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> It has been suggested that seeds are unpredictable things and consequently it would be a waste of time to try to perceive laws of behavior among such erratic individuals. . . . But other disciplines have shown us quite clearly that, although the behavior of any individual in a population may be quite unpredictable, the behavior of populations of individuals can often be defined very accurately.
>
> *Eric Roberts (1973)*

1 Introduction

Seeds are the remarkable achievement of evolution to convey most terrestrial plant species from one generation to the next. In some cases, this happens immediately after the seeds are released from the mother plant, while in others, seeds are the vehicle to transport species from one satisfactory growing

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environment to the next, for example, through winter freezes or summer droughts, or to a new location entirely. Increasingly, as human impacts on the natural environment mount, we will be attempting to aid species in adapting to changing conditions by replanting them or establishing them in locations more amenable to their future success. On the other hand, some species will be able to take advantage of the changing conditions and become invasive in new locations through the assistance of their seeds. In addition, critically for feeding humans and their domesticated animals, seeds are the delivery system for transferring improvements incorporated by breeders into new varieties into the field for crop production. If the seeds do not fulfill their role by germinating and producing viable seedlings, that value is not transferred and productivity is less than it could be. Efficiency in crop production will be essential for reducing the expansion of agriculture's footprint and maintaining natural ecosystems (Folberth et al., 2020). At the same time, biological approaches to controlling weeds in crop production are increasingly focusing on managing or eliminating soil weed seed banks (Ramesh, 2015). Thus, understanding what seeds pay attention to and how they respond to environmental signals is critical to sustaining plants that we wish to promote (crops and native species) and restricting those that we wish to suppress (weeds and invasive species) (Long et al., 2015; Klupczyńska and Pawłowski, 2021).

This chapter will focus on approaches to understand seed behavior by modeling germination responses to key factors in their environment, particularly temperature (T) and water potential (ψ), that are being impacted by climate change. Seeds' responses to these environmental signals are also influenced by their physiological state, such as dormancy to time germination to coincide with appropriate conditions for seedling success and seed age or vigor that limits the useful lifetime of a given seed. Our attention will be primarily on what we have learned from the application of these population-based threshold (PBT) models to quantify and predict seed behavior. It is not expected that farmers need to become mathematicians or statisticians in order to optimally manage their planting dates or seedbed preparation. However, they do need to understand intuitively how seeds respond to their environments and how to extract the best performance possible from the seeds that they plant. Similarly, seed companies focus primarily on developing new varieties, but in the end, their product is a seed and its performance in the greenhouse or field determines the likelihood of a repeat customer. In addition, as some herbicides that have been relied on for weed control become less effective due to plant adaptation through genetic and metabolic resistance mechanisms (Perotti et al., 2020), managing weed seed production and persistence in the soil seed bank is assuming greater importance. Ecologists seeking to maintain or reestablish species in natural habitats must be able to characterize seed dormancy and germination patterns in order to schedule planting dates for

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successful plant establishment and regeneration (Larson and Funk, 2016; ten Brink et al., 2020). Thus, we will also emphasize how models quantifying seed behavior, particularly as a result of its population nature, can be used to assess, improve and maintain seed quality, and we will introduce new tools to facilitate the application of these models for practical purposes.

2 The importance of seed quality and behavior in crop production and weed control

It is hard to overemphasize the impact of seed quality and seedling emergence on crop production and management. In some highly domesticated and self-pollinating crops, such as wheat, rice or beans, seed production largely resembles crop production, with some additional precautions to maintain the identity of the variety and of the seeds produced. The seeds retain little dormancy from their pre-domestication ancestors and generally have relatively few problems in stand establishment that cannot be overcome by planting a bit more seed or adjusting planting dates. In addition, these crops can tiller or branch and fill spaces left by the failure of emergence. The situation is less simple for maize or many vegetables, including tomatoes, peppers, melons, onions, carrots and Brassicas, as almost all modern highly productive varieties in these crops are F1 hybrids, requiring specialized seed production methods to produce separate male and female parent plants and then enable them to cross specifically to produce the F1 hybrid seeds. In addition, each seed produces one plant in the crop field, and modern varieties are bred to perform best at specific plant populations per hectare. Thus, emergence failure means a missing production unit, and even delayed emergence can handicap the tardy plant due to shading by its neighbors, making it essentially a weed by using resources (water, nutrients) but not producing a harvestable plant.

These issues are particularly critical for vegetable crops. In lettuce or cabbage, for example, each plant produces one harvestable head, so if seeds are planted at the desired population density, emergence failure means lower yield per hectare. In addition, the optimal harvest time is dependent upon plant development to a specific state, so delay in emergence by even 2 or 3 days may mean additional costly harvest operations. Overseeding followed by thinning can assure an optimal plant population but with additional costs for seeds and labor for thinning at an early stage. Transplanting seedlings directly into the field solves these problems for the farmer but transfers them to the transplant producer. Flats of transplants must be fully filled, and all seedlings should be at an identical stage of development to satisfy customers. If seed germination is uniform and complete, this is simple. But if the seed lot germinates less than 100% or is not uniform in its emergence, this creates major and costly problems for the transplant producer to sort seedlings and assemble new trays of usable

transplants. The requirements and demands of both field and transplant customers are therefore transferred to the company producing the seeds to not only provide a variety that grows well in the intended climate and meets consumers' desires and demands but also deliver that variety in seed lots of extremely high quality and uniformity. This is in addition to the fact that most of these crops are also hybrids and may require hand pollination and greenhouse or off-season production in diverse locations and may take 2 years to produce one seed crop, as in the biennials such as cabbage, onion or carrot. We will therefore briefly identify some of these issues and indicate how understanding seed behavior can contribute to assuring the high seed performance that is expected in modern agriculture.

2.1 Seed production, conditioning and enhancement

Seed quality begins with seed production and, in particular, with the seed maturity at harvest. In a number of crops, it was once thought that when the seeds had attained maximum dry weight, seed development was completed and harvest could begin. However, it has become clear now that the final stages of seed maturation after full dry weight accumulation but prior to desiccation are important for maximum seed quality, particularly with respect to the development of seed longevity (Egli, 2017; Leprince et al., 2017; Ellis, 2019). This creates a particular problem for indeterminate crops in which flowering and seed development continue sequentially so that a range of seed maturities are present simultaneously (Still and Bradford, 1998). This issue is exacerbated by the fact that many of these same crops retain the seed-shattering trait of their undomesticated ancestors, which has been eliminated in most domesticated grain and pulse crops (Maity et al., 2021). When seed shattering is present, the more mature seeds will be released from the fruit and lost before the later seeds are mature (Steiner and Akintobi, 1986). Thus, the timing of harvest is always a trade-off between optimal quality and highest yield, and the inevitable mix of maturities creates additional problems for seed conditioning and performance. PBT models have been used to quantify seed germination characteristics of seeds of different maturities in cabbage and identify features that could be useful for guiding decisions on when to harvest such crops (Still and Bradford, 1998). New developments in seed conditioning and image analysis are creating opportunities to identify and separate seeds on a number of criteria, potentially enabling the upgrading of such lots (Galletti et al., 2020; Mortensen et al., 2021). Correlating these performance indices with physical features of seeds can be applied to guide seed conditioning equipment to remove lower-quality fractions from the seed lot (Bruggink and van Duijn, 2017; Bello and Bradford, 2021; https://www.seed-x.com/product/sorter/; https://videometer.com/videometerlab/; https://www.vibeia.com/qm3-seeds-phenotyping-analyzer). Physiological enhancement

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of seed performance via priming treatments can also be optimized with the use of PBT models (see Section 4.5).

2.2 Crop management and yield

As indicated above, it is difficult to recover during the growing season from the consequences of poor stand establishment. Some crops can spread to fill empty spaces, but others cannot, while weeds consider such gaps as opportunities. The lack of uniformity among plants creates problems for the timing of management activities, such as pest control, pollination, growth regulator applications or harvest dates. The most critical factor in stand establishment in the field is also the least controllable: the weather immediately after planting. In most crops, guidelines are well established with respect to the minimum soil temperatures at planting. Crop management is also now largely guided by degree-day (or thermal time) thresholds associated with different developmental stages. This enables the management of plants or pests in relation to actual growth and development rates rather than calendar days. The importance of a well-prepared seedbed, precision control of planting depth and optimal water management are also widely appreciated, even if achieving such goals can be thwarted by soil conditions or unexpected changes in weather.

Understanding and modeling seed behavior can have multiple applications for optimizing stand establishment. The degree-day models mentioned above are based on the cardinal temperatures (minimum, optimum and maximum) for seed germination, and the models for determining these values and the associated germination rates will be explored in detail in Section 4.1. A key parameter of germination models is related to the uniformity of germination among the seeds in the lot. As standard germination tests are often conducted under optimal conditions, the variation observed sets an upper limit for the uniformity that can be expected in the field. However, standard germination tests focus on total viability and seldom quantify uniformity, so this information is generally not available. It is also useful to understand and appreciate the sensitivity of seeds to the T and ψ conditions of the seedbed. Even relatively slight reductions in water availability can have considerable negative effects on germination speed and uniformity. As climates are changing, the ability to match seed lots with marketing environments in which they will be successful is a considerable advantage for a seed company and its customers (https://climate.ai). The speed of germination is the first component of seed vigor that decreases in association with seed aging, and it can be quantified by PBT models (Section 5.2). Knowing where a seed lot is in its progression from high vigor and viability toward death is critical for managing seed inventories for sale or for germplasm conservation. In addition to focusing on the crop, the management

of weed populations is a critical component of efficient agriculture. Germination and dormancy modeling of weed seed banks and their behavior in response to weather patterns can enable the prediction of weed emergence patterns and target management actions to control them (Section 5.1).

2.3 Transplant production

Given the difficulties and uncertainties noted above for field planting, it is no surprise that transplanting is becoming more popular for crop stand establishment, particularly for high-value crops. In many cases, seeded flats are placed in climate-controlled rooms so that optimal temperature and moisture conditions can be maintained during germination and emergence to increase uniformity. Even so, problems can persist, such as in coordinating the emergence of rootstock seedlings of wild species accessions for grafting with hybrid scion varieties in tomatoes (Mahmoud, 2020). Machines have been developed specifically to image young plants and sort them into trays with uniform seedlings for automated grafting (https://www.tta.eu/equipment/selecting/flexsorter). However, given the imperatives of scheduling and the financial risks of failure to achieve a required standard of usable plants, it is not uncommon to do pretests of seed lots to confirm the seeds' response to the planned conditions and assess uniformity before large-scale plantings. Doing these pretests in such a way that they can be assessed by germination models would improve both their accuracy and utility. For example, doing a test at two temperatures enables estimation of the germination rates across the entire temperature range likely to be employed, enabling the selection of a temperature that assures complete germination in the shortest time possible. The same approach can be applied to testing seeds prior to applying priming treatments that can speed germination and improve the uniformity of emergence (Section 4.5). Appreciation of the high sensitivity of seeds to water availability will encourage the use of irrigation systems that can apply water very uniformly and accurately to minimize variation among individual seeds or trays. Using transplants has reduced the risks of stand establishment for the farmer, and it has moved the critical germination and seedling emergence phase into more controlled conditions. However, it has also increased the expectations for seed performance.

These considerations indicate why it would be beneficial for seed companies and seed testing organizations to more fully adopt population-based approaches to assessing and managing seed quality. The models discussed below are based on knowing the time courses of germination, on every seed tested if possible; this will require further automation of seed testing procedures. The labor required to do repeated observations (at least daily, often more frequently) to capture adequately the full germination time

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course is unsupportable using human analysts. However, various methods are now available to collect such information robotically and process it in a form usable for model fitting and evaluation (Ducournau et al., 2005; Bello and Bradford, 2016; Colmer et al., 2020). Thus, while using germination-based models may have been applicable only for research in the past due to the labor requirements, advancements in imaging and robotics soon will routinely and accurately acquire data for complete germination time courses, enabling full use of the capabilities of the models described in the following sections.

2.4 Seed dormancy and germination characteristics in weed control and plant population ecology

Our primary focus in this chapter will be on crop seeds, but understanding and characterizing seed dormancy and germination behavior are also critical for managing unwanted plants (weeds) and encouraging the survival or reestablishment of plants in natural locations. Weed management entails the study of the soil seed bank (Schwartz-Lazaro and Copes, 2019), which persists as a result of seed dormancy mechanisms (Soltani et al., 2019). PBT models have been applied effectively to understand weed seed bank persistence and emergence in relation to environmental factors (Boddy et al., 2012; Batlla and Agostinelli, 2017). In ecological contexts, PBT models have been utilized in combating invasive species (Meyer and Allen, 2009; Hawkins et al., 2017; Liu et al., 2019), enabling revegetation (Larson and Funk, 2016) and characterizing species demographics over time (Gremer et al., 2016; Liu et al., 2020). Given the wide diversity of seed dormancy types and plant life cycles (Saatkamp et al., 2019; Fernández-Pascual et al., 2021), we can only cover a few examples of the possible applications of PBT approaches in seed ecology. However, we note that the population-based understanding of seed biology we describe is especially useful in ecological and plant diversity contexts due to the more prominent dormancy mechanisms present in wild species compared to domesticated plants and their importance in bet-hedging adaptations (Rubio de Casas et al., 2015; Gianella et al., 2021).

3 Understanding the population-based behavior of seeds

3.1 Basis of population-based approaches to seed biology

It is a fundamental fact of seed biology that seeds can be regarded simultaneously as individuals and as members of a larger population or cohort. Seeds are designed for dispersal, with each seed on its own to assess the environment in which it finds itself and to determine whether and when to commit itself to

germination (Bassel, 2016). That commitment is a life-or-death decision for the individual, as once a seed has initiated germination to the point of protrusion of the embryo from its enclosing tissues, it cannot go back. If it has sensed and interpreted its external and internal conditions correctly, its embryo/seedling has a higher probability of success in growing to maturity. If it misinterprets the conditions or fails to heed negative signals, the risk of failure for that seedling is increased. The evolutionary pressures on seeds to make the correct choices have resulted in the myriad of strategies embodied in seed dormancy and environmental signaling systems represented among the diversity of plant species and climates around the globe (Baskin and Baskin, 2014, 2021).

However, while it is individual seeds that face the dilemma of whether to germinate now or wait until later, it is the collective action of populations of seeds that ultimately determines the success of the species or crop, whether in nature or in agricultural fields (Mitchell et al., 2017; Gremer et al., 2020). In nature, species whose seeds can more accurately predict the optimal time to germinate in their environment have a greater probability of long-term success in that environment (Larson and Funk, 2016; ten Brink et al., 2020). There are still risks involved, however, due to variability in environmental conditions, leading to bet-hedging strategies in which the seed population as a whole consists of subpopulations responding differently in variable environments (Gremer et al., 2016; Gianella et al., 2021). Some seeds in the population will be less dormant and will germinate immediately when adequate conditions (e.g., temperature, water) are present, possibly reaping the advantage of early emergence ahead of competitors, but also risking death if the favorable environmental conditions do not persist. Other seeds in the same population may take the opposite strategy, bypassing apparently adequate opportunities for years and representing a bank account of seeds invested in the future. Success for the species incorporates the likelihood of failure for most seeds but sufficient spreading of risk to assure that a sufficient fraction of individuals establish and grow to reproductive maturity.

Once humans began collecting seeds and purposefully replanting them, the selective pressures changed and traits such as retention of mature seeds on the mother plant (nonshattering) and absence of dormancy were associated strongly with successful propagation. Rather than the wide diversity in individual responses often characterizing seed populations in native species (e.g., bet-hedging strategies), farmers prefer seed populations to germinate immediately and simultaneously after planting, taking the responsibility on themselves to select the optimal timing for successful emergence and subsequent growth and yield (Finch-Savage and Bassel, 2016). At the same time, cultivation activities changed the selective pressures on non-crop species, resulting in the selection of weeds whose seed dormancy characteristics enable them to both persist for long periods in the soil and to emerge at times that enhance the

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likelihood of their seedlings to both compete with the crop and avoid removal (Malavert et al., 2021a).

3.2 Characterizing germination timing of seed populations

When studying seeds, whether in natural or agricultural systems, we must appreciate the roles of both the individuals and the populations that they collectively constitute. The range of seed germination behavior observed in a given population is not an indication of measurement error, stochastic noise or uncertainty but simply the reality of their inherent biological diversity. The most common measure of seed performance is the percentage of germination (or viability) in the population after being exposed to a suitable condition of hydration and temperature for a period of time. Here, we will be interested also in the cumulative increase in the percentage of seeds germinated with time after initiation or the germination time course (Fig. 1). Whenever a measurement is expressed as a percentage, it immediately indicates that there are individuals in the population that can vary in that particular measure. Thus, the total population is composed of individuals or subpopulations that can differ in their characteristics or responses, which themselves are often of a quantal or digital nature (i.e., living or dead, dormant or nondormant, germinated or not, etc.).

Despite its persistent usage in the scientific literature, the term 'germination rate' is incorrect when applied to data measured in percentages. In physics, a 'rate' is a measure of a change over time, or a velocity, and it should be also in seed biology. A germination 'rate' could therefore be either the increase in percentage germination per unit of time or the time it takes a given percentage of seeds to germinate after imbibition. The former term is equivalent to the slope of the cumulative germination time course, which approaches linearity around 50%, but is actually sigmoid (Fig. 1). However, rather than representing a rate, the slope is actually determined by the variation among seeds in their individual times required to achieve embryo (generally radicle) emergence. Therefore, the term 'germination rate' should refer to the time required for a given percentage of the seed population to germinate and will therefore be defined at a specific percentage, for example, 50% to indicate the median time to germination in the population (Fig. 1). As the germination time course is not symmetric around 50%, that percentage should be termed the 'median', that is, half of the seeds germinating faster and half slower. The mean (average) time to germination is shifted to longer times due to the asymmetry of the time courses. In a symmetric distribution (such as the normal distribution), the mean and the median are equivalent.

The inverse of this time (t) to a specific germination percentage (g) (i.e., $1/t_g$) is considered to be the germination rate for that percentage (GR_g) with

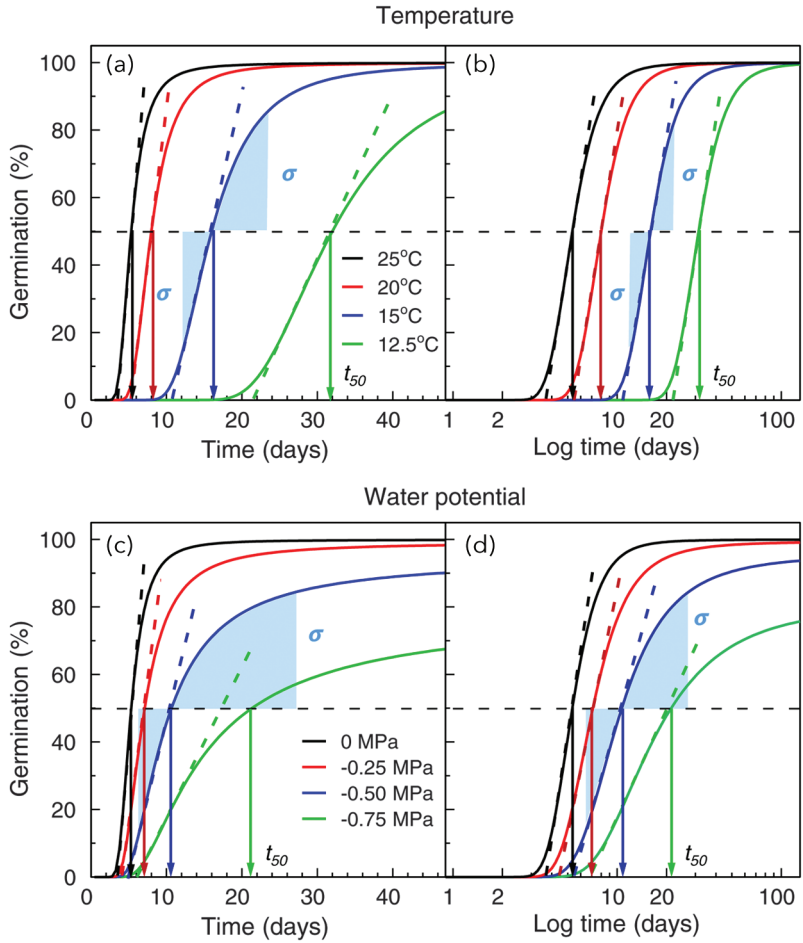

Figure 1 General features of seed germination time courses in response to temperature (T) (a, b) and water potential (ψ) (c, d) on a linear time scale (a, c) and a logarithmic time scale (b, d). (a) The black curve shows a standard germination time course on water (0 MPa) at 25°C with a base temperature (T_b) of 10°C. The red, blue and green curves show the time courses for the same seed lot at 20°C, 15°C and 12.5°C on water. The sides of the shaded areas on an example time course indicate the time from 16% to 50% or 50% to 84% germination, which is ±1 standard deviation (σ) in the seed population, assuming a normal distribution. Note that the period on the time axis is not symmetrical around the median (50%) but rather is much shorter for 16–50% compared to 50–84%, indicating that the time courses are not symmetric but rather are right skewed. The dashed lines indicate the initial slopes of the curves, which decrease as the time to germination increases. The times to 50% germination (t_{50}) are also shown on each curve (colored arrows). (b) Plotting these time courses at different temperatures on a $\log_{10}$ scale results in parallel slopes for different temperatures and more symmetric sigmoidal curves. Note also that at suboptimal temperatures, it is generally observed that all seeds can eventually germinate even at lower temperatures (above T_b) when the incubation period is long enough. (c) The black curve shown is under the same initial conditions as in (a), but the temperature

units of hour^{-1} or day^{-1}. The slope of the cumulative germination curve is a result of the variation in germination times in the population, with highly uniform populations having steep germination time course curves while less-uniform populations have shallower curves with larger times between successive germination events. The slopes of the time courses are also sensitive to environmental conditions, particularly T and ψ (Fig. 1). It is evident that on a population basis, the key descriptive parameters in relation to germination are the percentages of seeds that can complete radicle emergence, also termed germination *sensu stricto* (Nonogaki et al., 2007), the times when individual seeds did so (or their inverses, the germination rates for a specific cumulative percentage) and variation in those times among individual seeds in the population (Fig. 1).

The analysis of seed behavior in population terms has a long history, but Eric Roberts was a key innovator in applying population-based approaches to quantify seed characteristics (Roberts, 1960). In 1961, he demonstrated that the increase in germination percentage as rice seeds lost dormancy during storage could be described by a cumulative normal distribution or a sigmoidal curve (Roberts, 1961). This showed that the after-ripening period required to satisfy the dormancy requirements varied among seeds, with the highest frequency around the mean duration and lower frequencies earlier and later. The required after-ripening periods were distributed normally among seeds in the population, which resulted in a symmetrical sigmoidal curve of increasing germination percentage with increasing storage duration. Roberts illustrated that plotting this data on a probability (or probit) scale based on standard deviation units (Bliss, 1934) resulted in a straight line, the slope of which is inversely proportional to the standard deviation of the distribution of individual after-ripening requirement times in the seed population. Consistent with the terminology discussed above for germination rates, he also noted that 'the gradient of the curve showing percentage germination plotted against time does not indicate the rate of breaking dormancy, as has sometimes been implied in the literature. A steep gradient shows that the spread or standard deviation of the distribution of dormancy periods among the seeds of a population is small, whereas a shallow gradient represents a wide spread of dormancy periods' (Roberts, 1961).

