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Water Partitioning Between Environment and *Setaria faberi* Seed Exterior-Interior Compartments

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Abstract: Experiments were conducted modeling changes in *S. faberi* seed moisture loss, (drydown) to elucidate the roles water partitioning between seeds and environment might play in germination. Changes in moisture content during drydown were estimated by modeling the behavior of 2 seed structures: the exterior hull (palea, lemma) and the interior caryopsis. Water saturates the hull by adhesion and the formation of surface films. In contrast, water typically enters and hydrates the gas and water-tight caryopsis only by passing through the placental pore. By analyzing the rate of water loss during a drying period, it was possible to determine the initial moisture content of the 2 anatomical structures and to reveal how the loss of water from the hull (partition 2) and caryopsis (partition 3) varied with moisture availability and thermocycle conditions in the local environment (partition 1). The drying procedure removed water in 3 distinct phases. In the first, water was lost quickly from the hull surface and more slowly from the caryopsis via the narrow placental pore. In the second, water continued to be lost from the caryopsis alone. After some time the interior water was tightly bound in the seed, the final phase. The moisture incubation experiments demonstrated that *S. faberi* seeds did not absorb water in the manner expected if it was a homogeneous material. Hydration of dry *S. faberi* seeds began with preferential partitioning of water to the interior seed caryopsis and the embryo. With additional local moisture availability the hull and caryopsis compartments absorbed moisture in a similar manner until the moisture content becomes sufficiently high to saturate the caryopsis. The caryopsis saturated at a lower local water availability content than did the seed hull. With saturation of the caryopsis, the hull preferentially absorbed the excess moisture. Hull water was spread uniformly over the seed surface forming a water film (partition 2). It is speculated that the water film changes the manner in which, the seed interfaces with the external soil microsite environment (partition 1), the locus of oxygen exchange. The delivery rate of oxygen to the embryo is a function of its phase, partial pressure gradient and diffusion rate to the embryo (partition 3). The thickness of the seed surface water film is therefore a powerful mechanism regulating the pool of dissolved oxygen pool (partition 2) directly available to the seed embryo (partition 3), hence, its crucial role in determining subsequent seed behaviors. Evidence that hull surface water quantity, in the physical form of boundary layer film thickness, controls germination was provided by the observation that maximum seed germination occurs in conjunction with intermediate levels of moisture availability in the soil environment. Soil moisture levels above and below this optimal hydration level resulted in lower germination. Consistent with the theory of oxygen-water control of *S. faberi* germination, these observations support the concept that optimal water film thicknesses on the seed surface are those that maximize the trade-off between hull surface area available for oxygen diffusion and water availability to support and sustain germination metabolism. These experiments indicate a robust, dynamic system of germination control by the transduction of external moisture and thermocycle signals from the soil to the seed embryo as modulated by the characteristics of the seed hull surface and the placental pore. The seed in unable to control its temperature, but the morphology of its exterior surface provides a means by which, the seed is able to regulate the other 2 requisites for germinative growth, moisture and oxygen.

Key words: Seed dormancy, germination, soil microsite, soil seed banks/pools, seedling recruitment, seed hull

INTRODUCTION

Setaria faberi Herrm. is a ubiquitous invasive weed species of the north temperate regions of the globe (Dekker, 2003, 2004a; Warwick, 1990). The seed qualities it possesses, notably seed dormancy and the ability to form enduring soil-seed pools, are important life history traits affording it success in disturbed habitats.

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Harper (1965) indicated that Seed polymorphisms seem particularly likely to be sensitive indicators of evolutionary change in alien invaders. A long-standing question in evolutionary biology is how proximate ecological selective pressures and ultimate evolutionary forces shape phenotypic diversity and species patterns. Seed morphology therefore, seems to be a ripe area of investigation, in particular the morphology of seed surfaces as the interface, the 'antenna', linking the soil-borne weed with its immediate microsite environment.

Setaria faberi dormancy is induced in seeds during embryogenesis (Dekker *et al*., 1996), resulting in the dispersal of a diverse array of dormancy phenotypes (heteroblasty) from a single synflorescence (genotype) at abscission. *Setaria* heteroblasty refers to seeds with different dormancy capacities (Dekker *et al*., 2003). This dormancy or germination capacity is based on the interaction of 3 morpho-physiological mechanisms within the *Setaria* seed: hull water oxygenation, transfer aleurone cell layer membrane diffusion and symplastic oxygen scavenging (Dekker *et al*., 1996; Dekker and Hargrove, 2002). Thus, germination capacity is an inherent quality of a *Setaria* seed, which is retained for its entire life. The formation of long-lived, heterogeneous soil-seed pools is the inevitable consequence of the dormant seed rain.

The environmental signal regulating weedy *S. faberi* behavior is the amount of oxygen dissolved in water received by the embryo over time and temperatures conducive to both growth and oxygen solubility (i.e., oxy-hydro-thermal-time, Dekker *et al*., 2003). Three interrelated morpho-physiological mechanisms influence the delivery of water and gases, constraining and controlling *Setaria* sp. seed embryo behavior. The first is the seed hull and outer envelopes that act to attract and accumulate water, enhance gas solubility in that water by means of its surface rugosity and channel that gas-laden water to the placental pore, the basal opening to the seed caryopsis interior (embryo, endosperm, aleurone layer and caryopsis coat), the only water entry point into the interior portions of the caryopsis. The extremely narrow Placental Pore (PP, primarily residual xylem and tracheary tissues from the dead pedicle) terminates with the Transfer Aleurone Cell Layer (TACL), a membrane whose diameter and function regulates the free diffusion of gas-laden water in and out of the symplast (Rost, 1971, 1972, 1973, 1975; Rost and Lersten, 1970, 1973). The *Setaria* sp. seed is gas- and water-tight except at this very narrow pore due to the enveloping Caryopsis Coat (CC). Together the PP, TACL and CC provide the second controlling mechanism: modulation of water and gases between embryo and the external environment. The third element is an oxygen-scavenging heme-containing protein that acts to buffer the seed against premature germination by sequestering oxygen (Dekker and Hargrove, 2002; Sareini, 2002). These controlling mechanisms are represented schematically in Fig. 1.

