Quantitative estimation of seed desiccation sensitivity using a quantal response model: application to nine species of the genus *Coffea* L.

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Abstract

Seed desiccation sensitivity was studied in nine species of the genus *Coffea* by measuring seed viability after equilibration over various saturated salt solutions. A quantal response model based on the logistic distribution was developed in order to describe the typical S-shaped patterns observed. The closeness of fit of the desiccation sensitivity model was shown, and the assumption that seed desiccation sensitivity follows a continuous distribution within species was verified. For each species, the water content at which 50% of initial viability was reached, WC_{50}, and a specific parameter describing the intra-specific variability, β, were calculated using a non-linear regression. A simplified water sorption model was developed which allowed easy calculation of water activity and water potential corresponding to WC_{50} (a_{w50} and Ψ_{50}) for relative humidities ranging between 10 and 100%. Distribution of WC_{50} and Ψ_{50} (or a_{w50}) in the genus *Coffea* was homogeneous within the following intervals: from 0.05 to 0.38 g $H_2O.g^{-1}$ dw for WC_{50} and from -168 to -11 MPa for Ψ_{50}. Different classifications of the coffee species studied as regards to their desiccation sensitivity were obtained depending on whether WC_{50} or Ψ_{50} was used for classification. The continuum for desiccation sensitivity observed within the nine species studied confirmed that coffee is an appropriate material for studying desiccation sensitivity.

Keywords: *Coffea*, seed, desiccation sensitivity model, sorption isotherm

Introduction

The term 'recalcitrant' was introduced by Roberts (1973) to characterize seeds which cannot withstand

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much desiccation and thus cannot be stored under the dry conditions used for orthodox seeds. Recalcitrant seeds are also characterized by their very short lifespan in the hydrated state. Since this definition was proposed, various degrees of desiccation sensitivity, hydrated storage lifespan and chilling sensitivity have been observed in numerous recalcitrant seed species, and it has thus been suggested that the recalcitrant category includes a continuum of different seed storage behaviours (Farrant *et al.*, 1988). In addition, Ellis *et al.* (1990) defined a third category of seed storage behaviour, termed 'intermediate', which does not fall under the orthodox or recalcitrant categories, based on storage experiments performed with *Coffea arabica*.

Thus, considering that 'recalcitrance is not an all-or-nothing situation', Berjak and Pammenter (1994) made several suggestions to researchers working on seed desiccation sensitivity in order to facilitate, in the future, the understanding of seed desiccation responses. Two of these recommendations concerned the determination of the water content, and the corresponding water activity at which 50% of the original viability (V_{50}) is reached when dehydrating seeds under specified ambient conditions. Berjak and Pammenter (1994) underlined notably that the drying rate should be specified and that relative humidities should be used for determining water activities (a_w). However, no statistical procedure was proposed to determine the water content corresponding to V_{50}, and a simple method for its determination is still needed.

For desiccation-sensitive species, typical patterns of response to desiccation are the characteristic 'S-shaped' curves, such as those presented by Hong and Ellis (1996) in their protocol to determine seed storage behaviour. S-shaped patterns are characteristic of quantal response models, which have been widely used and described in medical research for drug tolerance (Finney, 1978). In quantal response models, it is assumed that each individual of the population studied has its own tolerance level. This leads to the

concept of a continuous distribution of tolerance in the population studied, which is often approximated by a normal or logistic distribution. The choice between normal and logistic distributions is not crucial since both approximate very well the distribution of tolerance (Finney, 1978). In our study, the logistic distribution was chosen for its numerical tractability which allowed easy testing of the model with the least square regression.

Seed desiccation tolerance studies involving quantal response models have been rarely reported: to our knowledge, the logistic distribution has never been tested, and a normal distribution of desiccation tolerance within the seed populations studied was assumed with *Araucaria hunsteinii* (Tompsett, 1982), *Dipterocarpus turbinatus* (Tompsett, 1987), *Quercus rubra* (Pritchard, 1991) and *Zizania palustris* (Kovach and Bradford, 1992) only. This allowed, through the probit transformation, the statistical estimation of the relationship between viability and water content and the precise determination of the water content corresponding to V_{50}. However, in other studies, even though a highly significant correlation was observed between probit-transformed viability and water content, the hypothesis of a continuous distribution of desiccation tolerance was rejected, and the goodness of fit of the model was linked to the very high coefficients of variation observed for water content (Probert and Longley, 1989; Dickie *et al.*, 1991; Finch-Savage, 1992).

In the present study, a mathematical model that allowed estimation of seed desiccation sensitivity was established using the logistic distribution. The desiccation sensitivity of seeds of nine coffee species was studied. The model proposed was then applied to determine V_{50} in these species. A simplified water sorption model was developed and applied to calculate the water activities corresponding to V_{50}.