Figure 1 (*Continued*)

is maintained at 25°C while the ψ is reduced to −0.25 (red), −0.5 (blue) or −0.75 MPa (green) relative to a median base water potential ($\psi_b(50)$) of −1 MPa with a standard deviation (σ_{ψ_b}) of 0.3 MPa. The skewness in the time courses is even more evident as ψ decreases as shown by the shaded areas indicating apparent σ values. (d) Plotting germination time courses at different ψ on a log scale does not completely normalize the slopes or the skewness of the time courses. See Sections 4.1 and 4.2 for further discussion of the features of these time courses.

Richard Ellis and Eric Roberts subsequently extended this approach to quantify seed longevity in relation to the temperature and seed moisture content (or water activity) at which seeds were stored (Ellis and Roberts, 1981; Roberts and Ellis, 1989). After an initial lag period of variable duration, the percentage of seeds retaining viability over storage time also closely matched a normal distribution, with the (negative) slope of the regression line on a probability scale indicating the standard deviation of the variation in individual seed lifetimes within the population under the specified conditions. This parameter is sensitive to the temperature and seed moisture content during storage, enabling Ellis and Roberts to develop their seed longevity equation (Ellis and Roberts, 1980; see Section 5.2). The parameters of this equation (seed viability constants) can be used to estimate the potential seed longevity under combinations of temperature and moisture content (Ellis, 1988) and are compiled (when known for a species) at the Kew Seed Information Database (Royal Botanic Gardens, 2021). The Ellis–Roberts viability equation and other models to predict seed longevity in storage or under natural conditions (Walters et al., 2005; Long et al., 2015; Solberg et al., 2020) represent critical insights and achievements in the development of mathematical models in seed science but will not be addressed in detail in this chapter. In general, these models of seed longevity or persistence address only final percentages of germination/viability in a seed population rather than the kinetics of germination over time following imbibition, which will be the primary focus here due to its greater relevance to seed performance in agriculture. We will discuss the effects of seed storage and aging on germination rates in Section 5.2.

A final general point can be made before examining the details of seed responses to different factors. The timing of seed germination for a seed lot is often calculated using mean germination times (MGT) according to a commonly used formula (ISTA, 2020), which multiplies the number of newly germinated seeds (n) at a given time by that time (t), sums those values, and divides by the total number of germinated seeds, or

$$\mathrm{MGT} = \Sigma(nt) / \Sigma(n). \tag{1}$$

While these MGT values match fairly closely with the median time to germination when germination is rapid and complete in the population, it becomes increasingly skewed to longer times when germination is delayed (Fig. 1). The exponential increase in time to germination of the slower seeds increasingly shifts the MGT values to longer times relative to the median when germination is delayed by environmental conditions. This issue is compounded when total germination is less than 100%, as the population (n) is often defined as only the number of seeds that actually germinated, regardless of the number of seeds tested. This error is not too problematic when most of the

seeds germinate (e.g., 90% or more), but as the total germination percentage goes down, this measure becomes increasingly unreliable as a description of the total population (Bewley et al., 2013; Gianinetti, 2020). In the extreme, if only two seeds in a seed population germinated, the MGT calculation based on germinated seeds would be the average of the times to germination of those two seeds. If this MGT value was compared to that of a seed lot that germinated 100%, it would be comparing the best two seeds in one population to the average for the other, which is clearly a misleading comparison. It is true that an MGT value based only on the viable seeds will give an estimate of when those seeds will germinate, but it must be paired with the germination percentage to give an accurate description of the seed lot performance. Thus, in general, for modeling germination, the complete seed population should be considered, and comparisons between seed lots should be based on the same fractions in the entire population, not upon values for only the fraction of the population capable of germinating under a given condition. For example, the times at which seed lots achieved a given germination percentage that they all exceeded, say 25%, could be used to compare across those lots. The importance of comparing seed lots with these population principles in mind will become evident as we explore the patterns that seed populations exhibit when germinating in response to different environmental and physiological factors.

3.3 Population-based threshold models describing seed germination behavior

In addition to approaching the analysis of seed behavior through population-based concepts, another key feature is the seeds' responses to different levels or dosages of a given factor influencing germination. The approach here has been to utilize thresholds, which imply that there is a minimum dosage or factor level required to initiate the response. This concept has a long history in biological responses to T, for example, as characterized by a minimum or base T (termed T_b) below which the response does not occur. Above this T_b, the process or response progresses at a rate dependent upon the amount by which the current T exceeds T_b, as will be explored in detail in Section 4.1. This is the basis for the degree-days or thermal time approach to normalizing developmental processes across variation in T noted previously. This combination of a minimum threshold sensitivity and an increasing rate of progress as the dosage increases above this threshold is fundamental for describing seed behavior in response to many factors. However, while in thermal time calculations, the minimum thresholds are often considered to be constant among individuals, in the case of seeds, these thresholds can vary among individuals. As shown earlier for seed dormancy loss and seed aging, the variation in these thresholds

is often normally distributed among seeds in the population. Thus, instead of a single threshold, there is a population distribution of thresholds that can be characterized by its median and standard deviation. Each seed has a specific sensitivity threshold, and once it has been exceeded by the dosage level of the factor of interest, progress toward germination can begin (or will be prevented in the case of inhibitors), and it will proceed at a rate proportional to the amount by which the factor level exceeds the threshold (negatively in the case of inhibitors, i.e., slowing germination). It turns out that this fundamentally very simple PBT model can describe seed behavior in response to essentially all of the factors that influence germination, as will be illustrated in detail in the following sections.

3.4 General features of germination time courses and their characterization for seed populations

The remainder of this chapter will focus on the description and application of these PBT models that characterize, quantify and predict seed germination rates and percentages across a wide range of conditions (Bradford, 1995). They are based on the concept that seeds require particular environmental (e.g., T, ψ, O_2) or physiological (e.g., after-ripening, chilling, light, hormones) factors to exceed a certain minimum level or threshold in order to proceed toward germination. In addition, the PBT models explicitly incorporate the concept that as the amount by which a given factor level exceeds its threshold (or base) level required for a response, the time to exhibit the response will be shortened proportionately. That is, the time to germination is inversely proportional to the difference between the current factor level and the threshold for response to it. This can be generalized by the following equation for a given factor X that promotes germination:

$$\theta_X = \left(X - X_b\right)t \tag{2}$$

$$\text{or, } GR = 1/t = \left(X - X_b\right)/\theta_X \tag{3}$$

where X is the level or concentration of factor X, X_b is the base (or threshold) level for a response to X, θ_X is a time constant, and t is the time to germination. Since θ_X is a constant, this means that the difference between X and X_b is inversely proportional to the time required for the response to occur (t) or directly proportional to the germination rate ($GR = 1/t$). If X is a germination-promoting factor, then once X exceeds X_b, germination can occur, and the greater the amount by which X exceeds X_b, the shorter the time required to complete germination, so that θ_X remains constant. If X is a germination-inhibiting factor, then X_b refers to the concentration or level that will prevent germination, and the difference term can be reversed (i.e., $X_b - X$) so that as the

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level of X increases toward X_b, the difference between them decreases and the value of t increases until germination is prevented (i.e., $t = \infty$) at $X = X_b$:

$$\theta_X = \left(X_b - X\right)t. \tag{4}$$

As an intuitive understanding of how the components of this model interact is helpful for appreciating how different factors influence germination, we present a simple analogy based on a ball rolling down a hill and then up and over a second hill onto a flat surface (Fig. 2). In this model, the height of the second hill represents the threshold that the ball (representing the seed) must climb before it can germinate (i.e., reach the flat surface beyond). The factor levels are represented as the starting heights of the ball on the first hill; when $X \leq X_b$, the ball will not have sufficient momentum to climb the second hill, and germination is prevented. As X (starting height) increases above X_b for germination promoters, the ball will have sufficient momentum to overcome the threshold hill and proceed further distances past it, the latter representing the germination rate (GR) or $1/t$ (Fig. 2a). The more momentum gained (higher factor level), the farther the ball can roll past the threshold hill (higher GR or faster germination). These relationships are reversed for inhibitors: GR is reduced as increasing levels (downward on the scale in this case) approach X_b, the level that prevents germination completely (Fig. 2b). The time constant (θ_X) is represented by the slope of the first hill; at the same factor level, the final momentum will be the same, so the threshold hill can be overcome, but the speed of the ball will be slowed (GR reduced) if the first hill is less steep (θ_X is increased) (Fig. 2c). A change in θ_X will proportionately affect all factor levels. This analogy aspires to make the relationships among the three major parameters of the PBT models clear. However, if the ball and hill analogy is not intuitive for the reader, an alternative hydraulic analogy has also been presented (Donohue et al., 2015).

Equations 2 and 4 describe the response of an individual seed to promoters or inhibitors relative to its own X_b or threshold. To extend this to a population basis, either the time constant (θ_X in the case where X is temperature) or the threshold values (X_b for other factors) can vary among individual seeds (models for specific factors influencing germination will be discussed in detail below). In the ball and hill model, this is represented as a range of X_b thresholds, each representing a specific germination fraction within the total population (Fig. 2d). For a given factor level (starting height), how far the ball will eventually roll is determined by how much momentum is required to climb the second hill (threshold). As these thresholds vary among individual seeds (i.e., $X_b(g)$, where g refers to a specific germination fraction in the population), they must be described in population terms. In most cases, this variation among seeds in their individual thresholds will occur in a normal distribution, which can be defined by its mean and standard deviation (Fig. 3a). The larger the standard deviation in sensitivity thresholds among seeds, the larger the range of dosages

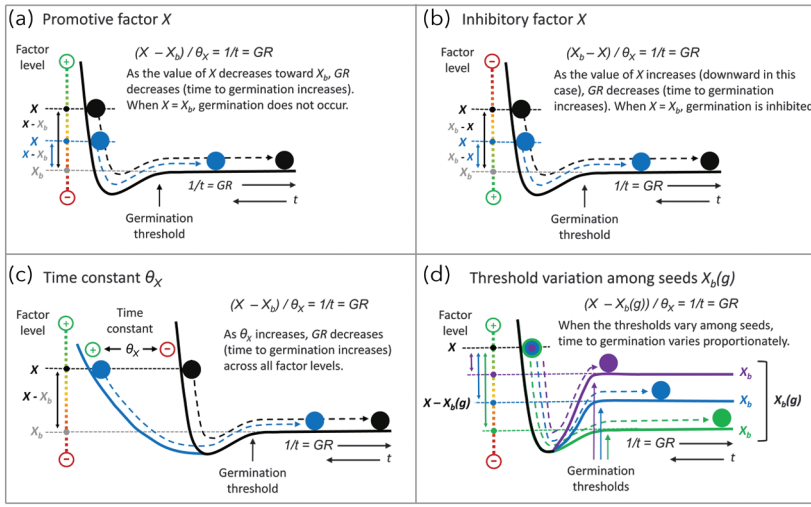

Figure 2 Rolling ball analogy to population-based threshold models. To conceptually illustrate the features and parameters of population-based threshold (PBT) models, consider the case of a ball rolling down an incline and then rolling up over a subsequent hill (threshold). The distance that the ball would roll on the flat after surmounting the hill represents the germination rate (*GR*, or 1/*t*). (a) In the case of a factor promoting germination (e.g., temperature or GA), decreasing the factor level would lower the starting height of the blue ball, giving it less momentum to overcome the hill (threshold), and it would subsequently roll a shorter distance, indicating a slower *GR*, or longer time to germination; increasing the factor level would have the opposite effect, speeding germination. If the promotive factor level is reduced to equal X_b, germination is prevented as the ball will not have sufficient momentum to overcome the threshold. (b) To illustrate the effect of germination inhibitors (e.g., more negative ψ or ABA), we reverse the scale on the factor level; that is, lower factor levels increase the height of the ball and *GR* is increased. As the inhibitory factor level increases (downward on the scale; blue ball) it approaches X_b, reducing *GR* and preventing germination when $X = X_b$. (c) The time constant (θ_X) has the effect of speeding or slowing germination at all factor levels; a shallower slope of the incline illustrates the effect of increasing θ_X. It does not affect the final momentum, so the ball can still overcome the threshold (second hill), but it will delay germination proportionately across factor levels. In the case of suboptimal temperatures, the value of θ_T varies among seeds in the population (see Fig. 4). (d) For most factors, it is the threshold that varies among seeds, illustrated as variation in the height of the second hill that the ball must climb. Thus, for a given factor level *X* (starting height on the first hill), the ball will use increasing amounts of momentum to scale the second hill, leaving it with less to proceed on the flat, or reducing *GR*. When the value of X_b increases to equal *X*, germination is prevented, that is, the ball would not have sufficient momentum to climb the second hill. To view an animated version of panels in this figure, see www.animation.pbtmodels.org.

of a given factor that is required to permit (or prevent) germination of the entire population. A consequence of this model is that the effect of a given change in the level of factor *X* will not have an equal effect on all seeds in a population.

This is why germination time courses are not symmetrical, with the time from the beginning of germination to 50% being shorter than the time from 50% to 100%, or right skewed in time (Fig. 1a and c). This becomes increasingly evident as the time to germination is extended, such as by lowering the T or ψ. In the case of suboptimal temperatures, seeds often share a common T_b, so a given change in T has the same proportional effect on all seeds, but its effect is still more pronounced on the slower germinating seeds. This is illustrated in Figure 1a, where a reduction in temperature slows germination of all seeds, but has a greater effect on the slower seeds in the population, resulting in the shift in the median slope of the latter curve on a linear scale. When these curves are plotted on a logarithmic time scale, however, the median slopes of these time courses are essentially parallel (Fig. 1b). Thus, the effect of lowering the temperature has the same proportionate effect on all seeds, but as the differences in times to germination increase logarithmically within the population, the differences become progressively larger with time on a linear scale.

If those seeds were exposed to reduced ψ rather than lower T, times to germination are also delayed; the differences between the early and late germinating seeds are increased and are not fully compensated by plotting on a log scale (Fig. 1c and d). This is because rather than sharing a common base water potential threshold (ψ_b), seeds vary in their thresholds with respect to ψ. This results in a much greater effect of a given change in ψ for some seeds compared to others, depending upon their position in the sensitivity distribution (Fig. 3a). When the promotive factor level (e.g., high ψ) exceeds the response thresholds of all seeds, germination of all seeds is rapid and the asymmetry in the germination time course is hardly noticeable (Fig. 3b, black curve). As the level of the promotive factor is reduced (or the inhibitor level increases, e.g., ψ becomes lower or more negative), seeds with higher thresholds (right side of the distribution) are more strongly affected than the seeds with lower thresholds (left side of the distribution). This is reflected in the greater delays in germination at higher percentages compared to lower percentages (Fig. 3b). As the factor level is further reduced, an increasing fraction of seeds are unable to germinate as the factor level falls below their individual thresholds, and the remaining seeds take proportionately longer to germinate. It is also important to note that as the model is driven by the difference between X and X_b, the same effect occurs if the factor level is held constant and the threshold distribution is increased or decreased. Thus, as we will see, dormancy can be alleviated by reducing inhibitors, increasing promoters, or by shifting the cellular sensitivity thresholds to either or both of these regulators. This basic pattern has been identified in seed responses to essentially all environmental and hormonal factors that influence germination, indicating that population variation in

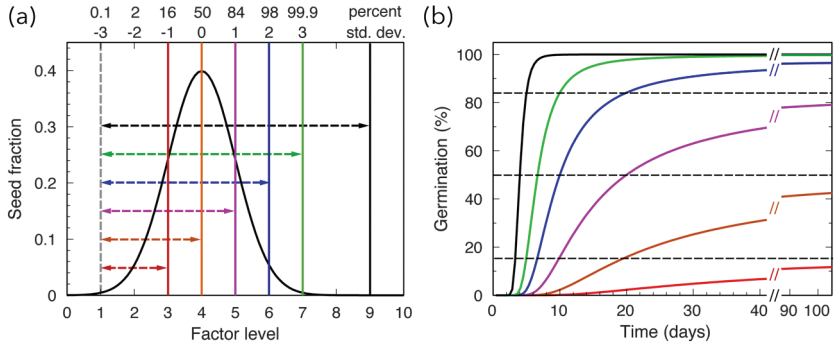

Figure 3 Sensitivity distribution in a seed population and consequent effects on germination time courses. (a) The distribution of sensitivities to a given factor affecting germination can be described in most cases as a normal distribution (black curve). This distribution is centered at the median (same as the mean for a normal distribution) or the 50th percentile in the population (upper scale). Percentages can be converted to probit values, which are in standard deviation units from the mean (lower scale above panel). The percentage values corresponding to increments of one standard deviation are shown, which is nonlinear; 68% of the population lies within one standard deviation below and above the median. The colored vertical lines indicate different levels of a factor that promotes germination (e.g., high water potential or GA). The dashed arrows indicate the difference between the factor levels and the minimum level that must be exceeded for any seeds to germinate (dashed vertical line). It is apparent that a given reduction in the factor level (e.g., from level 9 to level 6) has a much greater effect on seeds with higher thresholds (right side of the distribution) than on seeds with lower thresholds (left side of the distribution). Also, as the factor level falls below the threshold of a given fraction of the population, those seeds are unable to germinate. (b) The germination time courses predicted at each factor level shown in panel (a). Due to the relationship described above, the effect of a given reduction in factor level is much greater for the higher percentages (which have higher response thresholds) than for the lower percentages (with lower thresholds), although all fractions will respond proportionately to their own thresholds. This results in the right skewing of germination time courses and in the reduction in final germination percentages as the level of the required promotive factor is reduced. The time axis is broken at the right to indicate the extended time required for the slowest germinating seeds to approach the final percentages predicted by the model.

response thresholds is a fundamental feature of seed biology and likely of many other aspects of biology (Bradford, 2018).

3.5 Implications of population-based models for the management of stand establishment

Understanding these population-based features of germination time courses and thinking of seeds in population terms is essential in managing them to achieve optimal performance in agricultural or ecological contexts. From an agricultural point of view, we want to minimize the variation in the time of germination and maximize the emergence percentage. Already from the

simple examples shown, it is clear that T and ψ are critical factors for uniform germination and that the farther they are from optimal, the greater the impact on uniformity. Thus, providing optimal conditions during the initial imbibition and germination phase after planting is critical for achieving maximum uniformity. In addition, reducing the variation in thresholds within the seed population is an important criterion for seed quality. A fundamental physiological feature of a seed population (i.e., a given seed lot) is its sensitivity distribution, as characterized by its median and standard deviation (σ). It is this inherent variation within the seed lot that we need to focus on, as its effects are magnified under suboptimal environmental conditions. In addition, these population features can change during seed maturation and storage, so optimizing them during harvest, storage and delivery to the grower is critical for the highest performance. As seed companies have tools to improve seed uniformity (e.g., grading, sorting, priming, etc.) (Halmer, 2004; Bruggink and van Duijn, 2017; Mortensen et al., 2021), understanding these basic behavioral principles of seed populations and using population-based models to characterize these enhancement operations provide opportunities for advancements in seed technology.

In ecological applications, seed lots are often collected in nature and are much less uniform than agricultural seeds. They also often exhibit diverse types of dormancy that may require exposure to specific conditions to enable germination (Baskin and Baskin, 2020). On the other hand, uniformity in germination after planting may be less critical or even detrimental, depending upon the planting conditions and potential lifetime of seeds in the soil (Burghardt et al., 2015). For example, the dormant fraction of a seed lot planted for restoration purposes may be able to survive in the seed bank and emerge in a subsequent season. However, the low success rates of such ecological interventions (Commander et al., 2020) indicate that better knowledge about and prediction of native seed behavior is sorely needed.

4 Population-based models for environmental factors affecting seed germination

4.1 Temperature (thermal time model)

The population-based models for describing seed germination time courses are based upon the threshold concept described above (Bradford, 1995). In general, a threshold indicates the magnitude or intensity of some requirement or input that must be exceeded for a certain reaction, phenomenon, result or condition to occur. In the case of a seed, a threshold is the minimum effective level or dosage of the relevant factor required for the seed to respond by initiating germination (or inhibiting germination for repressors). This is the case

for thermal time models, for example, in which there is a base (or minimum) temperature below which germination will not occur (or will take infinitely long). Above this T_b, germination is possible, and its rate (inverse of time to a specific percentage germination percentage) will increase linearly with temperature (Fig. 4b). This is the thermal time model, which states that the thermal time required for germination (θ_T) of a specific percentage or fraction (g) of the seed population ($\theta_T(g)$) is equal to the degrees in excess of T_b (or $T - T_b$) multiplied by the time to germination of percentage g (t_g) (Bierhuizen and Wagenvoort, 1974; Alvarado and Bradford, 2002).

$$\theta_T(g) = (T - T_b)t_g. \tag{5}$$

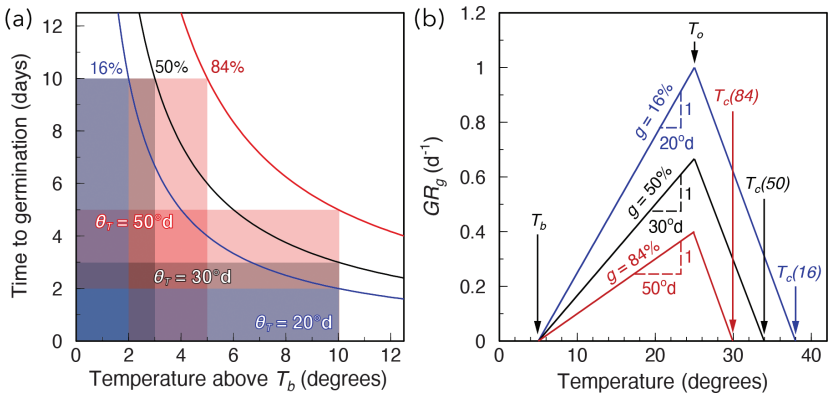

Figure 4 Temperature and thermal time relationships of seed germination. (a) The thermal time concept is based upon the fact that the time to germination for a given percentage fraction of the seed population is inversely proportional to the degrees above the base temperature (T_b). The blue, black and red curves show this relationship for the 16%, 50% and 84% fractions or one standard deviation below and above the median. As is indicated by the shaded rectangles, any such rectangle constructed below these curves encloses the same area, for example, 20, 30 and 50 degree-days (°d) for 16%, 50% and 84%, respectively. Thus, as the temperature increases above T_b, the time to germination reduces proportionately so that their product remains constant. This fundamental feature of thermal time enables developmental time to be normalized across temperatures by putting time and temperature on an accumulated thermal time basis, i.e., $(T - T_b)\,t$. Note that the differences between the curves (and between the areas of the thermal time boxes) are not equal, due to the skewing of germination time courses as percentage increases. (b) Plotting the inverse of the times to germination from panel (a) (i.e., the germination rates or GR_g) results in linear relationships with temperature. The accumulated thermal time ($\theta_T(g)$) is now represented by the inverse of the slopes of the lines for each fraction (g), and T_b (arbitrarily set at 5°C) is their common intercept on the x-axis. This provides a convenient method to determine T_b. These linear relationships only hold up to the optimum temperature (T_o, at which germination is most rapid). Increasing temperature above T_o generally results in parallel decreasing GR values with different ceiling temperatures ($T_c(g)$) at which germination is prevented (intercepts on the x-axis).

