), assessing seed viability before and after drying, through germination experiments. This approach implies that seed dormancy breaking and germination studies should be carried out before starting a research on seed storage responses (Hong and Ellis 1996). However, this protocol uses thousands of seeds, which may be a constraint when working with wild tree species (Pritchard et al. 2004). In order to overcome this limitation, Pritchard et al. (2004) developed a screening approach which deals with only the effect of drying and short-term storage at the initial mc (%) on seed viability assessed through seed germination, called the ‘100-seed test’. This test was initially developed for palm seeds and then recommended by the Royal Botanic Gardens, Kew as a suitable method for identifying desiccation sensitive seeds in developing ex situ conservation strategies (Gold and Hay 2014) and, as such, adopted worldwide (e.g. Hamilton et al. 2013; Lan et al. 2014).

Even though seed desiccation responses of tree species have been investigated in Central America (e.g., Daws et al. 2005), information is still deficient for the Caribbean Islands. The Jardín Botánico Nacional ‘Dr. Rafael M. Moscoso’ of Santo Domingo (JBSD) in the Dominican Republic is working with the Royal Botanic Gardens, Kew for the conservation of the threatened forest of Hispaniola in the framework of “The Global Tree Seed Bank Project” (https://brahmonline.kew.org/msbp/Projects/Trees). Through this collaboration, a new seed bank was recently opened in the JBSD (Mattana et al. 2017). This included the setup of a screening programme, based on the 100-seed test protocol (Gold and Hay 2014; Pritchard et al. 2004), for all the tree seed lots entering the seed bank (Mattana et al. 2017).

Therefore, the objectives of this study were to: (1) identify the native tree species entering the JBSD Seed Bank for which the 100-seed test protocol could assess seed desiccation responses and those for which further studies on seed germination requirements are needed; and (2) discuss the results of the 100-seed test in the light of those of the BRT (Wyse and Dickie 2018) and TSW–MC (Lan et al. 2014) predictive models.

Materials and methods

Study area

The island of Hispaniola, administratively divided between the Dominican Republic and Haiti, is the second largest island, after Cuba, of the Caribbean Islands Biodiversity Hotspot (Acevedo-Rodríguez and Strong 2008). The altitude of the island ranges from sea level to the 3175 m a.s.l. of Pico Duarte in the Dominican Republic, which is the highest peak of the whole Antilles. The climate is tropical, altered by the Atlantic trade winds and the topography of the island. The annual average temperature is 25 °C, with little variation during night or day and few seasonal fluctuations (Cano Carmona et al. 2010). The Island experiences two distinct seasons: a dry season from November through April and a wet season with a mid-summer drought, resulting in a bimodal cycle of precipitation (May/June and August/October; Karmalkar et al. 2013). The flora of the Island comprises over
4,000 species, with ca. 40% of them being endemic (Acevedo-Rodríguez and Strong 2008). According to the most recent estimates, 1,060 tree species, belonging to 94 families, are reported for the Dominican Republic (Botanic Gardens Conservation International 2019).

**Seed collecting**

Ninety-seven seed lots of 91 native woody species belonging to 36 families were collected during the period 2015–2017 in different localities of the Dominican Republic, as detailed in Table S1 and stored at the Jardín Botánico Nacional ‘Dr. Rafael M. Moscoso’ of Santo Domingo (JBSD) Seed Bank. The choice of the optimal timing for seed collection, as well as harvesting methods and quantity of material to be collected, were regulated by ethical and scientific criteria that guarantee a high quality of the collected material (Way 2003) and minimised the impact on the in situ genetic resources (Menges et al. 2004). Collected seed lots were delivered to the JBSD Seed Bank up to seven days from collecting and processed following international protocols (ENSCONET 2009; ISTA 2007; Smith 1995; Terry et al. 2003). Priority for the cleaning was given to fleshy fruits, for which seeds and pyrenes are separated from the pulp under running tap water and then left to dry. After cleaning, seed lots included whole dry fruits, woody endocarps, and seeds, but we will refer to them as “seeds” through the manuscript. Taxonomic nomenclature follows The Plant List (2013).