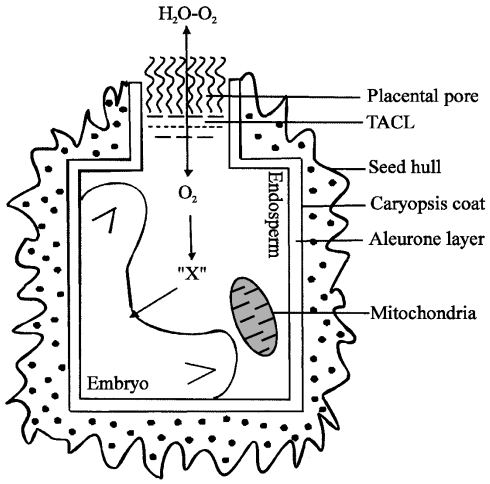

Fig. 1: Schematic diagram of the *Setaria* sp. seed and the morpho-physiological components responsible for seed dormancy and behavior. The symplast (aleurone layer, Transfer Aleurone Cell Layer (TACL), endosperm, oxygen-scavenging protein (X) and the embryo) is surrounded by two enclosing structures, the hull (lemma, palea) and the gas- and water-tight caryopsis coat. Water and dissolved gas entry into the symplast is restricted to the placental pore at the basal end of the seed

Temperature, oxygen and moisture signals are exchanged between three continuous partitions: The soil matrix, the surface of the seed (hull) which, includes the placental pore and the interior of the seed (Fig. 2). The soil matrix in contact with the seed surface is the local source of moisture and gases required by the seed to initiate germination. The re-supply rate of this external water/gas pool varies over time in delayed response to macroscopic hydrological and thermal cycles (weather). Germination requires conditions favorable for rehydration and sustained metabolism. For a variety of plant species, lower temperatures, causing increased dissolved oxygen content and lower metabolic demands in seeds, can play an important part in seasonal germination timing (Dekker and Hargrove, 2002). It is therefore, logical that the presence of surface films or excessive moisture as supplied by the environment can alter the degree to which, a given internal moisture content and temperature are favorable for embryonic germination.

The hull surface and internal structure of the placental pore influence the effective exchange rate

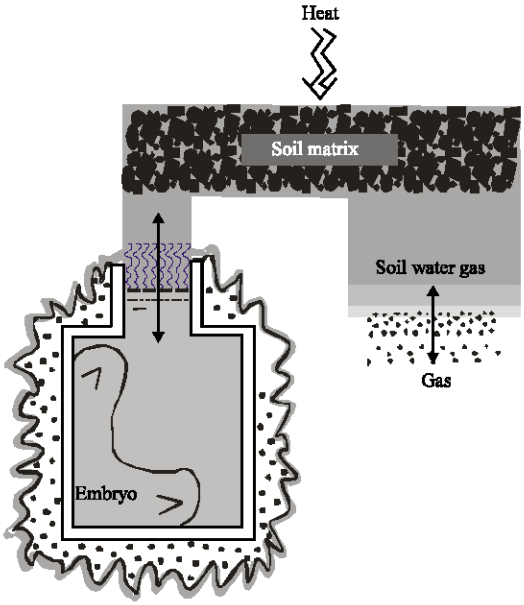

Fig. 2: Schematic diagram of the transduction of water-gas (e.g., H_20-O_2) and temperature (HEAT) signals from the soil matrix to the *Setaria* sp. seed symplast. Soil water-gas mixtures can be present in 3 continuous partitions (gray shaded areas): Soil matrix water-gas (top), seed exterior (hull) and apoplast (bottom left gray) and the seed symplast (bottom right gray). Heat from the environment affects oxygen solubility in water and metabolic behaviors (germination)

between the external environment and the embryo. These structures strongly impact the rate at which water and dissolved gases diffuse into and out of the seed via the action of the transfer aleurone cell layer membrane and the structure of the placental pore (Rost, 1971, 1975). Unlike water and gas, temperature consistently maintains equilibrium between the seed interior and the external environment.

It is hypothesized that the change in moisture content of the *Setaria* sp. seed is driven by the dynamics of two anatomical structures: The hull and the caryopsis. Water saturates the hull by adhesion and the formation of surface films. In contrast, for caryopses with intact suberized coats, water enters and hydrates the caryopsis only by passing through the placental pore. The external partial pressure of water determines the equilibrium hydration levels of the seed and hull. Under situations of time-varying water potential, the hydration of both anatomical structures will oscillate; however, the rapidity with which water enters and exits the different structures depends both on the size of the gradient in water potential and the effective surface area over which moisture may be exchanged (Fick's Law). It stands to reason that it may be possible to differentiate between water lost from each of the 2 structures by modeling changes in the seed moisture content as they dry in this 2-stage process.

The fundamental hypothesis is that knowledge of the temperature-water-gas mechanism, in combination with seed heteroblasty, will be sufficient to forecast the response of *Setaria* seed pools to macroscopic weather variables (Dekker *et al*., 2001, 2003; Dekker and Hargrove, 2002; Jovaag *et al*., 2006). Here support is provided for the notion that the signal from the external environment is more complex than that of a thermal or simple hydrothermal accumulator. It is demonstrated that variations in moisture availability result in heterogeneous distribution of moisture within the seed; when the moisture potential of the environment rises sufficiently, relatively more water is partitioned to the seed surface. Thus, the anatomy of the seed hull may function as more than a simple protective structure for the *Setaria* seed; it may represent a mechanism by which, the seed has tuned its germination timing by indirectly altering the frequency of its internal moisture and gas availability.

MATERIALS AND METHODS

Seeds: Two different dormant seed accessions were used in separate experiments to determine seed water content and the effects of this water and temperature on germination. Different *S. faberi* seed accessions and thermocycle conditions, were utilized to ensure that generalizations about the partitioning of moisture between seed compartments, especially those concerning the influence of variable thickness hull water films, would be robust and applicable to another *S. faberi* genotype. A dormant *S. faberi* seed population was used to determine the seed interior (primarily symplast) and exterior (apoplast) water content. Lot #3776 (J99-35) was derived from seed in soil collected August 28, 1999 (Julian week 35) from an agricultural field on the Johnson Farm, Iowa State University Agriculture Research Farm, Ames, Iowa, USA (41°58'88" N latitude, 093°38'49" longitude; Atchison, 2001). A second dormant *S. faberi* seed population was used to determine the effect of moisture availability on seed germination. Lot 3781 (K99-40) was derived from seed collected October 5, 1999 (Julian week 40) from an agricultural field at the Southeastern Research Farm, Iowa State University Agriculture Research Farm, near Crawfordsville, Iowa, USA (41°12'26"N latitude, 091°29'68" longitude; Atchison, 2001). Immediately, after harvest, the seeds of both accessions were air-dried separately overnight on screens at 19°C. After air drying, bulk samples of both seed accessions were stored separately at -20°C until the time of the experiments (March, 2002).

