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Quantifying the oxygen sensitivity of seed germination using a population-based threshold model

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Abstract

Seeds vary widely in the sensitivity of germination to oxygen (O_2) partial pressure, depending upon the species, temperature, dormancy state and physiological status of the seeds. Most analyses of the O_2 sensitivity of germination have focused on final germination percentages and estimated the O_2 percentage in air that is required to reduce germination to a given percentage (usually 50%). In contrast, we have applied a population-based threshold model utilizing time courses of germination to quantify three parameters related to seed germination sensitivity to O_2 availability: the median base (or threshold) O_2 percentage, the standard deviation of O_2 thresholds among seeds in the population, and an oxygen–time constant that relates O_2 percentage to germination timing. The model fits germination responses accurately across a wide range of O_2 concentrations. The response to O_2 was logarithmic in all cases, with the O_2 percentage required for 50% germination ranging from 21% to as low as 0.005%, depending upon the species, the temperature and the seed dormancy level. Modelling indicated that some seeds can adapt to low O_2 percentages and shift their thresholds to lower values over time. Lower temperatures decreased the minimum O_2 threshold, as did after-ripening. Seed priming generally reduced the oxygen–time constant and increased the standard deviation of germination responses, but had relatively little effect on the O_2 sensitivity *per se*. The population-based threshold model can be used to quantify the O_2 sensitivity of seed germination and to predict germination rates and percentages when O_2 availability is limiting.

Introduction

During seed imbibition, the quiescent dry seeds rapidly resume metabolic activity, including respiration, which can be measured within a few minutes of hydration (Hourmant and Pradet, 1981). It is not, then, surprising that O_2 supply has a major impact on germination, as has been reviewed previously (Al-Ani *et al.*, 1985; Corbineau and Côme, 1995). Sensitivity of seed germination to oxygen partial pressure (pO_2) depends on the species (Corbineau and Côme, 1995). Al-Ani *et al.* (1985) identified two groups of seeds according to their responsiveness to low pO_2: seeds with high lipid content (group I) are more sensitive to O_2 deprivation than are seeds with high starch content (group II). Sensitivity to O_2 has also been expressed in terms of mean germination rates (speed), which had linear relationships with the logarithm of pO_2 (Al-Ani *et al.*, 1985), and the minimum O_2 thresholds calculated by extrapolation of these linear relationships differed among species. Differences in sensitivity to O_2 supply observed between carrot (*Daucus carota*) cultivars (Corbineau *et al.*, 1995) also suggest that there may be a genetic component in this variation. In addition, Finch-Savage *et al.* (2005b) demonstrated that sensitivity of *Brassica oleracea* seeds to O_2 deprivation varied among genotypes and segregated with a continuous distribution in a doubled haploid population. In addition, the differences observed between genotypes or seed batches might also result from the conditions of seed production. For example, in both carrot (Corbineau *et al.*, 1995) and *B. oleracea* (Finch-Savage *et al.*, 2005b), the greater the mean seed weight, the higher the sensitivity of the seeds to hypoxia. Differences in seed weight in carrot are largely associated with the position of the seed on the mother plant (umbel order) even within the same genotype (Oliva *et al.*, 1988).

The O_2 requirement for seed germination is also strongly modulated by other environmental factors (e.g. temperature, water potential and light) (Corbineau and Côme, 1995, and references therein). Generally, the sensitivity of seeds to O_2 deprivation decreases with decreasing temperature, because of reduced respiratory activity and the higher solubility of O_2 in water (Côme and Tissaoui, 1973; Corbineau and

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Côme, 1995). The acquisition of responsiveness to light of *Oldenlandia corymbosa* seeds during imbibition requires at least 3.5% O_2 (Corbineau and Côme, 1985), while continuous white light reinforces the sensitivity to O_2 deprivation of negatively photosensitive seeds (Corbineau *et al.*, 1992; Gutterman *et al.*, 1992).

Seed physiological status also influences germination responses to O_2. In different species, dormant seeds are more sensitive to O_2 deprivation than are non-dormant ones. This has been clearly demonstrated with cereal (Corbineau and Côme, 1980; Corbineau *et al.*, 1981; Benech-Arnold *et al.*, 2006), sunflower (*Helianthus annuus*) (Gay *et al.*, 1991; Corbineau and Côme, 1995) and Douglas fir (*Pseudotsuga menziesii*) seeds (Corbineau *et al.*, 2002). Priming (hydration and dehydration) treatments also seem to improve germination of seeds in suboptimal O_2. Osmoprimed seeds of tomato (*Solanum lycopersicum*, formerly *Lycopersicon esculentum*) (Özbingöl *et al.*, 1998) and leek (*Allium ampeloprasum* var. *porrum*) (Corbineau *et al.*, 1994b) are less sensitive to O_2 deprivation than are the control non-primed seeds.

A population-based threshold model has been applied to quantify seed germination responses to temperature, water potential, hormones, light, ageing, dormancy and after-ripening (Covell *et al.*, 1986; Gummerson, 1986; Ni and Bradford, 1992, 1993; Bradford *et al.*, 1993; Bradford, 1995, 2005; Larsen *et al.*, 2004; Bair *et al.*, 2006). The model is based upon the effects of these various factors on the time courses of germination across a range of factor levels. The model quantifies the median base or threshold level of the factor that allows germination, the variation in these threshold values among seeds in the population, and a time constant that relates the speed of germination to the factor level in relation to the thresholds. It has proven to be a robust method to quantify sensitivity thresholds for seed germination and to assess the effects of various influences on those thresholds, such as priming, alternating temperatures, dormancy and after-ripening (Dahal and Bradford, 1990, 1994; Alvarado and Bradford, 2005; Huarte and Benech-Arnold, 2005; Bair *et al.*, 2006). In addition to providing quantitative parameters that characterize germination responses, the model allows the prediction of germination time courses at any level of the quantitative factor and the possibility of modelling the outcomes of interactions among two or more factors (Ni and Bradford, 1992, 1993).