**Likelihood of seed desiccation sensitivity**

Probability of recalcitrance (PoR) was assessed for the investigated species using the three BRT models developed by Wyse and Dickie (2018). These models predict the likelihood of seed desiccation responses based on habitat and traits information for the species, and the seed desiccation responses of close relatives (either member of the same genus, family or order, depending on the model). PoR ranges from 0 (likely desiccation tolerant species) to 1 (likely desiccation sensitive species), with a threshold value of 0.5 to distinguish between the two categories (Wyse and Dickie 2018).

**TSW–MC characterization**

Seed desiccation responses categories were assigned a priori for the collected seed lots according to the TSW–MC criteria (Hong and Ellis 1996). According to Lan et al. (2014), when seeds had an initial mc > 30% and a TSW > 500 g, we considered the seed lot as “Desiccation Sensitive” (DS); in contrast, when the initial mc < 30% and TSW < 500 g, we considered them as “Desiccation Tolerant” (DT). When seeds had only one of the two traits (mc or TSW) compatible with DS, we considered the seeds as “Non Desiccation Tolerant” (NDT) and, in particular, NDT1 when mc > 30%, but TSW < 500 g and NDT2 when TSW > 500 g, but mc < 30%.

**100-seed test protocol**

Seed physiological responses to desiccation were assessed for the investigated seed lots (see Table S1) by applying the “100-seed test protocol” (Pritchard et al. 2004), modified by Gold and Hay (2014) and further adapted as detailed below and summarized in Figure S1.
At least 100 seeds per seed lot were divided in three sublots named: “fresh seeds” (at least 36 seeds); “dried seeds” (at least 32 seeds); and “moist seeds” (at least 32 seeds).

The equilibrium relative humidity (eRH) of the “fresh seeds” was determined immediately after cleaning, using a temperature and relative humidity logger (Tinytag View 2 TV-4505 with probe; Gemini Data Loggers, Chichester, UK). At the same time, mc (%) was determined gravimetrically through oven-drying at 103 °C for 17 h (ISTA 2007), using at least ten seeds. When working with large (> 7 mm) or hard coated seeds, they were cut in a half or pieces < 7 mm, before weighting and oven-drying (ISTA 2007). Fresh weight of the seeds before oven-drying was used to estimate their TSW in grams.

“Fresh seeds” were incubated for germination at 25 °C, with a photoperiod of 12 h of irradiance per day, using two replicates of at least 13 seeds each, sown on paper soaked with distilled water in 90 mm diameter plastic Petri dishes or in transparent closed plastic boxes (174 × 115 × 60 mm). When seed size was excessively large for the plastic boxes, germination on compost in the nursery was preferred. Seeds of species belonging to families reported to have physical dormancy, as those listed in Baskin et al. (2000), were scarified before incubating. Germination was recorded weekly and the germinated seeds removed. Germination tests lasted a minimum of four weeks. If germination occurred, the test was terminated two weeks after the last seed germinated. The remaining seeds were evaluated by a cut-test to ascertain whether they were full (looking healthy and fresh), empty, infested or mouldy. Final germination percentage was calculated based on the total number of viable seeds sown per replicate.

“Dry seeds” were kept at room temperature in a sealed glass container for a week, with a 1:1 ratio in weight of seeds: silica gel; then moved to another container with a 1:4 ratio for another week; and then in a paper or cloth bag kept into a sealed plastic drum with an excess of silica gel for another week (or more, until the 15% eRH was reached). The ratios of 1:1 and 1:4 for silica gel drying correspond to 30 and 15% eRH, respectively (Probert 2003), leading to a progressive increment of drying level, in order to limit seed viability loss due to fast drying up to the excess of silica gel in the drum. Meanwhile, “moist stored seeds” were kept in a semi-closed glass container in a ventilated room for the same length of time. At the end of this 3-weeks (or more) period the eRH and mc% values of the two seed lots (“Dry seeds” and “Moist seeds”) were reassessed as described for “Fresh seeds”, using at least six seeds instead of ten for moisture content. Also, for each seed lots, after one day at room conditions, two replicates of at least 12 seeds each were incubated for germination, as above described for “Fresh seeds”.