Seed assays: Seed water content was evaluated in 30 mL gas-tight vials, with 20 mm outside diameter mouths (Wheaton Science Products, Millville, NJ, USA). Two disks of Anchor Blue germination blotter paper (Anchor Paper Co., St. Paul, MN, USA), 32 mm in diameter, were placed in and completely covered, the bottom of the vials. Seeds stored at -20°C prior to use in these experiments. Ten *S. faberi* seeds were weighed (water content assay only) and placed into a 30 mL vial containing 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, or 3 mL distilled, de-ionized water (the available local moisture) per vial (0.25-1.5 mL only for the germination assay). After placing seed and water in the vials they were immediately sealed: a neoprene stopper and an aluminum seal was crimped (Wheaton hand crimper, model 22430; Wheaton Science Products) around the vial neck to ensure a gas- and watertight seal (Dekker and Hargrove, 2002). Five vials (replications) were used for each vial water content (8) and temperature regime (3) in the water content assay. Twelve vials (replications) were used for each water vial content (6) and temperature regime (3) and after-ripening period (4, see assay below) in the seed germination assay.

Diurnal thermocycles

Seed water content assay: The seeds were allowed to equilibrate with the water in the vials at 3 different, alternating, diurnal (12 h: 12 h), temperature regimes (thermocycles; 5-15; 15-25; 25-35°C) for 7 days. After this time the vials were opened and the 10 seeds weighed immediately. They were then surface dried on paper towels at ambient room temperature (20°C) and weighed repeatedly during the process of drydown at 10 min, 1, 2, 4, 8, 24 and 48 h after removal from the vials. After these air dry weights were obtained, 48 h post-vial removal, the seed were dried for 24 h in a 54°C oven to determine their dry seed weight. Seeds were weighed immediately after removal from the oven to reflect the oven-dried weight before resorption of moisture from the air.

Seed germination assay: Germination determination was conducted at 3 different, alternating, diurnal (9 h dark, 15 h light) temperature regimes (thermocycles): constant 25°C, 18.75-28.75°C (diurnal mean temperature 25°C, diurnal temperature difference 10°C), or 12.5-32.5°C (diurnal mean temperature 25°C, diurnal temperature difference 20°C) for 8 days. The accumulated diurnal heat units (600° h, 0°C base temperature) were identical in all thermocycle conditions. The seed vials were placed in either a controlled environment chamber for after-ripening at 4°C constant temperature for 18 day, after which they were moved to controlled environment seed germination cabinets to evaluate germination. When, the germination assays were completed the vials were removed and the numbers of germinated seeds per vial (of 10) were recorded.

Initial moisture content determination: The general shape of seed moisture loss (drydown) curves has received a great deal of attention for crop seeds (e.g., Parti, 1993). In a comparison of the model performance of different mathematical models of the drydown process, Casada (2002) found the Page equation (Eq. 1) to perform well in describing seed moisture content dynamics during drying. In contrast to crop seeds, many weed seeds have relatively thick hulls. The enveloping structures of cereal grains can influence calculation of moisture content, but this effect can estimated by examining water loss over time. The hull-associated water tends to be lost very quickly due to its large exposed surface area and thus, may be subtracted from the more gradual process of caryopsis moisture loss. The so-called thin-layer drying models described above could be used to describe the drydown process and consequently the moisture content of *S. faberi*. We used the methodology to estimate hull moisture content as it relates to a factorial set of intial environmental incubation treatments (8 incubation water contents x 3 temperature regimes).

The page equation describes the time course of the moisture ratio, MR:

$$MR = e^{-k \cdot t^n} \quad (1)$$

where:

k = A rate constant
n = A shape parameter
MR = Defined to be

$$MR = \frac{MC_t - MC_{eq}}{MC_0 - MC_{eq}} \quad (2)$$

The moisture content, MC, is calculated on a dry-weight basis and the moisture content at equilibrium was assumed negligible after prolonged oven-drying at 56°C. It follows from Eq. 2 that changes in the total seed weight can be described with the function:

$$W_i(t) = S + A_i e^{-b_i \cdot t^n} \quad (3)$$

If it is assumed that seed moisture leaves the seed through 2 processes, one very rapid, (surface evaporation) and one slow (caryopsis moisture loss), both of which follow different forms of the page equation, then Eq. (3) becomes:

$$W_i(t) = S + A_i \left(p_i e^{-a_i \cdot t^{n1}} + (1 - p_i) e^{-b_i \cdot t^{n2}} \right) \quad (4)$$

Here,

S = The dry weight of the seed
A = The total initial quantity of moisture
p = The proportion of the total moisture associated with the hull
a and b = Are rate constants that may depend on prior environmental conditions
n_1 and n_2 = Are shape parameters

The modified Page Eq. (4) can be made linear in the parameter b after all surface moisture has evaporated (t >10 min) and before the seed is extremely dry (t <48 h). These times are clearly functions of the drydown environment imposed and are best determined post-hoc by examining the data. The functional form of the linearized equation is:

$$\text{Log}(W_i(t) - S) = \text{Log}\left(A_i\left(1 - p_i\right)\right) - b \cdot t^n \qquad (5)$$

The fundamental premise of this model of seed behavior is that the coefficients A and p can play a strong role in regulating the progress of germination of the *Setaria* sp. seed. The relationship between these treatment-specific parameter estimates and the behavior of the *Setaria* sp. seed were developed in another study (unpublished) on the inter- and intra-specific morphology of the seed hull. Here, the goal is only to establish a procedure to estimate model parameters when seeds are subjected to a series of controlled environmental conditions. In particular, in the rate at which water is lost through the placental pore under strong gradients in the partial pressure of water, for knowledge of this rate will allow an estimation of how the water contained on and in the seed is distributed. The initial Page Eq. (2) was fit to all data sets (10 min<t <48 h) with non-linear regression using Mathematica (Research Inc., vers. 4.0, Champaign) Wolfram (1999) to determine a common shape parameter, n. Eq. (5) was then fit with non-linear regression to estimate a treatment-specific intercept and slope. The slope was exponential rate of water loss and the intercept was the initial caryopsis water content. The resulting table of parameter values was then analyzed for the factorial design using standard ANOVA procedures.

