

Population-based threshold models describe weed germination and emergence patterns across varying temperature, moisture and oxygen conditions

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Summary

1. Opportunities for diversifying the management of weedy populations may be enhanced through accurate predictions of seedling emergence, because the timing and success of control measures often hinges on the timing of weed emergence. We used population-based threshold models to establish the temperature, moisture and oxygen conditions for optimum germination of herbicide-resistant and -susceptible *Echinochloa phyllopogon*, a weed of temperate paddy rice, and applied them to predict emergence from field soil.

2. We combined hydrothermal time for germination, accounting for within-population variation in base water potentials (Ψ_b), with thermal time for early seedling growth to predict the quantity and proportional size of the emergence flushes that constitute final recruitment.

3. Emergence in field soils was reduced by moisture stress and flooding, especially for the resistant population. In all populations, germination rates increased between 9.5 and 31 °C, Ψ_b was <-1.0 MPa, and there was no sensitivity to oxygen supply.

4. *Synthesis and applications.* Population-based threshold models produced physiologically meaningful germination parameters, which are useful in defining the environmental constraints to germination, and predicting *Echinochloa phyllopogon* germination and emergence in field soils. By exploring the effects of temperature, water stress and flooding on germination and emergence, we predict irrigation regimes for optimising recruitment and the timing of weed control.

Key-words: base water potential, flood tolerance, hydrotime, metabolic herbicide resistance, moisture stress, seed burial depth, seedling recruitment, stale seedbed, thermal time

Introduction

Seedling emergence is considered as the most important phenological event for defining whether an annual plant will grow, compete and reproduce successfully (Forcella *et al.* 2000). Precise estimates of the timing of emergence can be critical to the success or failure of weed control measures (Grundy *et al.* 2000). For an individual seedling, time to emergence is determined by three separate but consecutive processes: dormancy release, germination and growth to the surface (Vleeshouwers & Kropff 2000), which are each partly controlled by environmental factors like temperature, moisture and soil air composition (Forcella *et al.* 2000). When seed populations are subjected

to gradients of these factors, physiologically based parameters can be obtained and used to gauge dormancy release and predict germination (Allen *et al.* 2007). Accordingly, population-based threshold models (PBTM) have been developed to describe germination responses to temperature, water potential (Bradford 2002) and oxygen (Bradford, Côme & Corbineau 2007) and have been used to predict crop seedling emergence (Finch-Savage, Rowse & Dent 2005). Field predictions of weed seedling emergence for adequate timing of weed control must involve a mechanistic integration of soil microclimate variables (Forcella *et al.* 2000), account for interpopulation variability in both germination and shoot growth to the soil surface (Grundy 2003), and consider the effects of seed burial depth (Grundy, Mead & Burston 2003).

The temperate region summer annual weed *Echinochloa phyllopogon* (Stapf) Koss can germinate and initiate shoot

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growth under hypoxia in flooded paddies (Yamasue 2001), causing up to 50% rice yield losses in California (Hill *et al.* 1985). Heavy reliance on thiocarbamate herbicides for *E. phyllopogon* control has enabled the selection and spread of biotypes with resistance to most available grass herbicides selective to rice (Fischer *et al.* 2000). An accurate prediction of germination and emergence in herbicide-resistant (R) populations could bolster the efficacy of alternative control measures. For example, it could help define the optimum timing for herbicide application in the stale seedbed technique, which entails recruiting and treating weeds with herbicides, for which resistance does not exist, prior to planting the crop (Johnson & Mullinix 1995). Accurate estimates of emergence timing are essential if growers are to adopt this technique (Fischer *et al.* 2009). While R *E. thinsp;phyllopogon* plants are smaller and shatter seeds earlier than herbicide-susceptible (S) plants (Tsuiji *et al.* 2003; Boddy *et al.* 2012), information is lacking on differences between R and S germination and emergence dynamics that could further contribute to control of R populations. The existence of such differences can be evaluated by studying multiple populations from diverse origins to assess whether those differences are consistently expressed through emergence variability (Vila-Aiub, Neve & Roux 2011).