Data analysis

Seed desiccation sensitivity is not a binary variable, but a gradient between two extremes (Walters 2015). Therefore, these data were analysed as a continuous factor and quantified using the Viability Loss Index (VLI), calculated as follows:

\[
VLI = \frac{\text{Fresh germination}\% - \text{dry germination}\%}{\text{Fresh germination}\%}
\]

VLI values theoretically vary from 0 (desiccation tolerant seeds) to 1 (desiccation sensitive seeds), although negative values were obtained when dried seeds germinated to higher level than fresh seeds. However, in order to assign the seed lots to qualitative categories, seeds with VLI > 0.95 were considered as “desiccation sensitive” (vDS), while those with 0.95 > VLI > 0.5 as “potentially desiccation sensitive” (vDSp). Seeds with VLI < 0.05 as
“desiccation tolerant” (vDT) and those with 0.05 < VLI < 0.5 were considered as “potentially desiccation tolerant” (vDTp).

VLI was calculated only for those seed lots for which fresh germination, considering its variance calculated as standard deviation, was higher than 50% and the seed moisture content after drying lowered below 20% (Table S2). Three seed lots for which standard deviation of dry germination was higher than 35% were also excluded from the VLI calculation (Table S2).

Statistical analysis

All analyses were carried out in R version 3.5.1 (R Core Team 2013). All graphs were plotted using the “ggplot2” package (Wickham 2016).

In order to confirm that the applied drying protocol significantly affected the seed moisture content of the investigated seed lots, the effect of treatment (3-level factor: control, fresh; dry; and moist) and of the “a priori” TSW–MC categories (4-level factor: DS, DT, NDT1 and NDT2) on moisture content values were tested using a linear regression model (“lm” function), with seed lots as replicates (N = 97). Parametric assumptions were checked using the “car” (Fox and Weisberg 2011) and the “ggplot2” packages. As the assumptions of homogeneity of variance, non-multicollinearity, and normal distribution of residuals were not met, a robust non-parametric linear regression was carried out by bootstrapping (2000 repetitions), using the “boot” package (Canty and Ripley 2017).

In order to assess the germination responses to the applied drying protocol, the effect of treatment on seed germination (3-level factor: control, fresh; dry; and moist) was tested for each a priory TSW–MC category by generalized linear mixed models with binomial distribution (“logit” link function), using the “glmer” function of the “lme4” package (Bates et al. 2014). Germination responses were recorded for each replicate (petri dish) as number of germinated and non germinated seeds. The seed lot was considered as random factor to avoid pseudo replication (Crawley 2012).

Results

Seed collections variability

Seeds of the 91 investigated species varied in their mass by five orders of magnitude, ranging from a TSW < 0.1 g for Elekmania buchii and Exostema caribaeum to values > 10 kg for Juglans jamaicensis, Mammea americana, and Mora abbottii (Fig. 1 and Table S2). Seed collections varied also in the macro-morphology, including true seeds, woody endocarps, and whole dry fruits (see Fig. 1 for a selection).

Likelihood of seed desiccation sensitivity

The three BRT models developed by Wyse and Dickie (2018) allowed for prediction of the likelihood of seed desiccation sensitivity for all the investigated species (Table S2). For one species (Canella winterana) the applied predictive model was at order level, for 42 species at family level, and for 25 at genus level, while information on seed storage behaviour was already available for 23 species (meaning a PoR value of 0 or 1 for orthodox or recalcitrant
species, respectively; Table S2). These models allowed identifying 65 species (70 collections) as likely orthodox and 26 (27 collections) as likely recalcitrant (Table S2).