Despite the importance of O_2 in regulating germination in many situations (Corbineau and Côme, 1995), the threshold model has not previously been applied to analyse seed germination responses to O_2. We sought to test whether the threshold model was applicable to quantifying seed germination responses to O_2 availability and, if so, whether new insights might be gained from its application. We have therefore utilized the threshold model to analyse a number of published and previously unpublished datasets representing 15 species and a number of temperature, priming and dormancy conditions. The results demonstrate that the threshold model can be extended to analyse germination responses to O_2 and can provide new information about seed O_2 requirements.

Methods

Germination at different O_2 percentages

When data were obtained from published reports, details of the methods used are in the original citations (see Table 1). Unpublished experiments of Corbineau and colleagues of germination in different O_2 percentages utilized the apparatus and methods described previously (Côme and Tissaoui, 1968). Gas mixtures containing from 0 (pure nitrogen) to 21% O_2 (air) and from 21 to 100% O_2 (pure O_2) were obtained through capillary tubes from compressed air and nitrogen, or from compressed air and O_2, respectively. The gaseous atmospheres thus obtained were passed continuously through germination chambers at a constant flow rate ($4\,l\,h^{-1}$). Each assay was performed with two germination chambers (replicates) containing 30 (*Araucaria*), 100 (tobacco) or 50 seeds (all the other species) placed on a layer of cotton wool moistened with deionized water.

A seed was regarded as germinated when the radicle had pierced the seed-covering structures. Germination counts were made daily, up to 7, 14 or 21 d, depending on the species. The results presented are the means of the germination percentages obtained in the two replicates.

Seed treatments

For osmopriming, seeds were placed on a polyethylene glycol (PEG) 8000 solution at −1.0 MPa at 15°C (tomato) (Özbingöl *et al.*, 1998) or 20°C (carrot, pepper) for 3 or 7 d. After the osmotic treatments, seeds were rinsed with deionized water for 30 s and dried for 3 d at 20°C and 55% relative humidity prior to germination.

For wheat (*Triticum aestivum*) and sunflower, experiments were carried out with freshly harvested (dormant) seeds and seeds that were stored dry at ambient temperature for 1 year (wheat) or at 5°C for 9 months (sunflower) in order to release their dormancy.

The oxygen–time threshold model

The oxygen–time threshold model was adapted from the hydrotime model originally described by Gummerson (1986) and subsequently developed by Bradford and others (Bradford, 1990, 1995;

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Table 1. Oxygen response parameters of seed germination derived from the population-based threshold model

Seed	Species	Treatment or condition	Log $Ox_b(50)$ (log % O_2)	$Ox_b(50)$ (% O_2)	σ_{Ox} (log % O_2)	θ_{Ox} (log % O_2 h)	r^2	Reference
Cauliflower cv. Nautilus	*Brassica oleracea* var. *botrytis*	25°C	0.499	3.15	0.172	18.7	0.959	F. Corbineau, unpublished
Calabrese, broccoli	*Brassica oleracea* var. *italica*	20°C	0.395	2.48	0.137	31.2	0.935	Finch-Savage *et al.* (2005b)
Radish cv. Fakir	*Raphanus sativus*	20°C	0.366	2.32	0.243	18.5	0.927	Al-Ani *et al.* (1985)
Rape seed cv. Falcon	*Brassica napus*	25°C	0.574	3.75	0.226	9.4	0.877	F. Corbineau, unpublished
Carrot cv. Nantucket	*Daucus carota*	20°C	0.723	5.29	0.233	50.4	0.935	Corbineau *et al.* (1994a)
Carrot cv. Senior	*Daucus carota*	20°C, not primed	0.652	4.49	0.162	43.2	0.902	F. Corbineau and C. Faquet, unpublished
Carrot cv. Senior	*Daucus carota*	20°C, primed	0.561	3.64	0.214	30.0	0.843	F. Corbineau and C. Faquet, unpublished
Fennel	*Foeniculum vulgare*	20°C	0.451	2.83	0.448	26.4	0.893	F. Corbineau and N. Özbingöl, unpublished
Witloof chicory cv. Zoom	*Cichorium intybus*	20°C	0.659	4.56	0.352	13.2	0.945	F. Corbineau, unpublished
Sunflower cv. Mirasol	*Helianthus annuus*	25°C, dormant	0.929	8.49	0.446	22.8	0.913	F. Corbineau and E. Roussey, unpublished
Sunflower cv. Mirasol	*Helianthus annuus*	25°C, non-dormant	0.733	5.41	0.282	16.8	0.845	Gay *et al.* (1991)
Soybean cv. Essor	*Glycine max*	25°C	0.480	3.01	0.232	23.3	0.923	F. Corbineau and M. Posmyk, unpublished
Tomato cv. Elko	*Solanum lycopersicum*	25°C	0.502	3.17	0.155	40.8	0.914	Özbingöl *et al.* (1998)
Tomato cv. Marmande	*Solanum lycopersicum*	25°C	0.568	3.70	0.168	24.0	0.927	Corbineau and Côme (1995)
Tomato cv. Elko	*Solanum lycopersicum*	15°C	−0.794	0.161	0.512	412	0.942	Özbingnöl (1998)
Tomato cv. Elko	*Solanum lycopersicum*	15°C, not primed	−0.501	0.315	0.445	336	0.944	N. Özbingnöl and F. Corbineau, unpublished
Tomato cv. Elko	*Solanum lycopersicum*	15°C, primed 3 d	0.012	1.03	0.420	101	0.886	N. Özbingnöl and F. Corbineau, unpublished
Tomato cv. Elko	*Solanum lycopersicum*	15°C, primed 7 d	−0.695	0.202	0.853	96.0	0.925	N. Özbingnöl and F. Corbineau, unpublished
Pepper cv. Zyngaro	*Capsicum annuum*	25°C	0.975	9.45	0.084	31.2	0.834	F. Corbineau and N. Lemonnier, unpublished
Pepper cv. Zyngaro	*Capsicum annuum*	25°C, primed	0.947	8.86	0.102	16.8	0.780	F. Corbineau and N. Lemonnier, unpublished
Tobacco cv. 2601	*Nicotiana tabacum*	20°C, light	0.254	1.80	0.393	118	0.770	F. Corbineau and J.P. Koltalo, unpublished
Tobacco cv. 2601	*Nicotiana tabacum*	30°C, light	0.074	1.18	0.684	93.6	0.863	F. Corbineau and J.P. Koltalo, unpublished
Tobacco cv. 2601	*Nicotiana tabacum*	30°C, light + GA	0.408	2.56	0.453	50.4	0.683	F. Corbineau and J.P. Koltalo, unpublished
Tobacco cv. 30	*Nicotiana tabacum*	20°C	−0.760	0.17	0.340	187	0.934	F. Corbineau and J.P. Koltalo, unpublished
Tobacco cv. 30	*Nicotiana tabacum*	30°C	0.260	1.82	0.264	36	0.744	F. Corbineau and J.P. Koltalo, unpublished
Araucaria	*Araucaria angustifolia*	25°C	0.715	5.19	0.400	170	0.928	Salmen Espindola (1995)
Lamb's lettuce cv. Verte de Cambrai	*Valerianella olitoria*	20°C	0.717	5.21	0.190	40.8	0.922	F. Corbineau, unpublished
Lamb's lettuce cv. Verte de Cambrai	*Valerianella olitoria*	25°C	1.32	21.0	0.453	69.6	0.734	F. Corbineau, unpublished
Rice cv. Cigalon	*Oryza sativa*	25°C	−1.80	0.0160	0.860	135	0.870	Al-Ani *et al.* (1985)
Wheat cv. Champlein	*Triticum aestivum*	20°C, dormant	0.357	2.27	0.727	82.3	0.904	Corbineau *et al.* (1981)
Wheat cv. Champlein	*Triticum aestivum*	20°C, stored	−2.30	0.005	0.766	118	0.860	Corbineau *et al.* (1981)

