Effect of reduced water potential on seed germination of a forest tree: a hydrot ime approach

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ABSTRACT: Hydrot ime (θH) models provide information on seed tolerance to low water potential and time to germination under different conditions. Here it was evaluated the capacity of graphic and probit model to describe germination parameters and germination times (t) in a tropical legume (Peltophorum dubium). Germination tests were conducted under reduced water potentials (polyethylene glycol solutions from 0 to -1.2 MPa) at 25 °C. Regression lines were applied to investigate the relationship between germination rates (1/t) and water potential for different germination percentages (fractions 10, 30, 50 and 70%). Those regressions were used in the graphic model to calculate θH (1/slope) and determine the base water potential (Ψb) as the point which the line intercepts the x-axis (G% = 0). In the probit model, germination percentages were transformed to probit units and plotted against Ψb-values to describe germination response under a single regression line. Values for θH varied from 1.8 to 2.0 MPa day in both models, and Ψb showed a normal distribution, as presupposed by the probit model. Predicted germination times (t10 and t50) mostly fell within observed times, thus showing biological relevance of the models to describe the effects of water potential on seed germination.

Index terms: osmotic potential, Peltophorum, probit, psi-base, hydrot ime model.
**INTRODUCTION**

Water uptake is the principal factor starting the germination process, promoting cell respiration, DNA synthesis and growth (Bewley et al., 2013). Therefore, water potential (Ψ) strongly drives seed germination, by regulating the amount of water able to realize work in a solution. In a physiological sense, the decrease of water potential reduces germination capacity (G%), as well as the rate of the germination process (Gummerson, 1986; Bradford, 1995). Therefore, the germination rate (GR, inverse of germination time, t) seems to linearly decrease with negative Ψ-values, until the point at which seeds stop the germination process due to low water potential (base water potential, or Ψb).

Because seeds can only accomplish germination above a Ψb-value, threshold models can be developed to describe seed germination responses to water potential (Bradford, 1990; Alvarado and Bradford, 2002; Finch-Savage, 2004). Above Ψb, seeds require an accumulated Ψ (MPa) through time (hydrot ime, or θ_H) to germinate and, therefore, hydrot ime models were used to describe germination responses to reduced water potentials, mostly in crops (e.g. Gummerson, 1986; Dahal and Bradford, 1994; Windauer et al., 2007). Concerning native species, few studies in current literature investigated such threshold models regarding water relations and seed germination of tropical trees (Daws et al., 2008). Some studies evaluated the role of Ψ on seed germination of Brazilian species (Botelho and Perez, 2001; Fonseca and Perez, 2003; Rego et al., 2007), but rarely explaining whether germination would fit the presupposes of hydrot ime models (Cardoso and Pereira, 2008; Oliveira et al., 2019). Most attention regards to the role of temperature, rather than water potential, as described by thermal time models in neotropical species (Cardoso and Pereira, 2009; Pires et al., 2009; Daibes and Cardoso, 2018; Duarte et al., 2018).

Furthermore, seed germination can be expressed in different germination fractions (percentages), given the distribution and variation of time to germinate within seeds in a population (Garcia-Huidobro et al., 1982; Gummerson, 1986; Alvarado and Bradford, 2002). Hence, GR (germination rate) decreases with Ψ, but also varies according with the percentiles of seeds (i.e. seeds within the 10% percentile do germinate faster than the seeds within the 50 or 70% percentile). Likewise, θ_H requirements are related to GR and different Ψb-values might be expected for different fractions of seed germination (Bradford, 1990; Alvarado and Bradford, 2002). This might be important to understand the proportion of seeds which can be recruited in seasonal environments, where seeds are subjected to desiccation during the dry season, facing low water potentials in the soil seedbanks (Cavallaro et al., 2016).

Therefore, this study aimed to investigate the role of water potential in seed germination of *Peltophorum dubium*, a tropical tree legume typically occurring in South American seasonal forests. Specifically, it was addressed how hydrot ime models (graphic model and probit model) would describe seed germination of the species, explaining hydrot ime requirements and Ψb. Using non-dormant seeds (previously alleviated from physical dormancy), it was hypothesized that seed germination would behaved such as predicted by the model, following presupposes of graphical model (linear relationship of germination rate with decreasing of Ψ-values) and repeated probit model (normal distribution of Ψb with a single value of hydrot ime requirement).

**MATERIAL AND METHODS**

**Seed harvesting**

Seeds were obtained from a certified producer located in the municipality of Porto Ferreira (21°3’S; 47°2’W; state of São Paulo, Brazil). The harvesting site shows average temperatures from 19 to 25 °C and mean annual precipitation of 1500 mm (Daibes and Cardoso, 2018). The species has a widespread distribution throughout South America, mostly occurring in seasonal forests from the Paraguai-Paraná Basin to the state of Bahia (Barneby, 1996). The harvesting was performed in May 2011, in different mother plants and seeds kept stored within the pods (indehiscent fruit) inside paper bags under low temperatures (~5 °C) until their use in the experiments, few months later. Dispersal period may range from April to December, and ripened fruits remain attached to the trees for several months (Carvalho, 2002). The total
viability of seeds was high by the beginning of germination trials (~90%) and no light requirement was detected in *P. dubium* seeds (Perez et al., 2001).