**TSW–MC characterization**

Initial moisture content (mc, %) of fresh seeds and their log (TSW) were slightly positively correlated ($y = 8.06x + 7.05; R^2 = 0.24; p < 0.001$; Fig. 2). The TSW–MC criteria allowed identifying a priori 59 seed lots as desiccation tolerant (DT; fresh mc < 30% and TSW < 500 g; Fig. 2) and 17 seed lots as desiccation sensitive (DS; fresh mc > 30% and a TSW > 500 g; Fig. 2). Twenty-one seed lots were considered as non desiccation tolerant (NDT): 12 as NDT1 (i.e. mc > 30%, but TSW < 500 g; Fig. 2) and nine as NDT2 (TSW > 500 g, but mc < 30%; Fig. 2).

**100-seed test protocol**

The 100-seed test was carried out for all the 97 seed lots. Fresh mc (%) of the different categories identified a priori through the TSW–MC criteria, ranged from $15.26 \pm 6.28\%$ (DT) to $45.35 \pm 9.29\%$ (DS; Fig. S2). Seed drying performances of the different TSW–MC categories highlighted how the adapted 100-seed test protocol applied in this study allowed to get an average dry mc close to 5% for DT ($6.25 \pm 3.84\%$), NDT2 ($6.00 \pm 4.03\%$) and NDT2 ($8.09 \pm 3.43\%$) while for DS species this value was $12.82 \pm 10.97$ (Fig. S2). Moist treatment led to final mc% ranging from $13.02 \pm 6.98\%$ for DT to $35.27 \pm 14.19\%$ for DS (Fig. S2).
The bootstrapped linear model confirmed that the drying method applied in this study led to statistically significant different values among treatments as well as among categories (Table 1). All interactions between treatment and category for dry seeds were statistically significant except for NDT1 seed lots (Table 1).

Seed germination performances of the different TSW–MC categories highlighted a higher germination of fresh seeds for DS and NDT1 (ca. 65%) respect to DT species (ca. 54%), while NDT2 had the lowest value (ca. 45%; Fig. S3). Not surprisingly, seed germination of dry seeds did not vary drastically from the fresh germination for DT species (ca. 44%), whereas it dropped to ca. 12% for DS species, while NDT1 and NDT2 showed values of 18 and 24%, respectively (Fig. S3). Species categories showed differences on their final germination of moist seeds with this value being similar to that of dry seeds for DT species (ca. 42%), intermediate between those of fresh and dry seeds for NDT1, NDT2 and DS (ca. 25, 38 and 28%, respectively; Fig. S3). The generalized linear mixed models carried out for each species category highlighted that treatment had a statistically significant negative effect with respect to the control fresh seeds except for the moist seeds of the NDT2 category (Table 2) However, all models showed a large overdispersion and therefore estimated parameters should be considered with caution.

For 40 of the investigated seed lots, the criteria identified for calculating the VLI (i.e., fresh germination > 50% and dry mc < 20%) were met (Table S2). Fourteen seed lots (corresponding to the same number of species) were identified as desiccation sensitive (vDS; VLI > 0.95), one (Sapindus saponaria) as probably desiccation sensitive (vDS; VLI > 0.95), one (Sapindus saponaria) as probably desiccation sensitive.

Fig. 2 Seed storage behaviour categories assigned a priori to the seed collections (N = 97) according to the TSW–MC model criteria, where desiccation tolerant (DT) species have an initial seed moisture content <30% and a TSW < 500 g, while desiccation sensitive (DS) species have higher values for both traits (Lan et al. 2014). Seed lots which had a mc > 30%, but a TSW < 500 g and vice versa were identified as NDT1 and NDT2, respectively. Points with different colours correspond to different plant families whereas their size is a function of their seed mass. Blue line corresponds to the fitted linear model (y = 8.06x + 7.05; R² = 0.24; p < 0.001) for the relationship between initial mc (%) and log(T SW) (g), and the grey area to the 95% confidence interval. Fitted linear model confirmed by bootstrapping. (Color figure online)
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Table 1 Coefficient values of the non-parametric bootstrapped linear model (2000 repetitions) for the effect of treatment (3-level factor: control, fresh; dry; and moist) and of the a priori TSW–MC categories (4-level factor: DS, DT, NDT1 and NDT2) on moisture content