GA, gibberellic acid, 1 mM.

Finch-Savage, 2004; Allen *et al.*, 2007). The model can be defined by the equation:

$$\theta_{Ox} = [Ox - Ox_b(g)]t_g \quad (1)$$

where θ_{Ox} is the oxygen-time constant, Ox is the O_2 percentage in the air surrounding the seed, $Ox_b(g)$ is the base or threshold value of Ox just allowing germination of percentage g of the viable seed population, and t_g is the time to germination of percentage g. Since θ_{Ox} is assumed to be constant, as the difference between Ox and $Ox_b(g)$ decreases, t_g increases proportionately, and vice versa. In the case of O_2 effects on germination, it was found that the response was proportional to the logarithm of the O_2 percentage, so the actual model used was:

$$\theta_{Ox} = [\log Ox - \log Ox_b(g)]t_g. \quad (2)$$

Since the value of $Ox_b(g)$ varies among seeds in the population, a probit regression method was used to estimate the median and standard deviation of what was assumed to be a normal distribution of O_2 thresholds within the seed population (Bradford, 1990). This can be performed in a spreadsheet by regressing together the probit-transformed germination percentages at each observation time and Ox level on the values of $\log Ox_b(g)$ [calculated as $\log Ox - (\theta_{Ox}/t_g)$] for each observation. The value of θ_{Ox} is varied until the best fit is obtained (highest r^2 value for the regression). Linear relationships in this plot support the assumption of a normal distribution of thresholds. The median base O_2 percentage [$\log Ox_b(50)$] is the value of $\log Ox_b(g)$ when probit $g = 0$ (50%), and the inverse of the slope of the regression is the standard deviation of $\log Ox_b(g)$, or σ_{Ox}. Together, the median and the standard deviation define the distribution of $\log Ox_b(g)$ values, which, along with θ_{Ox}, allows the generation of predicted germination time courses at any value of $\log Ox$. Thus, the three parameters θ_{Ox}, $\log Ox_b(50)$ and σ_{Ox} can characterize the germination response at any value of Ox. More sophisticated weighted probit regression models can also be utilized if confidence limits or mean separations between treatments are required, but the parameter values obtained generally vary little from those obtained by the simple regression approach. Graphs and predicted time courses were generated using CoPlot software (www.cohort.com).