The impact of incubation water content on moisture partitioning. Estimates of mean hull and caryopsis moisture contents were regressed against initial vial water content using a common saturation model, the Boltzman sigmoidal function:

$$W(x) = c_0 + \frac{\left(c_1 - c_0\right)}{\left(1 + \text{Exp}\left(\frac{v_{50} - x}{d}\right)\right)} \qquad (6)$$

where:

W = The water content of the seed compartment (hull, caryopsis)
c_0 = The lower bound
c_1 = The maximal level
v_{50} = The amount of vial moisture (x) resulting in a 50% change in water content
d = A slope parameter

Parameter estimates, function confidence intervals and goodness-of-fit statistics were reported for each compartment and thermocycle treatment.

Estimating seed hull surface area: The hull surface area of *Setaria* sp. seed used to estimate water boundary layer film thickness (μm) was calculated by approximating its shape as a smooth ellipsoid with 3 unequal axes (4.7, 1.2 and 1.1 mm; Dekker, 2004a) using a formula from Wolfram (1999). In reality, the *Setaria* sp. seed is neither perfectly ellipsoid nor smooth.

RESULTS

Water loss from the seed: The fit of all data sets to the modified Page equation (Eq. 1) resulted in an estimate of a common shape parameter n of 0.60 with a 95% confidence interval ranging from 0.59-0.61 and an overall r^2 of 0.95. The linearization of the Page equation fit all data sets remarkably well ($r^2 > 0.9$ in all cases). To illustrate the concept of how the model was used to estimate moisture with the different parts of the seed, example data are shown in Fig. 3. The linear region (for the transformed variables of Eq. 5) is clearly visible.

The fast (hull) and the slow (caryopsis) water loss functions were exponential (i.e., the processes approximated simple diffusion when the seed is hydrated) (Fig. 3). While, more complicated models would improve accuracy under low-moisture conditions (e.g., Casada 2002), the exponential decay model works sufficiently well (with coefficients of determination of >0.9) to describe generally the manner in which water accumulates on and in the hull and caryopsis of the foxtail seed.

The drydown data (Fig. 3) reveal three phases of water loss: a combination of 2 exponential processes acting at different rates, a single exponential process and a low moisture limit that asymptotically approaches an equilibrium with the oven environment. The rate of water loss during the second phase, as well as the transition from phase 2-3 is determined by the material composition of the seed and drying conditions. In phase 1, water is being lost from both the hull and caryopsis. Water loss from the seed hull occurs quickly. For times greater than some critical time (t*), the hull water is entirely depleted while, the water continues to exit from the caryopis via the placental pore. In phase 2, water is lost from the caryopsis

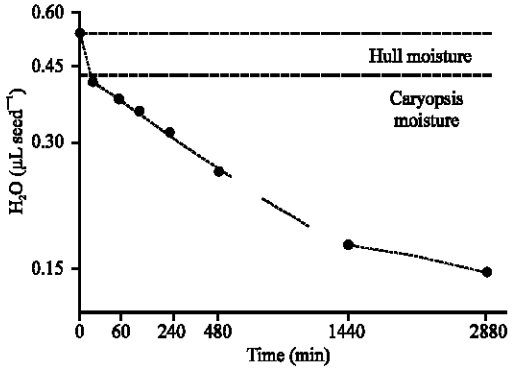

Fig. 3: Example drydown curve showing changes in *S. faberi* seed water content (µL H_2O $seed^{-1}$) with time for one replicate of the one week 1 mL incubation with a 15:25°C thermocycle. The y-axis is log-scale and is power-law scaled (exponent of 0.6). Following their pretreatment conditioning, seeds were removed from vials and transferred to drying ovens. Initial moisture loss was primarily due to rapid evaporation from the hull. A subsequent linear (in the transformed coordinates) phase follows, which eventually asymptotically approaches equilibrium with the conditions within the oven. When extrapolated to the initial time, the fit of the linearized phase is used to infer the water content of the hull and the remainder ascribed to the caryposis

alone. After a sufficiently large amount of time (t**), the interior water becomes tightly bound and the shape of the drydown curve departs from exponential. In phase 3, water concentration approached equilibrium with the air in the oven (54°C). For the air drying methodology, the first phase was completed within the first 10 min of drying and after 8 h, the seed entered phase 3 drying.

Effect of incubation moisture and temperature on rate of water loss. The rate of available vial water loss was a dynamic process and depended on the temperature and moisture conditions. The estimates of initial water content and loss-rate from Eq. (5) demonstrated that not all temperature or moisture treatments had equivalent loss rates ($p<0.0001$; Table 1). An interaction plot of incubation thermocycle and vial moisture content treatments on the rate of phase 2 water loss is shown in Fig. 4. In general, the rate of water loss increased with increasing available vial water content, from 0.25 mL to about 0.75. The rate of water loss for all three thermocycles was similar for available water vial contents from about 0.75-1.5 mL. The rate of water loss was slowest for many incubation vial moisture amounts at

Table 1: ANOVA model results for the dependence of the decay rate (k) of the page equation on incubation treatment

Treatments	Dof	SS	MS	F	p-value
Intercept	1	0.018449	0.018449	20694.48	<0.0001
Temp	2	0.000093	0.000047	52.32	<0.0001
Water	7	0.000259	0.000037	41.44	<0.0001
Temp*water	14	0.000009	0.000001	0.71	0.7575
Error	96	0.000086	0.000001		
Total	119	0.000446			

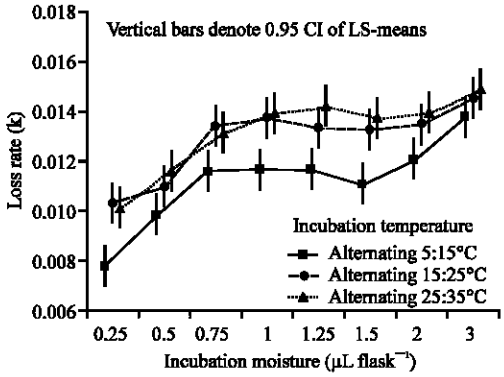

Fig. 4: Rate of water loss (estimated k parameter from Eq. 1) during phase 2 for *S. faberi* seed as a function of incubation vial moisture content (µL $flask^{-1}$) and incubation temperature treatment (5:15, 15:25 and 25:35°C). Vertical bar correspond to the 95% confidence intervals of the least-square means for each combination of treatments within the factorial design. The rate of loss was slower for the coldest thermocycle treatment and when vial water content was extreme

the coolest temperatures (5:15°C) and fastest at most of the warmer thermocycles (15:25 and 25:35°C).