| Coefficients                  | Estimate | Bias | SE  | 2.5% CI     | 97.5% CI     |
|-------------------------------|----------|------|-----|-------------|--------------|
| (Intercept)                   | 45.35    | 0.03 | 2.21| 41.14       | 49.90        |
| Treatment dry                 | −32.53   | −0.07| 3.48| −38.52      | −24.90       |
| Treatment moist               | −10.08   | −0.06| 4.08| −19.11      | −2.63        |
| Category DT                   | −30.09   | −0.03| 2.37| −35.19      | −25.57       |
| Category NDT1                 | −5.87    | 0.02 | 2.92| −11.73      | −0.22        |
| Category NDT2                 | −25.62   | 0.07 | 3.26| −32.44      | −19.40       |
| Treatment dry: category DT    | 23.52    | 0.09 | 3.62| 15.90       | 29.93        |
| Treatment moist: category DT  | 7.68     | 0.10 | 4.25| −0.21       | 17.15        |
| Treatment dry: category NDT1  | 0.53     | 0.04 | 4.06| −7.93       | 8.035        |
| Treatment moist: category NDT1| −5.65    | −0.14| 6.36| −17.09      | 7.08         |
| Treatment dry: category NDT2  | 19.14    | −0.03| 4.45| 10.23       | 27.89        |
| Treatment moist: category NDT2| 8.37     | −0.01| 6.01| −2.21       | 21.80        |

In bold coefficients for which the confidence interval did not cross zero, confirming the validity of the estimates

Table 2 Model parameters of generalized linear models for each a priori TSW–MC category for the effect of treatment (fixed factor with 3 levels: control, fresh; dry; and moist) on seed germination with seed lot as random factor

| Coefficient | Estimate | SE  | z value | Pr(>|z|) | 2.5% CI     | 97.5% CI     | Residual deviance | Random factor |
|-------------|----------|-----|---------|----------|-------------|--------------|------------------|---------------|
| DT          | (Intercept) | −0.05 | 0.38 | −0.14 | 0.88 | −0.84 | 0.70 | Dev 1330 | Dev 7.80 |
|             | Dry      | −0.72 | 0.11 | −6.3  | 2.14e−10 | −0.95 | −0.50 | df 345  | SE 2.79  |
|             | Moist    | −0.74 | 0.11 | −6.4  | 1.23e−10 | −0.97 | −0.51 |
| DS          | (Intercept) | 1.22 | 0.52 | 2.36 | 0.02 | 0.12 | 2.30 | Dev 485.3 | Dev 4.11 |
|             | Dry      | −4.52 | 0.30 | −15.03 | <2e−16 | −5.13 | −3.95 | df 96  | SE 2.03  |
|             | Moist    | −2.43 | 0.20 | −11.84 | <2e−16 | −2.84 | −2.03 |
| NDT1        | (Intercept) | 0.564 | 0.85 | 0.66 | 0.51 | 1.44 | 2.34 | Dev 269.7 | Dev 7.98 |
|             | Dry      | −3.63 | 0.35 | −10.42 | <2e−16 | −4.34 | −2.97 | df 66  | SE 2.82  |
|             | Moist    | −3.00 | 0.32 | −9.37 | <2e−16 | −3.65 | −2.39 |
| NDT2        | (Intercept) | −0.42 | 0.60 | 0.69 | 0.49 | 1.86 | 0.88 | Dev 249.4 | Dev 2.96 |
|             | Dry      | −1.76 | 0.27 | −6.53 | 6.45e−11 | −2.30 | −1.24 | df 50  | SE 1.72  |
|             | Moist    | −0.43 | 0.23 | −1.86 | 0.06 | −0.89 | 0.02 |

In bold coefficients for which the confidence interval did not cross zero confirming the validity of the estimates

(vDSp; 0.5 < VLI < 0.95), 11 seed lots/species as probably desiccation tolerant (vDTp; 0.5 > VLI > 0.05), and 14 seed lots/species as desiccation tolerant (vDT; VLI < 0.05; Table S2). The identified categories showed differences on their seed mass and initial mc,
with increasing values of both traits from vDT to vDS species (Figure S4). In particular, vDT seed lots ranged in TSW from 1.22 to 427 g (with a mean value of 87.78 g) while vDS from 63 to 58,402 g (mean 6145 g). Initial mc (%) varied from 13.6 to 20.22% (mean value of 13.65%) and from 25.36 to 64.38% (mean value of 41.33%), for vDT and vDS, respectively (Table S2).