The thermal time (θ_T) is in degree-day (°C d) or degree-hour (°C h) units, indicating that it is the product of the temperature and the accumulated time. As there is an inverse relationship between T in excess of T_b and t_g (see curves in Fig. 4a), and $\theta_T(g)$ is a constant for a given fraction g, this means that any combination of T and t_g will result in the same rectangular area under the T versus t_g curve for fraction g (see shaded rectangles in Fig. 4a), which is equal to $\theta_T(g)$. In seed germination tests, it is generally possible to control the temperature to a specific value for the duration of the test, so that $T - T_b$ multiplied by the time to germination of a given percentage (t_g) equals the thermal time for that percentage ($\theta_T(g)$). However, in the field, the temperature varies daily during the germination and emergence period, so the thermal time can be accumulated for each day (i), and germination will occur when the total thermal time requirement is achieved.

$$\theta_T\left(g\right) = \Sigma_i \left(T - T_b\right)_i \left(t_g\right)_i. \tag{6}$$

As temperature varies diurnally, the daily mean temperature is often used for thermal time calculations. This also works for seed germination, with the caveat that some seeds require alternating temperatures for germination, so germination performance will not be the same if the seeds are held at the mean temperature constantly (Batlla et al., 2003). In general, developmental timing varies across temperatures for most plants, insects and poikilothermic (cold-blooded) animals and can be normalized on a thermal time scale, resulting in its widespread use in modeling and predicting crop growth and maturity, pest management and many other biological processes in addition to seed germination (Trudgill et al., 2005).

It is often, but not always (see Section 5.1), the case that all seeds in the population will have the same (or very similar) value for T_b. In this case, the value of $\theta_T(g)$ varies depending upon the physiological variation in t_g in the seed population. When plotted as germination rates ($1/t_g$) versus time, the lines representing different fractions of the population will vary in slope but with a common intercept at T_b (Fig. 4b). The inverses of the slopes of these lines will be equal to $\theta_T(g)$, as seen by rearranging Equation 5:

$$GR_g = 1/t_g = \left(T - T_b\right)/\theta_T\left(g\right), \tag{7}$$

where GR_g refers to the germination rate (GR) for fraction g. Between the base and optimal (T_o) temperatures, this equation generally works well to describe the rates of germination for a given seed population across temperatures (as in Fig. 1a). Recommendations for many crops will include minimum soil temperatures at planting and the expected times to seedling emergence based upon the thermal time requirements for germination and subsequent seedling growth to the soil surface.

Equations 5 and 7 predict that the time to germination for a given fraction g would continue to decrease indefinitely as $T - T_b$ increased. However, this generally occurs only until an optimum temperature (T_o) is reached; with further increases in T, the time to germination (or other biological process) slows down and eventually stops or is prevented when a maximum or ceiling (T_c) temperature is reached (Fig. 4b). In many cases, this T_c varies among different seeds in the population ($T_c(g)$), resulting in the characteristic triangular shapes of complete GR temperature responses shown in Fig. 4b (Covell et al., 1986; Alvarado and Bradford, 2002; Bakhshandeh et al., 2020). This figure reemphasizes that each seed in a population represents a particular fraction g in that population and will have a unique time to germination (t_g) or rate (GR_g) determined by its physiological state (potential speed of germination) and its base, optimum and ceiling temperatures. These latter temperatures are known as the cardinal temperatures for seed germination and are critical for understanding seed performance in relation to temperature (Alvarado and Bradford, 2002; Rowse and Finch-Savage, 2003). Figure 4 also illustrates that for a given factor (T in this case), seeds can share a common threshold within a population (as for T_b) or can vary in their thresholds (as for $T_c(g)$). It is also evident that the earliest germinating seeds have the greatest GR values at suboptimal temperatures and the highest T_c values, or more generally, the most rapidly germinating seeds are also the most vigorous and stress tolerant.

The simplest description of germination in response to temperature therefore has two components, one for temperatures between T_b and T_o (suboptimal region) (Equations 5 and 7) and another for temperatures between T_o and T_c (supraoptimal region) (Equations 8 and 9) (Ellis et al., 1986):

$$\theta_T = \left(T_c(g) - T\right)t_g, \tag{8}$$

$$\text{or, } GR_g = 1/t_g = \left(T_c(g) - T\right)/\theta_T. \tag{9}$$

For this supraoptimal region, it is the T_c that varies with g ($T_c(g)$), while θ_T (with a value distinct from $\theta_T(g)$) is a constant for all seeds. Above T_o, increasing temperature inhibits rather than promotes germination rates, so the terms are reversed on the right side of the equation; as T increases above T_o toward T_c, the difference between them decreases and the time to germination increases proportionately to maintain θ_T constant. This results in the parallel linear relationships with decreasing GR_g between T_o and $T_c(g)$ (Fig. 4b). There also are modifications to this bilinear model that use continuous variables to describe this change in direction of germination speed rather than two linear components (Grundy et al., 2000; Rowse and Finch-Savage, 2003), which is appropriate if the germination behavior exhibits a plateau or curve near T_o rather than a sharp reversal (Watt and Bloomberg, 2012).

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The models in Equations 8 and 9 also assume that thermal time would continue to accumulate as T increased above T_o. However, Alvarado and Bradford (2002) found that the models fit their data better if thermal time did not continue to accumulate above T_o, that is, if Equation 8 was modified in the supraoptimal temperature region to Equation 10, capping thermal time accumulation to that achieved at T_o.

$$\theta_T = \left(T_c(g) - T_o\right)t_g. \tag{10}$$

On the other hand, Rowse and Finch-Savage (2003) proposed that thermal time should continue to accumulate but decreasing germination rates should start to occur below T_o. This will be discussed further in Section 4.3 after the introduction of the hydrotime and hydrothermal time (HTT) models.

4.2 Water (hydrotime model)

In addition to a temperature between its T_b and T_c, a seed also requires water in order to germinate. Pure water represents the highest water potential (ψ) value (0 MPa) at a given temperature, so reducing ψ (to more negative values) has a proportional effect in delaying germination, just as reducing temperature toward T_b slows germination (Fig. 1) (Bradford, 1990, 1995). At the minimum or base threshold ψ (or ψ_b), the time to germination becomes infinite, or germination is prevented. A critical development in modeling seed germination was based on the insight of Gummerson (1986) that individual seeds could differ in the ψ_b thresholds that must be exceeded to allow them to proceed toward germination. Combining these two components resulted in the hydrotime model for germination:

$$\theta_H = \left(\psi - \psi_b\left(g\right)\right)t_g, \tag{11}$$

$$\text{or, } GR_g = 1/t_g = \left(\psi - \psi_b\left(g\right)\right)/\theta_H, \tag{12}$$

where θ_H is the hydrotime constant (MPa h or MPa d), $\psi_b(g)$ represents the base water potential of fraction g of the seed population (MPa), and t_g is the time to germination of fraction g (h or d) (Bradford, 1990). This pattern is displayed in Fig. 3b if we use ψ as the factor, that is, factor level 9 represents pure water and the other factor levels represent progressively lower ψ values. All of the features of the germination time courses at different ψ values, the delays in germination, the skewness of the curves, the final germination percentages, and so on, are generated by this simple model that requires only three parameters: the value of θ_H, the mean ($\psi_b(50)$) and standard deviation (σ_{ψ_b}) of the $\psi_b(g)$ distribution among seeds in the population.

In contrast to the common T_b in the suboptimal temperature region (Fig. 4b), ψ_b values vary for different fractions of the population (Figs. 2d and

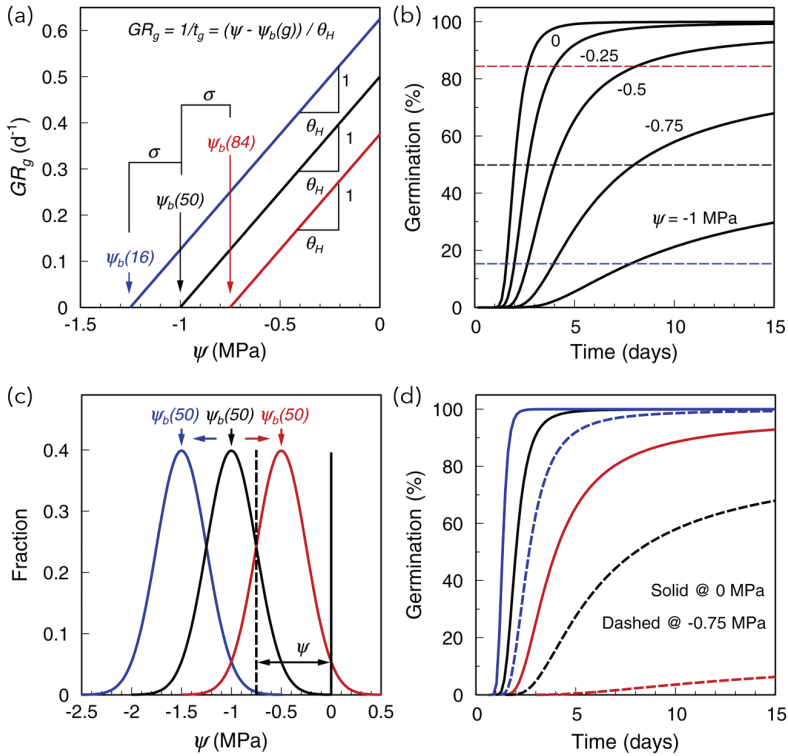

Figure 5 Key characteristics of the hydrotime model of seed germination. (a) Plotting the germination rates to different percentages (GR_g or $1/t_g$) shows that while the hydrotime constant (θ_H or the inverse of the slope of the lines) is the same across percentages, the intercepts on the *x*-axis (the base water potentials or $\psi_b(g)$) differ among percentages. When the ambient water potential (ψ) is 0 MPa, GR_g is at its maximum for each percentage of the population. As ψ decreases, the GR_g values fall and become zero when $\psi = \psi_b(g)$ for that fraction of the population. Values are plotted for 16%, 50% and 84%, representing the population mean and one standard deviation (σ) above and below it. (b) Germination time courses are illustrated for the seed population in panel (a) at different ψ values between 0 and −1 MPa (the latter is the $\psi_b(50)$ used in this illustration along with σ_{ψ_b} = 0.25 MPa and θ_H = 2 MPa d). The horizontal dashed lines indicate the 16%, 50% and 84% germination percentiles for comparison with panel (a). Note that relatively slight reductions in ψ notably delay germination, particularly in the slower fractions of the population. The curves for −0.75 and −1 MPa will asymptotically approach 84% and 50% germination, as indicated by the $\psi_b(50)$ values for these percentages (panel a), after very long times (more than 200 days). (c) This panel illustrates shifts in the $\psi_b(g)$ distributions from the values used in panels (a) and (b) (black curve) to 0.5 MPa higher (red curve) or lower (blue curve), without changing the σ_{ψ_b} or θ_H values. The vertical solid line indicates the relationship of the environmental ψ to these distributions when ψ = 0 MPa, and the vertical dashed line indicates when ψ = −0.75 MPa. (d) Germination time courses for the seed populations indicated in panel (c) at ψ = 0 MPa (solid lines) or −0.75 MPa (dashed lines). Reductions in environmental ψ have a much greater effect in delaying germination in populations with higher $\psi_b(g)$ distributions (red lines) than in those with lower $\psi_b(g)$ distributions (blue lines).

5a). As ψ decreases toward ψ_b, GR_g decreases (or t_g increases) until germination is prevented when ψ is equal to or less than $\psi_b(g)$. The parallel slopes of the GR_g versus ψ lines indicate that the hydrotime constant (θ_H, or the inverse of the slope; Eqn. 12) is the same across all seeds in the population. This is similar to the situation for germination at supraoptimal temperatures (Eqn. 8; compare Fig. 5a with Fig. 4b); the reason for this will become clear in the next section. Depending upon the mean and spread of the ψ_b values in the seed population, germination will occur over time in a characteristic pattern at different water potentials (Fig. 5b). When the $\psi_b(50)$ is fairly high, for example, −1 MPa in the example shown, the time to germination increases markedly with relatively slight reductions in ψ, particularly in the slower fractions of the seed population. For example, −0.03 MPa is an approximate value for the water potential of soil at field capacity, when bulk water has drained from its pores. For seed germination, this is essentially the same as the 0 MPa curve shown. However, depending upon the soil type and weather conditions, the soil ψ near the surface can drop considerably lower over a few days. A reduction even to −0.25 MPa considerably slows germination and increases the spread in germination times for a population with a $\psi_b(50)$ of −1 MPa (Fig. 5b). In addition, although half of the seed population should be able to complete germination at −1 MPa, it would take approximately 300 days for the germination percentage to approach 50% at that ψ due to the exponential increase in the time to germination as the ψ approaches the ψ_b for a particular seed fraction, 50% in this example (Fig. 5b; c.f., Fig. 3b showing extended time axis). Seeds in the population with $\psi_b(g)$ values more positive than the ψ of the environment are prevented from germinating, that is, t_g becomes infinitely long.

In addition to the environmental conditions (primarily T and ψ) of the seedbed, the physiological status of the seeds can also dramatically affect their response to a given environment. For example, seed lots can differ in their hydrotime parameters. If the population has a $\psi_b(50)$ of either −1.5 or −0.5 MPa, two standard deviations below and above the $\psi_b(50)$ in the example above (Fig. 5c), the germination performance at a given ψ will be markedly affected (Fig. 5d). If the $\psi_b(50)$ is lower, germination will be more rapid and uniform, while if it is higher, germination will be delayed and more dispersed, even at 0 MPa (Fig. 5d, solid lines). If the soil ψ decreases, such as to −0.75 MPa (Fig. 5c), the effect on germination will be much greater on the population with the higher $\psi_b(50)$ (Fig. 5d, dashed lines). As will become evident below, $\psi_b(g)$ distributions of seed populations do change, particularly in relation to seed dormancy. Thus, both environmental conditions (soil type, rainfall, irrigation, salinity) and physiological characteristics (the value of ψ_b and its variation among seeds) determine the pattern of seed germination and subsequent seedling emergence.

These features of seed germination responses to ψ have some important implications for stand establishment. First, the speed and particularly the

uniformity of germination are quite sensitive to even relatively slight drops in ψ. Thus, to achieve the most rapid and uniform germination and emergence, the water status of the seeds must be kept as high and constant as possible, but not submerging the seeds for extended periods, which leads to oxygen deprivation and poor performance (see Section 4.4). This is well known in the transplant industry, which utilizes uniform temperatures near the optimum and frequent light applications of water following seeding to maintain high and constant ψ during germination and seedling emergence. In the field, growers aim to plant seeds into adequate soil moisture and irrigate after planting if possible. Without irrigation, Fig. 5b illustrates the impacts that drying soil will have on germination rates and uniformity. However, if rainfall occurs at any time, seeds will immediately respond to the increase in ψ and shift up to a higher germination curve. In practice, this can result in a split emergence pattern, with some seeds emerging early due to the initial soil water present, followed by a second flush of emergence if a subsequent rain event occurs. This presents a very difficult management situation for the grower, as plant uniformity in crop stands is almost entirely dependent upon the uniformity of seedling emergence. After emergence, each plant grows at an initially exponential rate that becomes linear with leaf canopy closure, so later emerging plants do not actually catch up with their peers until late in the development process (Loomis and Connor, 1992). If they are shaded by neighbors, their growth rates are even further reduced. This results in problems for virtually all crop management decisions that depend upon the developmental stage, including flowering, pollination and maturity as well as fertilization and pest management (Finch-Savage and Bassel, 2016). Similarly, seed performance in a given hydration environment is dependent on the physiological quality of the seed lot, particularly the mean of the threshold distribution and the variance around it (Fig. 5c and d). A seed lot with a more negative and a narrow $\psi_b(g)$ distribution will be much more robust to reductions in soil ψ during the germination period. Understanding the hydrotime behavior of seed populations instills an appreciation for the high sensitivity of seeds to their hydration environment and the consequences of failing to provide optimal conditions during the earliest step in crop production.

4.3 Water and temperature (hydrothermal time model)

Temperature and water are two inescapable and variable environmental factors that are critical for seed germination. Thus, combining the thermal and hydrotime models into a single HTT model is an important step that also was conceived by Gummerson (1986). This was done for suboptimal temperatures by combining Equations 5 and 11.

$$\theta_{HT} = \left(\psi - \psi_b(g)\right)\left(T - T_b\right)t_g, \tag{13}$$

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$$\text{or,}\quad GR_g = 1/t_g = \left[\left(\psi - \psi_b(g)\right)\left(T - T_b\right)\right] / \theta_{HT}. \tag{14}$$

The HTT constant (θ_{HT}, MPa °C h or MPa °C d) incorporates the effects of ψ on germination on a thermal time basis. Although it was noted that germination responses to temperature are often analyzed in relation to the logarithm of time (Fig. 1b), the HTT equation uses actual times to germination (t_g). This is because the variation in $\psi_b(g)$ thresholds in the hydrotime model accounts for the skewness present in the time courses (Fig. 1c). It is more appropriate to think of the HTT model as being based on the hydrotime model but expressing the time in thermal time units to normalize for the effect of temperature. This model has proved to be a broadly applicable and quite accurate model for describing germination rates and percentages in response to both T and ψ (Dürr et al., 2015), with to date nearly 50 publications using the term 'hydrothermal' in their titles and nearly 300 total publications addressing various aspects of application of this concept in seed physiology, crop production, weed management and ecology. In the suboptimal temperature range, these equations account well for the increasing germination rate as T increases above T_b and the opposite effect as ψ decreases toward the ψ_b thresholds of different seed fractions (Figs. 4b and 5a).

The HTT model can also provide an explanation for the reversal in germination rates and inhibition of germination at supraoptimal temperatures (Fig. 6a) (Alvarado and Bradford, 2002). This behavior at supraoptimal temperatures can be explained by an increase in $\psi_b(g)$ as T exceeds T_o. That is, the $\psi_b(g)$ distribution shifts to progressively higher values as T increases above T_o (Fig. 6c). When the value of $\psi_b(g)$ reaches 0 MPa for a given fraction g, that temperature is the T_c value for that fraction (Fig. 6b and c). This progressive inhibition of germination rates as the threshold distribution shifts upward toward 0 MPa explains why the slopes of these curves are parallel above T_o and why unlike T_b, which is often a single value for a seed lot, T_c values vary among seed fractions. This behavior can be added to the HTT model by including a constant (k_T, MPa °C^{-1}) that indicates the slope of the increase in ψ_b per degree above T_o.

$$\theta_{HT} = \left[\left(\psi + k_T\left(T - T_o\right)\right) - \psi_b(g)\right]\left(T_o - T_b\right)t_g, \tag{15}$$

$$\text{or,}\quad GR_g = 1/t_g = \left[\left(\psi + k_T\left(T - T_o\right)\right) - \psi_b(g)\right]\left(T_o - T_b\right) / \theta_{HT}. \tag{16}$$

These equations reduce the difference between $\psi - \psi_b(g)$ by the value of k_T for each degree that T exceeds T_o, which is equivalent to shifting the entire $\psi_b(g)$ distribution to more positive values (Fig. 6c; Alvarado and Bradford, 2002). The larger the value of k_T, the steeper the slope of this increase would be, and

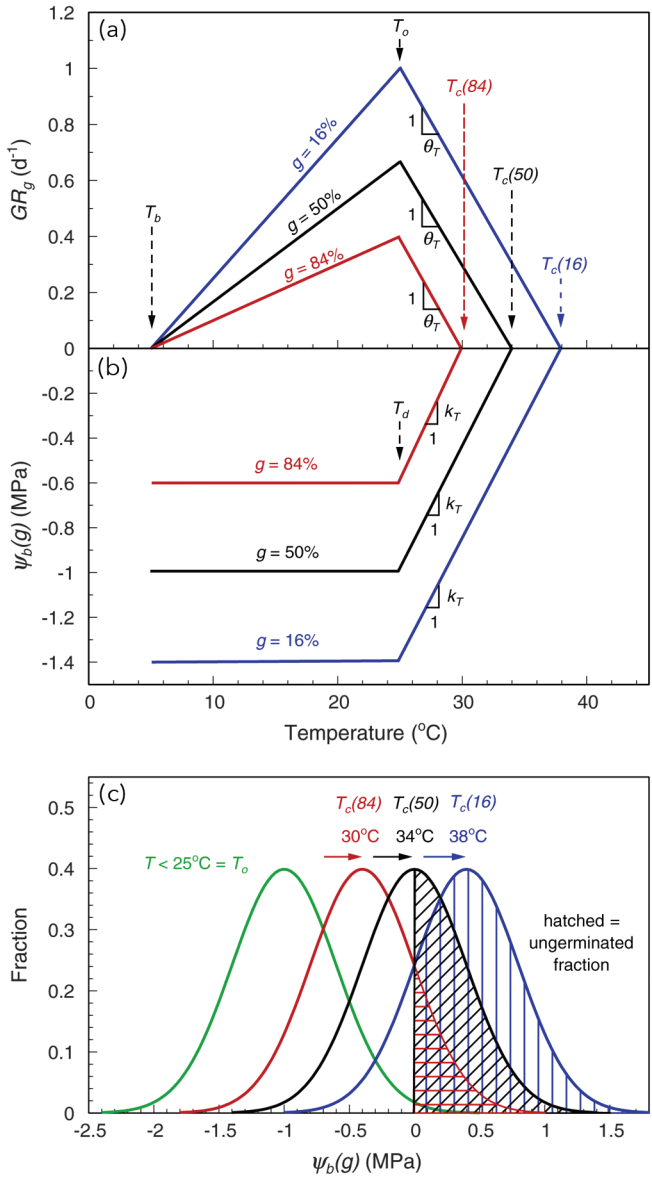

Figure 6 Relationships of germination rates to temperature and $\psi_b(g)$ distributions. (a) Illustration of the germination rates (GR_g) for g = 16%, 50% and 84% germination between T_b and $T_c(g)$ (as in Fig. 3b). (b) The $\psi_b(g)$ values of the seed population remain relatively constant below T_o, then increase with a slope of k_T per degree Celsius (Eqn. 15). The decreasing GR_g values at $T > T_o$ in panel (a) are due to this shift in the $\psi_b(g)$ distribution to higher (more positive) values. When the $\psi_b(g)$ values of a fraction of the seed population exceed 0 MPa, that fraction is unable to germinate at that temperature (GR_g = 0), which is the T_c value for that fraction. T_d indicates the T at which the $\psi_b(g)$

the sharper the drop in GR_g above T_o (Fig. 6a and b). In some cases, this can result in an inhibition of germination within a few degrees above the optimum temperature, also known as thermoinhibition (Bradford and Somasco, 1994).