To describe germination patterns and make physiologically based weed emergence predictions under varying temperatures and irrigation regimes, we (i) applied PBTM to define nondormant *E. phyllopogon* seed responses to temperature, water potential and oxygen availability in soil; (ii) used model parameters to assess germination differences among R and S populations; (iii) combined a hydrothermal germination PBTM with a thermal time-driven seedling growth model adjusted for soil depth to predict seedling emergence from field soil; and (iv) used this approach to identify an irrigation regime that will optimise seedling emergence. This research demonstrates how a PBTM approach to analysing germination and emergence, grounded on readily obtainable environmental data, can describe the effects of temperature, moisture and oxygen on germination and emergence, might be used to manipulate field germination and emergence patterns, and could inform the weed control decision-making process.

Materials and methods

PLANT MATERIAL

Echinochloa phyllopogon seed populations, representing the range of phenotypic variability previously reported in California (Tsuiji *et al.* 2003), were collected in rice fields from across the Sacramento Valley of California, tested for herbicide resistance and subsequently classified as S (populations CR and HR) or R (populations KS, RD and SW) (Boddy *et al.* 2012; Appendix S1). Seeds of each population were harvested from 38 plants in early fall and thereafter stored dry at 3 °C. Three months before exper-

imentation in late spring, seeds were transferred to water-filled containers and dark stored at 3 °C to break dormancy by simulating winter moisture and temperature (Baskin & Baskin 2001). Thus, the experiments described here address germination responses in nondormant seeds. Seeds were counted as germinated whenever radicle or coleoptile growth exceeded 1 mm. Unless otherwise noted, plants from populations CR, HR, KS and SW were used in all studies described herein.

GERMINATION EXPERIMENTS

Germination response to temperature

Temperature effects on germination was determined in 2010 on isothermal lanes of a thermogradient table set at 15, 22, 25, 28, 31, 35 and 40 °C, assuming sensitivity to temperature fluctuations was removed during dormancy breakage (Batlla, Verges & Benech-Arnold 2003). For each population, sets of approximately 55 seeds were placed in three 3-cm Petri dishes and covered in 2-mL DI water. Petri dishes were sealed with Parafilm and exposed to 14-h photoperiods under 18 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD; water was replenished as needed. Germinated seeds were tallied and removed every 12 h over ten days.

Germination sensitivity to moisture and oxygen

The effects of water potential (Ψ) or oxygen concentrations (O_x) on germination were assayed in 2008 in two concurrent experiments. Because *E. phyllopogon* has evolved in flooded environments (Yamasue 2001), we assayed germination under fairly moist conditions using polyethylene glycol 8000 (PEG; Fisher Scientific, Pittsburgh, PA, USA) solutions of 0 (pure DI water), -0.2, -0.4 and -0.7 MPa prepared according to Michel (1983). The Ψ levels were verified with a dewpoint water potential meter (WP4 Dewpoint Potential Meter, Decagon Devices, Pullman, WA) at the beginning and end of the experiment. The experimental unit was a 14 × 14 × 5 cm transparent plastic container placed inside a 3.8-L airtight resealable clear plastic bag (Fig. 1). In each container, one set of 35 seeds for each population was attached to a 2.5 × 2.5 cm section of double-sided acrylic-based adhesive that was fixed to the bottom surface, which allowed seeds to remain in place under 300 mL of either DI water or PEG solution per container. Pressurised 21% oxygen flowed at a rate of 600 mL min^{-1} through containers. Inflow of gas into containers created a positive pressure inside the sealed bag that permanently pushed air outwards through an air-pressure release assembly (Fig. 1).

Using the same experimental arrangement, O_x treatments were bubbled into DI water as described above. Following Al-Ani *et al.* (1985), concentrations of 21, 10, 1, 0.01 and 0.001% were created with pressurised 21% O_2 and pre-mixed O_2/N_2 ratios (Airgas NCN, Sacramento, CA, USA). Immediately before inflow into the containers, O_2 concentrations in gas mixtures were verified with a headspace trace oxygen analyser (Pac Check 650, Mocon Inc., Minneapolis, MN, USA).

Both the Ψ and O_x experiments were conducted in a growth chamber set at a constant 25 °C and 12-h photoperiods of 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD (halogen lights) and were replicated six times with populations randomly arranged within each container. Germinated seeds were counted and removed daily for the first 5 days of the experiment and every 2–3 days thereafter until day 24.