Materials and methods

Plant material

Batches of mature fresh seeds of *Coffea brevipes* Hiern, *C. canephora* Pierre, *C. eugenioides* Moore, *C. humilis* Chevallier, *C. liberica* Hiern, *C. pocsii* Bridson, *C. pseudozanguebariae* Bridson and *C. stenophylla* G. Don. were provided from the field collections of ORSTOM-IDEFOR, Divo and ORSTOM, Man, Côte-d'Ivoire (water contents at receipt were 0.88, 0.82, 0.99, 0.64, 0.79, 0.79, 0.81 and 0.72 g $H_2O.g^{-1}$ dw, respectively). *C. arabica* L. (*typica* variety) seeds were provided from CATIE, Turrialba, Costa Rica (water content at receipt, 0.68 g $H_2O.g^{-1}$ dw). Before desiccation treatments, seed lots were stored in the dark at ambient temperature for 1–2 weeks.

Desiccation

After the testa was removed, seeds were desiccated by equilibration for 2 weeks at 25°C over 13 different saturated salt solutions (BaCl$_2$, K$_2$CO$_3$, K$_2$SO$_4$, KNO$_3$, KOH, Mg(NO$_3$)$_2$, MgCl$_2$, Na$_2$CO$_3$, NaNO$_3$, NaOH, NH$_4$Cl, NH$_4$NO$_3$ and (NH$_4$)$_2$SO$_4$). Saturated salt solutions were prepared according to the method described in the Merck Index (Budavari *et al.*, 1989). Seed sorption isotherms were established for each species using all saturated salt solutions. The effect of desiccation on seed viability was determined by using 6–12 dehydration treatments, depending on the species. For each saturated salt solution, the water content (expressed in g $H_2O.g^{-1}$ dw) was estimated for 10 individual seeds. The dry weight was measured after 2 d of drying in an oven at 105°C. For each species, the effect of 2 weeks of equilibration at 25°C over a saturated salt solution on the intra-treatment variance for seed water content was estimated by the observed *F* values, as calculated by the initial variance (Var_i):final variance (Var_f) ratio. For each treatment, the initial and final variances for seed water content were calculated on 10 individual seeds.

Desiccation rate

A preliminary experiment was carried out with one species, *C. canephora*, to determine the time necessary for seeds to equilibrate when placed over the various saturated salt solutions employed and to establish the corresponding desiccation rates. The water content of 10 individual seeds was measured as described previously after 0–14 days of equilibration.

Viability assessment

For each dehydration treatment, seed viability was assessed on 50 seeds. With six coffee species, *C. brevipes*, *C. eugenioides*, *C. humilis*, *C. pocsii*, *C. pseudozanguebariae* and *C. stenophylla*, seed viability was also measured immediately upon receipt (i.e. before any dehydration treatment). Seed culture was carried out according to the method described by Dussert *et al.* (1998). The emergence of the hypocotyl and radicle and opening of cotyledons after 3 months in culture were used as criteria for measuring the viability.

Desiccation sensitivity model

The quantification of desiccation sensitivity was carried out by considering the following probabilistic model, which assumed that within each species, desiccation sensitivity of individual seeds followed a logistic distribution function, F, with $F(x) = \Psi(\alpha + \beta x)$ where α and β were specific parameters, with

$-\infty < \alpha < \infty$, $0 < \beta < \infty$, $\Psi(t) = [1 + \exp(-t)]^{-1}$ and $-\infty < t < \infty$.

In our study, the viability, V, was the response and the water content, WC, was the stimulus, thus, $V = [1 + \exp(-\alpha - \beta WC)]^{-1}$.

The estimated initial viability, V_i, was introduced in the model which could thus be written

$$V = \frac{V_i}{1 + \exp(-\alpha - \beta WC)}. \tag{1}$$

If WC_{50} is defined by the water content at which 50% of initial viability was reached (V_{50}),

$$V_{50} = \frac{V_i}{2} = \frac{V_i}{1 + \exp(-\alpha - \beta WC_{50})}, \text{ thus}$$
$$\exp(-\alpha - \beta WC_{50}) = 1 \text{ and} \tag{2}$$
$$WC_{50} = -\alpha/\beta$$

From (1) and (2), desiccation sensitivity was thus described by the following quantal response model:

$$V = \frac{V_i}{1 + \exp(-\beta(WC - WC_{50}))}$$

The desiccation sensitivity model was tested using the least square regression as computed by the Quasi-Newton method. The proportion of variance explained, R^2, and the residual variance, σ_e^2, were used for evaluating the fit of the model.

The interval between WC_{90} and WC_{10}, i.e. the water content at which 90% and 10% of initial viability were reached, respectively, was calculated to describe in a more tangible manner the intra-specific variation described by the parameter β. From the equation describing the desiccation sensitivity model, it could be easily deduced that

$$WC_{90} - WC_{10} = \frac{2 \ln(9)}{\beta}.$$

Sorption model

A numerical model of sorption isotherms was necessary to determine mathematically the water activity corresponding to WC_{50}. D'Arcy and Watt's (1970) sorption model was developed to describe mathematically water sorption, and its appropriateness for describing seed sorption isotherms has been demonstrated by Vertucci and Leopold (1987).