Examples of how changes in the parameters of the HTT model influence germination speed are illustrated by plotting the percent germination after 5 days of imbibition across a matrix of temperatures and water potentials (Fig. 7). In an agricultural context, these examples reinforce the importance of maintaining near-optimal conditions in the seedbed or transplant tray during the initial imbibition and germination period. They also illustrate the expansion of the permissive hydrothermal space when T_b is lower or T_o is higher, or when the $\psi_b(g)$ distribution is more negative. In an ecological sense, these figures represent different possible germination niches for seeds having different HTT parameters (Liu et al., 2020). This will be discussed further subsequently in relation to seed dormancy (Section 5.1).

In Equation 15, and as discussed in relation to supraoptimal temperatures (Section 4.1), the thermal time component is accounted for by the $T_o - T_b$ term, which in essence caps the accumulation of thermal time at the amount associated with T_o. It would be expected that increasing T above T_o will continue to increase the speed of germination due to increasing thermal time accumulation. However, the germination rate is actually decreasing above T_o due to the upward shift in the ψ_b distribution. Capping thermal time accumulation at T_o solves this problem in some cases (Alvarado and Bradford, 2002). However, an alternative model has been proposed in which the increase in $\psi_b(g)$ begins at a temperature below T_o (termed T_d; Fig. 6b) and thermal time continues to accumulate with increasing T in the supraoptimal range even as the rise in $\psi_b(g)$ slows germination (Rowse and Finch-Savage, 2003; Finch-Savage et al., 2005). This results in a more gradual transition from increasing to decreasing GR rather than a sharp peak and reversal at T_o (Fig. 6). Both types of patterns have been observed (Bloomberg et al., 2009; Hardegree et al., 2015; Bakhshandeh et al., 2017), and both approaches give similar results with respect to the base and maximum temperatures and hydrotime parameters.

A key feature of thermal time models is that germination time courses at different temperatures (e.g., Fig. 1a) all collapse to a single time course when

Figure 6 (*Continued*)

distribution begins to increase, which is used in some models in which T_o is not a single T but can represent a more gradually changing transition (Rowse and Finch-Savage, 2003). (c) As the $\psi_b(g)$ distribution shifts toward 0 MPa in response to $T > T_o$, increasing fractions of the population will have ψ_b values equal to or exceeding 0 MPa; that fraction of the population is prevented from germinating (hatched areas of the $\psi_b(g)$ distributions). Thus, the slower germinating seeds (e.g., 84%) are the first to be inhibited at lower temperatures, while the faster-germinating seeds with lower ψ_b values can germinate to higher temperatures.

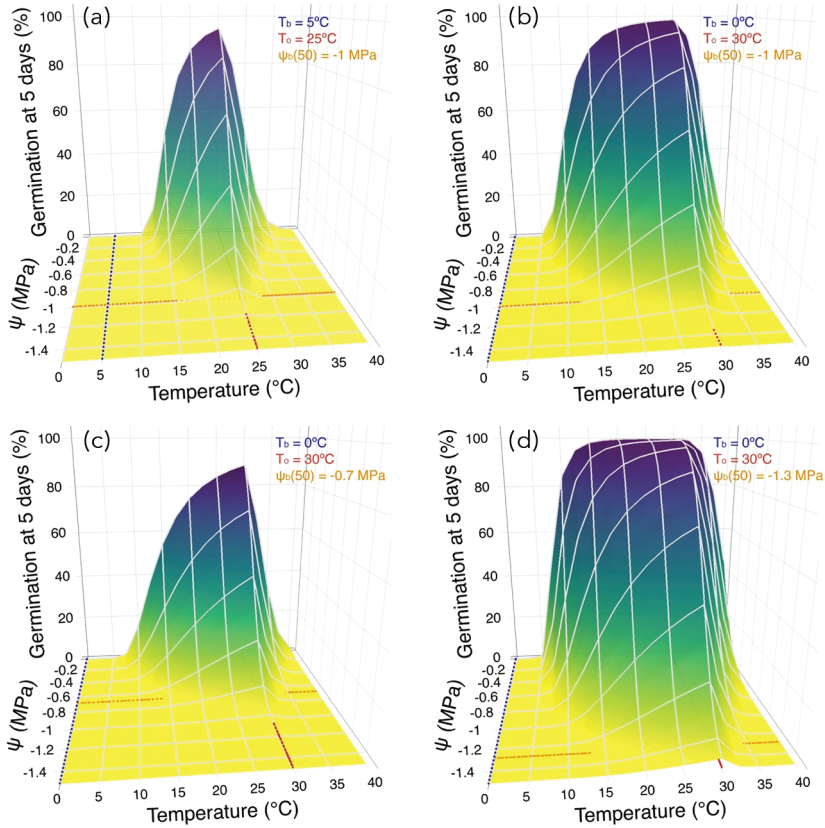

Figure 7 Examples of the effects of combinations of temperature (T) and water potential (ψ) on the percentages of seeds germinating within 5 days of planting. (a) Seeds with relatively higher T_b (5°C; blue dashed line on temperature axis) and low T_o (25°C; red dashed line on T axis). The hydrotime parameters are θ_{HT} = 50 MPa °C d; $\psi_b(50)$ = −1.0 MPa (dashed orange line on ψ axis); σ_{ψ_b} = 0.3 MPa; k_T = 0.1 MPa °C^{-1}. The narrow temperature range between T_b and T_o results in relatively slow germination except near the optimum temperature and germination decreases as ψ declines. (b) Using the same hydrotime parameters but reducing T_b to 0°C and increasing T_o to 30°C results in increased germination within 5 days at higher ψ and over a broader range of T. (c) Using the same cardinal temperatures as in panel (b), but raising the $\psi_b(50)$ to −0.7 MPa (equivalent to one standard deviation) slows germination despite a more favorable temperature range. (d) Using the same cardinal temperatures as in panel (b), but lowering the $\psi_b(50)$ to −1.3 MPa (equivalent to one standard deviation) speeds germination and expands the range of T and ψ over which high germination can occur within 5 days. Note the overall interchangeability of T and ψ in their effects on germination timing and their synergistic effects in expanding the hydrothermal germination space when both factors positively affect germination (low T_b, high T_o and low $\psi_b(50)$).

plotted on a thermal time scale (e.g., Dahal et al., 1990). This is expected from the concept of thermal time in which the time to the developmental event requires the same amount of thermal time accumulation at any suboptimal temperature (Fig. 4a). Similarly, germination time courses at different water potentials (e.g., Fig. 4b) can also be normalized using the simple function $[1 - (\psi/\psi_b(g))]\ t_g$, which will convert times to germination at any ψ to the corresponding time to germination at 0 MPa (Bradford, 1990; Dahal and Bradford, 1990), that is, on a normalized hydrotime scale. If effects of temperatures are accounted for by thermal time and those of water potentials by normalization of hydrotime, then germination time courses across all T and ψ can be plotted on a common normalized HTT scale (Dahal and Bradford, 1994; Alvarado and Bradford, 2002; Rowse and Finch-Savage, 2003; Alvarado and Bradford, 2005). Thus, the two main factors affecting germination and seedling emergence can be understood as acting on the 'biotime' scale at which germination occurs after imbibition (Bradford and Trewavas, 1994).

It is useful to think in terms of normalized HTTs, that is, the combination of T and ψ effects, in understanding and predicting seedling emergence patterns in both agricultural and ecological situations (Bradford, 1995; Finch-Savage et al., 2005; Meyer and Allen, 2009; Finch-Savage and Bassel, 2016). While the models have generally been developed using constant T and ψ, both of these vary daily in the field. Degree-day models generally use the average daily temperature, often also putting a cap on the maximum temperature, as the degree-days relationship for plant development also does not increase linearly above certain upper limits. Soil ψ also varies diurnally, daily and seasonally, with the magnitude varying with soil type and planting depth. Between irrigations or rainfall, soil ψ will decline, with a steeper gradient near the soil surface. On the other hand, soil ψ near the surface will increase quickly after irrigation or rainfall, and seeds previously prevented from germinating by low soil ψ will then germinate quickly (Finch-Savage et al., 2005). Lower water potentials are also experienced by seeds in saline soils, as the dissolved ions lower the osmotic potential of the soil solution. Halophytes (plants that can grow and reproduce in saline environments >200 mM NaCl, equivalent to ~−1 MPa) can germinate at low water potentials, and a halothermal time model based on the HTT model can describe their germination behavior in saline solutions (Seal et al., 2018). While the seed coat of the halophyte *Suaeda maritima* largely excludes the NaCl from the embryo (Szymansky et al., 2021), nonhalophytic species such as chicory (*Cichorium intybus* L.) may absorb significant quantities of salt ions, contributing to the osmotic adjustment of the embryos and enabling germination to somewhat lower water potentials (Bakhshandeh et al., 2020). The extent of this osmotic adjustment could be estimated by analyzing germination time courses using the halothermal time model. By lowering the osmotic potential of the soil solution, salinity will delay germination and

can cause major problems for stand establishment in drying soils, as salts are concentrated near the surface.

4.4 Oxygen (oxygen-time model)

Another environmental factor influencing germination in the soil is the availability of oxygen (Corbineau and Côme, 1995). As the diffusion of oxygen through water is much slower than through air, seeds of some semiaquatic species, such as rice, are adapted to germinate at quite low oxygen concentrations (hypoxia), but germination of most seeds begins to be inhibited when oxygen percentage in the gaseous phase falls below 10% (Yentur and Leopold, 1976). Thus, saturation of soils by flooding can prevent germination due to restrictions of oxygen availability. Oxygen consumption rates of seeds are closely related to their germination rates and are affected by T and ψ in similar ways (Dahal et al., 1996; Bello and Bradford, 2016). It is therefore unsurprising that seed germination responses to oxygen are also described by the PBT model (Bradford et al., 2007). In this case, germination varies with the logarithm of the oxygen percentage in the gaseous phase according to the following equation:

$$\theta_{Ox} = \left(\log Ox - \log Ox_b(g)\right) t_g, \tag{17}$$

where θ_{Ox} is the oxygen–time constant [log (O_2%) d or log (O_2%) h], Ox is the percentage of O_2, and $Ox_b(g)$ is the base oxygen percentage that prevents germination of fraction g of the population. $Ox_b(50)$ values for different species varied from less than 1% to over 9%, with rice having the lowest value at 0.02%, or anaerobic. After-ripening of dormant barley grains lowered their median oxygen threshold from 36% (only 30% germination in ambient 21% O_2) to 0.3% by both increasing the permeability of the hull surrounding the grain and lowering the O_2 threshold of the embryo (Bradford et al., 2008). As hypoxia had been shown to increase the sensitivity of dormant grains to inhibition of germination by abscisic acid (ABA) (Benech-Arnold et al., 2006), PBT analyses of ABA and gibberellin (GA) effects on germination (see Section 5.5) found that sensitivity to these hormones also was influenced by oxygen availability (Bradford et al., 2008). The ability to quantify the effects of these factors and their interactions on germination revealed the complex relationships among internal (ABA and GA) and external (hull O_2 permeability) changes associated with after-ripening and dormancy loss (see also Section 5.1).

4.5 Priming and seed enhancement (hydrothermal priming time model)

Observations that seeds that had been hydrated to levels not allowing completion of germination could be dried without damage and subsequently

would germinate more quickly when rehydrated led to the development of the practice of seed 'priming' (Heydecker et al., 1973; Hegarty, 1978). Seeds can be hydrated, incubated and dehydrated in a controlled manner, then distributed and planted in the normal way. Upon rehydration, these primed seeds will germinate more quickly and uniformly, resulting in improved stand establishment. Different approaches have been developed to control and limit seed water absorption during the priming treatment, using osmotica (osmopriming), clay particles (solid matrix priming) or simply limiting the amount of water provided (hydropriming) (Halmer, 2000; Halmer, 2004). Priming has also been used to pretreat seeds to break dormancy (Valdes and Bradford, 1987) or inoculate them with biological agents (biopriming) (Lutts et al., 2016). Seed priming has become a standard commercial practice in a number of higher value crops, including lettuce, tomato, pepper and celery.

A limitation for seed priming in practice is that the specific conditions for optimal treatment, primarily the T, ψ and duration of the treatments, vary among species and even seed lots within a species or variety. Thus, a range of trial treatments must be conducted empirically to identify the optimal conditions prior to treating a large commercial lot. The risk of failure is considerable, as if the ψ is too high and/or the duration is too long at the selected T, some fraction of the seeds can complete germination during the treatment and will be damaged upon dehydration. As priming is used primarily on high-value vegetable seeds, such mistakes can be quite costly. Thus, a method to consistently pretest seed lots to determine their sensitivity characteristics and their responses to test conditions is valuable.

The germination kinetics of primed seeds can also be characterized by HTT models (Dahal and Bradford, 1990; Dahal et al., 1990; Bradford and Somasco, 1994; Cheng and Bradford, 1999; Patanè et al., 2016; Tatari et al., 2020). The improvements in germination rates and uniformity are primarily due to smaller values for θ_{HT} rather than to the lowering of $\psi_b(50)$. Effectively, the hydration period at reduced ψ contributes to the total HTT required for germination, so the time required after rehydration is reduced. As a lower θ_{HT} requirement shortens the time to germination for all fractions of the seed population, the variation in time of germination becomes smaller, or germination is more synchronous. Initial experiments indicated that up to a point, increasing durations of priming increased the subsequent GR_{50} (Tarquis and Bradford, 1992). In this case, since priming occurs at ψ levels below the lowest ψ_b values for the seed population (to prevent germination), but metabolic advancement can still occur, a new threshold term is required (ψ_{min}), which is the lowest ψ at which the priming effect will occur; that is, incubation at lower ψ values does not result in the advancement of GR. The same is the case for temperature, with priming time accumulating above a minimum threshold, or T_{min}. Thus, the

GR_{50} of the seed population after priming can be described on the basis of hydrothermal priming time as follows:

$$GR_{50} = GR_i + \left[\left(\psi - \psi_{min}\right)\left(T - T_{min}\right)t_p\right] / \theta_{HTP}, \quad (18)$$

where GR_i is the initial GR_{50} of the seed lot before priming (h^{-1} or d^{-1}), t_p is the duration of priming (h or d) and θ_{HTP} is a hydrothermal priming time (HTP) constant (MPa °C h or MPa °C d) (Bradford and Haigh, 1994). In practice, a matrix of test priming treatments at two or three T and ψ values and durations are conducted, the seeds are then dried, and the GR_{50} values for each test condition are determined in germination tests. With these data, regression methods can be used to estimate the effective values of ψ_{min}, T_{min} and θ_{HTP} for the seed lot. There can be complications in some cases. For example, some seeds can shift their ψ_b distribution to more negative values during extended incubation at reduced water potentials, potentially resulting in unexpected germination during the treatment (Dahal and Bradford, 1994). Some seeds are also induced into secondary dormancy by extended incubation at reduced water potentials (Gianinetti and Cohn, 2007; Hawkins et al., 2017; Malavert et al., 2021b).

While fitting PBT models requires additional preliminary pretests for each seed lot, an advantage of doing these in a systematic way and calculating the HTP parameters is that similar seed performance will be obtained from different combinations of T, ψ and t_p conditions if the total hydrothermal priming time, or $\theta_{HTP} = (\psi - \psi_{min})(T - T_{min})t_p$, is the same. That is, T, ψ and t_p are interchangeable as long as the total θ_{HTP} is the same. This allows optimization of materials, equipment and time for conducting priming treatments on a large scale. For example, adjusting ψ may require an osmoticant that is expensive or results in waste for disposal, so priming at higher ψ may be more economical. Similarly, raising T may shorten the actual priming time required and increase product throughput. As long as pregermination is prevented by keeping the ψ below the lowest ψ_b in the seed population, or keeping t_p shorter than the time needed for any seeds to complete germination under the chosen conditions, modification of the parameters to achieve a desired HTP time should increase GR by the predicted amount, and improved uniformity automatically accompanies increased GR. Thus, conducting the pretest systematically and using the results to calculate the HTP parameters would enable adjustment of the key parameters of the priming treatment to achieve optimal results for each seed lot with the greatest efficiency and least risk. Experience with this approach could perhaps identify consistent HTP parameters for a given species or variety of interest, allowing reduction in the number of pretest conditions required to characterize a specific lot, but as seed germination characteristics can change due to development, harvest and storage conditions, it is likely that such pretests will continue to be a component of successful priming operations.

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5 Population-based models for physiological and other factors affecting seed behavior: dormancy, aging, seed vigor, respiration and other characteristics

5.1 Dormancy (hydrothermal time and temperature limits models)

A reduction or elimination of seed dormancy is one of the early hallmarks of the domestication of crop species. A plethora of dormancy mechanisms have been described that enable species to be successful and reproduce in their native environments (Baskin and Baskin, 2014; Willis et al., 2014). Once humans began to harvest seeds and purposefully replant them, dormancy was strongly selected against, as seeds that failed to germinate did not contribute progeny to the subsequent harvest, and it has been completely eliminated in some of our most important agricultural species. In wheat, for example, research on seed dormancy is now focused primarily on how to restore sufficient resistance to germination after development to prevent precocious sprouting in the event of untimely rain before harvest (Schramm et al., 2010; Nonogaki and Nonogaki, 2017). However, this domestication process is still in progress for many crop species, particularly those in which the seed is not the primary food product, such as the biennial vegetables. Many of these can exhibit seed dormancy and can require specific conditions to alleviate it, such as dry after-ripening, imbibed chilling or light. This may be as simple as holding seeds in a warehouse until they have after-ripened sufficiently, as for sunflowers or melons, or may involve priming treatments, such as those used to prevent the expression of thermoinhibition in lettuce seeds. It is evident that the presence of dormant seeds in a seed lot will reduce the percentage of usable transplants or affect the intended plant populations in direct-seeded crops.

Seed dormancy is a critical factor in ecological applications, where undomesticated species are being seeded into natural conditions. Native species are often exquisitely attuned to their environments, moving into and out of dormancy while in a seed bank that constitutes the future of the species (Donohue et al., 2010; Rubio de Casas et al., 2015; Finch-Savage and Footitt, 2017; ten Brink et al., 2020; Gianella et al., 2021). Successful reintroduction of species requires not only seeds from genotypes adapted to the environment of the location but also the timing of seed distribution to match seeds' expectations or requirements (Baskin and Baskin, 2020). In many cases, very low success rates are observed in such restoration efforts, while conversely, seed dormancy characteristics may play a critical role in the competitive advantages of invasive species (Meyer and Allen, 2009; Commander et al., 2020; Gioria et al., 2021).

A functional definition of dormancy is when a viable seed fails to germinate when provided conditions under which a nondormant seed of the same

species would be able to complete germination. This self-referential definition is not entirely satisfactory, but it is the case that seed dormancy is generally a transient developmental state that eventually disappears to allow the seed to germinate. Between a fully dormant state in which a seed will not germinate under any conditions and a fully nondormant state in which the seed will germinate across a wide range of conditions, there are many intermediate states in which germination is conditional on the environment being within certain ranges (e.g., T or ψ) or providing certain signals (e.g., light). PBT models can therefore be useful for quantifying the status of a seed population in terms of the environmental thresholds permitting germination at a given time, and how these thresholds change in response to dormancy-breaking conditions.

The population-based HTT models have been effectively employed for both physiological and ecological analyses of seed dormancy. With respect to physiological aspects of dormancy, both the water potential and the thermal components can contribute. An early demonstration of this showed that after-ripening and dormancy loss in *Bromus tectorum* L. seeds were associated with decreases in the mean ψ_b of the seed population (Christensen et al., 1996; Bair et al., 2006), which resulted in more rapid germination and higher final percentages. Subsequent work confirmed that the loss of dormancy in seeds was associated with the progressive shift of the $\psi_b(g)$ distribution to more negative values, which enables more seeds to germinate and to do so more quickly (Alvarado and Bradford, 2005; Meyer and Allen, 2009). Manipulation of hormonal regulators of dormancy, such as ABA or fluridone (an ABA biosynthesis inhibitor), had the expected corresponding effects on the $\psi_b(g)$ threshold distribution. This is essentially the reverse of the upward shift in $\psi_b(g)$ when the temperature exceeds T_o (Fig. 6c); instead, as after-ripening (or other dormancy-breaking treatment) continues, $\psi_b(g)$ moves progressively more negative, allowing more seeds to germinate and speeding germination. Germination time courses as seed populations leave dormancy resemble those shown in Fig. 5b, but going in the direction from lower to higher ψ. As the $\psi_b(g)$ distribution shifts to more negative values, seeds with ψ_b thresholds more negative than the current ψ will start to germinate, with the time to germination inversely proportional to the difference between the soil ψ and the seed's ψ_b threshold. This gradually makes more seeds germinable over time, while still retaining sensitivity to current hydration conditions.