Models’ predictions and experimental results

When comparing the VLI categories with those assigned a priori, according to the TSW–MC criteria and the likelihood of seed desiccation sensitivity predicted at species level by the three boosted models developed by Wyse and Dickie (2018), 31 seed lots/species yielded consistent results (Table S2); three of them (Colubrina elliptica, Krugiodendron ferreum, and Magnolia hamorii) showed VLI results which did not correspond to BRT models results and the TSW–MC criteria (Fig. 3); and five of them (Coccothrinax fragrans, Cojoba zanonii, Mora abbottii, Roystonea borinquena, and Sapindus saponaria) showed BRT predictions which did not correspond to TSW–MC criteria and VLI experimental categories (Table S2).

When plotting the distribution density of the DT seed lots according to the TSW–MC criteria in a grid constituted by their PoR and VLI values, the highest density of seed lots is correctly located in the area of the grid that corresponds to the DT species, with the exception of the three species identified as vDS by the experimental results (i.e. VLI higher than 0.95; see Fig. 3), but correctly predicted as likely orthodox by the predictive models.
of Wyse and Dickie (2018) and for two species (C. fragrans and R. borinquena), which were identified as vDT and vDTp, respectively, by their VLI values (see Table S2), but that according to the predictive models should be considered as likely recalcitrant (Fig. 4).

**Discussion**

**The 100-seed test as a tool to assess seed desiccation responses**

This study allowed gathering new data on seed desiccation responses for seed lots of 40 native trees of the Caribbean region and identifying those species for which more studies should be carried out. Overall, the 100-seed test is a reliable screening tool for seed desiccation responses of a diverse flora, as claimed by Gold and Hay (2014) and reported in previous studies (e.g., Hamilton et al. 2013; Lan et al. 2014). However, it should be taken into account that “moist seeds” had a high mortality (data not shown) with final germination percentages generally lower than that of fresh seeds despite their response to desiccation. This was due to the “moist seeds” being kept at high levels of temperature and relative humidity (Gold 2014). Therefore, when both “dried seeds” and “moist seeds” died, storage time could not be removed as a factor in the desiccation experiments (Pritchard et al. 2004).
Seed dormancy issues

The 100-seed test was applied here as an overall screening for all tree seed lots entering the seed bank, in a flora for which information on seed germination ecology is still under-investigated. Baskin and Baskin (2005) reported that the percentage of trees in climax tropical forests with dormant seeds range from ca. 43 to ca. 66% for evergreen and montane forests, respectively. Sautu et al. (2007), in their study on a seasonal moist tropical forest of Panama, reported ca. 48% of tree species with dormant seeds. Ray and Brown (1994) found that only 17 of the 29 investigated tree species from a Caribbean dry forest in the Virgin Islands germinated well (> 80% of final germination), under shade-house conditions.

In this study, the only applied seed dormancy breaking treatment was the manual scarification for seeds with physical dormancy according to Baskin et al. (2000). Other pre-treatments to overcome morphological and/or physiological components of seed dormancy, such as cold and/or warm stratifications, dry after ripening or exogenous GA3 (Baskin and Baskin 2014a) were not carried out. In some cases, seed germination of dry seeds was higher than that of fresh seeds suggesting the presence of physiological seed dormancy which was broken by drying (Baskin and Baskin 2014b). Low germination was recorded for most species of Arecales, Rubiaceae and Sapotaceae families (see Table S2) for which morphophysiological (Arecales), physiological (Rubiaceae) and physiological dormancy due to mechanical constrains (Sapotaceae) have been previously reported (e.g., Baskin and Baskin 2005; Baskin and Baskin 2014b; Finch-Savage and Leubner-Metzger 2006).