D'Arcy and Watt's model can be written as follows:

$$WC = \frac{KK'(p/p_0)}{1 + K(p/p_0)} + c(p/p_0) + \frac{kk'(p/p_0)}{1 - k(p/p_0)}$$

where WC is the seed water content (expressed in grams of sorbed water per gram of dry weight), p/p_0 is the relative vapour pressure, K, K', c, k and k' are specific parameters. The first term of D'Arcy/Watt's model describes sorption of strong binding sites, the second term relates to weak binding sites, and the third term describes sites where water condenses as a collection of molecules (multimolecular sorption).

In using D'Arcy and Watt's model, the expression of p/p_0 as a function of WC consists in resolving a cubic equation, which is not formally feasible. It is thus proposed to simplify the D'Arcy/Watt model for values of p/p_0 between 0.1 and 1 by replacing the first term by the constant K'. Indeed, between 0 and 1, the first term $\dfrac{KK'(p/p_0)}{1 + K(p/p_0)}$ tends to $\dfrac{KK'}{1 + K}$ as p/p_0 tends to 1, but the convergence occurs most rapidly for values of p/p_0 close to zero and, at the p/p_0 value of 0.1, the first term is very close to its asymptotic value. Since K is high compared with 1, it was assumed that the first term tends to K'.

Thus, the following simplified sorption model

$$WC = K' + c(p/p_0) + \frac{kk'(p/p_0)}{1 - k(p/p_0)}$$

was tested using the least square regression for p/p_0 values between 0.1 and 1. The proportion of variance explained, R^2, and the residual variance, σ_e^2, were used for evaluating the fit of the model.

Both water activity, a_{w50}, and water potential, Ψ_{50}, corresponding to WC_{50} were calculated mathematically after expressing $a_w(p/p_0)$ as a function of WC, K', c, k and k'. Expressing a_w as a function of WC consisted of resolving a quadratic equation. Because of its high number of terms, the resulting expression is not developed here. Water potentials were calculated from water activities from the equation $\Psi = RT \ln(a_w)/V_w$ (Pa), with $R = 8.314$, $T = 298$ K and $V_w = 18.07 \ 10^{-3}$ l.

Results

Desiccation rate

The seed water content as a function of the dehydration period for *C. canephora* seeds over a saturated NH_4Cl solution (equilibrium RH = 78%) is presented in Figure 1. It illustrates the curvilinear change of seed water content during equilibration over saturated solutions (an exponential model has been used to fit the observed data in Figure 1) and showed that the drying rate was not constant. However, since in most desiccation treatments at least 8 d of equilibration were required to reach the final water content, the drying rate of seeds by
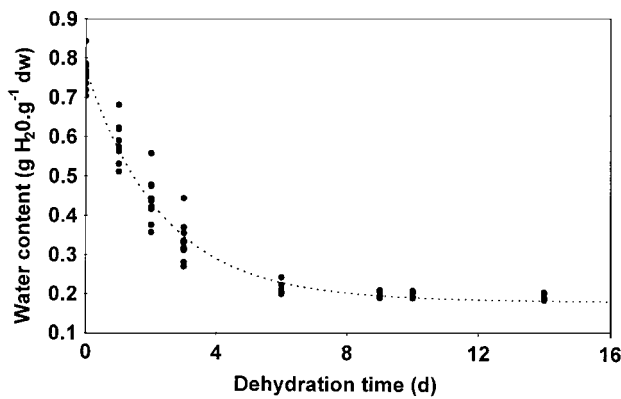
Figure 1. Effect of desiccation time on *C. canephora* seed water content (g $H_2O.g^{-1}$ dw). Seeds were desiccated by equilibration over a saturated NH_4Cl solution. Points (individual seed water content) were fitted by an exponential curve in order to illustrate the non-linear evolution of seed water content with time.

equilibration over a saturated salt solution could be considered very low.

Effect of the desiccation procedure on the intra-treatment variance for seed water content

Observed *F* values for seed water content obtained after 2 weeks of equilibration with six saturated solutions are presented in Table 1 for the nine coffee species. With the exception of two treatments (*C. brevipes* and *C. stenophylla* over a saturated K_2SO_4 solution), since observed *F* values were higher than the $F_{(9,9)} = 3.18$ for $P = 0.05$ ($F_{(9,9)} = 5.35$ and 10.11 for $P = 0.01$ and 0.001, respectively), there was always a significant reduction of the intra-treatment variance for seed water content during the dehydration period.

This decrease of intra-treatment variance for seed water content in line with increasing dehydration periods can also be observed in Figure 1.

Effect of desiccation on seed viability

For the nine coffee species studied, a decrease in the percentage of normal seedlings was observed when seeds were dehydrated (Fig. 2). With the exception of *C. arabica* and *C. pseudozanguebariae,* for which a rapid decline in viability was observed at low water content only, typical S-shaped patterns were generally observed. The nine coffee species showed a large variability as regards the water content at which seed viability started to decline or that at which seed viability was completely lost. Moreover, in some species, the difference between these two effective water contents could be very high: for example, viability of seeds of *C. humilis* started to decline at about 0.6 g $H_2O.g^{-1}$ dw, and complete viability loss was noted at about 0.1 g $H_2O.g^{-1}$ dw.