In addition to this mechanism based on shifts in $\psi_b(g)$, dormancy is also controlled by shifting of the temperature thresholds for germination (Batlla and Benech-Arnold, 2015). The cardinal temperatures discussed earlier (Fig. 4) assume that the seeds tested lack dormancy and that the T_b, T_o and $T_c(g)$ values are reasonably constant within a species. However, seeds of many species will not germinate across this entire temperature range when they are dormant. Instead, these cardinal temperatures represent end-point values that can

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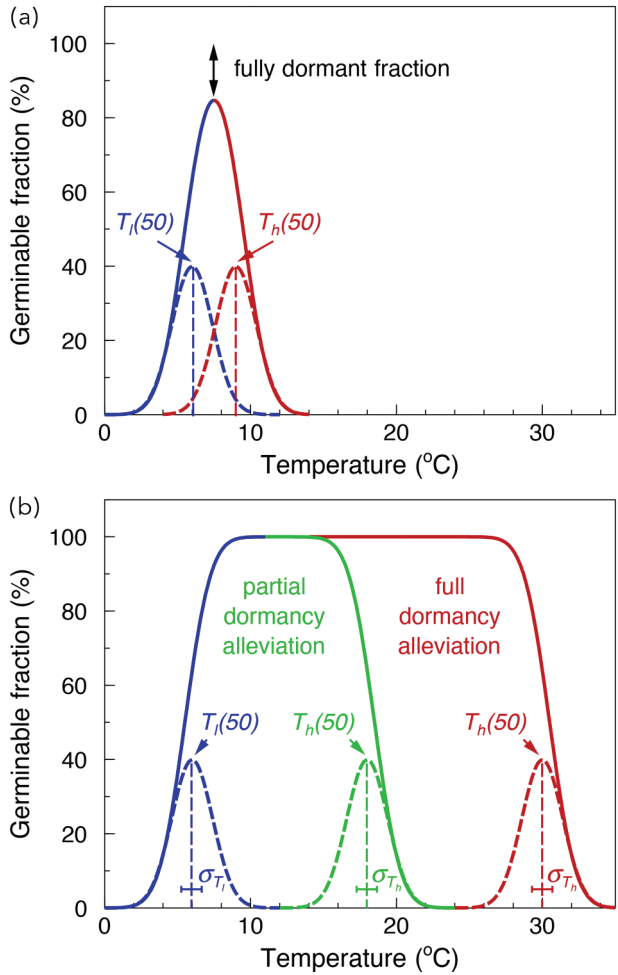

Figure 8 Dormancy alleviation by shifting of upper-temperature thresholds in a winter annual species. (a) A seed lot exhibiting dormancy due to a low high-temperature limit (T_h) for germination such that its distribution overlaps that of the lower temperature limit (T_l). This results in a narrow temperature range in which some seeds are above their T_l but still below their T_h, but a fraction of seeds are unable to germinate at any temperature. $T_l(50)$ and $T_h(50)$ indicate the medians of the lower and higher temperature limit distributions. (b) After being exposed to conditions that alleviate dormancy (e.g., chilling or after-ripening), the T_h distribution shifts to higher temperatures (green) and eventually to near the maximum temperature for germination (red curve, i.e., $T_c(g)$ for the species) when dormancy is fully alleviated. The opposite occurs in summer annual species, with inhibition of germination occurring initially at lower temperatures, and the T_l distribution shifts to lower temperatures in response to dormancy-alleviating conditions. Adapted from Batlla and Benech-Arnold (2015).

be approached as dormancy is lost. Dormant seeds may exhibit lower-limit (T_l) or higher-limit (T_h) temperatures that are considerably higher or lower, respectively, than T_b or T_c and that vary among seeds in the population (Fig. 8) (Batlla and Benech-Arnold, 2015). These T_l and T_h thresholds can shift seasonally in seeds in the soil seed bank and in response to various dormancy-breaking or dormancy-inducing conditions. In general, dormancy-breaking conditions tend to expand the temperature range between T_l and T_h, which move toward T_b or $T_c(g)$, respectively, in nondormant seeds (Fig. 8b). In contrast, conditions that induce dormancy in these species narrow the range of temperatures at which germination is permitted and can merge together and prevent germination at any temperature (Fig. 8a), as in the induction of secondary dormancy (Malavert et al., 2017). This approach has been applied successfully to understand the emergence differences between summer annual and winter annual weeds and their responses to additional environmental inputs (Fernández Farnocchia et al., 2019; Fernández Farnocchia et al., 2021; Malavert et al., 2021a).

PBT models have also been applied more generally in plant development and ecology (Donohue et al., 2015). The threshold component often is described in terms analogous to the thermal time model, in which the accumulation of developmental time above a threshold (such as degree-days) enables the organism to transition to the next developmental phase of its life cycle. This sense of the word 'threshold' evokes a doorway leading to the next developmental phase after accumulating sufficient developmental time in the prior phase. For plants, this developmental cycle is often summarized as seed, vegetative growth, flowering and seed/fruit development. However, this cycle omits another important checkpoint, which is the transition of a seed from dormant to germinable. As noted above, except for highly domesticated crops, seeds are generally dormant when shed and lose dormancy in response to environmental or physiological conditions that are associated with successful seedling survival in a given climate. Thus, it has been proposed that the transition of seeds from dormant to germinable is a legitimate developmental transition for plants, akin to vernalization that enables the vegetative to flowering transition (Huo et al., 2016). It is clearly the case that the initiation of germination is a singular once-in-a-lifetime event for an individual seed and that it triggers a massive cascade of gene expression and growth (Cadman et al., 2006; Finch-Savage et al., 2007). There are important follow-on consequences, as earlier germinators tend to have better access to light, while later emerging seedlings are likely to be shaded by neighbors and stunted. Environmental and seasonal germination niches have been identified for diverse species, which interact with dormancy thresholds to restrict germination to more favorable seasons or to take advantage of highly favorable conditions (Larson and Funk, 2016; Saatkamp et al., 2019). As temperature and water availability are the two

most critical environmental factors for seedling success, it is no surprise that hydrothermal parameters characterizing seed dormancy loss and germination (e.g., as in Fig. 7) were highly correlated with species demographics in the field over decades in a desert plant community (Huang et al., 2016; Liu et al., 2020). Seed germination responses to light are also amenable to analysis by PBT models (Batlla and Benech-Arnold, 2005; Bradford, 2005; Malavert et al., 2021a). Similar approaches could be extended to apply to chilling treatments to break dormancy in both seeds and tree buds (Penfield and Springthorpe, 2012; Tarancón et al., 2017; Martínez-Berdeja et al., 2020), vernalization to promote flowering (Angel et al., 2015) and other aspects of plant growth and development (Bradford, 2018).

5.2 Aging and seed vigor (aging time model)

Another application of PBT models is to quantify seed aging and loss of vigor following harvest and during storage. The Ellis–Roberts seed viability equation is fundamental to modeling the deaths of increasing fractions of a seed lot over time when stored under specific conditions of T and ψ (Ellis and Roberts, 1981; Roberts and Ellis, 1989). Its population basis is integrated into the model, which is as follows:

$$v = K_i - p / \sigma, \tag{19}$$

where v is the probit of percent viability after storage period p, K_i is the initial viability (on a probit scale; see Section 6.1) and σ is the standard deviation of the normal distribution of seed deaths over time (days or years). As for germination, the most critical factors affecting seed aging rates are temperature and water content (Ellis and Roberts, 1981). These factors are accounted for by adjusting the value of σ depending upon the seed moisture content (m, fresh weight basis) and temperature (T) during the storage period according to the following equation:

$$\log\sigma = K_E - C_W \log m - C_H T - C_Q T^2, \tag{20}$$

where K_E is a species constant that accounts for the differences in inherent storability among species and C_W, C_H and C_Q are constants determined empirically in storage trials across a range of m and T conditions. It is important to note the logarithmic effect of m and the quadratic effect of T and that increases in either of these reduce storage life. These nonlinear effects of m and T on seed longevity are consistent with the earlier work of Harrington, whose 'thumb rules' for seed storage posited that every 1% increase in m or 10°F (6°C) increase in T reduced storage life by half, with compounding additive effects (Harrington, 1972).

It is common in the seed trade to measure seed moisture content (m) as a percentage of the initial fresh weight, while researchers generally express it as a percentage of dry weight. On either scale, the seed moisture content at a given ψ varies among species, primarily due to differences in seed oil content (Cromarty et al., 1982). Thus, Roberts and Ellis (1989) modified their longevity equation to be based on equilibrium relative humidity (*eRH*, %) rather than moisture content:

$$\log\sigma = K_E - C_W eRH - C_H T - C_Q T^2, \quad (21)$$

where the C_W constant has its meaning as earlier but with different values. This modification has not been widely adopted for the longevity equation, but there are considerable advantages to measuring seed water content as its equivalent *eRH* (see http://www.dryingbeads.org/tools for a conversion tool). While optimal seed moisture contents have to be adjusted among species, their storage characteristics are more similar on an *eRH* basis. That is, while a starchy seed (e.g., maize) might have a moisture content of 12% (fresh weight basis) at 50% eRH, an oily seed (e.g., sunflower) at the same *eRH* would have a moisture content of only 8%. However, their aging behaviors would be much more similar at the same *eRH* compared to being at the same moisture content (Bradford et al., 2018). Thus, it is standard practice in seed banks to pre-equilibrate seed lots at low RH (15%) prior to storage (see Section 7.3). In addition, while measuring seed moisture by heating in an oven requires at least several hours, electronic water potential meters can measure water activity (=*eRH*/100) in less than a minute (e.g., https://www.metergroup.com/), allowing real-time monitoring of seed moisture content during packaging, for example. Even simpler and cheaper are humidity indicator papers that can be used without any instrumentation to measure *eRH* of a seed batch for estimating harvest maturity or checking storage conditions (Bradford et al., 2016; Thompson et al., 2017).

The general pattern of seed aging begins with an initial plateau period of variable length during which the final viability remains essentially constant but the time to germination increases steadily (Fig. 9a and b). Eventually, the viability declines in a sigmoidal pattern and times to germination increase more rapidly (Fig. 9c). This pattern indicates that all seeds do not age at the same rate or die at the same time. Rather, seeds in a seed lot have a range of potential lifetimes that match well to a normal distribution, as demonstrated by the importance of σ in the Ellis–Roberts equation (Eqns. 20 and 21). However, as valuable as it is, the viability equation only predicts the fraction of seeds that are capable of germinating after a given storage period and does not address directly the variation among seed lots in storage times before viability begins to decline (see, e.g., Walters et al., 2005; Fleming et al., 2019).

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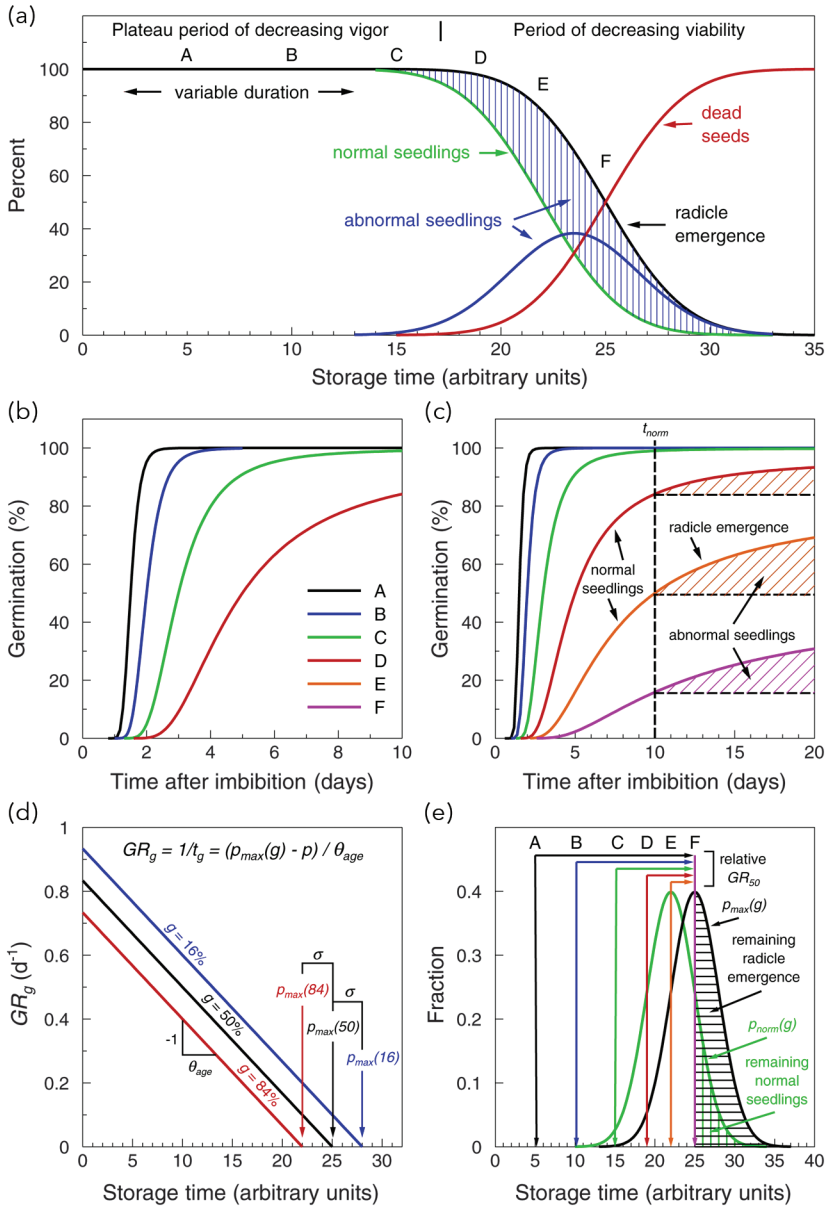

Figure 9 Threshold model for the effects of storage time on seed vigor and viability. (a) During seed storage, there is often an initial plateau period of variable length in which viability remains constant but vigor decreases followed by a period of relatively rapid loss of viability. The ability to produce normal or viable seedlings is lost first (green curve), followed by the loss of ability to even achieve radicle emergence or germination *sensu stricto* (black curve). The difference between the two curves indicates the percentage of

In addition, it does not account for the gradual decrease in *GR* during the initial plateau phase (Fig. 9d), which is a key indicator of the decline in seed vigor prior to the loss of viability (Bradford et al., 1993). As should be apparent by now, the timing of germination is a critical feature of seeds with respect to their success, whether in a crop field or in nature. Thus, we can ask whether the principles of PBT behavior, as revealed in seed germination rates, as well as percentages, can be used to quantify seed aging and potentially predict seed storage life.

In most cases, germination tests of stored seeds only record final viability. Thus, Walters and coauthors (Fleming et al., 2019; Walters et al., 2020) stated, 'Ageing is "asymptomatic" before P80 [80% viability] is reached, meaning that changes in germination do not occur or are difficult to reliably detect'. While this is the case for viability, germination is delayed with increasing storage time prior to the loss of viability, exhibiting a pattern strikingly similar to that of seeds exposed to reduced ψ (or whose $\psi_b(g)$ values are increasing) (Fig. 9b; compare

Figure 9 (*Continued*)

abnormal seedlings (i.e., seeds that produced a radicle but not a viable seedling) that can be expected during this period (blue hatched area and frequency curve). Seeds unable to protrude a radicle are considered to be dead and their fraction increases over time (red curve). (b) Germination time courses predicted by the aging time PBT model at the storage times indicated (A–D) during the plateau phase in panel (a). As the storage period approaches the radicle emergence distribution, the time to germination increases proportionately to each seed's $p_{max}(g)$ value (Eqn. 22). (c) The germination time courses are shown on an extended time scale to illustrate germination patterns as storage time increases into the viability loss phase of aging. The vertical dashed line indicates the time predicted by the model after which no additional normal seedlings will be produced under the storage conditions (t_{norm}; see Eqn. 23). That is, the percentage of normal seedlings will decrease with increasing storage times (A–F in panel a), although radicle emergence may continue longer, with the production of abnormal seedlings (hatched regions). (d) Germination rates ($GR_g = 1/t_g$) associated with predicted time courses decline linearly with increasing storage time with a negative slope equal to θ_{age} (with a value of 30 storage units, days in this example). Lines are shown for g = 16%, 50% and 84%, or the mean ($p_{max}(50)$) and one standard deviation ($\sigma_{p_{max}}$) above and below it. Where these lines intersect the x-axis (i.e., $GR_g = 0$) represents the maximum lifetime under the storage conditions for radicle emergence of fraction g. (e) Relationships of normal seedling and radicle emergence threshold distributions to germination patterns. The black curve indicates the $p_{max}(g)$ distribution of the seed population for all examples in this figure ($p_{max}(50)$ = 25 storage units, $\sigma_{p_{max}}$ = 3 units). The green curve indicates the $p_{norm}(g)$ distribution for the production of normal seedlings, which in this example occurs one standard deviation earlier in the storage period than the radicle emergence distribution. As the storage time (p) increases from A to F (vertical arrows), the difference between p and $p_{max}(g)$ decreases and t_g increases proportionately (Eqn. 22), resulting in the decreasing GR_g values shown in panel (d). At storage time F (= $p_{max}(50)$), only half of the seeds would be able to complete radicle emergence (eventually), and only 16% would be able to produce normal seedlings (c.f., panel c), as indicated by the hatched areas under the two distributions. Adapted from Bradford et al. (1993).

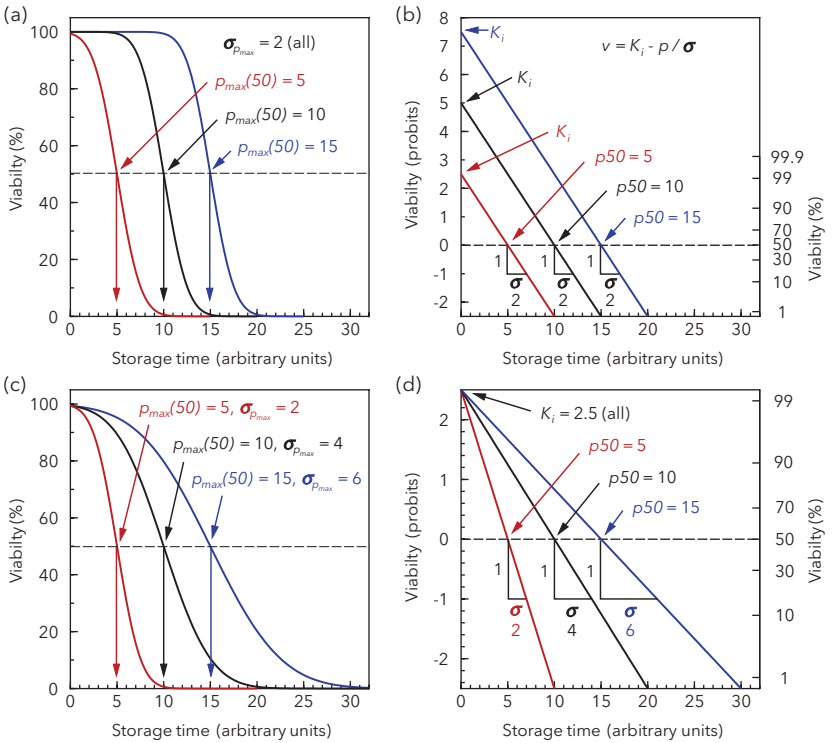

Figure 10 Comparison of the aging time model and the Ellis–Roberts longevity model parameters for the viability loss period of seed aging under different conditions. (a) For cases in which there is a plateau period prior to the beginning of viability loss (as in Fig. 9a), three examples are shown having different $p_{max}(50)$ values but the same $\sigma_{P_{max}}$ values. This could be the case for seed lots of a given species differing in initial quality but stored under the same storage conditions. Seeds with higher initial quality would have a longer plateau period (blue curve) than seeds with a lower initial quality (red curve). (b) The same viability loss time courses as in (a) analyzed by the Ellis–Roberts method, using only data after viability loss had begun. The data are plotted on a probit scale (left *y*-axis), which uses standard deviation units and transforms sigmoidal distributions into straight lines; the corresponding percentage values are shown on the right *y*-axis. The Ellis–Roberts *p50* values correspond to the $p_{max}(50)$ values in the aging model and the σ values are all equal, as the storage conditions are the same (Eqn. 20). Higher K_i values indicate the relative differences in initial seed quality among the lots (i.e., different lengths of their plateau phases), although the actual percentage values are not relevant, as the probit scale continues to approach 100% indefinitely. (c) Examples of the effect of different storage conditions on viability loss of subsamples of the same seed lot, in this case without a plateau period. Both the $p_{max}(50)$ and $\sigma_{P_{max}}$ values would increase under better storage conditions (lower *T* or *eRH*; blue curve) compared to poor storage conditions (higher *T* or *eRH*; red curve). (d) Ellis–Roberts analysis of the data in panel (c) indicates a common K_i value (it is the same seed lot in this case), which is equal to the initial viability percentage. The slopes of the lines increase (viability loss is more rapid) as the storage conditions become less favorable for seed longevity. In addition to the same information about the viability loss distribution as the Ellis–Roberts model, the aging time model also provides information on the germination kinetics during both the plateau and viability loss phases (Fig. 9b and c).

to Fig. 5b and d). Observing these similarities, Bradford et al. (1993) proposed the following threshold model of seed aging:

$$\theta_{age} = \left(p_{max}\left(g\right) - p\right)t_g, \tag{22}$$

where θ_{age} is an aging time constant (units of storage time and germination time, e.g., year days or year hours), $p_{max}(g)$ is the normal distribution of maximum lifetimes under the conditions of storage (days or years), p is the period or duration of aging (days or years) and t_g is the time to germination of fraction g under a given standard condition (hours or days). [Note: Equation 22 is modified from the original published version to avoid negative θ_{age} values, as $p_{max}(g)$ is always larger than p. Equation 22 is in the 'inhibitor' form of the model (Fig. 2b), as increasing p delays or prevents germination.] This model reproduces well the actual germination time courses of seeds aged for increasing periods of time (Fig. 9b and c), in which GR_{50} values decrease linearly with aging period (Fig. 9d; Bradford et al., 1993). The $p_{max}(g)$ values estimated by the model represent the storage periods under the given conditions required for fraction g of the seeds to lose viability (i.e., to be unable to achieve radicle emergence) (Fig. 9e). It can thus be used to characterize and compare the potential storage lives of seed lots, while $\sigma_{p_{max}}$ (the standard deviation of $p_{max}(g)$) indicates the spread in time over which seeds will become abnormal or die (Fig. 9a, c and e). We have informally termed this approach to quantify seed aging as the 'metronome' rule. It suggests that at maturity and harvest, a given seed has a fixed p_{max} period before death that is dependent upon the conditions during its maturation and in which it is stored subsequently. The seed can therefore be considered to have a fixed number of clicks of a metronome in its lifespan; however, like a metronome, those clicks can occur very slowly or very rapidly in time, depending upon the storage conditions (primarily *T* and *eRH*). Thus, the goal of the seed conservator is to enable the seed to play out its lifetime at an *adagio* (low *T* and *eRH*) rather than a *presto* (high *T* and *eRH*) tempo.

As the Ellis–Roberts model has been widely used and verified for a large number of species (https://data.kew.org/sid/viability/), it is relevant to compare it to the aging time model. With respect to the period of viability loss, the aging time model can identify the time until 50% viability loss ($p_{max}(50)$) and the standard deviation around it ($\sigma_{p_{max}}$; Fig. 10a and c). The Ellis–Roberts model uses probit analysis to identify the K_i (initial viability percentage on a probit scale), *p50* (the time to loss of 50% viability) and the slope of the probit viability loss line (the inverse of σ) (Fig. 10b and d). These values, except for K_i, are the same in both models. The K_i values initially assumed that they are the intercept of the probit lines with the *y*-axis, or initial viability, which works well when there is no plateau period of storage (Fig. 10d). However, when there is a plateau period of constant viability, only the data from the viability loss period should be used

in the Ellis–Roberts analysis, and the K_i values for seed populations exhibiting a plateau phase will be unreasonably high when converted to percentages (Fig. 10b). This is not particularly a problem, as the differences in K_i values still indirectly indicate the relative differences in initial plateau periods among the seed lots. Thus, with respect to identifying useful parameters applicable to characterize the viability loss period of seed aging, both models provide similar information. However, the aging time model also characterizes the changes in germination time courses throughout the aging period, providing an aging phenotype for the period when viability alone is uninformative.