Effect of incubation moisture and temperature on caryopsis and hull moisture content. Each estimate of initial moisture content was used to calculate the fraction of initial vial moisture that was associated with the caryopsis (Fig. 5) and the hull (Fig. 6). These estimates provide insights into the relationship between local moisture availability (partition 1) and both hull (partition 2) and caryopsis (partition 3) seed moisture content with changes in temperature. Caryopsis absorption was greater at the higher temperatures than at lower temperatures when averaged over vial water content availability (0-3.0 mL; Table 2 and Fig. 5). Caryopsis water content increased with increasing local water availability (vial water content) from 0.25 to about 0.75 mL (Table 3 and Fig. 5). The caryopsis became saturated at about 0.75-about 1.25-2 mL, with a small increase at about 1.5-3.0 mL. Seed hull moisture absorption increased with increasing incubation thermocycle temperature from 5:15-25:35°C when averaged over vial water content availability

Table 2: Mean caryopsis-associated moisture content (as proportion of seed Dry Weight (DW); g H_2O g^{-1} DW) averaged over all incubation vial moisture contents (0.25-3.0 mL H_2O per flask) for each of 3 incubation thermocycles (5:15°, 15:25°, 25:35°C); means with the same letter are not significant using the duncan's multiple range test

Thermocycle (°C)	Mean g H_2O g^{-1} seed DW
5:15°C	0.252C
15:25°C	0.244B
25:35°C	0.239A

Table 3: Mean caryopsis-associated moisture content (as proportion of seed Dry Weight (DW); g H_2O g^{-1} DW) versus incubation vial moisture content (0.25-3.0 mL H_2O per flask) averaged over 3 incubation thermocycles (5:15°, 15:25°, 25:35°C); means with the same letter are not significant using the duncan's multiple range test

H_2O per Vial (mL)	Mean g H_2O g^{-1} seed DW
0.25	0.213D
0.50	0.227C
0.75	0.246B
1.00	0.248B
1.25	0.247B
1.50	0.263A
2.00	0.253B
3.00	0.265A

Table 4: Mean hull-associated water content (g H_2O $\times 10^{-4}$) averaged over all incubation vial moisture contents (0.25-3.0 mL H_2O $flask^{-1}$) for each of 3 incubation thermocycles (5:15, 15:25 and 25:35°C); means with the same letter are not significant using the duncan's multiple range test

Thermocycle (°C)	Mean g H_2O g^{-1} seed DW
5:15°C	0.000203A
15:25°C	0.000181B
25:35°C	0.000167C

(0-3.0 mL; Table 4 and Fig. 6). The hull water content increased with increasing local water availability (vial water content) from 0.25 to about 2. The exterior seed hull was saturated at about 2 mL, with no further increases with additional water availability. Therefore, the caryopsis (partition 3; Fig. 5) saturates at a lower local water availability content than does the seed hull (partition 2; Fig. 6).

Seed hull surface water film thickness: If the water present on and in the hull was spread uniformly, it would create a water film with the thickness displayed by the right-hand y-axis scale of Fig. 6. Film thickness is approximately proportional to vial water content until the filter paper begins to saturate with water (approximately, 2 mL per vial). No increase in film thickness occurred at vial water content of 2 mL or greater, indicating that the film thickness was saturated at 2 mL, the maximum. Some small fraction of the moisture was held inside the hull structural matrix and would not contribute to the surrounding layer, but in any case, the thickness would be proportional to the amount of surface water.

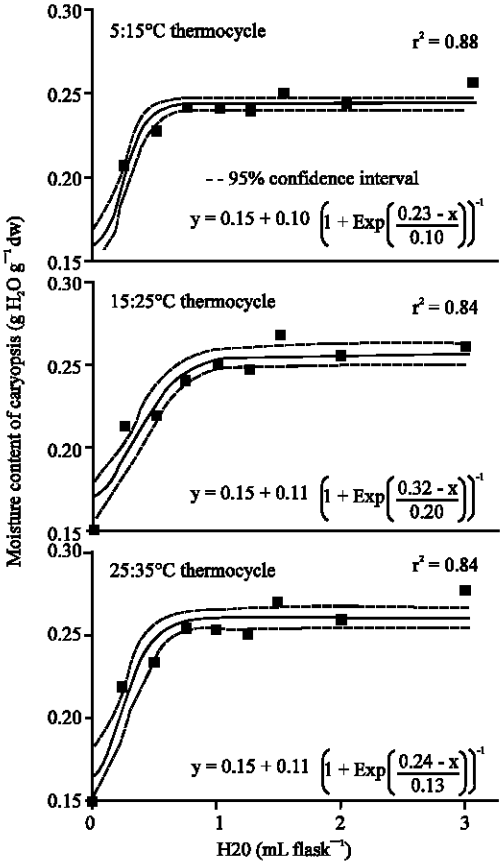

Fig. 5: Caryopsis-associated moisture content (as proportion of seed dry weight; g H_2O g^{-1} seed dry weight) as a function of incubation vial moisture content (mL H_2O $flask^{-1}$) for each of 3 thermocycles (5:15, 15:25 and 25:35°C). Each data point shown is the mean of 5 replicates, though the curve and associated confidence limits and statistics were calculated from the raw data (before averaging replicates within treatment). Dashed lines correspond to the 95% confidence band for the regression, which explained 84-88% of the variance. Below a certain level, lowering incubation moisture conditions lowers the initial caryopsis water content and above that level a maximal content is achieved