Results

Application of the threshold model to quantify germination sensitivity to oxygen

Data from germination of cauliflower (*Brassica oleracea* L. var. *botrytis*) illustrate the application of the population-based threshold model to quantify germination sensitivity to O_2. Germination time courses were recorded at 25°C in 21, 15, 10, 5 and 3% O_2. Reducing O_2 percentage to 10% slightly delayed germination, but further reductions to 5 or 3% both delayed and reduced the final germination percentage (Fig. 1). The oxygen–time model fit these data well, based on the logarithm of the O_2 percentage ($r^2 = 0.96$). The model indicated that germination

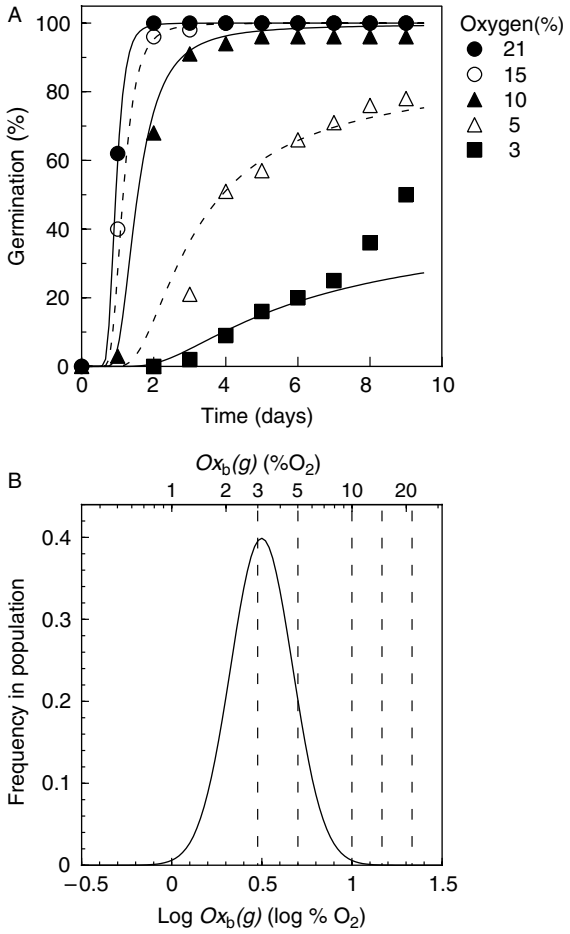

Figure 1. Germination time courses of cauliflower (*Brassica oleracea* L. var. *botrytis*) seeds at 25°C in a range of O_2 percentages. (A) Germination (symbols) at 21, 15, 10, 5 and 3% O_2 and time courses (solid and dashed lines) predicted by the threshold model using the parameter values in Table 1. (B) The distribution of $Ox_b(g)$ values based upon the median and standard deviation of $Ox_b(g)$ values. The vertical dashed lines indicate the O_2 percentages represented in panel A. As the difference between a given O_2 percentage and the threshold for a particular seed fraction decreases, the time to germination increases. If the O_2 percentage intercepts the distribution, the fraction of seeds represented by the relative area under the curve to the right of the O_2 level will not be able to complete germination; so the final germination percentage also declines as pO_2 decreases. (Previously unpublished data of F. Corbineau).

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would be reduced to 50% at 3.15% O_2. Once the median base O_2 percentage ($\log Ox_b(50)$), the standard deviation of the base oxygen percentage (σ_{Ox}), and the oxygen–time constant (θ_{Ox}) were determined (Table 1), the model predicted initial germination time courses at all O_2 percentages with good accuracy (Fig. 1). The distribution of $Ox_b(g)$ values in the seed population is shown in Fig. 1B, along with dashed lines indicating how different O_2 percentages relate to the threshold distribution. The model predicts that only a few seeds would be able to germinate at 1% O_2. Interestingly, seeds imbibed at low O_2 percentages initially exhibited the germination time course predicted by the model, but after incubation for over a week at 3% O_2, additional seeds apparently developed the capacity to germinate at this low pO_2 (Fig. 1A). This pattern was observed in several of the datasets analysed, and may indicate that adaptation to low O_2 availability can occur in seeds to shift their response thresholds to lower values (discussed further below). Additional datasets for other *Brassicaceae* species were also analysed and gave values similar to those shown in Fig. 1, with $Ox_b(50)$ values between 2.3 and 3.8% (Table 1).

As a further example, carrot (*Daucus carota*) seed responses to O_2 percentage were also described well by the threshold model (Fig. 2, Table 1) (Corbineau *et al.*, 1994a). Carrot seeds had a slightly higher median O_2 threshold than did *B. oleracea* seeds, with $Ox_b(50)$ values of 4.5 to 5.3% (Table 1). Fennel (*Foeniculum vulgare*) seeds (also in the *Apiaceae*) were apparently somewhat more tolerant of low O_2, with a $Ox_b(50)$ value of 2.8% (Table 1). Seeds of witloof chicory (*Cichorium intybus*), soybean (*Glycine max*), tomato, *Araucaria angustifolia* and lamb's lettuce (*Valerianella olitoria*) also had $Ox_b(50)$ values in the 3–5% O_2 range (Table 1). Pepper (*Capsicum annuum*) seeds were much more sensitive to O_2 limitation, with $Ox_b(50)$ values of *c.* 9%, while tobacco (*Nicotiana tabacum*) seeds were less sensitive, with median O_2 thresholds of 1–2% (Table 1).