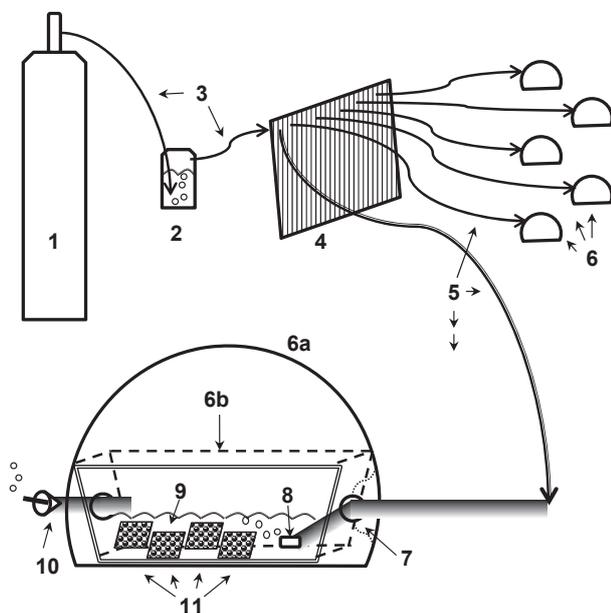


Fig. 1. Experimental set-up for evaluating moisture and oxygen effects on germination: 1) 6.1 m³ cylinder with pre-mixed concentration of O₂ (remainder N₂); 2) water-filled flask providing moisture; 3) clear Nalgene tubing; 4) flow board; 5) 4.8-mm inner diameter, 9.5-mm outer diameter latex tubes; 6) experimental units consisting of 6a) a 3.8-L clear airtight resealable plastic bag surrounding 6b) a 14 × 14 × 5 cm transparent plastic container; 7) airtight grommet-lined opening; 8) aquarium bubbler; 9) 300 mL of DI water or polyethylene glycol (PEG) solution; 10) air-pressure release assembly: airtight grommet, 4-cm tubing segment, rubber stopper and syringe needle; 11) one 2.5 × 2.5 cm cut section of Velcro adhesive industrial strip with 35 seeds for each population.

SEEDLING EMERGENCE EXPERIMENTS

Early seedling growth

Data for predicting early seedling growth under fully hydrated aerobic conditions were obtained by placing single pre-germinated seeds in 9 × 9 cm pots and growing them hydroponically in a glasshouse under 28/14 °C day/night temperatures, 50% relative humidity and natural light supplemented by 16 h of 900 μmol m⁻² s⁻¹ PPFD, to approximate late-May conditions in mid-Sacramento Valley rice fields (UCIPM 2011). Seedling height was measured at 3-day intervals during the first 2 weeks of growth, in five replicate plants per population arranged in a completely randomized design. The experiment was repeated.

Seedling emergence from soil

The efficacy of combining the germination and early growth models to predict seedling emergence was tested on data from a 2009 outdoor experiment. Two Sacramento Valley rice soils: HR (Yolo clay loam, fine-silty, mixed, nonacid, thermic Typic Xerorthents, 36% clay, 1.8% OM) and RD (Castro clay, Fine, thermic Typic Calciaquolls, 28% clay, 2.8% OM) were exposed to natural *E. phyllopon* seed rain in September 2008, followed by 35cm rainfall, and average daily temperatures of 4–23 °C, from 1 October 2008 to 30 April 2009 (UCIPM 2011), levels typical of inland northern California winters. Soils were then collected from