The aging time model also reveals other interesting features. For example, the parameters of this equation during the plateau period of the viability time course shifted to different values once viability started to decline (Bradford et al., 1993). That is, θ_{age} and $p_{max}(50)$ had larger values during the plateau period when viability was constant and had smaller values (more rapid loss with time) once viability began to decline. This is consistent with observations that once viability begins to decline, improving the storage conditions by lowering T is less effective than it is during the plateau period (Walters et al., 2004). The transition from the plateau to the viability loss period seems to be accompanied by increases in the rate of damage accumulation and/or decreases in the ability to repair such damage upon hydration, denoted by the change in aging time parameters. While it has been standard to use 50% viability ($p50$) as the germination percentage for characterizing longevity, it may in fact be more appropriate to use a higher value nearer to this transition point. In seed conservation, a primary objective is to preserve genetic diversity, so regeneration of accessions is recommended before viability falls to 85% of the initial value (De Vitis et al., 2020; Solberg et al., 2020). In the case of commercial vegetable seeds, even a fall in germination percentage below 95% can be too much for competitive markets. With either the viability equations or the aging time model, the time to any viability percentage g can be estimated, as the distribution in time is related to $p_{max}(50)$ and $\sigma_{p_{max}}$ or to $p50$ and σ (Fig. 9a and e and 10). When σ is large, the time for viability to fall from 85% to 50% can be many years under optimal seed bank storage conditions. It may therefore be more appropriate to standardize longevity predictions at the threshold percentage for regeneration of accessions rather than the median of the population (Nagel and Börner, 2010). As it is the identification of when this phase of rapid deterioration and loss of viability begins to occur that is critical for both germplasm and agricultural seed storage, periodic measurements of germination rates under the storage conditions being used and fitting of the PBT aging model to the data could be used to predict approximate storage times when the rapid loss of viability would begin to occur on a lot by lot basis. This predictive ability for a given seed lot is not present in the Ellis–Roberts approach, which can only be determined retroactively after a significant fraction

of viability has been lost, and the potential duration of the plateau period cannot be predicted. On the other hand, change in the parameters of the aging time model once viability begins to be lost suggests that its predictions during the plateau phase will somewhat overestimate the actual $p_{max}(50)$ and $\sigma_{p_{max}}$ values; that is, the rate of viability loss once it begins may be quicker than estimated from the decline in *GR* during the plateau phase. This again recommends putting emphasis on estimating the storage period at which viability begins to decline rather than when it has reached 50%.

The change in seed aging kinetics after an initial plateau period of storage suggests that during the plateau period, physiological or molecular damage (oxidation) is occurring but is largely repairable after imbibition (Zinsmeister et al., 2020). The delay in radicle emergence likely represents the additional time required for such repair before germination *per se* begins (Matthews and Khajeh-Hosseini, 2007). For example, molecular mechanisms assess DNA damage upon seed imbibition, and germination is delayed until such damage has been repaired (Waterworth et al., 2016). At some duration of aging, the accumulated damage is too great to repair fully upon imbibition, and seeds either produce abnormal seedlings or do not germinate (Fig. 9). Aged seeds that do produce normal seedlings, however, showed no reductions in root growth rates after radicle emergence (Tarquis and Bradford, 1992), indicating that the delay in germination allows repair to occur and that there are no persistent consequences for seedling growth. However, in a standard seedling growth vigor test, all seeds are planted at the same time and harvested at the same time a number of days later. In this case, the delayed germination will translate into reduced seedling size, not because the seedlings grow more slowly or are permanently handicapped but simply because they had a shorter time to grow after emergence due to their delayed germination. In fact, such root or seedling growth assays are actually indirect measures of the timing and variation in germination times. As discussed previously, delayed germination itself can result in reduced yield or management issues, but this is primarily due to the slower emergence rather than to the persistent effects of aging *per se* on the subsequent growth potential of the individual plant.

Another useful feature of the PBT aging model is that it allows the prediction of when abnormal seedlings will begin to appear in a germination time course. It is generally the case that the curves for loss of capacity to produce normal seedlings and loss of radicle emergence ability are parallel, and the difference between them represents the percentage of abnormal seedlings that will be observed after that aging time (Fig. 9a; Bradford et al., 1993). For species that produce more abnormal seedlings (e.g., soybean), this difference is large, while for other species (e.g., lettuce), relatively few abnormal seedlings are observed, and the difference in storage time between these curves is comparatively small. Once the aging properties of a seed lot have been determined using the aging

time model, it is possible to calculate the time after which no additional normal seedlings are expected to appear (Fig. 9b and c) (Bradford et al., 1993),

$$t_{norm} < \theta_{age} / \left(p_{max}(g) - p_{norm}(g)\right) \quad (23)$$

where t_{norm} is the imbibition time after which normal seedlings will not appear (Fig. 9c) and $p_{norm}(g)$ is the distribution of aging times at which fraction g of normal seedlings occurs (green curves in Fig. 9a and e). This makes sense, as the normal distribution of seed deaths in time also represents the relative quality of seeds in the lot, with the higher quality seeds (having longer p_{max} values, lower ψ_b values, etc.) germinating earlier than the poorer quality seeds. Thus, extending the aging time model into seed testing could potentially reduce reliance on subjective scoring of seed abnormalities and instead use germination rates (i.e., percentage of seeds germinated within a specific time period) to rank seed lots for their vigor. This is essentially the same as the early count in seed testing (Ilbi et al., 2020), but the timing of such observations could be chosen based on the aging time model to approximate the actual normal seed percentage that would result from extending the test period and subjectively evaluating all of the seeds for normality. It is, of course, possible that some abnormal seedlings could appear prior to t_{norm}, which would likely be due to physical damage or some cause other than aging *per se*.

The similar patterns of germination time courses in response to aging, T and ψ (Figs. 1, 5 and 9) suggest that these could be combined into a 'hydrothermal aging time' model. Of course, the ψ values for optimal seed storage are in the range of −200 MPa (15% *eRH*), while few seeds of any species can germinate below −2 MPa (Walters et al., 2002; Bewley et al., 2013), so quite different mechanisms apply. However, we already understand fairly well how T and ψ (*eRH*) affect seed aging (Roberts and Ellis, 1989; Hay et al., 2003; Ellis and Hong, 2006; Ellis and Hong, 2007; De Vitis et al., 2020), so we can anticipate that conducting germination time course experiments after storage at multiple T and *eRH* conditions would enable incorporation of their effects on germination kinetics into the aging time model by adjusting $p_{max}(g)$ values as a function of T and *eRH*. Accelerated aging or controlled deterioration conditions (i.e., higher T and *eRH* than would be used for storage) have long been used to speed seed deterioration and assess potential longevity in much shorter times (Zhang and McDonald, 1997; Elias et al., 2012). However, the type of aging occurring at the very high eRH range (at or above equilibrium with saturated NaCl, or 75% *eRH*) is different from that occurring at lower values typical for seed storage (Schwember and Bradford, 2010). Controlled deterioration at more moderate ψ levels (e.g., in equilibrium with ~60% relative humidity) and elevated temperatures (up to 50°C) can result in aging time courses within a reasonable time (a month or so) that are likely to be transferrable to storage conditions using known relationships of aging rates to T and *eRH* (Walters et al.,

2020; Zinsmeister et al., 2020). That is, equations comparable to Equations 20 and 21 could be developed to adjust the aging model parameters (θ_{age}, $p_{max}(50)$ and $\sigma_{p_{max}}$) in response to the *T* and *eRH* conditions of the storage environment as in the Ellis–Roberts model. Further research is needed to confirm this, but the practical consequences for germplasm storage would be significant (see Section 7.3).

5.3 Seed respiration rates (all models)

While this chapter is more about modeling seed behavior than identifying the underlying causes, it is worth noting that the PBT models described here can also be applied to seed respiration rates. This requires measurements on a single-seed basis, as the models describe population behavior, not simply average behavior obtained from bulk seed measurements. However, equipment is available for measuring respiration (oxygen consumption) rates of individual seeds during imbibition and germination (Bradford et al., 2013). When this is done, the resulting time courses of oxygen consumption rates match very closely to those of germination across all of the factors described above, and the same PBT models can be applied to the respiration rate data (Bello and Bradford, 2016). This provides the opportunity for automating the collection of germination time course data, the major limitation to the application of PBT analyses.

These results also raise interesting questions for further study. While it is relatively straightforward to imagine how changes in *T* could affect metabolic rates through energy considerations, this is less clear for ψ effects at the quite high range at which effects on *GR* are evident. The close correspondence between germination rates and respiration rates in individual seeds suggests that seed metabolic rates are adjusted in response to environmental inputs and physiological states such as dormancy. For example, oxygen consumption rates of lettuce seeds imbibed at a high temperature that prevents germination (thermoinhibition) drop almost immediately to essentially zero (Ardura, 2017). It makes perfect sense that dormant seeds must conserve their resources even when hydrated, as in many climates, the soil seed bank will be hydrated essentially continuously, yet seeds can persevere in a dormant state for years and retain sufficient reserves for germination (Finch-Savage and Footitt, 2017). Despite early work on respiratory inhibitors and a possible role of the pentose phosphate pathway in dormancy (Roberts and Smith, 1977), we have relatively little understanding of metabolic rates in imbibed dormant seeds or how they are regulated, although some leads may be emerging from research on a metabolic 'power saving mode' in dormant plant vegetative buds (Martin-Fontecha et al., 2018). ABA is involved in dormancy in both buds and seeds, and the Sucrose-Non-Fermenting-1-Related Protein Kinase complex, which coordinates energy balance, anabolic versus catabolic metabolism and growth

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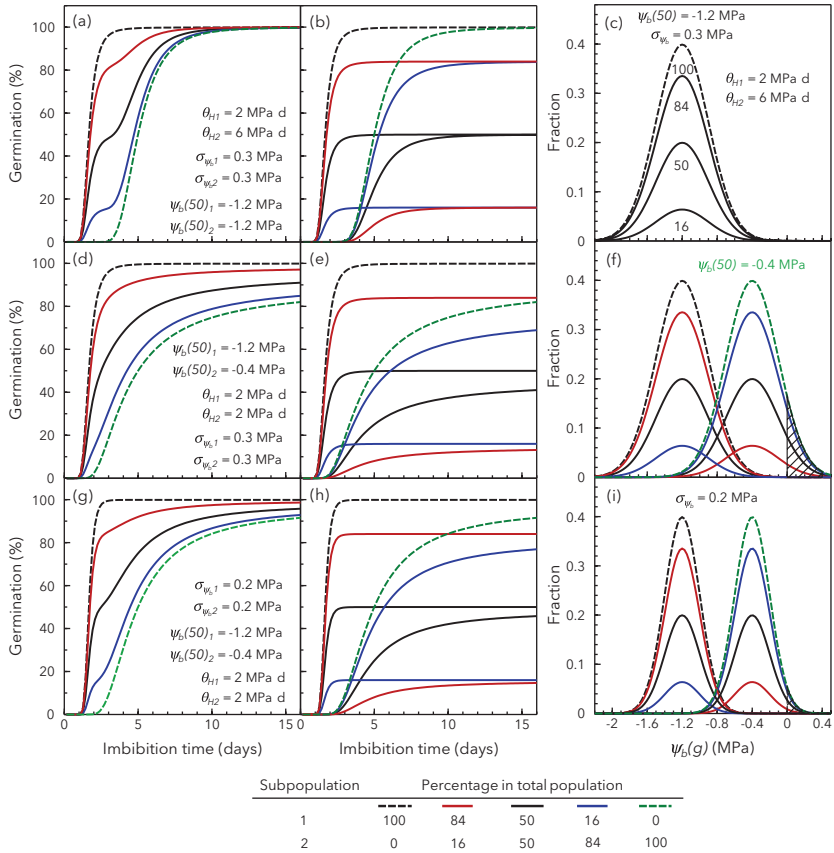

Figure 11 Illustration of the effects of subpopulations of seeds in a seed lot on germination time courses. Panels (a), (d) and (g) show the predicted germination time courses of the total populations, panels (b), (e) and (h) show the corresponding time courses of the component subpopulations composing the blended lots (same colors), and panels (c), (f) and (i) show the underlying $\psi_b(g)$ distributions of the two subpopulations. In panels (a) to (f), the dashed black line is for a single seed lot with the following hydrotime parameters: θ_H = 2 MPa d, $\psi_b(50)$ = −1.2 MPa and σ_{ψ_b} = 0.3 MPa (e.g., panel c), and all seeds were imbibed at 0 MPa. The solid curves represent seed lots in which 16% (red), 50% (black) or 84% (blue) of the seeds in population 1 are replaced by seeds from population 2 (a second seed lot; dashed green line). In (a) and (b), the two subpopulations have the same $\psi_b(g)$ distribution (c), but the θ_H value of subpopulation 2 seeds is increased to 6 MPa d. In (d), (e) and (f), the θ_H and σ_{ψ_b} values are the same in the two subpopulations, but the $\psi_b(50)$ value is changed to −0.4 MPa in subpopulation 2 (f). In (g), (h) and (i), an additional change of σ_{ψ_b} to 0.2 MPa is made in both populations (i). The hatched regions in (f) and (i) indicate that the $\psi_b(g)$ thresholds of seeds in this region are above 0 MPa, and thus that fraction of seeds would be unable to germinate on water. The examples in (a), (d) and (g) indicate the diversity of shapes of germination time courses that can be generated by changing the values in the hydrotime model and the fraction of seeds in each subpopulation. See Section 5.4 for further discussion.

in plants and many other organisms (Baena-González et al., 2007), is also involved in seed maturation, dormancy, germination and longevity (Yang et al., 1997; Rosnoblet et al., 2007; Bolingue et al., 2010).

5.4 Subpopulations (all models)

The discussion to this point has tacitly assumed that a seed population can be characterized by a single distribution of thresholds for a given factor. This is not always the case. For example, it is common in the seed industry to blend lots from different productions, which may have different germination characteristics, into a single lot. In the case of indeterminate plants, the early developing seeds can experience conditions that are quite different from those of the later maturing seeds (Donohue, 2009), and environmental conditions during seed development can have significant consequences for seed dormancy and quality (Auge et al., 2017; Penfield and MacGregor, 2017). Seeds of native species are often collected from diverse plants or fallen fruits or cones and may contain different subpopulations with distinct germination behaviors. This can result in apparent non-normal distributions of thresholds and less than optimal fits of the models described above, which has spurred studies to test other statistical models that might better fit these non-normal distributions and debates about which models should be used (see Section 6.3).

While there is no logical requirement that seed populations exhibit normal distributions of properties, in our experience, they most often do so, as illustrated by the wide applicability of the Ellis–Roberts longevity equation. When they do not, the simplest explanation is that it is due to the tested seed lot containing a mixture of different source populations. While these can be modeled by more complex functions and adjustment of additional empirical parameters, it makes more sense biologically to look for evidence of mixtures of subpopulations and test for their presence. For example, it is not uncommon in germination time course studies to observe what appear to be breaks in the time course pattern (e.g., Fig. 11a and g) or curves that are less sigmoidal (Fig. 11d). These will be evident only with closely spaced observations or, as in the case of single-seed respiration measurements or imaging, with data for each seed. It is very difficult to distinguish subpopulations with widely spaced observations during the germination time course, but if such subpopulations are known to be present, predictions based on them can fit such data better (Bello and Bradford, 2016). If we assume that there are two populations of seeds mixed together, we can fit two PBT models simultaneously, using the following general equation for a factor X:

$$\begin{aligned} D(g) = f_1\left[\left[X - \left(\theta_{X1} / t_{g1}\right) - X_b(50)_1\right] / \sigma_{X1}\right] \\ + \left(1 - f_1\right)\left[\left[X - \left(\theta_{X2} / t_{g2}\right) - X_b(50)_2\right] / \sigma_{X2}\right], \end{aligned} \tag{24}$$

where $D(g)$ is the sensitivity distribution of the total population, f_1 is the fraction of the total population in subpopulation 1, X is the level of factor X, θ_{X1} and θ_{X2} are the time constants for subpopulations 1 and 2, t_{g1} and t_{g2} are the germination times of individual seeds in subpopulations 1 and 2, $X_b(50)_1$ and $X_b(50)_2$ are the medians of the distributions of X_b values for subpopulations 1 and 2, and σ_{X1} and σ_{X2} are the standard deviations of X_b values among seeds in subpopulations 1 and 2. The values of f_1 and the parameters of both subpopulation models can be optimized by adjusting the parameters to minimize the deviation of the total population model from the actual data (Bello and Bradford, 2016) (see Sections 6.1 and 6.2).

Even relatively small differences in the hydrotime parameters of two populations can result in changes in the time courses, dependent also on the fraction of each population present (examples in Fig. 11). The predicted time courses of each subpopulation (Fig. 11b, e, and h) illustrate how these can overlap and meld together into relatively normal-looking time courses (Fig. 11d). For temperature (Bello, 2020) and aging (Bello and Bradford, 2016), this approach is capable of matching the actual total germination time courses of the mixed populations, while also accurately determining the model parameters for each subpopulation. It is also the case that seed populations can change their PBT parameters during a germination test, such as during extended incubation at lower ψ values (Dahal and Bradford, 1994). Testing data for the presence of additional subpopulations could identify such shifts and quantify their parameters.

This approach to deconvolute non-normal threshold distributions into multiple subpopulations with normal distributions is analogous to Fourier transformation of complex frequency patterns into their underlying sinusoidal components (Rahman, 2011). Theoretically, additional subpopulation terms could be added to the equation above (i.e., $f_3 = 1 - f_1 - f_2$, etc.) if justified by the data. Of course, larger seed populations and frequent data collection would be required to identify more subtle modifications of germination time course patterns. By varying the fraction and the parameters of each subpopulation, a wide diversity of total germination time course patterns can be generated, some of which will better match more complex or skewed distributions. For example, priming primarily decreases the value of the time constant, while aging increases the time constant; if subpopulations are present that did not respond similarly to priming or were aged differently, the patterns shown in Figure 11a can result (Bello and Bradford, 2016). If the median threshold value is different between the subpopulations, more normal-looking curves can result, but in fact, two distinct but overlapping subpopulations of seeds generate the time courses in Figure 11d and g. Changing the standard deviations of the threshold distributions can also result in distinct time courses (Fig. 11g and i). Mixed populations of seeds with different temperature thresholds can also be

resolved from their germination time courses (Bello, 2020), a situation that likely occurs in species in which dormancy is regulated by shifts in such thresholds (Batlla and Benech-Arnold, 2015).

Thus, for understanding the biological meaning of such patterns, it is more appropriate to assume that they are caused by multiple normally distributed subpopulations than to seek a specific function to empirically fit each novel pattern. The latter may fit the observed data well, but the biological meaning of the additional parameters required for such models can be obscure (Watt et al., 2010; Mesgaran et al., 2013; Chen et al., 2021). In contrast, the subpopulation approach is directly amenable to practical use, for example, if there is a less vigorous minor subpopulation in the seed lot (e.g., Fig. 11a, population 2). Characterizing it in terms of the fraction that it represents in the total population and the time at which germination of the seeds in each population is most distinct (Fig. 11b) could guide sorting equipment to remove those seeds from the lot. This is particularly feasible if imaging approaches can also be applied to identify unique characteristics of the two populations by scanning them prior to germination testing, so that the timing of each seed's germination is known and can be associated with detectable physical characteristics for sorting (Bello and Bradford, 2021; Nehoshtan et al., 2021).

5.5 Hormone time or biotime models

The focus of this chapter is to address factors in seed behavior that impact seed quality and influence seedling emergence. However, seed dormancy and germination are largely controlled by plant hormones, particularly by GA and ethylene acting to promote germination and ABA to inhibit germination (Bewley et al., 2013; Cao et al., 2019; Nonogaki, 2019). Thus, we will briefly point out that PBT models can also be used to quantify the sensitivities to hormones that influence seed germination (Ni and Bradford, 1992; Ni and Bradford, 1993; Dutta and Bradford, 1994; Bradford et al., 2008). For ABA, a germination inhibitor, the relevant equation is as follows:

$$\theta_{ABA} = \left(\log\left[ABA_b(g)\right] - \log\left[ABA\right]\right)t_g, \tag{25}$$

where θ_{ABA} is the time constant (log (M) h or log (M) d), $ABA_b(g)$ is the distribution of base ABA concentrations that prevent germination of fraction g of the population (M), ABA is the concentration of ABA (M) applied in the test. For the germination promoter GA, the equation is expressed as follows:

$$\theta_{GA} = \left(\log\left[GA\right] - \log\left[GA_b(g)\right]\right)t_g \tag{26}$$

where θ_{GA} is the time constant (log (M) h or log (M) d), GA is the concentration of GA (M) applied in the test and $GA_b(g)$ is the distribution of base GA

concentrations that just permit germination of fraction *g* of the population. For ABA, increasing the concentration applied to seeds will result in a series of time courses similar to those in Fig. 5b, with delayed and inhibited germination as the ABA concentration increases. For example, the ABA–time PBT model was used to quantify the ABA sensitivity of embryo growth and germination of celery seeds (Walker et al., 2021). When GA was applied to tomato seeds that were mutated to prevent GA biosynthesis, the GA–time PBT model closely matched the more rapid germination and higher final percentages as the GA concentration increased (Ni and Bradford, 1993).

PBT models specifically quantify the sensitivity of seed germination to these factors and the speed of response to them once their sensitivity thresholds have been exceeded. They therefore incorporate the two key aspects of hormonal regulation, the level of the hormone and the sensitivity of the cells or tissue to it, into a 'biotime' model that addresses both the recruitment of additional individuals (proteins, cells, seeds) and rate effects on turnover or growth/development as hormone levels increase (Bradford and Trewavas, 1994; Trewavas, 2012; Bradford, 2018). In addition, it was possible to model the effects and interactions of multiple different factors simultaneously, such as ψ and ABA or GA, by combining the t_g predictions of their PBT models for a given fraction and subtracting the t_g for that fraction in water (Ni and Bradford, 1992, 1993). As tools are now available to investigate hormonal regulation of germination on a gene-by-gene and cell-by-cell basis (Topham et al., 2017; Xu et al., 2020; Abley et al., 2021), it is important to understand the inherent variation among seeds in their sensitivity thresholds. For example, it is often the case that a given hormone dosage (e.g., of GA) might alleviate dormancy only for a specific fraction of the seed population. It is not uncommon to see this referred to as being 'partially effective' in breaking dormancy (e.g., Xu et al., 2020). However, as germination is an all-or-none response for an individual seed, this actually indicates that the hormone was 100% effective in alleviating dormancy in a certain fraction of the population; higher dosages could potentially recruit more seeds to germinate, or if not, the remaining seeds require a different signal to trigger germination.