Seed desiccation sensitivity model

The proportion of variance explained by the desiccation sensitivity model was always very high (96–100%) and the residual variance very low (1–46), which demonstrated the goodness of fit of the model (Table 2). No correlation was found between WC_{50} and the specific parameter β ($P = 0.1302$, $R^2 = 0.2956$). The initial viability, V_i, and WC_{50} were also independent ($P = 0.7257$, $R^2 = 0.0187$), as well as β and V_i ($P = 0.4617$, $R^2 = 0.0797$). With the six species for which seed viability was measured upon receipt (i.e. before dehydration), the estimated initial viability V_i was highly correlated with viability measured at that time ($R^2 = 0.6475$; $P = 0.0332$).

Table 1. Effect of 2 weeks of equilibration at 25°C over various saturated salt solutions ($MgCl_2$, $Mg(NO_3)_2$, NH_4NO_3, NH_4Cl, KNO_3 and K_2SO_4) on the intra-treatment variance for seed water content: observed *F* values, as calculated by the ratio of the initial variance (Var_i) and the final variance (Var_f). For each salt, the corresponding relative humidity (%) at 25°C is indicated between brackets

|	F_{obs} (Var_i: Var_f ratio)					
	$MgCl_2$ (34%)	$Mg(NO_3)_2$ (53%)	NH_4NO_3 (62%)	NH_4Cl (78%)	KNO_3 (92%)	K_2SO_4 (97%)
C. canephora	124.3	70.7	87.0	48.0	16.1	5.9
C. liberica	472.8	271.0	13.4	161.6	111.4	6.2
C. brevipes	76.9	6.7	99.8	20.8	110.3	2.3
C. eugenioides	821.4	660.6	712.3	200.9	323.5	18.6
C. humilis	8028.7	133.4	40.7	207.4	5.5	20.4
C. pseudozanguebariae	502.4	112.4	302.7	83.5	65.8	15.3
C. pocsii	158.5	17.6	61.5	27.5	101.1	9.4
C. stenophylla	65079.9	25.7	51.1	2724.3	9.5	1.9
C. arabica	15.3	37.0	31.7	5.7	14.2	15.8
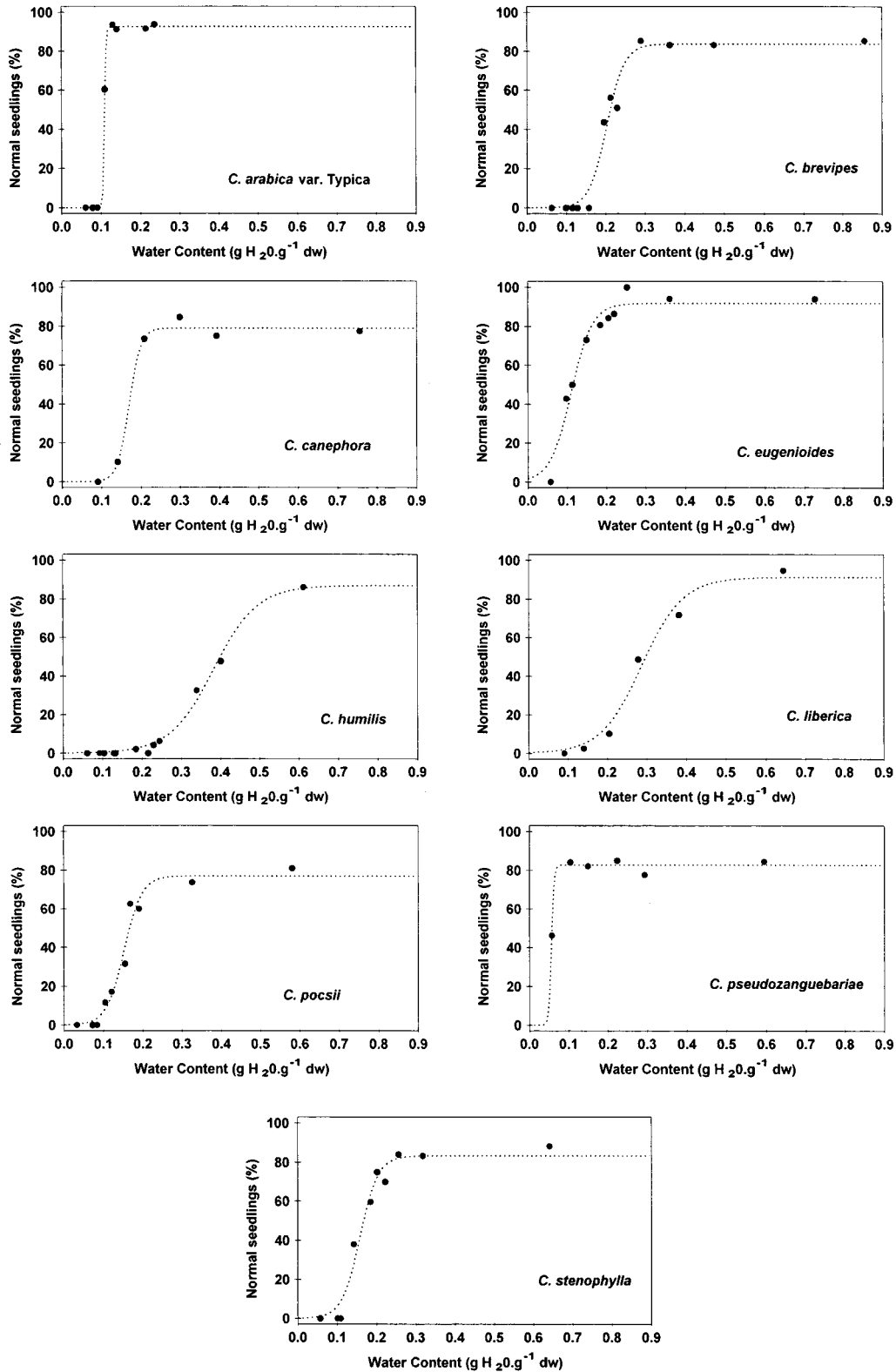
Figure 2. Observed viability of seeds at various water contents (●) and fitted pattern of the desiccation sensitivity model as computed by the Quasi-Newton method for nine coffee species.