Low numbers

When working with native trees, particularly from tropical and semitropical regions, collecting large amounts of seeds may be difficult, due to low density of individuals of each species, technical challenges in reaching the crown of tall trees, and poor seed quality, including high levels of seed parasitism (Finkeldey and Hattemer 2007). In addition, when working with collections made for species conservation purposes, and of rare or threatened species, the number of seeds used for experimental purposes should be carefully considered (Pritchard et al. 2004).

The original protocol in Pritchard et al. (2004) and in Gold and Hay (2014) uses two replicates of 13 seeds for germination experiments and 6–10 seeds for moisture content calculation. When applying this protocol, researchers may consider increasing these numbers (e.g. 3–5 replicates with 10 seeds each; Hamilton et al. 2013) in order to increase its statistical power. In our study, we used two replicates as indicated in the original protocol, and, when seed availability was not a constraint, we increased the number of seeds sown per replicate. However, in some cases, the number of germinable seeds (i.e. germinated plus non germinated filled seeds) was even less than 13 per replicate due to the presence of empty/infested seeds (data not shown). We applied statistical tools to handle the data analysis of low seed numbers. Bootstrapping was applied to fit robust linear regressions in order to deal with parametric assumptions issues (Field et al. 2012). Binomial generalized mixed models (GLMMs) using the number of germinated vs non germinated seeds per replicate, were carried out in order to get a weighted regression, using the individual sample seizes as weight (Crawley 2012). However, the models were overdispersed and therefore the outcomes of the GLMMs should be taken with caution.
Species for which further studies are needed

Three species were identified as desiccation sensitive by experimental results which however, according to their seed lot traits and the results of BRT models, should be considered desiccation tolerant. *Colubrina elliptica* (Rhamnaceae) has been reported to have seeds that retain 100% viability following drying at 15% eRH and freezing at −20 °C for more than one year (Royal Botanic Gardens Kew 2019) and therefore it is considered as likely desiccation tolerant by Wyse and Dickie (2018). The initial mc and the TSW of the seed lot analysed in this study fell within the thresholds for desiccation tolerant seeds (Hong and Ellis 1996; Lan et al. 2014). However, seed viability dropped drastically after both drying and moist storage at moist conditions, suggesting a desiccation sensitive behaviour.

*Krugiodendron ferreum* (Rhamnaceae) seed lot could be described as desiccation tolerant, according to both initial mc and TSW. The BRT models of Wyse and Dickie (2018) assessed this species as likely orthodox at family level. However, the experimental results clearly identified the tested seed lot as desiccation sensitive as previously suggested by a technical report (Gann et al. 2016).

Finally, *Magnolia hamorii* (Magnoliaceae) seed lot had traits compatible with a desiccation tolerant behaviour and this was confirmed by the results of the BRT model at genus level. According to the experimental results this seed lot should be considered as desiccation sensitive. However, the high germination percentage reached by fresh seeds (> 80%) was calculated on a reduced number of seeds due to the presence of empty seeds in both replicates.

The experimental results achieved for these three species could have been determined by a premature collecting. The majority of orthodox seeds acquire full desiccation tolerance shortly before natural dispersal (Hay and Smith 2003) and full ripe seeds should be used in desiccation tolerance screening tests (Gold and Hay 2014). Therefore, the full Hong and Ellis (1996) protocol should be carried out for these three species, before confirming their seed physiological responses to desiccation.

Providing new data for the predictive models

Five species showed BRT predictions which did not correspond to TSW–MC criteria and VLI experimental results: *Coccothrinax fragrans* and *Roystonea borinquena* (Arecales), *Cojoba zanonii* and *Mora abbotii* (Leguminosae), and *Sapindus saponaria* (Sapindaceae). According to the BRT model *C. fragrans* and *R. borinquena* are likely to be recalcitrant, while both seed lot traits and experimental result converged through a desiccation tolerant behaviour. The BRT prediction was made at family level and based on a probability of recalcitrance of 0.7 and 0.6, respectively and therefore close to the threshold of 0.5 between the two categories. This is not surprising, considering that Arecales and Arecales are, respectively, ones of the families and orders with the highest incidence of recalcitrant species (Dickie and Pritchard 2002; Wyse and Dickie 2017).