depths ≤ 10 cm, mixed for uniform aggregate size and seed distribution, dried for 3 weeks and transferred to 26 × 26 × 5.5 cm nursery flats as a 5-cm-deep layer where most germinable seeds reside (Forcella *et al.* 2000). Flats were subjected to: (i) daily application of 0.5-L water to approximate irrigation in a dry-seeded rice stand establishment system; (ii) intermittent moisture stress by irrigating with 1 L of water every 3 days to explore the effects of periodic field drainage on weed recruitment; or (iii) flooding to 10 cm above the soil surface to impose hypoxia and simulate typical flooded rice conditions. Soil temperature was measured 1 cm below the surface at 15-min intervals using temperature probes (Spectrum Technologies, Inc., Plainfield, IL, USA). Soil moisture probes (Hydrosense, Campbell Scientific, North Logan, UT, USA) were inserted horizontally 3-cm deep into the soil through perforations on the side of the flat. Daily soil moisture readings from each soil were converted to Ψ using a calibration curve obtained by measuring a set of twelve soil samples with both the moisture probe and the dewpoint water potential meter (Rundel & Jarrell 1989). Experimental temperature and moisture conditions are presented in Fig. 2. Five 3 × 1.2 × 0.5 m outdoor basins (blocked replications) were split into three sections; each was randomly assigned a water regime (main plots), within which the two soils (subplots) were randomly arranged. Seedlings were counted as emerged at 2 cm in height above the soil surface, which approximates the growth stage for stale seedbed weed control with glyphosate (Fischer *et al.* 2009).

DATA ANALYSIS: GERMINATION

Final germination was expressed as a percentage of total treatment seed number. Based on germination rate analysis (Appendix S2), models were calculated for the temperature range 15–31 °C, which encompasses average daily springtime soil temperatures in rice cropping areas of the Northern Sacramento Valley in California (UCIPM 2011). We used the Microsoft Excel (2003–2010, Redmond, WA, USA) Solver tool to derive model parameters by minimising the root-mean-square error (RMSE) between simulated and observed data (Huarte & Benach-Arnold 2010). Sets of model parameters were derived by replication and subjected to ANOVA using JMP 8.0 software (SAS Institute Inc. Cary, NC, USA), protected LSD_{0.05} values were calculated therefrom, and averages ± SE are presented (Tables 1 and 2).

Germination and thermal time

Thermal time to germination for g fraction of the population [$\theta_T(g)$] can be expressed as:

$$\theta_T(g) = (T - T_b)t_g \quad \text{eqn 1}$$

where T is experimental temperature, T_b is base temperature for germination, which is assumed to be constant across all seed fractions within each population and t_g is time (days) to germination for fraction g of the population (Covell *et al.* 1986). Because $\theta_T(g)$ (expressed in thermal time units, TU, °C day) is assumed to follow a normal distribution, we used probit analysis to obtain model parameter values (Dahal, Bradford & Jones 1990):

$$\text{probit}(g) = \{[\log(T - T_b)t_g] - \log \theta_T(50)\} / \sigma_{\theta T} \quad \text{eqn 2}$$

where $\text{probit}(g)$ is the probit transformation of cumulative germination that linearises its cumulative normal