Seed moisture partitioning between hull and caryopsis: Moisture absorption by and partitioning between, the seed exterior hull and the interior caryopsis was not equal. At low local water availability (0.25-0.5 mL) the caryopsis preferentially absorbed water (about 27-30% on a seed dry-weight basis (dwb), 18-20% on a wet-weight basis (wwb); Fig. 7). At high local water availability (1.5-3 mL) the seed hull preferentially absorbed water, presumably

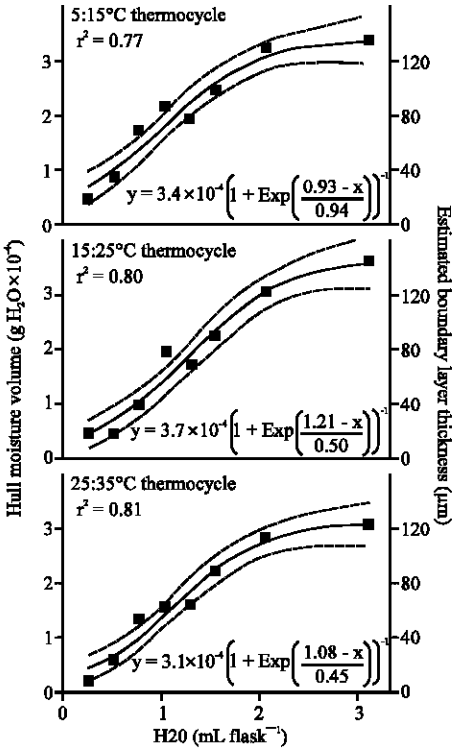

Fig. 6: Hull-associated water content (g $H_2O \times 10^{-4}$) and estimated hull boundary layer thickness (μm) as a function of incubation vial water content (mL H_2O per flask) for each of three incubation thermocycles (5:15, 15:25 and 25:35°C). Each data point shown is the mean of 5 replicates, though the curve and associated confidence limits and statistics were calculated from the raw data (before averaging replicates within treatment). Dashed lines correspond to the 95% confidence band for the regression, which explained between 77 and 81% of the variance. In contrast to the dynamics of the caryopsis moisture shown in Fig. 5, increasing the amount of moisture during incubation continued to raise the water content of the hull, at least over the critical range from approximately 1.5-2 mL of incubation moisture

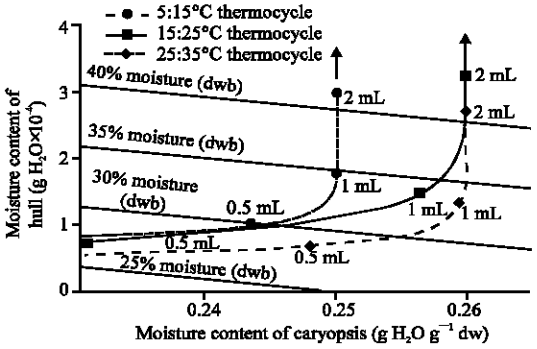

Fig. 7: Relationship of seed moisture partitioning between the hull (g $H_2O \times 10^{-4}$) and caryopsis (as proportion of seed dry weight; g H_2O g^{-1} seed dry weight) in 3 incubation thermocycles (long-dashed line, round symbol, 5:15°C, solid line, square symbols, 15:25°C, short-dashed line, diamond symbol, 25:35°C); symbols reflect the mean of each imbibition moisture level from 0.25-3 mL, the 3 curves are cures fit to Miterlisch-type saturation equations with non-linear regression; straight, sloping diagonal lines (gray) correspond to the approximate moisture content on the dry-weight basis (dwb) of the entire seed (caryopsis and hull). The plot synthesizes the information contained in Fig. 5 and 6 and shows its relationship to moisture content on a whole-seed, dry-weight basis

because the caryopsis was already saturated or nearly so (about 40% and greater (dwb), 27-29% wwb). Absorption and presumably loss, of moisture at intermediate local water availability (0.75-1.25 mL) from the hull and caryopsis was similar (about 35% moisture (dwb), 23-25% water (wwb)). Hydration of dry *S. faberi* seeds begins with preferential partitioning of water into the interior seed caryopsis and the embryo. With additional local moisture availability the hull and caryopsis compartments absorbed moisture in a similar manner. With greater moisture and saturation of the caryopsis, the hull preferentially absorbed moisture. Over the range of 0.25-3 mL vial moisture availability contents, the moisture content of seed ranged from about 27 to >40% on a dry weight basis (18-29% wet-weight basis).

Effect of moisture availability on seed germination. Overall germination varied from 7-76%, depending on vial moisture availability and thermocycle conditions (Fig. 8). Most of the seed germination occurred in 0.75 mL (constant 25°C), 0.75-1 mL (alternating 10°C temperature difference), or 0.75-1.25 mL (alternating 20°C temperature difference) vial water quantity. Seed germination was greater in alternating thermocycle conditions than in constant temperatures for all vial water quantities from 0.5-1.5 mL and the separation between alternating and constant temperature increased with increasing water content. Seed germination was similar between the 2 alternating thermocycles for all vial water quantities except at 1.25 mL, where greater germination occurred in the 20°C temperature difference relative that with a difference of 10°C.

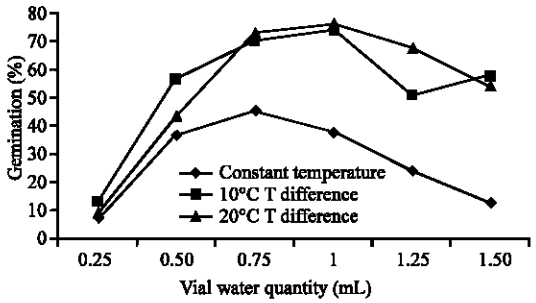

Fig. 8: The effect of vial water quantity and after-ripening time (4°C for 18 days) on percent *S. faberi* seed germination in three diurnal light and temperature conditions (9 h dark, 15 h light diurnal thermocycles: constant 25°C, 18.75-28.75°C (10°C diurnal temperature difference), or 12.5-32.5°C (20°C diurnal temperature difference). Means in the same column with the same capital letter, or in the same row with the same lower case letter, are not statistically different (t-test, p = 0.05)

DISCUSSION

Oxygen dissolved in water imbibed by the seed and temperatures conducive to growth, are the environmental signals regulating weedy *Setaria* sp. behavior in the soil (Dekker, 2003, 2004b). These oxygen-moisture-temperature signals are exchanged between three continuous partitions: the soil matrix, the surface of the seed hull and the embryo inside the gas- and water-tight caryopsis (Fig. 2). The soil matrix in contact with the seed surface is the local source of moisture and gases required by the seed to initiate germination. The re-supply rate of this external water-gas pool varies over time in delayed response to macroscopic hydro and thermal cycles (weather). Germination requires conditions favorable for rehydration and sustained metabolism.