**Germination trials**

To conduct germination tests under different water potentials, seeds were removed from the pods and carefully screened to remove malformed and/or predated seeds. Then, seeds were individually scarified with a sandpaper to overcome physical dormancy. Once scarified, seeds were expected to behave as non-dormant seeds in the germination trials (Daibes and Cardoso, 2018). Different water potentials were obtained by aqueous solutions of PEG 6000 (polyethylene glycol), ranging from zero (distilled water) to -1.5 MPa (0; -0.3; -0.5; -0.7; -0.9; -1.1; -1.3; -1.5). PEG solutions were prepared according to Villela et al. (1991), as adapted from Michel and Kaufmann (1973). Seeds were then set to germinate on a double layer of filter paper soaked in at least 6 mL of the corresponding PEG solution in Petri dishes (90 mm), sealed with plastic film to prevent evaporation of the solution.

Three replicates of twenty seeds were used in each water potential treatment, and germination tests were conducted in germination chambers under the constant temperature of 25 °C, considered within the range of optimal conditions for seed germination of the species (Daibes and Cardoso, 2018). Because *P. dubium* seeds are non-photoblastic, all germination trials were conducted in the dark. Seed germination (radicle protrusion) was daily counted for one month or until the germination of all seeds in the plate. At each counting, Petri dishes were carefully re-wrapped again in the plastic film. By the end of the trials, remaining seeds were visually inspected to attest viability and scored as dead.

**Data analysis and hydrotime modeling**

Prior to hydrotime modeling, the germination capacity (%) and time (t) of germination through the different water potential treatments were evaluated. Germination capacity was statistically compared using GLMs with a binomial distribution in lme4 package (Bates et al., 2015) in R software (R Core Team, 2018), considering distilled water (Ψ = 0) as the control (baseline) in the analysis. Observed t was calculated to different germination percentiles (10, 30, 50 and 70%) by linear interpolation of two nearest points in the germination curves, then obtaining the x-axis interception to the corresponding fraction (Steinmaus et al., 2000). Model parameters were obtained for two hydrotime models: graphic model and probit model. In the graphic model, the germination rates for the different germination fractions (GR (g) = 1/t) were regressed against the treatments to assess the linearity among GR and water potential (Bradford, 1990). Base water potential (Ψb) was estimated as the point which the regression lines intercepted the x-axis (GR = 0), while hydrotime (θ_H) was obtained as the inverse of regression line slope (θ_H = 1/slope), according to Gummerson (1986).

In the probit model, germination percentages were transformed to probit units and plotted as function of Ψb. Linear regression was used to evaluate the relationship between observed germination and the predicted line. The value of θ_H was probed repeated times and considered the best-fitting values which showed higher R² and least residual model (Dahal and Bradford, 1994; Bradford, 1995). Predicted germination curve was derived from the original probit regression line (Table 1) using a normal distribution in Excel® (Cardoso, 2011). Once obtained hydrotime parameters of both models, germination times were estimated by rearranging the basic equation: θ_H = (Ψ–Ψb).t(10), thus considering t(10) = θ_H/(Ψ–Ψb). Hence, the model-predicted germination times were calculated for the 10 and 50% percentiles (t10 and t50) and compared to observed t-values in relation to confidence intervals (95%). Because Ψb is plotted in the x-axis in the probit model, the following equation was considered: t(10) = θ_H/(Ψ–((probitG–a) / b), where “a” is the intercept, and “b” the slope of the probit regression.
Table 1. Parameters of hydrotime model regression lines: equation and $R^2$, base water potential ($\Psi_b$, MPa) and hydrotime values ($\theta_H$) for seed germination of *Peltophorum dubium*.

| Graphic model | Equation           | $R^2$ | $\Psi$-base | $\theta_H$ |
|---------------|--------------------|-------|-------------|------------|
| 10%           | $y = 0.559x + 0.526$ | 0.98  | -0.94       | 1.8        |
| 30%           | $y = 0.488x + 0.435$ | 0.97  | -0.89       | 2.0        |
| 50%           | $y = 0.480x + 0.390$ | 0.95  | -0.81       | 2.1        |
| 70%           | $y = 0.448x + 0.328$ | 0.99  | -0.73       | 2.2        |
| Probit        | $y = 5.480x + 3.866$ | 0.88  | -0.7 ± 0.18 | 1.8        |

The graphic model was performed for different germination fractions (10, 30, 50 and 70%), while $\Psi_b$ of probit model regards to 50% of seed germination ± standard deviation.