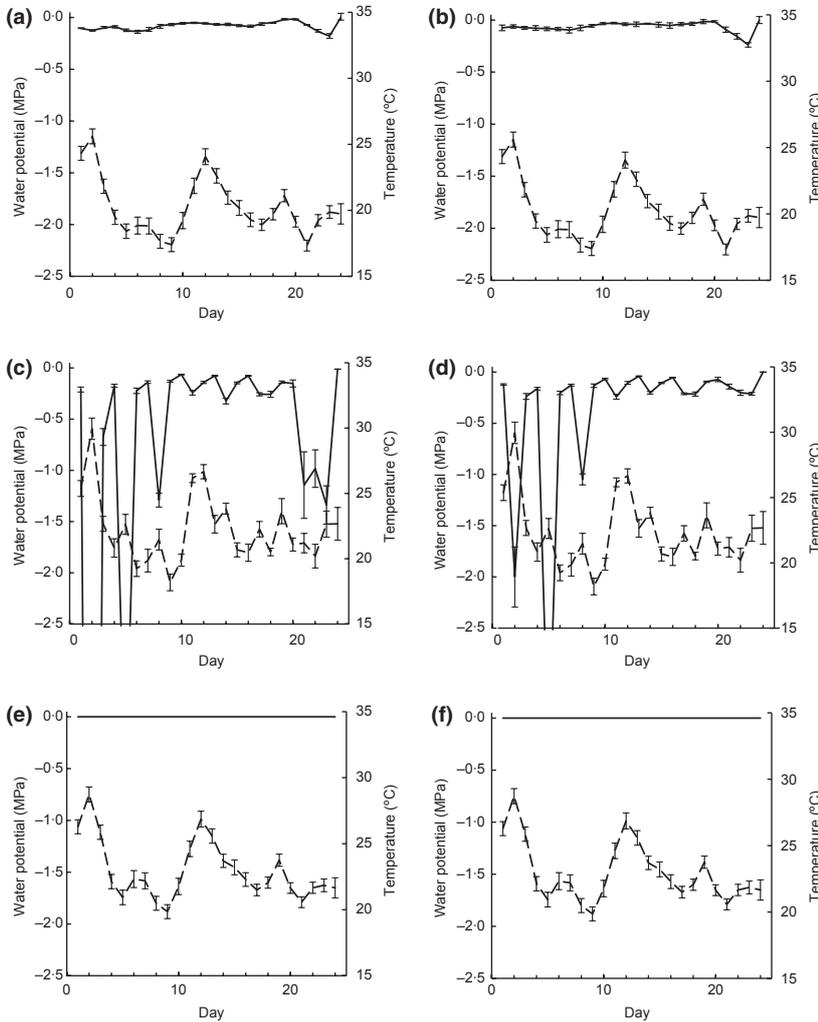


Fig. 2. Environmental conditions during field soil emergence experiment for daily irrigation, intermittent irrigation and flooding in soils HR (panels A, C and E, respectively) and RD (panels B, D and F). Ψ values for each soil (solid lines) are calculated as the average of moisture readings taken at 8 AM and 8 PM daily. Temperatures (dashed lines) represent 24-h averages pooled for both soils. Bars represent SE ($n = 5$).

distribution (on a logarithmic time scale), $\theta_T(50)$ is median thermal time to germination and $\sigma_{\theta T}$ is the standard deviation of thermal times to germination among individual seeds in the population (a measure of germination synchrony). These parameters were used to reproduce the original germination data as cumulative normal curves of

the function:

$$G = \{ \log(t_g) - [\log \theta_T(50) - \log(T - T_b)] \} / \sigma_{\theta T} \quad \text{eqn 3}$$

where G is cumulative germination percentage and was plotted against accumulated TU calculated as per eqn 1.

Table 1. Parameters of the thermal time model characterising the responses of *Echinochloa phyllopogon* populations to constant suboptimal temperature regimes, estimated by replication using eqn 2; T_b is the base temperature for germination, θ_{T50} is median thermal time to germination and $\sigma_{\theta T}$ is the standard deviation of the thermal time distribution. Values are averages \pm SE

<i>E. phyllopogon</i> population	T_b (°C \pm SE)	θ_{T50} (°C day \pm SE)*	$\sigma_{\theta T}$ (°C day \pm SE)
CR (S)	9.1 \pm 0.3	37.7 \pm 0.4	1.19 \pm 0.01
HR (S)	9.3 \pm 0.4	33.8 \pm 0.2	1.26 \pm 0.03
KS (R)	9.4 \pm 0.3	31.5 \pm 0.9	1.28 \pm 0.01
SW (R)	9.3 \pm 0.2	34.1 \pm 0.4	1.27 \pm 0.03
LSD _{0.05} †	NS	1.1	NS

* $\theta_T(50)$ is presented as $10^{\theta_{T50}}$.

†d.f. = 12.

Table 2. Parameters of the hydrotime model characterising the responses of *Echinochloa phyllopogon* populations to water potential (Ψ) regimes, estimated by replication using eqn 5; θ_H , hydrotime constant; $\Psi_b(50)$ is median base water potential and $\sigma_{\Psi b}$ is the standard deviation in Ψ_b among seeds

<i>E. phyllopogon</i> population	θ_H (MPa day \pm SE)	$\Psi_b(50)$ (MPa \pm SE)	$\sigma_{\Psi b}$ (MPa \pm SE)
CR (S)	2.91 \pm 0.13	-1.12 \pm 0.03	0.27 \pm 0.02
HR (S)	1.97 \pm 0.21	-1.13 \pm 0.03	0.17 \pm 0.07
KS (R)	1.81 \pm 0.10	-1.06 \pm 0.04	0.15 \pm 0.04
SW (R)	1.75 \pm 0.04	-1.07 \pm 0.01	0.11 \pm 0.02
LSD _{0.05} *	0.28	0.06	0.07

*d.f. = 24.