Change in moisture content of the *Setaria faberi* seed is driven by the dynamics of 2 anatomical structures: the hull and the caryopsis. Water saturates the hull by adhesion and the formation of surface films. In contrast, the suberized, gas- and water-tight, coat of the caryopsis restricts water-gas diffusion to the the placental pore and transfer aleurone layer leading to the embryo (Rost, 1975; Rost and Lersten, 1970).

The signal regulating *S. faberi* seed behavior from the external environment is more complex that a thermal or simple hydrothermal accumulator. Variations in locally available moisture results in a heterogeneous distribution of moisture between the seed hull surface and the interior of the caryopsis. The external partial pressure of water determines the equilibrium hydration levels of the seed and hull. Under situations of time-varying water potential, the hydration of both anatomical structures will oscillate. However, the rapidity with which water enters and exits the different structures depends both on the size of the gradient in water potential and the effective surface area over which moisture may be exchanged (Fick's Law). Differentiation of water lost from the caryopsis and hull was made possible by modeling changes in the moisture content of seeds as they dried (drydown) a compound process.

Water was lost from the seed in 3 phases, a process determined by the material composition of the seed, the available local water and the temperature. In the first phase, water was lost quickly from the hull surface and from the caryopsis via the placental pore. In the second phase water was lost from the caryopsis alone. After some time the interior water was tightly bound in the seed, the final phase. The rate of water loss from the seed was a dynamic process and varied with temperature and moisture conditions. These observations reveal the relationship of temperature and local moisture availability (partition 1) with both hull (partition 2) and caryopsis (partition 3) seed moisture content. The loss of water by both seed partitions varied with both moisture and thermocycle conditions. In the conditions used here, the rate of water loss was slower in the coolest condition (5:15°C) compared to warmer thermocycles (15:25 and 25:35°C) and the rate of water loss increased with increasing moisture at the lowest available water quantities (i.e., <0.75 mL), while, losses at higher moisture content were similar (i.e. >0.75 mL).

When exposed to different moisture potentials the *Setaria* seed absorbs water. However, it did not absorb as would be expected if it was a homogeneous material. Hydration of dry *S. faberi* seeds begins with preferential partitioning of water into the interior seed caryopsis and the embryo. With additional local moisture availability the hull and caryopsis compartments absorb moisture in a similar manner. When the moisture content becomes sufficiently high the caryopsis quickly becomes saturated. The caryopsis (partition 2) became saturated at a lower local water availability content than did the seed hull. Caryopsis water content increased with increasing moisture at low amounts (<1 mL) and became saturated in higher moisture conditions (1-3 mL). Caryopses absorbed more moisture at the higher temperatures than low, while concurrently seed hulls absorbed more moisture in cooler conditions than in the warmest thermocycle. With saturation of the caryopsis, the hull preferentially absorbed moisture. The hull water content increased with increasing moisture up to 2 mL and was saturated at that amount.

Hull water is spread uniformly over the seed surface forming a water film, the boundary functioning as the interface with the external environment. The physical properties of the hull water film (partition 2) play a functional role determining seed behavior as the site of oxygen exchange with the immediate soil microsite environment (partition 1). The delivery rate of molecular oxygen to the embryo is a function of its phase (gaseous in the soil atmosphere, liquid when dissolved in water films on soil particles and seed surfaces), partial pressure gradient and diffusion rate through whatever, anatomical structures separate the environment from the embryo. The seed hull surface water film is the immediate source of (dissolved) oxygen available to the seed embryo (partition 3), hence, its critical role in determining subsequent seed behaviors (Dekker and Hargrove, 2002; Dekker, 2003, 2004b). Using more sophisticated techniques for assessing the surface area, the range of seed hull surface areas and Surface-to-Volume ratios (S:V) were calculated for 5 different *Setaria* species and biotypes, both weeds and domesticated cultivars of foxtail millet (*Setaria viridis* subsp. *italica*). These studies revealed changes in seed hull qualities affecting surface water films: topography (rugose hull surface weeds, smooth hull surface crops), total surface area and elongation in weedy genotypes resulting in increased S:V's. These hull adaptations affect the area and thickness of water films, a powerful mechanism controlling the dissolved oxygen pool of the seed surface (partition 2) directly supplied to the seed embryo (partition 3). This regulation of the seed surface dissolved oxygen pool may provide mechanism for adaptation to local moisture and temperature conditions.

Moisture availability and temperature thermocycles play a dominant role in regulating *S. faberi* seed germination. Maximum seed germination occurred at specific moisture availability quantities (0.75-1.25 mL in our conditions), with lesser germination occurring at lesser (0.25-0.50 mL) and greater (1.5 mL) water contents. This observation provides a strong inference that hull surface water quantity in the physical form of boundary layer film thickness controls germination. Consistent with the theory of oxygen-water control of *S. faberi* germination (Dekker and Hargrove, 2002), these observations support the concept that optimal water film thicknesses on the seed surface are those that maximize the trade-off between hull surface area available for oxygen diffusion and water availability to support and sustain germination metabolism.

Relatively low hull surface water contents provide thinner water films for soil atmosphere gases to penetrate and therefore support greater gas diffusion but provide insufficient moisture for germinative growth. Thicker surface films provide greater resistance to gas diffusion but provide ample moisture for germination. Intermediate water availability optimizes the trade-off between water film gas diffusion (the dissolved oxygen pool) and moisture for germination.

The 'water sensitivity problem' of dormant barley (*Hordeum* sp.) in the malting industry provides some insight into the role played by water quantity and oxygen in seed germination (Essery *et al*., 1954; Pollack *et al*., 1955). The amount of available water had an important influence on the proportion of seeds which germinated: hyper-optimal and hypo-optimal water quantities inhibited germination relative to an optimal moisture amount. This water-sensitivity occurred during the early phases of germination of previously dormant barley seed lots. The effect was significant and occurred over a small range of water availability. Germination in pure oxygen completely relieved the effects of water-sensitivity on germination, but did not overcome reduced seed viability. Other studies did not support an alternative hypothesis, the possible role played by respiratory uncouplers, auxins or auxin-antagonists (Gaber and Roberts, 1969).