It may well be that within seed populations, particularly of undomesticated species or those in the process of meeting their dormancy requirements, multiple signals might be effective in stimulating germination, but only in certain subfractions of the seed population. Rather than being considered as stochastic noise (Johnston and Bassel, 2018), we could view this sensitivity variation as an integral feature of seed behavior and use it to partition the seed population into subfractions that exhibit specific responses to regulatory factors. For example, a 'partially dormant' seed lot might be imbibed to allow all of the nondormant seeds to germinate. Exposure of the remaining seeds to a hormone or signal (e.g., inducing expression of a specific gene) might then

stimulate an additional cohort of seeds to germinate, which could be collected and analyzed. Exposing a dormant seed population to increasing dosages of a dormancy-breaking factor, allowing all responding seeds to complete germination and removing them before applying the next increment of the factor dosage would be an experimental approach to achieve fractionation of the population according to seeds' threshold sensitivities. Our usual procedure of exposing the entire population to a treatment and then looking for an increase in the total germination mixes the dormant and nondormant seeds together in the germination time course and obscures the signal from those seeds that are specifically responding to the applied signal. The overall response of the seed population to dormancy-breaking factors is likely the sum of the behaviors of subpopulations that are waiting for different combinations of signals to trigger germination, enabling the variation in dormancy that is the basis of bet-hedging behavior (Willis et al., 2014; Gianella et al., 2021).

6 Applying population-based threshold models

6.1 A brief history of fitting population-based models to germination data

As noted in Section 3.2, Eric Roberts initiated the application of population approaches to seed biology (Roberts, 1960, 1961). He and Richard Ellis pioneered the application of probit analysis to quantify the distribution of seed deaths in time that underlies their seed longevity equation (Ellis and Roberts, 1980, 1981). The basis of probit analysis is to transform germination percentage (or fraction) into 'probability units', which are equal to the standard deviation of the distribution of the parameter values in the population. The median (50%) is converted to 0 probits, and increases and decreases in frequencies from the median are plotted in standard deviation units. This crowds together percentages near the median and expands the scale at both extremes (see scales at top of Fig. 3a or 10a and d). When plotted on a cumulative scale, this converts a symmetric sigmoidal curve into a straight line. Thus, for seed viability or thermal time data, converting germination fractions into probit units enabled the use of linear regression to identify the median (when probit = 0; equal also to the mean in a normal distribution) and standard deviation (the inverse of the slope) of the population. While this approach has its critics (see below), it is worth remembering that at the time in the 1960s and 1970s, electronic calculators and personal computers did not exist to do regression analyses or fit complex statistical models. The ability to visually or graphically use linear relations to solve such computational problems is underappreciated now but represented a major advance at the time (see, e.g., the nomograms developed to solve the longevity equation in Ellis, 1988).

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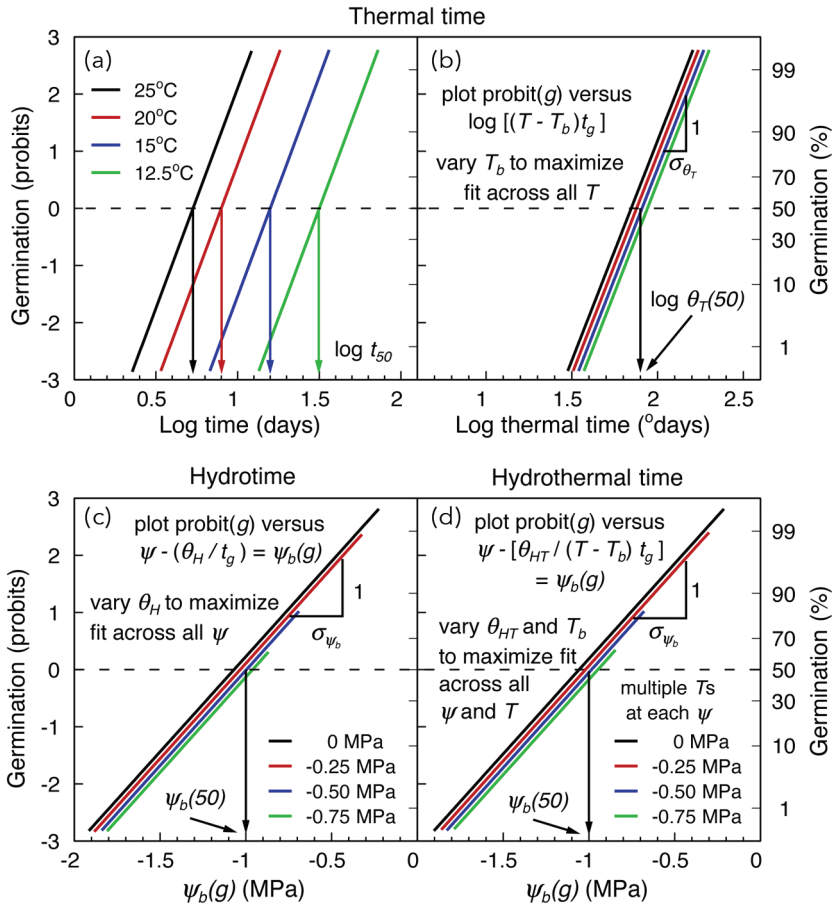

Figure 12 Application of repeated probit analysis to identify thermal time, hydrotime and hydrothermal time parameters of seed germination. (a) The germination time courses at different suboptimal temperatures shown in Fig. 1(b) are plotted here after transforming the percentage values into probits (*y*-axis). The units on a probit scale are standard deviations of a normal distribution (comparable percentages shown on right *y*-axis). The probit transformation converts normal sigmoidal cumulative distributions into straight lines. (b) Plotting the data of panel (a) on a log thermal time basis, or log [$(T - T_b)\ t_g$], results in a single common regression line when the optimal value of T_b is used. These are shown as parallel lines for illustration; with actual data, a data cloud will coalesce around the optimal regression line (least residual error) when the best T_b value is used. (c) The same approach can be used to estimate hydrotime parameters by plotting probit (*g*) (*y*-axis) as a function of $\psi - (\theta_H/t_g)$ for a series of time courses at different water potentials (Fig. 1c) and varying θ_H until the optimal regression is obtained. The value of $\psi_b(50)$ is where the regression crosses probit (50) = 0, and σ_{ψ_b} is the inverse of the slope. Note that unlike suboptimal temperatures, in which most viable seeds will eventually germinate, germination is inhibited for an increasing fraction of the population as ψ decreases, so the data available for more negative ψ values will be limited to lower values of *g*. This basic approach can be applied for all of the PBT models in which there is a distribution of

The loss of seed viability over aging time is generally normally distributed (Fig. 10b and d), so those data fit the expectations of probit analysis directly (when data from the initial plateau phase are omitted) (Bradford et al., 1993). Germination time courses, however, are generally right skewed in time but become more symmetric when plotted on a logarithmic time scale (Fig. 1) (Dahal et al., 1990). Plotting *GR* ($1/t_g$) rather than t_g can also make germination time courses more symmetric, as $1/t_g$ is normally distributed (Covell et al., 1986; Hay et al., 2014). Interestingly, the hydrotime model, in which it is the sensitivity thresholds of individuals in the population rather than the times to germination that are normally distributed, automatically compensates for the right skewness of the time courses on a linear time scale, as the effect of a given ψ on germination rate depends on $\psi_b(g)$, which varies among seeds in the population. That is, a small change in ψ has a much greater effect on t_g of seeds at the higher (more positive) side of the distribution than on the seeds with thresholds at the lower extreme (Fig. 5b). This automatically results in the right skewness in the germination time courses, which increases as ψ decreases. The same is the case for all of the PBT models in which the thresholds vary among seeds. As there has been some confusion on this point in the literature, it is important to repeat that the distribution of germination events over time (the germination time course) is seldom normally distributed or symmetric around the median; rather, it is the sensitivity thresholds among seeds that are normally distributed, and this, combined with the reciprocal effect of the factor level relative to the individual seed's threshold on germination timing, results in the skewness of germination time courses.

The use of probit regression for fitting PBT models is based on the normalization of time courses at different factor levels on the proper time scale. As discussed previously (Section 4.1), germination time courses conducted at different suboptimal temperatures and plotted on a log time scale become parallel sigmoidal curves (Fig. 1b). If the germination scale is transformed to probit values, which are in units of standard deviations, these sigmoidal curves are converted to straight lines (Fig. 12a). The concept of thermal time indicates that when expressed on a thermal time scale, all of these lines should coalesce onto a common line on a thermal time scale based upon a common value of T_b. Thus, if we plot probit (g) versus the log thermal time (log ($T - T_b$)) to germination for each observation time (t_g) (Fig. 12b), the result should be a single common regression according to the following equation:

Figure 12 (*Continued*)

thresholds among seeds in the population. (d) The hydrothermal time parameters can be determined as in (c), except that there can be multiple temperatures for each ψ value. The temperature is included as thermal time, by plotting probit (g) versus $\psi - \theta_{HT}/[(T - T_b)\, t_g]$. In this case, both θ_{HT} and T_b must be varied to find the optimal fit. A separate experiment conducted in water to determine T_b can give a good starting estimate of this value.

$$\text{probit}(g) = \left[\left(\log(T - T_b)t_g\right) - log\theta_T(50)\right] / \sigma_{\theta_T}. \quad (27)$$

By varying the value of T_b and conducting repeated regressions on the complete data set conducted at multiple temperatures, the value resulting in the minimum residual error, or maximum R^2 value, can be identified. The value of log $\theta_T(50)$ is the thermal time at which the line crosses probit (50%) = 0, and the inverse of the slope of the line is the value of σ_{θ_T}, indicating the variation in θ_T across germination fractions (Fig. 12b; Covell et al., 1986). Similarly, time courses at different constant ψ values can be normalized on a probit regression representing the sensitivity thresholds among seeds in the population (Fig. 12c):

$$\text{probit}(g) = \left[\psi - \left(\theta_H / t_g\right) - \psi_b(50)\right] / \sigma_{\psi_b}. \quad (28)$$

To determine the parameters of this equation, the term $\psi - (\theta_H/t_g)$ (which is equivalent to $\psi_b(g)$) is plotted on the *x*-axis versus the probit of the percentage *g* corresponding to t_g for each observed data point across ψ values. The value of θ_H is varied until the optimal regression across all data is attained, and the *x* value where the regression crosses probit = 0 is equal to $\psi_b(50)$ and σ_{ψ_b} is the inverse of the slope (Bradford, 1990). These can be combined into the HTT model

$$\text{probit}(g) = \left[\psi - \left(\theta_{HT} / \left(T - T_b\right)t_g\right) - \psi_b(50)\right] / \sigma_{\psi_b}, \quad (29)$$

which can be solved in the same manner by plotting the probit of *g* versus $\psi - [\theta_{HT}/(T - T_b)\, t_g]$ and varying both θ_{HT} and T_b until the regression across all tested ψ and *T* values is optimized (Fig. 12d). A good estimate of T_b values can be obtained by first analyzing only the data in water (ψ = 0 MPa) by the thermal time approach. This approach can be applied similarly to the other PBT models in which the threshold values vary among seeds in the population.

An important point can be made here regarding which data points are relevant for inclusion in fitting PBT models. The fundamental experimental data are observations of the additional seeds that have germinated since the last observation, resulting in a cumulative germination time course when summed over time. It is fundamental to the PBT model that the term t_g refers to the time at which cumulative fraction (or percentage) *g* was achieved. There is always some error in this, as one seldom observes the exact moment of radicle protrusion for a given seed. However, in the most sensitive period for data collection when germination percentage is increasing rapidly, this error is minimal when referring to the highest germination percentage achieved at that observation time. On the other hand, later in the germination time course, there are often observation times at which no additional germinated seeds are

recorded. In terms of the PBT model, these are not valid times for incorporation in fitting the model, as no additional germination occurred, so g has not increased and would therefore be the same as at the last observation. In terms of the model, each value of g is uniquely associated with a given time after imbibition, or t_g. We argue, therefore, that only observation times at which g has increased should be included in the data set for fitting PBT models. If this is not done, many germination time courses will end with a string of such null data points as germination does not increase as observation time is extended. This occurs often in hydrotime studies, as reducing the ψ will result in time courses that plateau at lower and lower percentages (e.g., Fig. 5). If null data points for the same percentage are included in probit analyses, for example, those points will skew the regressions to the right, resulting in inaccurately large values for σ_{ψ_b}. This can confound attempts to compare different distribution models that may handle these null points differently. The same is the case for not including viability data during an initial plateau phase in the calculation of parameter values for the Ellis–Roberts model (Fig. 10b). In addition to skewing fits to the data, the inclusion of these null data points can also inflate the number of data points in error estimations. Some statistical models automatically remove such null points by only considering the increase since the last observation time, and if this is zero, the observation effectively does not contribute to the estimation. It is a simple step to test whether a data point at time t is greater than at $t - 1$ and only including valid t_g values in fitting PBT models.

This approach to fitting data to these models involves identifying the parameter values of the sensitivity distribution and the time constant that result in all of the actual data points coalescing in a common linear regression on a probit scale. This has been done by iteration (i.e., 'repeated probit analyses') to identify the values that account for the greatest percentage of the variance or that minimize the residual error (Covell et al., 1986; Bradford, 1990; Bello and Bradford, 2016). Weighting should also be used in probit regressions, which gives greater weight to values nearer the median than to those at the extremes, and can be done automatically with statistical software. In fact, fitting of these models can now be done using nonlinear least-squares curve-fitting methods that do not require probit transformation of the germination fraction data (Hardegree, 2006; Hardegree and Winstral, 2006; Chantre et al., 2009). These routines compare the predicted values using an initial set of parameters to the actual values across all factor levels and iteratively adjust the parameter values to minimize the residual error across all data points. Thus, while still convenient (e.g., can be done easily in a spreadsheet) and of assistance in understanding the underlying basis of the models, the use of probit transformations and repeated regressions is no longer necessary to fit PBT models.

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6.2 R programs to fit PBT models

We have developed R scripts to fit germination data for all of the PBT models discussed above. The '*pbtm*' package combines existing PBT model calculations, parameter outputs and respective plots (www.pbtmodels.org). The package uses a nonlinear least-squares function (Bates and Watts, 1988; Bates and Chambers, 1992) to fit each model by comparing the raw data and respective treatments directly with the curve predicted by the selected model.

The R pnorm function computes the cumulative density function (cdf) which expresses the probability of a variable X to take a similar or lower value than x, respecting a normal distribution with a certain mean and standard deviation (sd). The pnorm function only requires three parameters (X, mean and sd) to output the probability but also provides options such as the direction of the evaluation (lower.tail) where the standard value (lower.tail=TRUE) assumes the probability of x to take similar or lower value (cdf for the lower or left area of the distribution) or this can be inverted to compute the probability of x to take a similar or higher value (cdf for higher or right side of the distribution). In the context of PBT models, we utilize the equations described above for each model but isolating the varying factor, such as $\theta_T(g)$ for the thermal time model or $X_b(g)$ for all other models. These equations are then used within the pnorm function with a varying mean, standard deviation and the direction of the distribution (lower.tail) depending on whether it is a promotive or inhibitory factor. The model can be optimized using a nonlinear least-squares algorithm (nls function in the R software) that refines all parameters involved by successive iterations, minimizing the sum of squares estimates of errors (SSE) or least squares when compared to the actual germination or respiration data. As discussed above, a tool in the R package also provides the option of removing null data observations when no additional germination occurred prior to model calculation.

6.3 Alternative approaches

There has been considerable debate regarding whether probit regression analysis is appropriate for analyzing seed germination data, and if not, what statistical approach should be used and what the underlying population distribution model should be (Watt et al., 2010, 2011; Mesgaran et al., 2013; Hay et al., 2014; Onofri et al., 2014; Hardegree et al., 2015; Mesgaran et al., 2017; Onofri et al., 2018; Gianinetti, 2020; Moltchanova et al., 2020). We have focused on the normal distribution here, but other models such as the Weibull, logistic and others have been proposed and tested (Watt et al., 2010; Mesgaran et al., 2013). There is no doubt that across diverse species and seed lots within those species, variation is present among the germination patterns observed. We have

argued above (Section 5.4) that in such cases, the differences could also be due to mixtures of seeds from multiple populations having different distributions, and in some cases, making that assumption and fitting multiple subpopulations can resolve the differences in goodness of fit. It has also been proposed that the experimental protocols used in seed germination violate assumptions of regression analysis, and other methods such as time-to-event or 'survival' analyses are more appropriate (Hay et al., 2014; Moltchanova et al., 2020; Romano and Stevanato, 2020). Gianinetti (2020) provides an in-depth analysis of these statistical issues and discusses the assumptions and complications inherent in seed germination data as well as advice on experimental design and execution in relation to the statistical approach utilized.

We will not go into the details of these discussions here, as there are valid arguments for different viewpoints, depending upon the statistical assumptions that are made and the parameters that are of most interest to the investigator, as well as the inherent variations in seed characteristics when comparing among species and seed lots. However, we are in agreement with Roberts (1999): "It is not often very useful to fit entirely arbitrary models to data: it is usually much more productive to seek solutions in which the coefficients have some biological meaning." Our focus has been on understanding seed behavior through the PBT modeling approach, and in most cases, all of the statistical approaches give quite similar values for the model parameters, with the differences among them being in the fraction of variation accounted for or in calculating the error terms to be used when comparing to other seed lots. Thus, for the purposes of estimating PBT model parameters and using them to inform us about the underlying seed behavior, the choice of method is less critical than when specific statistical comparisons (such as whether a given treatment significantly differs from another treatment) are the objective.

6.4 Experimental recommendations for fitting PBT models

In many cases in the literature, the quality of the original data is the limiting factor and makes the debate about different statistical models moot. As illustrated above in discussing subpopulations, discrimination among models describing germination time courses on the basis of their shape requires closely spaced observations. Often, the frequency of observations is simply too low or poorly spaced in time relative to germination frequency to have confidence in the ability to discriminate between alternative models (Bello and Bradford, 2016; Gianinetti, 2020). For this reason, and because we are primarily concerned with seed performance, we will discuss some experimental and technical factors that should be considered when collecting data for use in applying PBT models.

Fitting PBT models requires data for germination time courses under different conditions that must be known as accurately as possible. It is relatively

easy to maintain constant temperatures using growth chambers or temperature gradient tables, but it is critical to actually measure temperatures in the vicinity of the seeds rather than relying solely on the growth chamber's thermostat. Most researchers do not have the facilities to replicate multiple chambers at each temperature (Hardegree, 2006), so actual temperatures should be measured during experiments. It is not essential that a specific absolute temperature was maintained, but the actual temperature(s) during the experiment should be used when fitting the models. Similarly, using published recipes to prepare osmotic solutions to control ψ during germination experiments can result in significant errors, particularly with polyethylene glycol (PEG) solutions. Instead, the solution water potentials should be measured using an osmometer or similar instrument, and the seeds should be transferred to fresh plates every few days in order to assure that the ψ remains constant. For the most precise work, filter discs can be placed on the germination blotters with the seeds and moved directly into the osmometer for measurement. Again, it is less critical to maintain a specific target ψ value than it is to know what the actual value is during the experiment. When fitting the models, the factor level is essential information that is associated specifically with the seed lot's germination behavior. If that value is incorrect, it will cause error that is impossible for any statistical method to correct. Thus, if one wanted temperatures of 10°C, 15°C and 20°C or water potentials of −0.2, −0.4, and −0.6 MPa, and the actual values were 10.5°C, 14.8°C and 19°C or −0.24 MPa, −0.5 MPa and −0.7 MPa, that is no problem if those actual values are associated with the observed germination time courses, as the fitting routines will use those values in estimating the model parameters. However, using the expected values rather than the actual values will result in poor fits, incorrect parameter values and limited reproducibility. In our experience, seeds have a remarkable ability to sense their conditions, and when we have found anomalous behavior in a given experiment, we generally can trace it to human error or inaccurate control of the conditions; the seeds' responses across temperatures or dosages were virtually always accurate for the environment that they actually were experiencing.

The second important feature is to schedule observations to obtain at least 3–5 data points between the initiation of germination and ~80% of the final percentage for each condition. For example, lettuce seeds in optimal conditions can begin germinating within 12 h and be completed within 20 h; thus, an experiment planned for daily observations will have missed all of the relevant data before the first scheduled observation. Rather, experiments must be designed to obtain several observations during the time frame in which germination is occurring, such as by imbibing the seeds the previous evening and making observations frequently during the next day. This makes it clear why automated imaging, robotic scoring or correlated phenotypes such as respiration rates that can be frequently monitored are highly desirable. On the

other hand, at low T or ψ, or for more slowly germinating species, germination events will be delayed and more widely spaced in time and daily observations may be sufficient. It is often worth running a small-scale preliminary test to identify the key times for observations when planning a large experiment to be sure to schedule observations at the proper times for all of the treatment conditions. We note that some researchers fit observed germination data to arbitrary multiparameter models and then predict values for specific germination fractions (e.g., every 10%) for use in fitting PBT models. We are not in favor of this approach, as it can artificially increase the number of data points that are not associated with actual experimental observations. As statistical procedures are sensitive to the number of observations, this practice can artificially reduce estimates of residual error, for example.

On the other hand, in a seed testing lab that is familiar with a given species and the characteristics of its germination, it may be sufficient to collect only a few observations to place a given seed lot in a vigor ranking system based on its *GR*. Observation times could be identified and standardized such that germination percentages at only one or two time points could be converted to parameter values. If one is familiar with a species' characteristics, tests at only two temperatures and two water potentials are sufficient to estimate the HTT model parameters for a seed lot (Bradford and Still, 2004). It is not necessary or even desirable to estimate T_b or ψ_b by testing under conditions close to these values. This greatly extends the time required and allows extraneous factors to intervene (e.g., fungal contamination, evaporation from plates, etc.). Instead, temperatures near to T_o and 5°C below it or at 0 and −0.3 MPa may be sufficient since the response of *GR* is linear and the values of T_b or $\psi_b(50)$ can be determined by extrapolation to the intercept on the *x*-axis (Figs. 4 and 5). Similarly, if initial tests using controlled deterioration are used fit the aging time model, subsequent tests to check the vigor of stored seed lots would require only a single test under the same test conditions to estimate the seeds' aging rate by changes in *GR*.

7 Future trends in research

The demonstrated and potential utility of PBT models of seed germination have been discussed above. In this section, we mention some specific cases where the models, or the viewpoint on seed biology that they have revealed, can have beneficial applications. In addition, we note where additional research is needed to realize this potential.

7.1 Seed testing

There is a large literature on sampling, statistical comparisons, etc. for the seed testing industry (Elias et al., 2012). For universality, consistency and labeling,

these are valid and needed. However, despite much work on vigor tests (Baalbaki et al., 2009), and their widespread use in the seed industry, few have been incorporated into seed laws or adopted across species. Most vigor tests put seeds under some type of stress (e.g., cold, osmotic or salt stress), rapidly age the seeds (e.g., artificial aging, controlled deterioration) or measure seedling growth after some period of time. The case has been made here that all of these either directly impact the speed of germination (stress and age) or are indirect results of variation in germination timing (growth tests). Thus, the analysis of complete germination time courses would be the most direct approach to quantifying and comparing seed vigor. In fact, many companies use internal vigor tests that may utilize smaller sample sizes, focus on rates and uniformity in addition to total viability, and explore limits of environmental responses rather than optimal conditions, to select and create high-performing seed lots.