Simplified water sorption model

Water sorption isotherms for *C. liberica* (high sensitivity to desiccation) and *C. pseudozanguebariae* (low sensitivity to desiccation) are illustrated in Figure 3. The curves follow the expected shape in the range of relative humidities studied, i.e. a linear curve for p/p_0 values between 0.1 and 0.7, and a sharp upswing for higher p/p_0 values (0.7 and 0.97).

The very high proportion of variance explained by the model (98–100%) and the very low residual variance (0.0000–0.0003) demonstrated the closeness of fit of the simplified water sorption model for p/p_0 values between 0.1 and 1 (Table 3). The numerical expression of a_w (p/p_0) as a function of WC could thus be achieved.

Interspecific variability of seed desiccation sensitivity

The nine coffee species studied showed a broad variability for all parameters calculated: WC_{50}, a_{w50}, Ψ_{50} and $WC_{90}-WC_{10}$ (Tables 2 and 3; Fig. 4). WC_{50} ranged from 0.05 g $H_2O.g^{-1}$ dw for *C. pseudozanguebariae* to 0.38 g $H_2O.g^{-1}$ dw for *C. humilis*. The corresponding water activities and water potentials varied from 0.29 to 0.92 and from −168 to −11 MPa, respectively.

The distribution of WC_{50} and a_{w50} for the nine *Coffea* species studied was considered to be homogeneous since the presence of two modes in both distributions could not be tested statistically (Fig. 4). However, if two modes existed, corresponding to low-sensitivity and high-sensitivity species, respectively, the size of each mode was different, depending on the variable studied. Indeed, seven species presented a low sensitivity to desiccation on the basis of WC_{50}, whereas three species only were a little sensitive if a_{w50} was used as the criterion. With both models, *C. pseudozanguebariae*, *C. arabica* and *C. eugenioides* were the less sensitive species with WC_{50} values lower than 0.11 g $H_2O.g^{-1}$ dw and Ψ_{50} values lower than −94 MPa.

With WC_{50} values higher than 0.27 g $H_2O.g^{-1}$ dw and Ψ_{50} values superior to −28 MPa, *C. liberica* and *C. humilis* were the most desiccation-sensitive species with both classifications. By contrast, the ranking of two species, *C. canephora* and *C. pocsii*, varied depending on the classification employed.

Discussion

When desiccating coffee seeds by equilibration over saturated salt solutions for 2 weeks, seed water content followed a curvilinear decrease and, from the beginning of the second week onwards, it became very close to its final asymptotic value. This second week of equilibration may be considered as a short-term storage experiment. This assumption makes the interpretation of the results more difficult since the effect of desiccation can not be distinguished from that of short-term storage, especially for the most desiccation-sensitive coffee species. Therefore, if equilibration treatments confer the advantage of reducing intra-treatment variability for seed water content and, thus, of facilitating the establishment of statistical relationships between seed water content and viability, they present the potential drawback of confounding two distinct effects. However, since the duration of this storage treatment was very short, its possible effect was considered very low compared with that of desiccation and, in the following discussion, results are interpreted in terms of desiccation sensitivity only.