As might be expected from their ability to germinate under water, rice (*Oryza sativa*) seeds had very low O_2 thresholds (Fig. 3A; Al-Ani *et al.*, 1985). Germination rates were only slightly delayed at O_2 percentages down to 1%, and 10–15% of the seeds could germinate even in 0.005% O_2. The $Ox_b(50)$ value using data from all tested O_2 percentages was estimated to be 0.016% (Table 1). These data also exhibited the phenomenon mentioned above, where seeds incubated at very low O_2 percentages (e.g. 0.03 and 0.01%) showed unexpectedly high germination after an initial period of low germination (Fig. 3A), suggesting the possibility of adaptation to low pO_2. This was confirmed by separately modelling the time courses conducted in 0.1–21% O_2 and in 0.005–0.03% O_2. The parameters of the model changed

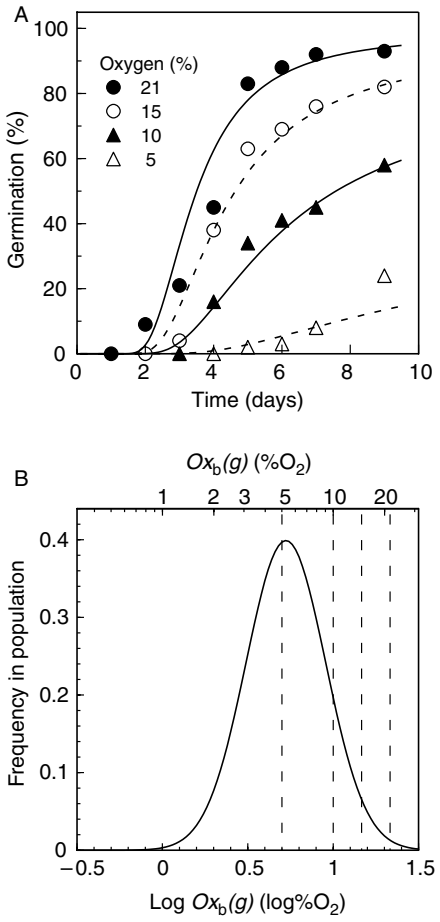

Figure 2. Germination time courses of carrot (*Daucus carota*) seeds at 20°C in a range of O_2 percentages. (A) Germination (symbols) at 21, 15, 10, and 5% O_2 and time courses (solid and dashed lines) predicted by the threshold model using the parameter values in Table 1. (B) The distribution of $Ox_b(g)$ values based upon the median and standard deviation of $Ox_b(g)$ values. Other details as in Fig. 1. (Data from Corbineau *et al.*, 1994a).

relatively little for the higher pO_2 range, with the $Ox_b(50)$ changing from 0.016 to 0.013% O_2 (Fig. 3B). For the lowest O_2 percentages, however, the model parameters exhibited a large increase in θ_{Ox} from 140 to 235 log % O_2 h, accounting for the initial delay in initiation of germination, and the $Ox_b(50)$ value decreased to 0.0043% O_2, threefold lower than the median threshold for seeds incubated at the higher O_2 range. The variation in O_2 thresholds among the seeds in the population (σ_{Ox}) also decreased from 0.86 to 0.55 log % O_2. In both cases, this range of variation in the thresholds predicted that 16% of the seeds (one standard deviation below the median) would germinate at a pO_2 of 0.0014%. Using these values, the predicted curves matched the germination time

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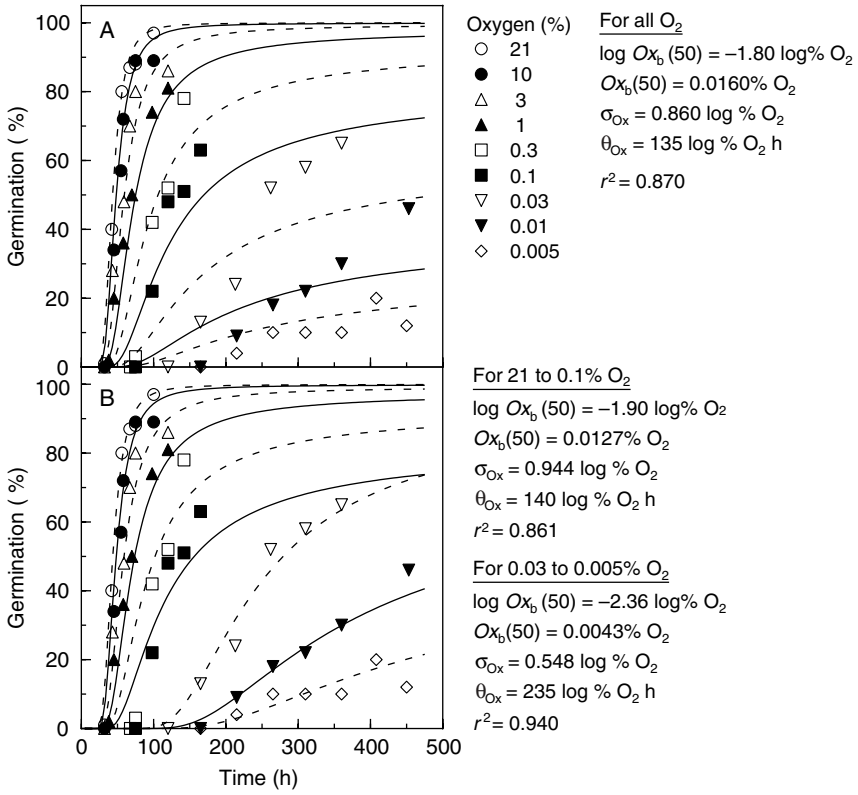

Figure 3. Germination time courses of rice (*Oryza sativa*) seeds at 25°C in a range of O_2 percentages. (A) Actual data (symbols) and predicted time courses (solid and dashed lines) and parameter values when data from all tested O_2 percentages are included in the model. (B) Actual data (symbols) and predicted time courses (solid and dashed lines) and parameter values when data from the six highest O_2 percentages and the three lowest O_2 percentages are analysed separately in the model. The improvement in predicted fits in the latter case is associated with a shift of $Ox_b(50)$ to a lower value, and a reduction in σ_{Ox} and increase in θ_{Ox} of seeds incubated at the lowest oxygen percentages. (Data from Al-Ani *et al.*, 1985).

courses in both high and low pO_2 regions very well ($r^2 = 0.86$ for higher percentages and 0.94 for lower percentages) (Fig. 3B).