Hydrotime to germination

The hydrotime model (Gummerson 1986) has a similar structure as the thermal model but different assumptions:

$$\theta_H = [\Psi - \Psi_b(g)]t_g \quad \text{eqn 4}$$

where hydrotime to germination (θ_H) is constant for all seeds in a population, Ψ is experimental water potential, the base water potential [$\Psi_b(g)$] below which germination is prevented for a given fraction g of the population follows a normal distribution and accounts for within-population variation in germination and t_g is time to germination of fraction g . The parameters of eqn 4 were estimated as (Dahal & Bradford 1990):

$$\text{probit}(g) = [\Psi - (\theta_H/t_g) - \Psi_b(50)]/\sigma_{\Psi_b} \quad \text{eqn 5}$$

where $\Psi_b(50)$ is median Ψ_b and σ_{Ψ_b} is the standard deviation in Ψ_b among seeds in a population. Combining germination data from a range of Ψ , we used eqn 5 to plot predicted germination as a function of thermal time by expressing t_g in TU.

Oxygen time to germination

Germination sensitivity to oxygen was analysed using an adaptation of the hydrotime model that assumes a normal distribution of base oxygen requirement (O_{x_b}) across the seed population and a constant oxygen time (θ_{O_x}) to germination for all seeds (Bradford, Côme & Corbineau 2007):

$$\theta_{O_x} = [\log O_x - \log O_{x_b}(g)]t_g \quad \text{eqn 6}$$

where O_x is daily average oxygen percentage in the micro-environment surrounding the seed, and $O_{x_b}(g)$ is the base or minimum level of oxygen just allowing germination of a given fraction g of the population.

PREDICTING SEEDLING EMERGENCE TIME COURSES

Emergence from field soils was predicted by modelling germination (using PBTM parameters) and early shoot elongation as consecutive phases of emergence, and adjusting for variation in seed burial depth. According to the hydrotime concept, each fraction of imbibing seeds in a population accrues hydrotime when soil $\Psi > \Psi_b(g)$ and $T > T_b$. Thus, combining responses to T and Ψ (eqns 1 and 4) into a single expression, a hydrothermal time constant (θ_{HT}) for suboptimal temperatures is defined (Gummerson 1986):

$$\theta_{HT} = (T - T_b)[\Psi - \Psi_b(g)]t_g \quad \text{eqn 7}$$

While thermal and hydrotime can accumulate concurrently in a fraction of the population once $\Psi > \Psi_b(g)$, germination may only occur once the full θ_H has accrued (Bradford 2002). To account for a normal distribution of Ψ_b among fractions of the population, we arbitrarily split the populations into 13 fractions and calculated Ψ_b values for each fraction to determine their corresponding starting points of θ_{HT} accrual (Fig. 3). To this end, we