The BRT model predicted a likelihood of desiccation tolerance for the two Leguminosae *C. zanonii* and *M. abbotii* at family level. However, the investigated seed lots resulted to be desiccation sensitive accordingly to both seed lot traits and experimental results. Although Leguminosae are reported to have a low incidence of seed desiccation sensitive species worldwide (1.2%; Dickie and Pritchard 2002), this percentage increases to 6% in tropical regions such as Sri Lanka (Jayasuriya et al. 2013).
Finally, *S. saponaria* was predicted to be likely orthodox by a model at genus level, with a probability of recalcitrance close to the threshold of 0.5, while the experimental results and the seed lot traits led towards a desiccation sensitive category. Royal Botanic Gardens Kew (2019) reports this species as “uncertain” highlighting the need of further studies.

These findings confirm how these models and their predictive success will benefit by the generation of new data (Wyse and Dickie 2018), particularly from tropical and subtropical regions, as those provided in this study.

## Conclusions

The adapted “100-seed test” protocol (Gold and Hay 2014; Pritchard et al. 2004) confirmed to be an effective screening tool for assessing seed physiological responses to desiccation at seed lot level, allowing gathering new data for tree species of the Caribbean region and identifying those species for which further studies are needed. Issues might arise during its practical set up, due to the poor knowledge on seed dormancy breaking and germination requirements, when working in a flora for which information on seed germination ecology is still under-investigated, and in the interpretation of the results, due to the low seed numbers used in the experiments. However, the availability of predictive models informed by seed lot traits (Hong and Ellis 1996; Lan et al. 2014) and seed and plant traits, such as the BRT models (Wyse and Dickie 2018) allows discussing the achieved results, highlighting those species for which further studies should be carried out in order to confirm their seed desiccation responses. These findings might guide the seed conservation of trees and their use in reforestation programmes in the Caribbean.

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Affiliations

Efisio Mattana\textsuperscript{1}\href{mailto:efisio.mattana@kew.org}{\textsuperscript{†}} \cdot Brígido Peguero\textsuperscript{2} \cdot Alice Di Sacco\textsuperscript{3}\href{mailto:alice.disacco@kew.org}{\textsuperscript{†}} \cdot Wilvin Agramonte\textsuperscript{2} \cdot Wilkin Rafael Encarnación Castillo\textsuperscript{2} \cdot Francisco Jiménez\textsuperscript{2} \cdot Teodoro Clase\textsuperscript{2} \cdot Hugh W. Pritchard\textsuperscript{4} \cdot Pablo Gómez-Barreiro\textsuperscript{5}\href{mailto:pablo.gomez-barreiro@kew.org}{\textsuperscript{†}} \cdot Elena Castillo-Lorenzo\textsuperscript{1}\href{mailto:elena.castillo-lorenzo@kew.org}{\textsuperscript{†}} \cdot Marianny Terrero Encarnación\textsuperscript{2} \cdot Michael J. Way\textsuperscript{3} \cdot Ricardo García\textsuperscript{2} \cdot Tiziana Ulian\textsuperscript{1}

1 Natural Capital and Plant Health Department, Royal Botanic Gardens, Kew, Wellcome Trust Millennium Building, Wakehurst Place, Ardingly, West Sussex RH17 6TN, UK
2 Jardín Botánico Nacional “Dr. Rafael M. Moscoso”, Santo Domingo, Dominican Republic
3 Conservation Science Department, Royal Botanic Gardens, Kew, Wellcome Trust Millennium Building, Wakehurst Place, Ardingly, West Sussex RH17 6TN, UK
4 Comparative Plant and Fungal Biology Department, Royal Botanic Gardens, Kew, Wellcome Trust Millennium Building, Wakehurst Place, Ardingly, West Sussex RH17 6TN, UK
5 Analytical Methods Department, Royal Botanic Gardens, Kew, Wellcome Trust Millennium Building, Wakehurst Place, Ardingly, West Sussex RH17 6TN, UK