The goodness of fit of the desiccation sensitivity model was shown for the nine coffee species tested. Several factors can explain the very scarce use of quantal response models in seed desiccation-tolerance studies (Tompsett, 1982, 1987; Pritchard, 1991; Kovach and Bradford, 1992). Indeed, quantal response models are often associated with the use of the normal distribution function. The mathematical complexity of the normal distribution function (the function contains

Table 2. WC_{50}: water content at which 50% of initial viability was reached; $WC_{90}-WC_{10}$: interval between water contents at which 90 and 10% of initial viability were reached respectively; V_i: initial viability; β: specific parameter; R^2: proportion of variance explained by the desiccation sensitivity model; σ_e^2: residual variance for the nine coffee species

	WC_{50}	$WC_{90}-WC_{10}$	V_i (%)	β	R^2 (%)	σ_e^2
C. arabica	0.109	0.009	92.6	490.5	100.0	0.8
C. brevipes	0.203	0.100	83.8	44.0	97.6	43.9
C. canephora	0.170	0.066	79.0	66.4	99.3	16.7
C. eugenioides	0.110	0.119	91.8	37.0	96.1	35.0
C. humilis	0.382	0.246	86.9	17.8	99.3	6.3
C. liberica	0.288	0.222	91.6	19.8	98.2	46.3
C. pocsii	0.153	0.101	80.0	43.6	97.0	41.0
C. pseudozanguebariae	0.056	0.015	82.7	297.3	96.8	12.3
C. stenophylla	0.158	0.101	83.2	43.3	97.2	49.6

Table 3. K', c, k, k': specific parameters of the simplified water sorption model; R^2: proportion of variance explained by the regression model; σ_e^2: residual variance for the nine coffee species. Calculated values of water activity (a_{w50}) and water potential (Ψ_{50}) corresponding to WC_{50}

	K'	c	k	k'	R^2 (%)	σ_e^2	a_{w50}	Ψ_{50}
C. arabica	0.050	0.092	1.038	0.021	99.7	0.000	0.444	−111
C. brevipes	0.081	0.010	0.983	0.037	99.6	0.000	0.766	−37
C. canephora	0.037	0.112	0.990	0.025	99.9	0.000	0.692	−50
C. eugenioides	0.056	0.098	0.994	0.020	99.7	0.000	0.409	−123
C. humilis	0.067	0.025	0.960	0.039	98.9	0.000	0.920	−11
C. liberica	0.038	0.094	0.976	0.029	99.9	0.000	0.874	−18
C. pocsii	0.038	0.073	0.985	0.022	98.9	0.000	0.744	−41
C. pseudozanguebariae	0.026	0.076	0.992	0.019	99.9	0.000	0.293	−168
C. stenophylla	0.061	0.072	0.983	0.025	99.9	0.000	0.670	−55

an integral) does not allow the use of common non-linear regression methods. Probit transformation allows testing of the model with a linear regression analysis, but few statistical softwares can perform this transformation and, in some cases, considerable
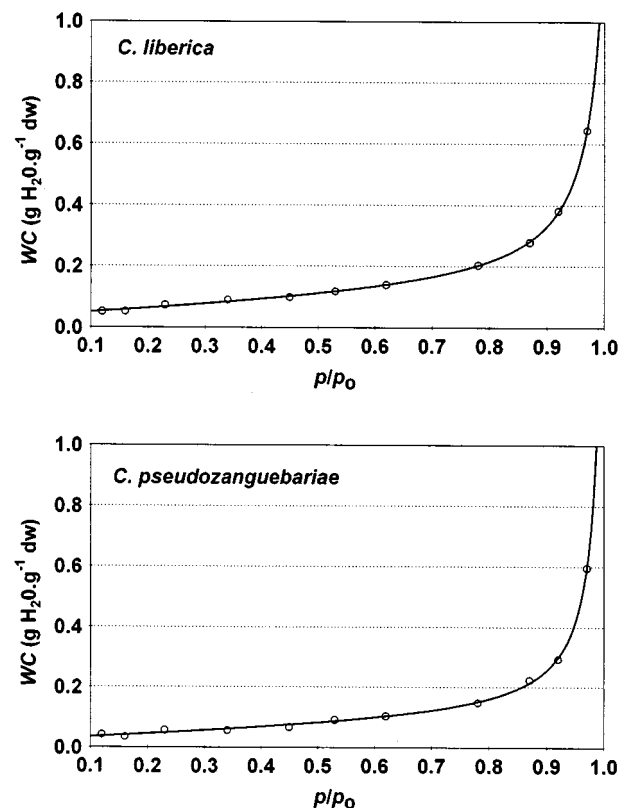