Alternating diurnal thermocycles stimulated greater seed germination than did constant temperatures for all moisture contents greater than 0.25 mL, given the same heat units in each condition. These results are consistent with the hypothesis that alternating diurnal temperature conditions provide additional oxygen to the seed embryo and interior caryopsis due to the de-gassing effect that occurs when water temperature is increased. When temperatures increase in a water solution with oxygen at equilibrium with the local atmosphere, gas solubility decreases and excess oxygen is forced out of solution as a gas to the adjacent environment as it reaches the new equilibrium. This pulse of gaseous oxygen is immediately available to the seeds' metabolic physiology, the acute oxy-hydro-thermal time signal (Dekker *et al*., 2001, 2003). The range of moisture quantities in which maximum seed germination occurred increased as temperatures changed from constant, to 10°C and then again to 20°C differences in diurnal thermocycles. Although, the surface water film thicknesses increased, this added resistance to diffusion may have been offset by the added oxygen available in the seed interior from the acute oxy-hydro-thermal time signal.

CONCLUSION

These observations indicate a robust, dynamic system of germination control by the transduction of external moisture and thermocycle signals from the soil to the seed embryo as modulated by the characteristics of the seed hull surface. The seed hull is the interface between the seed organism and its external environment.

The seed in unable to control its temperature, but morphology of its exterior surface provide a means by which the seed is able to regulate the other 2 requisites for germinative growth, moisture and oxygen.

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REFERENCES

Atchison, B., 2001. Relationships between foxtail (*Setaria* sp.) primary dormancy at abscission and subsequent seedling emergence. M.S. Thesis, Iowa State University, Ames.

Casada, M.E., 2002. Moisture adsorption characteristics of wheat and barley. Trans. Am. Soc. Agric. Eng., 45 (2): 361-368.

Dekker, J. and M. Hargrove, 2002. Weedy adaptation in *Setaria* sp. V. Effects of gaseous environment on giant foxtail (*Setaria faberi* R. Hermm.) (Poaceae) seed germination. Am. J. Bot., 89 (3): 410-416.

Dekker, J., J. Lathrop, B. Atchison and D. Todey, 2001. The weedy *Setaria* sp. phenotype: How environment and seeds interact from embryogenesis through germination. Proceedings of the British Crop Protection Conference-Weeds, pp: 65-74.

Dekker, J., 2003. The foxtail (*Setaria*) species-group. Weed Sci., 51: 641-646.

Dekker, J., 2004a. The Evolutionary Biology of the Foxtail (*Setaria*) Species-group. In: Inderjit (Ed.). Weed Biology and Management, Kluwer Academic Publishers, The Netherlands, pp: 65-113.

Dekker, J., 2004b. Invasive plant life history determinants of yield in disturbed agro-ecosystems. XIIth International Conference on Weed Biology, Dijon, France. Association Française pour la Protection des Plantes Annales, pp: 5-14.

Dekker, J., B. Atchison and K. Jovaag, 2003. *Setaria* sp. seed pool formation and initial assembly in agro-communities. Aspects of Applied Biol., 69: 247-259.

Dekker, J., B.I. Dekker, H. Hilhorst and C. Karssen, 1996. Weedy adaptation in *Setaria* sp. IV. Changes in the germinative capacity of *S. faberi* embryos with development from anthesis to after abscission. Am. J. Bot., 83 (8): 979-991.

Essery, R.E., B.H. Kirsop and J.R.A. Pollack, 1954. Studies in barley and malt: I. Effects of water on germination tests. J. Institute of Brewing, 60: 473-481.

Gaber, S.D. and E.H. Roberts, 1969. Water-sensitivity in barley seeds: I. A re-appraisal of the auxin hypothesis. J. Institute of Brewing, 75: 299-302.

Harper, J.L., 1965. Establishment, Aggression and Cohabitation in Weedy Species. In: Baker, H.G. and G.L. Stebbins (Eds.). Genetics of Colonizing Species. Proc. Academic Press, New York. First Int. Union of Biolog. Sci. Symposia on General Biol., pp: 243-265.

Jovaag, K., 2006. Weedy *Setaria* species-group heteroblasty blueprints seedling recruitment. Ph.D. Thesis, Iowa State University, Ames.

Parti, M., 1993. Selection of mathematical models for drying grain in thin-layers. J. Agric. Eng. Res., 54: 339-352.

Pollack, J.R.A., B.H. Kirsop and R.E. Essery, 1955. Studies in barley and malt: IV. Experiments with dormant barley. J. Institute of Brewing, 61: 301-307.

Rost, T.L., 1971. Structural and histochemical investigations of dormant and non-dormant caryopsis of *Setaria lutescens* (Gramineae). Ph.D. Thesis Iowa State University, Ames.

Rost, T.L., 1972. Ultrastructure and physiology of protein bodies and lipids from hydrated dormant and non-dormant embryos of *Setaria lutescens*. Am. J. Bot., 59: 607-616.

Rost, T.L., 1973. The anatomy of the caryopsis coat in mature caryopses of the yellow foxtail grass (*Setaria Lutescens*). Botanical Gazette, 134 (1): 32-39.

Rost, T.L., 1975. The morphology of germination in *Setaria lutescens* (Gramineae): The effects of covering structures and chemical inhibitors on dormant and non-dormant florets. Ann. Bot., 39 (159): 21-30.

Rost, T.L. and N.R. Lersten, 1970. Transfer aleurone cells in *Setaria lutescens* (*Gramineae*). Protoplasma, 71: 403-408.

Rost, T.L. and N.R. Lersten, 1973. A synopsis and selected bibliography of grass caryopsis anatomy and fine structure. Iowa State J. Res., 48: 47-87.

Sareini, H.T., 2002. Identification and characterization of the major seed peroxidase in *Setaria faberi*. M.S. Thesis, Iowa State University, Ames.

Warwick, S.I., 1990. Allozyme and life history variation in five northwardly colonizing North American weed species. Pl. Syst. Evol., 169: 41-54.

Wolfram, S., 1999. The Mathematica Book. 4th Edn. Cambridge University Press, Cambridge, UK.