RESULTS AND DISCUSSION

Seeds showed high germination capacity (90%) in water potentials of 0 and -0.3 MPa, with a significant reduction to ~70% at -0.5 MPa, decreasing to ≤ 10% at -0.7 and -0.9 MPa (Figure 1). Germination was null under the treatments of -1.1, -1.3 and -1.5 MPa. Germination rates showed a linear relationship with water potential, decreasing GR according to the reduction of $\Psi$ and showing a general parallel pattern among the different germination fractions (10, 30, 50 and 70%; Figure 2). Therefore, the interception points in the x-axis showed $\Psi_b$-values varying from -0.7 to -0.9 MPa among percentiles in the graphic model (Table 1). Regression lines for the different germination fractions in the graphic model showed slope ranging from 0.448 to 0.559 and $\theta_H$ values (1/slope) around 2.0 MPa day (Table 1). Because the parallel lines show a relatively similar slope (i.e., a similar hydrotime for different germination percentiles), it is the $\Psi_b$ which drives germination parameters of the germination fractions.

In the probit model, it was possible to clump the different germination curves (Figure 3A) into a single curve of germination percentages as function of $\Psi_b$ (Figure 3B). Base water potential followed a normal distribution in the probit model, which described 88% of germination parameters, showing $\theta_H = 1.8$ MPa day (Table 1). On the other hand, probit model might underestimate seed tolerance to low water potentials ($\Psi_b^{50} = -0.7$ MPa), whereas graphic model predicts a little more negative $\Psi_b$ value for the 50% fraction (-0.8 MPa; Table 1). Nevertheless, predicted times

Figure 1. Germination capacity (%; mean ± SD) of *Peltophorum dubium* seed germination under different water potentials ($\Psi$, MPa), showing significant decrease under reduced water potentials.
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Figure 2. Relationships of germination rate (GR) under different water potentials for different germination fractions (10, 30, 50 and 70%).

Figure 3. (A) Seed germination curves (cumulative germination percentage vs. time, in days) under different water potentials of 0, -0.3, -0.5 and -0.7 MPa. (B) Germination curve as function of $\Psi_b$ showing a normal distribution pattern (continuous line) predicted by probit model.
The germination was null under water potentials ≤ -0.9 MPa.

Table 2. Observed (mean ± 95% confidence interval) and predicted germination times (graphic model and probit model) for 10 and 50% of seed germination (t10 and t50) of *Peltophorum dubium* seeds in different water potentials.

| Ψ (MPa) | Observed | Graphic model | Probit model |
|---------|----------|---------------|--------------|
|         |          |               |              |
| 0       | 1.97 ± 0.31 | 2.17          | 1.92         |
| -0.3    | 2.61 ± 0.39 | 3.18          | 2.82         |
| -0.5    | 4.55 ± 1.43 | 4.63          | 4.10         |
| -0.7    | 8.0 ± 3.20  | 8.49          | 7.53         |
| -0.9    | –         | –             | –            |
| 0       | 2.67 ± 0.33 | 2.51          | 2.55         |
| -0.3    | 3.61 ± 0.17 | 3.97          | 4.44         |
| -0.5    | 8.28 ± 3.65 | 6.51          | 8.76         |
| -0.7    | –         | –             | –            |
| -0.9    | –         | –             | –            |

The germination was null under water potentials ≤ -0.9 MPa.
The results provide a preliminary approach on hydrotime models, by testing the basic assumptions of the models and linear relationships with water potential (Bradford, 1995). Likewise, most model presupposes were followed by P. dubium seeds under different temperatures, described by thermal time models (Daibes and Cardoso, 2018). Temperature and water potential show important interactions to describe germination parameters under laboratory conditions (Alvarado and Bradford, 2002). However, few studies were accounted for such interactions (hydrothermal time) in Brazilian species (Simão et al., 2010; Oliveira et al., 2019). Seedling emergence from soil seedbanks should also be examined, in order to validate such models under field conditions (Forcella et al., 2000).

Some critics were made to probit analysis due the lack of independency among germination counting through the days. Therefore, cumulative germination percentages would be temporally dependent, breaking the principle of independency among samples in a regression analysis (Hay et al., 2014). Solving this issue would require a considerable higher amount of seeds, often impossible to achieve from native populations. Analysis derived from semi-parametric distributions (survival analysis, for instance) could help us to fix such problems by taking in account the probability of individual seeds to germinate or fail (Onofri et al., 2010; McNair et al., 2012). Despite such relatively recent approaches proposed, their connection to hydrotime assumptions and usage in statistical software remains a matter of inquiry (Cao et al., 2013).

Nevertheless, the predictions of germination times from linear models showed to be useful, showing biological relevance (Bradford, 1995; Bradford and Still, 2004). Therefore, germination parameters may be drawn from relatively simpler equations, and the advantage of probit model is to derive predictions of germination time from a single line. The graphic model, on the other hand, requires different regression lines according to the desired germination fraction wished to describe (Daibes and Cardoso, 2018). Either way, the application of a model does not exclude the use of other methods to predict seed germination. Linear relationship of GR and water potential may be helpful to achieve general patterns of model presupposes and can serve as a support to evaluate germination behavior before running probit analysis.

CONCLUSIONS

Hydrotime models describe germination of a forest tree and the applicability of such models for seed testing and/or ecological purposes is still underestimated. Future studies should account for the variation in seed germination within and among species and the interactions of water potential with temperature. Field experiments would be warranted for validation of model descriptions under natural variation.

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