The threshold model was also capable of incorporating the effects on germination of O_2 percentages above ambient (21%). Witloof chicory seeds did not achieve 100% germination in 21% O_2, but did so when incubated in elevated pO_2 (Fig. 4). Including the data from all of the O_2 percentages resulted in poorer fits ($r^2 = 0.80$) than did including only the data from 21% and lower percentages ($r^2 = 0.94$). Both approaches predicted that O_2 percentages above ambient would speed germination and allow additional seeds in the population to germinate, but the latter gave better fits to germination time courses at lower O_2 percentages (Fig. 4). This model predicted the increase in total germination at higher pO_2, but also predicted that germination would be much more rapid than was observed at O_2 percentages above ambient (Fig. 4). However, the initiation of germination is also dependent upon the time required for imbibition and metabolic activation in these rapidly germinating seeds, so the minimum time to initiation of germination is likely limited by factors other than O_2 in these early stages.

Effects of temperature and priming on oxygen threshold values

It might be expected that O_2 requirements for germination would increase as temperature increased, due to higher respiration rates increasing the demand for O_2 and to lower solubility of O_2 in water. This was evident in data for lamb's lettuce seeds germinated at either 20 or 25°C (Fig. 5). At 20°C, germination was sensitive to O_2 percentage, with an estimated $Ox_b(50)$ of 5.21% (Fig. 5A, Table 1). At 25°C, germination was largely inhibited, and $Ox_b(50)$ was estimated to be 21% (Fig. 5B). A change of this magnitude cannot be due to reduced solubility of O_2 in water at the higher temperature, which changes only 10% between 20 and 25°C (from 0.031 to 0.028 ml O_2 ml^{-1}). The failure to germinate at the higher temperature is likely due to the

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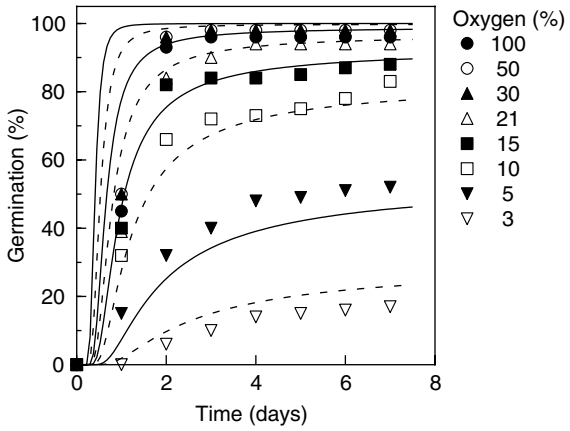

Figure 4. Germination time courses of witloof chicory (*Cichorium intybus*) seeds at 25°C in a range of O_2 percentages. The threshold model was fit using only data (symbols) at or less than 21% O_2, but time courses (solid and dashed lines) are predicted for all O_2 percentages. The model predicts the increased total germination at O_2 percentages greater than ambient, but underestimates the initial timing of germination. (Previously unpublished data of F. Corbineau).

imposition of thermodormancy, which could not be overcome solely by increasing the O_2 concentration, as the model predicts that only *c.* 80% of the seeds would complete germination even in 100% O_2 (not shown).

Tomato seeds, which are generally not subject to thermodormancy, also exhibited an increase in $Ox_b(50)$ values as temperature increased. At 15°C, tomato seeds germinated >80% at 5% O_2, and the threshold model predicted an $Ox_b(50)$ value of 0.16% (Fig. 6A, Table 1). At 25°C, however, only *c.* 60% of seeds could complete germination at 5% O_2, and an $Ox_b(50)$ value of 3.17% was estimated, or *c.* 20-fold greater than at 15°C (Table 1). The decrease in O_2 solubility over this range would account for an increase of only 0.21-fold in O_2 requirements. A similar increase in $Ox_b(50)$ with temperature was also evident in one variety of tobacco (cv. 30, Table 1). Thus, higher respiration rates at warmer temperatures, combined with limits to O_2 diffusion through seed covering tissues, apparently result in higher O_2 thresholds.

The data for germination of tomato seeds at 15°C contain some anomalous responses at low pO_2. While germination decreased regularly between 21 and 5% O_2, germination was inhibited to a much greater extent at 3% O_2 than was predicted (Fig. 6A). This suggests that the $Ox_b(50)$ estimated above (0.16%) is not actually achieved, and that tomato seeds exhibit a very sharp reduction in germination capacity between 5 and 3% O_2. This same pattern was evident in tomato seeds that had been primed for 3 d in −1 MPa PEG at 15°C (Fig. 6B). However, after 7 d of priming under these conditions, seeds incubated in 3% O_2 germinated essentially as the model predicted, with

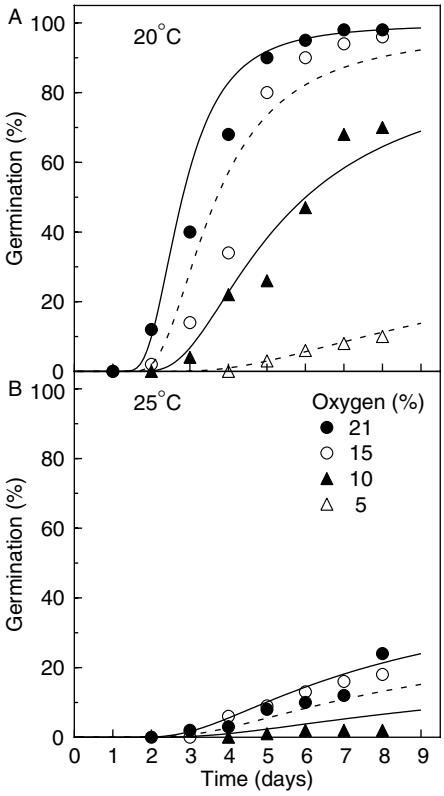

Figure 5. Germination time courses of lamb's lettuce (*Valerianella olitoria*) seeds at (A) 20°C or (B) 25°C in a range of O_2 percentages. Symbols are the actual data, and lines are the time courses predicted by the threshold model using parameter values in Table 1. (Previously unpublished data of F. Corbineau).