Nonetheless, germination time courses are seldom measured in standard seed testing due to the labor required to collect frequent observations to characterize such time courses in detail. However, this situation is rapidly changing. Methods for imaging of test plates and automated extraction of germination timing information have been available for a decade (Joosen et al., 2010), and improvements in both imaging and analysis methods are occurring rapidly (Silva et al., 2019; Colmer et al., 2020; Bello and Bradford, 2021; https://www.seed-x.com; https://www.vibeia.com/; https://videometer.com/). In most cases, however, it still has been necessary for a person to move the test plates individually under the imaging system to collect the data, again limiting observations to the times that workers are available. That is changing now with inexpensive electronic cameras that can be placed over each sample to collect images as frequently as desired and transmit the extracted data to a computer (Colmer et al., 2020). This fully automates the collection of germination time courses. Indirect approaches such as robotic single-seed respiration measurements can also be converted into time courses closely paralleling germination timing (Bello and Bradford, 2016). Thus, the impediments to using germination rates as a universal vigor test are being overcome, and the application of PBT models to such data allows precise quantification of the critical features of the seed population, including uniformity and stress sensitivity. Just as the data acquisition can be automated, the application of the PBT models could be incorporated into the software, reducing the labor requirements to setting up the experiments and subsequently receiving and interpreting the model parameters and graphs quantifying seed performance.

7.2 Seed production, conditioning and enhancement

A key factor in the production of high-quality seeds is their maturity at harvest. In general, seeds should be allowed to complete their full maturation program

and dry below the level of metabolic activity before harvest (Ellis, 2019). On the other hand, delaying harvest risks field weathering, preharvest sprouting and aging that will reduce seed quality. Postharvest technologies cannot reverse such damage but are able to remove immature, aged and damaged seeds, thereby upgrading seed lots. Methods to detect seed maturity specifically using chlorophyll fluorescence (high levels are associated with less mature seeds) can be used to upgrade seed quality in a number of species (Jalink et al., 1998; Kenanoglu et al., 2015; Bello and Bradford, 2021). Variation in seed maturity of indeterminate crops is a clear example of seed lots that contain multiple subpopulations (Still and Bradford, 1998), which is the foundation of standard seed conditioning equipment using seed size, shape, density, color or other physical characteristics to fractionate seed lots. More advanced imaging and association of spectral traits or internal structures (visible by X-ray) with quality features now enable sorting of seed lots on parameters directly associated with seed performance (Vanderburg et al., 1994; Bruggink and van Duijn, 2017; https://www.seed-x.com/product/sorter/). PBT models can be used to quantify seed lot performance and potentially to identify specific subpopulations of under-performing seeds for removal. These methods have also been applied to weed seed samples to estimate their germination potential and even herbicide tolerance (Matzrafi et al., 2017). Considerable research is needed to test and adapt these new technologies to diverse species and to identify the physical and/or physiological features that are closely associated with seed quality. The rapid deployment of artificial intelligence methods to extract such relationships from large data sets makes this a promising avenue for future research and commercialization. Structuring such algorithms to look for population-based relationships and patterns rather than simply independent stochastic correlations could make them even more powerful for identifying features for classifying seeds according to their performance potential.

7.3 Seed storage

Seed longevity models have been quite useful in identifying the storage characteristics of many species and improving seed storage conditions for commercial seed storage and germplasm conservation (Ellis, 1988). A major issue for seed bank managers is the variation in times at which seed lots, even of the same species, can lose viability (Fleming et al., 2019; Solberg et al., 2020). This requires periodic viability tests, which eventually consume seeds in the stored sample even if they are still viable. However, reducing the frequency of testing risks allowing viability to fall below the recommended 85% and potential loss of genetic diversity (Ellis et al., 2019). As stated by Hay and Whitehouse (2017), 'What a genebank manager ideally needs to manage collections better is a test at the time the seeds are placed into storage that

either predicts when the seed lots will reach the viability threshold [. . .] or that ranks the seed lots for relative longevity'. Despite the importance of estimating the length of the initial high viability period for individual seed lots, seed banks in general have not adopted routine procedures to estimate this when the sample has entered into storage. Instead, studies of seed longevity in seed banks are typically retrospective analyses of samples after extended storage periods (Walters et al., 2005; Nagel and Börner, 2010; Fleming et al., 2019; Solberg et al., 2020).

The PBT aging model shows that the reduction in *GR* during storage before any loss of viability can be used to estimate the maximum viability period under the conditions of the test (Section 5.2; Fig. 9). Pretests using controlled deterioration (CD) conditions and measuring germination rates under standardized conditions could be used to estimate the p_{max} values for a desired viability percentage. Conducting the same *GR* test after a storage period would reveal where a lot is in its progression toward loss of vigor and viability under the current storage conditions, calibrating the aging model to the actual storage conditions. Even if exact calibration is not possible, thresholds for reductions in *GR* in response to aging could be used to rank seed lots into potential longevity categories. Using the automated methods mentioned above, this could actually reduce the labor required for seed testing in seed banks, as it would allow prediction of when sampling during storage would be the most informative regarding potential loss of viability and reduce the number of uninformative tests during the plateau phase based only on total viability. In commercial seeds, the time frame is much shorter, but in both cases, it is 95–85% viability that is critical, not 50%, as the seed lot either needs to be salable or retain the desired genetic diversity. Thus, measuring *GR* during the initial phase of the aging period (which can be done on as few as 25 or 50 seeds per sample when using single-seed methods, saving the seed resources) can predict when viability will start to decline. The critical need is therefore to calibrate specific CD test conditions with subsequent longevity under the seed bank or commercial warehouse conditions. Seeds in the latter already are being tested periodically under current protocols, so the key step is to initiate the CD pretesting protocols to enable storage life prediction using the aging PBT model to be calibrated to the actual conditions. As noted previously (Section 5.2), these CD tests should be conducted at an *eRH* of 60% or less and the temperature can be increased up to 50°C, but tests at higher *eRH* values may not be good predictors of longevity under drier conditions. In addition, adding *GR* measurements to routine inventory tests (usually every 6 months in commercial seeds) would quickly build up a database relating changes in *GR* to both vigor and viability for different species and varieties and over time would provide useful information regarding production, harvest and storage effects on seed quality.

7.4 Breeding for improved seed quality and performance

Seed quality and performance can be improved by selection, as is evident in the seed traits that are characteristic of domestication, such as loss of dormancy. In reality, however, seed quality and performance generally are low on the list of breeder priorities, unless it is a limiting factor for sales. For example, seed shattering is still a problem for seed production in many crops, particularly vegetables (Still and Bradford, 1998), and only recently have commercial canola varieties become available with an engineered trait reducing shattering (Lambert et al., 2015). Priming is widely used to remove dormancy and increase uniformity in high-value seeds, as it is easier than breeding out inherited dormancy behaviors in these less-domesticated species. In addition, phenotyping for *GR* has been labor intensive and subject to variation from nongenetic factors (Morris et al., 2016). The increasing automation of seed testing should be helpful here, and the quantitative parameters characterizing seed quality from the PBT models could be useful as phenotypes for selection (Saux et al., 2020). Until improved seed quality traits become genetically fixed, the hydropriming time model can be useful for developing effective and efficient seed enhancement protocols. As physiological and molecular research is uncovering in increasing depth the mechanisms regulating seed dormancy and germination, we can anticipate that targets for gene modification can be identified more quickly and utilized to improve seed performance (Nonogaki and Nonogaki, 2017; Xu et al., 2020; Bizouerne et al., 2021).

7.5 Crop and weed emergence models for integrated crop management

Between T_b and T_o, all of the viable seeds should germinate eventually, but it can take a long time at lower T (Fig. 1). Thus, it is common practice to plant when soil T exceeds practical minimums so that germination and emergence can proceed without delay and avoid subjecting the seeds to risk from pathogens or pests. Planting at T above T_o also can occur, as in lettuce plantings in late summer for fall and winter production, leading to thermoinhibition and poor stand establishment that can be overcome by seed priming (Valdes et al., 1985). While crop management planning on a thermal time basis is routine in agriculture, we should think of lower ψ as being similar to low T; even relatively small reductions in ψ will delay and reduce uniformity. Priming is often claimed to improve tolerance to salinity, for example, but in most cases, it primarily affects the speed of germination under water or salt stress, which is valuable, but it has less effect on the minimum ψ for germination (Dahal and Bradford, 1990; Bradford and Somasco, 1994; Cheng and Bradford, 1999; Tatari et al., 2020). However, improvements in speed and uniformity of emergence due to

priming can be of significant value in the production of greenhouse crops or transplants.

Seed dormancy models are often associated with weed seeds, and emergence models are used to assist in controlling weeds (Allen et al., 2007; Boddy et al., 2012; Fernández Farnocchia et al., 2021). Management of weed seed production in crop fields and the associated seed banks is critical to extend the lifetime of herbicides and reduce reliance on them (Ramesh, 2015). The stand establishment period for the crop is the most critical time for weed control, so understanding the seed germination behavior of associated weeds may enable the implementation of management approaches. PBT models are being applied effectively for this purpose (Boddy et al., 2012; Batlla and Agostinelli, 2017; Matzrafi et al., 2020), and extension of this approach should be encouraged.

7.6 Ecological applications of PBT models

Ecologists studying germination, bet hedging, species demographics and climate change are also using PBT concepts to describe and model these processes (Huang et al., 2016; Larson and Funk, 2016; Gremer et al., 2020; Liu et al., 2020; Zhang et al., 2020). For example, the PBT models may be particularly useful in constructing ecological germination niche diagrams (Fig. 7). Conceptualizing seeds as populations, potentially with distinct functional subpopulations requiring different environmental signals to promote germination, could be particularly useful in studies of diverse dormancy and bet-hedging behaviors (Gianella et al., 2021). With ecological research now sharply focused on the implications of climate change, modeling approaches to identify 'climate clones' or locations around the world that have (or will have in the future) similar climates could be of considerable benefit for seed companies to extend the lifetimes of existing varieties adapted to specific climates and to conservationists seeking to identify locations to preserve species currently under threat (https://climate.ai/solutions/#seed).

7.7 Research on determinants of seed quality attributes

While remarkable progress has been made in understanding the genetic, molecular and physiological bases of seed vigor and dormancy, much research remains to be done to understand the specific signaling pathways translating environmental signals into plant metabolic, growth and developmental responses. We believe that progress in this area will require the incorporation of population-based thinking into experimental designs. In the early stages of such work, pooled samples of seeds at different stages of dormancy or germination can be adequate to identify major genes or the consequences of

their mutation or loss of function. However, the closer we get to understanding specific mechanisms and pathways at the cellular or biochemical levels, the more important it is to move to single-seed approaches and fractionation of seed populations into similar cohorts on a developmental time scale. For example, single-seed assays indicated that some germination-related enzymes increased in activity 1000-fold as the seeds initiated germination, while no activity was expressed in slower germinating or dormant seeds at the same time (Still and Bradford, 1997). Pooling all seeds and extracting them together obscures this fact and leads to the assumption that all seeds are gradually increasing expression of this enzyme. Similarly, if a seed lot is imbibed and the entire population is harvested and pooled together periodically for extraction and mRNA analysis, there appears to be a smooth progression from development/dormancy-related transcripts to the expression of germination/growth-related genes associated with the germination time course. However, if the seeds are separated into germinated and ungerminated fractions before extraction, the germinated fraction will contain almost exclusively transcripts from germination/growth-related genes, while the transcript profile of the ungerminated seeds is still almost identical to that of dry seeds (Yoong, 2015, and authors' unpublished results). This indicates that imbibed but ungerminated seeds maintain a physiological state similar to that of mature seeds until they commit to germination, followed by a massive loss of maturation-related transcripts and correspondingly increased expression of genes associated with germination/growth in a relatively short time on a seed-by-seed basis. This makes sense for dormant or ungerminated seeds, as they are constantly at risk for soil drying and need to retain the dehydration tolerance mechanisms developed during the latter stages of seed development. However, once a seed commits to germination, there is no going back, so the seed can fully initiate reserve mobilization and growth-related gene expression. Pooling seeds in different developmental time stages obscures such timing and makes it difficult to decipher or reconstruct causal connections among specific signaling and response pathways occurring in a given seed.

The same is true for studies of changes occurring in seeds in association with aging and loss of viability. Under genebank storage conditions, it can be years between the time that the first 10% of seeds lost viability and when 50% of seeds had died. Thus, measuring chemical changes in a seed lot at its p_{50} will mix together seeds that are still viable and have years to go before dying with other seeds that have suffered extensive damage and have been dead for years already (Fleming et al., 2017; Zinsmeister et al., 2020). As oxidation is the primary chemical process associated with aging (Bailly et al., 2008; Groot et al., 2015), there will be an increase in oxidized products in such samples compared to unaged seeds (Nagel et al., 2019). However, it is impossible in such a pooled sample to determine whether such oxidation is a cause or effect

of the loss of viability. That is, protective mechanisms may largely prevent such changes in viable seeds, and when these are exhausted, large-scale oxidation will occur; that is, some oxidation may be associated with the gradual loss of seed quality, but the large increase in such chemical signals over an aging time course may be primarily contributed by the increasing fraction of dead seeds in the sampled population. Thus, as we pursue the discovery of refined and specific physiological and biochemical mechanisms, pooling samples of disparate seed populations will prevent conclusively establishing causal relationships. Instead, it will be subject to the seed biology equivalent of what economists have called 'the flaw of averages' (Savage, 2012). One approach would be to cut seeds in half and save one half for extraction and analysis and assay the other half for viability, such as by the tetrazolium test. Once the viability status is known, the saved half-seeds of the known viability category could be extracted individually or pooled. In some species, such as soybean, one cotyledon can be removed and imbibed, and its subsequent color development is correlated with the viability of the embryonic seedling; this can be used to sort the individual seeds into viability categories for the analysis of the dry embryonic axis (Fleming et al., 2021). A more convenient solution could be advances in multispectral imaging that can presort seeds into viability categories and enable sampling of subpopulations of seeds with similar deterioration or viability characteristics (Olesen et al., 2015; ElMasry et al., 2019).

As seed and plant scientists adopt single-seed and single-cell methods to identify the actual causal factors of seed behavior and their underlying genes or regulatory pathways (Topham et al., 2017; Abley et al., 2021; Alamos et al., 2021), it will be important to also refine the experimental designs associated with the application of such methods. Those methods are very powerful, but practitioners are currently struggling to understand how to deal with the 'noise' in such studies. The PBT models described here imply that while seeds are highly variable, they form populations that are not randomly chaotic but rather are ordered based on their individual sensitivities to environmental and molecular signals. Such individual variation may be stochastic, but it is neither noise nor error, but rather is an integral component of how seeds fulfill their evolutionary function of introducing seedlings into environments conducive to their survival. Thus, although we have emphasized population-level analyses, the same considerations highlight 'the importance of individuality', which has been identified across levels of biological organization (Gilroy and Trewavas, 2001; Trewavas, 2014). We should therefore consider variation among individuals as a feature, not a bug, in the software of biology. Seed biology has been and can be a leader in illustrating how to utilize individual-based data to understand the emergent properties of cellular and molecular populations (Bradford, 2018; Alamos et al., 2021).

7.8 Broader implications

While we have focused here primarily on the population characteristics of seeds as revealed through PBT models, another interesting component is the ability to transform developmental processes occurring at different speeds onto a normalized time scale. This is quite familiar to agriculturalists through the use of thermal time or degree-days to normalize for changes in plant or insect development across variations in temperature. It seems natural for us to accept that warmer temperatures increase molecular motion and that physical or chemical processes speed up as temperatures rise. However, the actual basis of temperature sensing and response in plants remains largely obscure, and plants are able to compensate temperature differences if needed, such as to maintain an accurate circadian clock or accumulate chilling time for vernalization (Penfield, 2008; Penfield and MacGregor, 2014; Zhao et al., 2021). There is strong evidence that phytochrome B (PHYB) is involved in sensing temperature as well as light, but the underlying mechanisms are complex and still under study (Hernando et al., 2021). Nonetheless, seeds germinate almost perfectly in accordance with thermal time over most of the range between T_b and T_o. Similarly, their germination rates are also precisely adjusted by reductions in ψ, and in combination with temperature on the HTT scale, although the specific cellular mechanism responsible for sensing water potential also remains obscure (Dorone et al., 2021). Perhaps less expected is that the primary hormones regulating dormancy and germination, GA and ABA, also act in the same way, speeding or slowing the time to germination as their concentrations exceed thresholds that vary among seeds and in response to conditions such as chilling or after-ripening. Thus, through seeds, we can catch a glimpse of 'biotime', or biological time, that regulates metabolic and physiological processes and which may or may not coincide with clock time (Bradford and Trewavas, 1994). The relationships between dosages, thresholds and rates underlying PBT models (Fig. 2) may apply across biological scales and processes in a much more fundamental way than we currently appreciate.

In addition, the PBT models specifically incorporate the concept of sensitivity thresholds as being both a critical foundation for such biotime behavior and a source of variation among individuals that provide a wide range of flexibility in signal/response systems. As has been insightfully and entertainingly presented by Braitenberg (1984) in relation to neurological function, variation in the threshold sensitivities of simple autonomous units can be combined into surprisingly complex sensing and control systems. In essence, a normal distribution of sensitivities provides a reservoir of response units, some of which are highly sensitive and may be able to take

care of the situation, but if not, and signal levels rise, more units (e.g., cells, enzymes, transcription factors, etc.) can be recruited from the population (Powers et al., 2019). This provides a simple, automatic mechanism for monitoring and maintaining a status quo state in a changing environment or the ability to recruit help and respond quickly to large changes in inputs or developmental phase changes. Examples of apparent PBT behavior across all scales of biology have been described (Bradford, 2018). Thinking in PBT terms changes the way that experiments are designed and their outcomes are interpreted. We believe that a PBT-based viewpoint is essential for seed biologists and will be instructive for all biologists.

8 Where to look for further information

We have organized this chapter to provide a general introduction to PBT models and then to describe their application to an array of topics related to seed quality and performance. Thus, readers interested in a specific topic or application can refer to those specific sections and follow up with the citations therein. A detailed introduction to the initial development and application of the thermal, hydrotime and HTT models can be found in Bradford (1995). Further development of the models and illustration of the importance of the original data quality can be found in Bello and Bradford (2016). Approaches and applications for seed dormancy and ecology can be found in Allen et al. (2007), Batlla and Benech-Arnold (2015) and Liu et al. (2020). Different viewpoints on the statistical analysis of seed germination patterns and the fitting of PBT models can be found in several publications (Hay et al., 2014; Gianinetti, 2020; Moltchanova et al., 2020; Romano and Stevanato, 2020). R scripts and a website for fitting PBT models to germination data can be accessed at www.pbtmodels.org.

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10 Abbreviations

Term	Definition
ABA	Abscisic acid (concentration)
$ABA_b(g)$	Distribution of base ABA concentrations for germination fractions *g*
CD	Controlled deterioration
cdf	Cumulative density function
C_W, C_H and C_Q	Storability constants determined empirically in Ellis–Roberts equation
$D(g)$	Sensitivity distribution of the total population
eRH	Equilibrium relative humidity = water activity × 100
f_1	Fraction of the total population in subpopulation 1
g	Germination percentage
GA	Gibberellins (concentration)
$GA_b(g)$	Distribution of base GA concentrations for germination fractions *g*
GR	Germination rate
GR_g	GR for specific germination fraction *g* in the population
HT	Hydrotime
HTP	Hydrothermal priming time
HTT	Hydrothermal time
K_E	Species constant for storability in Ellis–Roberts model
K_i	Initial viability on probit scale in Ellis–Roberts model
k_T	Constant slope of the increase in $\psi_b(g)$ per degree above T_o
m	Seed moisture content fresh weight basis
MGT	Mean germination time
n	Germinated seeds or population
O_2	Oxygen
°C d	Degree-day
°C h	Degree-hour
Ox	Percentage of O_2
Ox_b	Base oxygen percentage that prevents germination
$Ox_b(50)$	Median Ox_b for population
$Ox_b(g)$	Distribution of Ox_b percentages of germination fractions *g*
p	Storage period
p50	Storage period to a loss of 50% viability
PBT	Population-based threshold
PEG	Polyethylene glycol
p_{max}	Maximum lifetimes under the conditions of storage
$p_{max}(50)$	Median p_{max} for population
$p_{max}(g)$	Distribution of p_{max} for germination fractions *g*

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Term	Definition
$p_{norm}(g)$	Distribution of aging times at which fraction g of normal seedlings occurs
probit	Probability unit = units of one standard deviation
ψ	Water potential, MPa
ψ_b	Minimum or base threshold ψ that prevents germination
$\psi_b(50)$	Median ψ_b for population
$\psi_b(g)$	Distribution of ψ_b for germination fractions g in the population
ψ_{min}	Lowest ψ at which the priming effect will occur
RH	Relative humidity
σ	Standard deviation
$\sigma_{P_{max}}$	Standard deviation for p_{max} distribution in population
σ_{ψ_b}	Standard deviation of ψ_b distribution in population
σ_{θ_T}	Standard deviation of θ_T distribution in population
σ_{X1} and σ_{X2}	Standard deviations of X_b values among seeds in subpopulations 1 and 2
T	Temperature
t	Time to germination
T_b	Minimum or base temperature
T_c	Maximum or ceiling temperature
$T_c(g)$	Distribution of T_c for germination fractions g in the population
t_g	Time to germination of percentage or fraction g
t_{g1} and t_{g2}	Germination times of fractions g in subpopulations 1 and 2
θ_{ABA}	ABA time constant
θ_{GA}	GA time constant
θ_H	Hydrotime constant
θ_{HT}	Hydrothermal time constant
θ_{HTP}	Hydrothermal priming time constant
θ_{Ox}	Oxygen–time constant
θ_T	Thermal time constant
$\theta_T(g)$	Distribution of θ_T for specific germination fractions g in the population
θ_X	Time constant for generic factor X
θ_{X1} and θ_{X2}	Time constants for subpopulations 1 and 2 of generic factor X
θ_{age}	Aging time constant
T_h	Dormancy higher limit T
T_l	Dormancy lower limit T
T_{min}	Lowest T at which the priming effect will occur
t_{norm}	Imbibition time after which normal seedlings will not appear
T_o	Optimum temperature

Term	Definition
t_p	Duration of priming
v	Percent viability on probit scale
X	Level or concentration of generic factor X
X_b	Base or threshold level for a response to X
$X_b(g)$	Distribution of X_b for germination fractions g in the population
$X_b(50)_1$ and $X_b(50)_2$	Medians of the distributions of X_b values for subpopulations 1 and 2

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