Figure 3. Observed seed water contents (○), *WC*, at various relative vapour pressures, p/p_0, and fitted pattern of the simplified water sorption model as computed by the Quasi-Newton method in a highly desiccation-sensitive species, *C. liberica*, and a weakly desiccation-sensitive species, *C. pseudozanguebariae*.
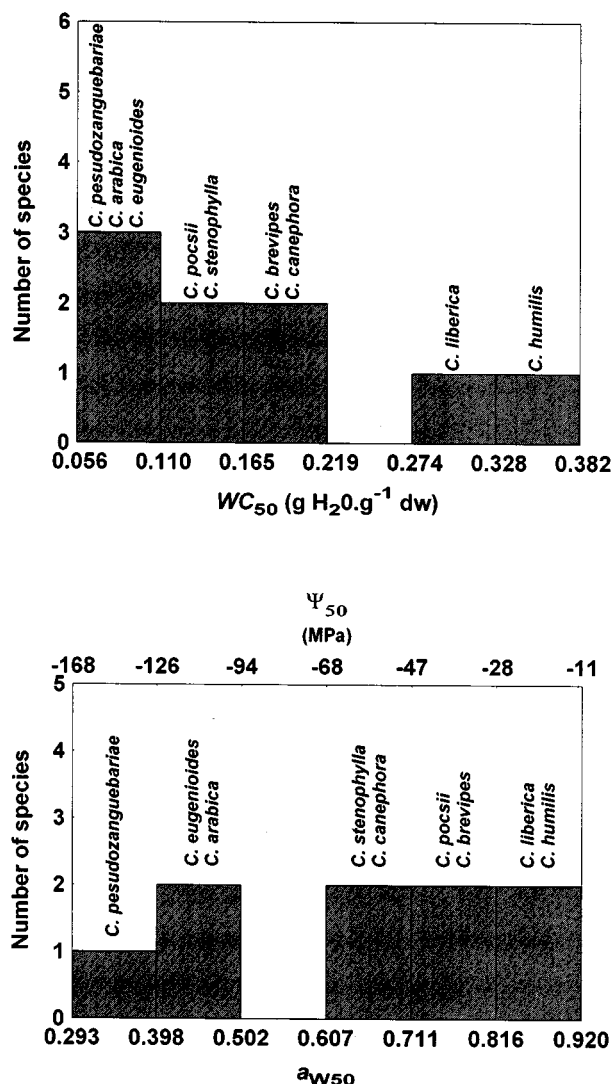

Figure 4. Distributions for the nine *Coffea* species of the water content at which 50% of initial viability was reached, WC_{50}, and the corresponding water activity, a_{w50}, and water potential, Ψ_{50}.

amounts of data are required to allow model fitting (Tompsett, 1987). Thus, the use of the logistic distribution and least square regression represents a very simple alternative method for quantifying desiccation tolerance.

The main assumption of the model was that desiccation sensitivity of individual seeds followed a continuous distribution within each species, i.e. that each seed possesses its proper critical water content below which it loses its capacity to germinate. Since the environmental and genetic factors controlling the development of individual seeds are highly variable, it could appear obvious to assume that within a seed population, representative of a species, desiccation tolerance follows a continuous distribution. However, in some cases (e.g. with *C. arabica* or in *C. pseudozanguebariae*), the variation in desiccation sensitivity in the seed population studied was so narrow that it could be assumed that a specific critical moisture content for desiccation damage existed. By contrast, with other coffee species (e.g. *C. liberica, C. humilis* and *C. eugenioides*), as well as with all species for which a typical S-shaped pattern was observed in response to dehydration (Tompsett, 1982, 1987; Kovach and Bradford, 1992), the data collected allowed verification of the model. The hypothesis that desiccation sensitivity of individual seeds followed a continuous distribution was rejected by some authors (Probert and Longley, 1989; Dickie *et al.*, 1991; Finch-Savage, 1992) who suggested that the high correlation rates obtained with probit transformation (normal distribution) were an artefact caused by the large variations in seed water content obtained within each treatment. In our experiments, intra-treatment variances for water content were extremely low after desiccation, and the hypothesis of a continuous distribution for desiccation sensitivity could not be rejected for this reason.

Interspecific variability for desiccation sensitivity could be appreciated on the basis of both quantitative parameters, WC_{90}–WC_{10} (or β), which describe the seed-to-seed variation for desiccation sensitivity and WC_{50}, which corresponds to the median value of the seed population. Species of the genus *Coffea* thus constitute relevant material for further research on seed desiccation sensitivity. On the one hand, investigations are needed to identify the potential sources of intra-specific variability. When seed lots are constituted in field genebanks by bulking seeds from several genotypes, as was the case in our study, experiments could be designed to test the effect of, for example, the genotype, the tree or the branch on the tree from which seeds are collected on their variability for desiccation sensitivity. The observed intra-specific variability could also be related to differences in the developmental stage of seeds at harvest. On the other hand, at both the intra- and inter-specific levels, all

current hypotheses reported in the literature concerning the mechanisms by which seeds tolerate desiccation (Vertucci and Farrant, 1995; Hong and Ellis, 1996; Berjak and Pammenter, 1997) could be investigated using species of the genus *Coffea*.