only a slight delay (Fig. 6C). Thus, extended priming appeared to eliminate the sharp O_2 requirement threshold between 3 and 5% O_2. However, pepper seeds exhibited only a relatively small reduction in $Ox_b(50)$ after 5 d of priming (Table 1).

Effects of after-ripening on oxygen threshold values

It is well known that the O_2 requirements for cereal seed germination are dependent upon the dormancy status of the seeds (Lenoir *et al.*, 1986; Lecat *et al.*, 1992). As illustrated here for dormant and non-dormant (after-ripened) wheat seeds (Corbineau *et al.*, 1981), the loss of dormancy shifted $Ox_b(50)$ values from 2.27% to 0.005% (Table 1, Fig. 7). Similar but less dramatic results were also evident for sunflower. Soon after harvest, sunflower seeds germinated rather slowly and only achieved about 85% final germination (data not shown). Germination was more rapid and to

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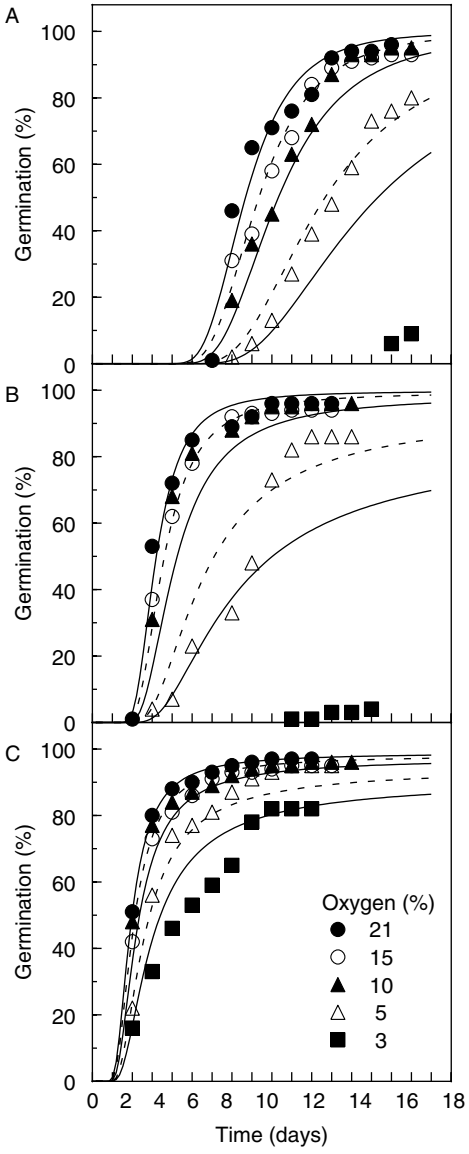

Figure 6. Germination time courses of tomato (*Solanum lycopersicum*) seeds at 15°C in a range of oxygen percentages. (A) Germination of untreated (control) tomato seeds. Note that the model does not predict the very low germination at 3% O_2. (B) Germination of tomato seeds primed in −1.0 MPa polyethylene glycol (PEG) for 3 d. Note that the model does not predict the very low germination at 3% O_2. (C) Germination of tomato seeds primed in −1.0 MPa PEG for 7 d. Note that the model does predict with reasonable accuracy the germination time course at 3% O_2. Symbols are the actual data, and lines are the time courses predicted by the threshold model using parameter values in Table 1. (Data of Özbingnöl, 1998; Özbingnöl and Corbineau, unpublished).

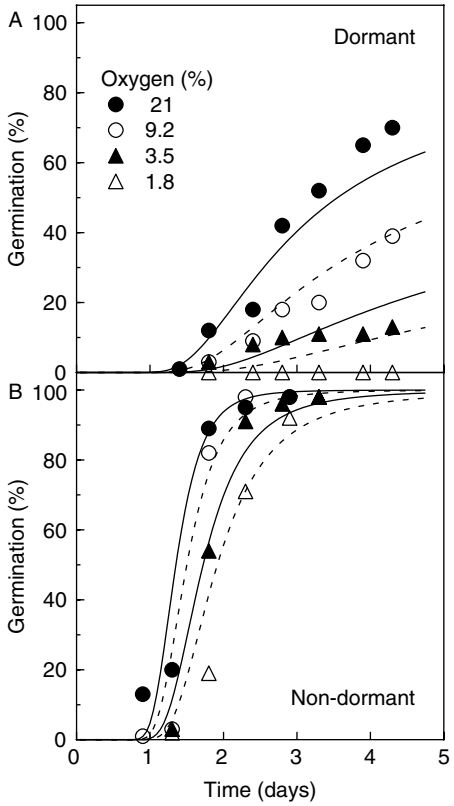

Figure 7. Germination time courses of wheat (*Triticum aestivum*) seeds at 20°C in a range of O_2 percentages. (A) Dormant seeds tested soon after harvest. (B) Non-dormant seeds tested after 1 year of dry storage at ambient temperature (after-ripening). Symbols are the actual data, and lines are the time courses predicted by the threshold model using parameter values in Table 1. (Data from Corbineau *et al.*, 1981).

higher percentages in pO_2 above ambient, and $Ox_b(50)$ values were relatively high (8.5%) (Table 1). Following after-ripening for 9 months at 5°C (Gay *et al.*, 1991), germination rates and percentages improved at all O_2 percentages except 5%, and the estimated $Ox_b(50)$ value was reduced to 5.4% (Table 1). Thus, in both monocot and dicot seeds, loss of dormancy was associated with a reduction in the threshold oxygen percentage.