Recently, Hong and Ellis (1996) have proposed a two-stage procedure to determine seed storage behaviour. The first stage relates to seed desiccation sensitivity and aims at predicting in which category – recalcitrant, intermediate or orthodox – a given species is the most likely to fall. These authors underlined that this first stage provides probability statements only and that determination of desiccation tolerance alone does not allow determination of seed storage behaviour. According to these authors, if most seeds are killed by desiccation to 0.18–0.25 g $H_2O.g^{-1}$ dw, they are likely to show recalcitrant seed storage behaviour. Thus, on the basis of the observed values for WC_{50}, *C. liberica* and *C. humilis* are most likely to be classified as recalcitrant. In a second step, species whose seeds withstand desiccation down to 0.18–0.25 g $H_2O.g^{-1}$ dw water contents, but are killed at water contents below 0.05 g $H_2O.g^{-1}$ dw, are likely to be intermediate. According to this second criterion, *C. arabica, C. canephora, C. eugenioides, C. pocsii* and *C. stenophylla* are most likely to fall in the intermediate category. Following the above criteria, the category in which *C. brevipes* and *C. pseudozanguebariae* are the most likely to fall could not be easily determined because their WC_{50} value is situated within the water content limits proposed by Hong and Ellis (1996). The existence of a continuum of WC_{50} values among coffee species underlines the difficulty of proposing rigid critical water contents for prediction of categorization as it is the case in the first stage of Hong and Ellis' procedure (1996). The seed storage behaviour of only three coffee species has been studied so far: *C. arabica, C. canephora* and *C. liberica* (Ellis *et al.*, 1990; Hong and Ellis, 1995). For these three coffee species, the above predictions of seed storage behaviour based on WC_{50} values only are consistent with the categorizations established by Ellis and co-workers: *C. arabica* and *C. canephora* showed intermediate seed storage behaviour and seeds of *C. liberica* were classified as recalcitrant. For the other species, further investigations on the survival of seeds after storage in different environments are needed, especially in the case of *C. pseudozanguebariae* which showed the lowest desiccation sensitivity.

To quantify seed desiccation sensitivity, various authors (Berjak and Pammenter, 1994; Vertucci and Farrant, 1995) have recommended reporting the water activity corresponding to V_{50}, rather than the water content, since the response of seeds to dehydration is related to the thermodynamic status of tissue water. In this study, we propose a simplification of D'Arcy and Watt's model (1970) which fits very well with water

sorption data within a restricted interval of vapour pressures (0.1–1) and allowed very precise determination of the water activity and water potential corresponding to V_{50}. The main advantage of this simplification is that it allows the numerical expression of a_{w50} as a function of WC_{50} through the resolution of a quadratic equation. Moreover, it lowers the number of parameters to be resolved during a non-linear regression, and thus reduces the amount of data needed for fitting. This simplification should be very useful in all cases where knowledge of isotherm characteristics in the 0–0.1 interval of p/p_0 values is not crucial, i.e. with species for which a_{w50} is higher than 0.1, which should be the case for most intermediate or recalcitrant species.

Vertucci and Farrant (1995) have proposed that there are critical water levels at which discrete changes in metabolic activity occur. These authors described five levels of hydration and, for each level, they reported the stresses resulting from water removal. They suggested that intact recalcitrant seeds die when Type 4 water (lower boundary, −3 MPa) is removed from seed tissues. The variation observed for Ψ_{50} within the genus *Coffea* was between −11 and −168 MPa. Thus, none of the studied *Coffea* species could be classified as recalcitrant according to this criterion. The hydration level 1 as defined by Vertucci and Farrant (1995) corresponds to water potential values below −150 MPa. These authors suggested that intermediate seeds rapidly lose viability when Type 1 water is removed. Based on this critical water potential, none of the coffee species studied, with the exception of *C. pseudozanguebariae*, would be intermediate. Since *C. arabica* was classified as intermediate (Ellis *et al.*, 1990) and since Vertucci and Farrant (1995) mentioned that the above critical water potential values were approximate, it appears that a very high variability could exist among species for the boundary water potentials between hydration levels 1 and 2. However, on the basis the assumptions of Vertucci and Farrant (1995), the nine coffee species studied would be intermediate, thus providing additional evidence for the existence of a continuum in seed desiccation sensitivity among intermediate species.

In conclusion, depending on the criterion used for prediction of categories in which species are the most likely to fall, WC_{50} or Ψ_{50}, the distribution of the nine coffee species studied between both recalcitrant and intermediate categories was not consistent. Determination of critical water contents or critical water activities to distinguish levels of seed desiccation sensitivity in species which do not present orthodox seed storage behaviour does not seem formally feasible. This study stressed the importance of quantifying desiccation sensitivity of a given species using standard statistical descriptors and

emphasized the recommendations of Berjak and Pammenter (1994).

Finally, in their recommendations to normalize data acquisition with desiccation sensitive species, Berjak and Pammenter (1994) also suggested determining WC_{50} for embryonic axes and storage tissues separately. Cryopreservation studies performed with zygotic embryos of some coffee species already provide some information on their desiccation sensitivity. In the case of *C. arabica* zygotic embryos (Abdelnour-Esquivel *et al.*, 1992), it was shown that V_{50} was reached at 0.11 g $H_2O.g^{-1}$ dw, a value equivalent to that obtained for whole seeds of this species in the present study. It was also observed with *C. liberica* that whole seeds and excised zygotic embryos could withstand equivalent desiccation levels (Normah and Vengadasalam, 1992). These preliminary results show the importance of investigating desiccation sensitivity of coffee zygotic embryos in several coffee species and of comparing the data collected with those obtained for whole seeds.

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