Discussion

The population-based threshold model has been applied to quantify seed germination responses to many environmental and physiological factors (Bradford, 1995; Finch-Savage *et al.*, 2005a; Bair *et al.*, 2006). It has proven to be a robust model that is simple to apply, and requires only three parameters to characterize seed germination responses to levels of various environmental or physiological factors. The median base threshold indicates the sensitivity of the population to the factor, the standard deviation of thresholds among

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seeds in the population indicates the variance or uniformity of the population, and the time constant is related to the overall speed of germination. Here, we demonstrate that this model can also be applied to quantify and characterize the germination responses of seeds to O_2 availability.

Using previously published data and our own unpublished results, the population-based threshold model was able to fit well to germination time courses across a range of pO_2 from 100 to 0.005%. The model provides quantitative estimates of the median O_2 sensitivity threshold [$Ox_b(50)$] and of the variation among seeds in their threshold values (σ_{Ox}). Seeds of a number of species have $Ox_b(50)$ values in the range of 2–5% O_2 (Table 1). Others, including pepper and dormant sunflower seeds, have $Ox_b(50)$ values in the 8–9% range, while some, particularly cereals, can have threshold values as low as 0.005% (Table 1). Although generally in the range of values reported for many of these species by Al-Ani *et al.* (1985), the values calculated by the threshold model are often several percent lower, even when the same data are analysed. This may be due to the methods used to calculate the germination rates in that work. It is unclear, for example, whether the rates used were the times to germination of 50% of the entire population or the times to germination of 50% of the fraction of seeds that completed germination. Although often used, the latter approach is not a valid comparison among seed lots or conditions, and can skew estimates of germination parameters when final germination percentages vary widely. Nonetheless, the clear differences between most dicot seeds and the cereals are consistent between the two methods. Temperature, after-ripening and priming also affected O_2 sensitivity thresholds, variances and time constants (Table 1).

Some data indicated that seeds could adapt to low O_2 percentages after a period of incubation following imbibition. This was clearly demonstrated in the case of rice, where application of the model to all data resulted in an acceptable (but not excellent) fit to the data (Fig. 3A). However, separation of the data into those at O_2 percentages above and below 0.1% resulted in good fits in both pO_2 ranges (Fig. 3B). Seeds initially incubated at O_2 percentages near the $Ox_b(50)$ value were delayed in initiating germination, but then germinated in a manner indicating that their $Ox_b(50)$ values had decreased 3- to 4-fold relative to seeds incubated at higher O_2 percentages (Fig. 3B). This may result from a metabolic adaptation of energy metabolism in hypoxia (Al-Ani *et al.*, 1985), inhibition of different biosynthetic processes (Geigenberger, 2003) or a progressive increase in permeability of the covering tissues to O_2 leading to better oxygenation of the embryo. Similar adaptive shifts after extended incubation were reported for tomato seeds in relation to water potential and abscisic acid thresholds for germination (Ni and Bradford, 1992; Dahal and Bradford, 1994). The threshold model provides a method to identify such shifts, as relatively poorer fits of the model are obtained when such adaptation occurs and all data are combined. Splitting the data into distinct subsets and fitting each factor range separately can identify such shifts and the factor levels at which adaptation is induced. It is difficult to distinguish such adaptation without a model to identify consistent patterns in the germination data.

An interesting feature of the threshold modelling approach is that it quantifies the variation among seeds in their sensitivities to various factors influencing germination. In the case of rice, for example, while the median O_2 threshold was quite low (0.013–0.016% O_2), the standard deviation of the thresholds in the population was 0.86–0.94 log % O_2 units, or a range of 3.44–3.76 log units (four standard deviations) between the 2nd and 98th percentiles of the population. Thus, some seeds required *c.* 3000-fold higher percentages of O_2 to germinate than did other seeds within the same seed population. Even a σ_{Ox} value of 0.25 log % O_2, near the lower end of those observed (Table 1), implies a tenfold range in O_2 sensitivity within the seed population.

The threshold model also assumes that there is an oxygen–time constant (θ_{Ox}) that is the same for all seeds in the population. That is, the product of the difference between the seed O_2 thresholds and the actual O_2 percentage, multiplied by the time to germination, is a constant for all seeds. Thus, at a given O_2 percentage, the higher the O_2 threshold value, the longer the time to germination. Alternatively, the time to germination increases as the O_2 percentage decreases relative to a fixed threshold distribution. This explains why the rate of germination decreases as O_2 percentage decreases, even for those seeds that will eventually germinate. Seeds whose thresholds are above the ambient O_2 percentage, on the other hand, will not complete germination (unless adaptation occurs to lower their thresholds below the ambient O_2 level).

In summary, a wide range of data for germination responses to O_2 availability could be analysed by the population-based threshold model approach. As has been reported previously (e.g. Al-Ani *et al.*, 1985), the response to O_2 percentage was logarithmic, and seeds exhibited a wide range of O_2 thresholds for germination, both between and within species. Temperature, after-ripening and priming can influence the estimated O_2 thresholds. This approach provides a consistent method for quantifying O_2 response thresholds for germination and for predicting germination rates and percentages at any O_2 level.

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