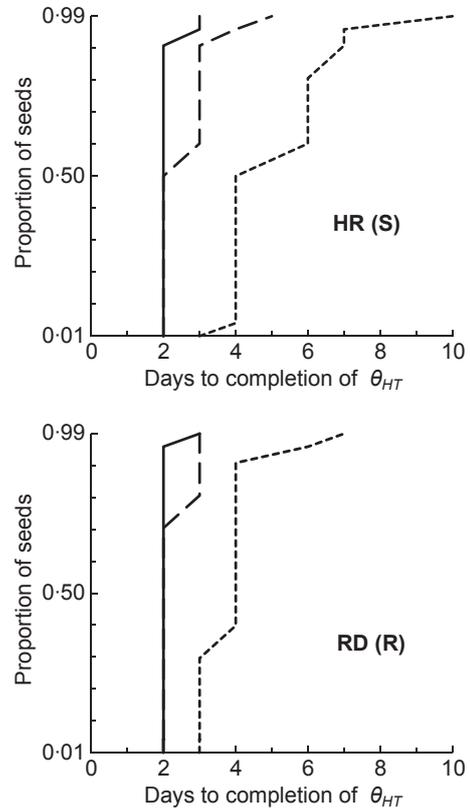


Fig. 3. Predicted germination and accrual of hydrothermal time (θ_{HT}), under daily irrigation (dashed lines), intermittent irrigation (dotted) and flooding (solid), for 13 fractions of seed populations HR and RD according to their respective base water potential [$\Psi_b(g)$] values. θ_{HT} were 30.93 for HR and 27.77 for RD calculated using eqn 7; $\Psi_b(g)$ values were calculated with eqn 8 and ranged from -0.67 to -1.60 MPa for HR and -0.71 to -1.41 MPa for RD. $\Psi_b(g)$ values were obtained for fractions 0.01, 0.05, 0.10, 0.20, 0.90, 0.95 and 0.99 of each seed population.

used the hydrotime parameters listed in Table 2, solved for Ψ_b in eqn 4 and substituted into eqn 5:

$$\Psi_b(g) = \Psi_b(50) + \text{probit}(g)\sigma_{\Psi_b} \quad \text{eqn 8}$$

For each day that $\Psi > \Psi_b(g)$, seeds of that fraction accrued a daily portion of hydrothermal time, calculated as $(T - T_b)[\Psi - \Psi_b(g)]$. Accrual was completed for fraction g upon reaching θ_{HT} , which was calculated as in eqn 7 with values of $[\Psi - \Psi_b(g)]t_g = \theta_H$ and T_b for populations HR and RD taken from Tables 2 and 1, respectively (values for population RD are averages of KS and SW); the value of T is that of the Ψ experiment (25 °C). Thus, predicted t_g for the onset of germination for each fraction of the population was calculated and converted to accumulated daily thermal units for germination (TU_G):

$$TU_G = \sum(T_a - T_b) \quad \text{eqn 9}$$

where T_a is ambient temperature on a given day.

Under well hydrated aerobic soil conditions, early shoot elongation (*ShE*) should be temperature driven (Wheeler & Ellis 1991); thus the thermal time for emergence of fraction g of the seed population [$TU_{E(g)}$] was predicted by adding the accumulated

thermal time for shoot elongation (TU_{ShE}) to the hydrothermal time required for germination:

$$TU_{E(g)} = TU_{G(g)} + TU_{ShE} \quad \text{eqn 10}$$

where $TU_{G(g)}$ is the accumulated thermal time for germination (eqn 9) of fraction g as determined by its accrual of θ_{HT} , and TU_{ShE} are TU required for shoot elongation.

To determine TU_{ShE} for each population, plant height was exponentially related to thermal time (Fig. 4); T_b for early growth was assumed to be the same as T_b for germination (Covell *et al.* 1986). Within our 5-cm-deep soil layer, limited burial inhibition of germination was assumed (Boyd & Van Acker 2003). Additional variability in time for emergence was estimated assuming normal distribution of germination response to soil depths (Grundy, Mead & Burston 2003); thus, TU_{ShE} was calculated as:

$$TU_{ShE} = TU_{ShE(x)} + [TU_{ShE(x)}(50)](\sigma)[\text{probit}(E)] \quad \text{eqn 11}$$

where $TU_{ShE(x)}$ is thermal time for shoot elongation to height x , $TU_{ShE(x)}(50)$ is median $TU_{ShE(x)}$, σ is the standard deviation of seed burial depths and $\text{probit}(E)$ is the inverse cumulative distribution of time to emergence. We modelled shoot growth from a median depth of 2.5 cm up to a final plant height of 2 cm above ground for a total average shoot elongation of 4.5 cm. Using the Solver routine as described earlier, a σ of 0.25 was iteratively determined to produce the best prediction for the daily irrigation regime (treatment 1, described earlier in the section on Seedling emergence from soil).

We applied this model to emergence from soils subjected to daily irrigation, intermittent irrigation and flooding using daily temperature and Ψ data (Fig. 2) to calculate hydrothermal time accrual. During periods where Ψ fell well below Ψ_b , we assumed that hydrothermal time accrual was interrupted (Finch-Savage & Phelps 1993). This approach does not account for hydrotime accrual below Ψ_b (Dahal & Bradford 1990) or for hydrotime effects on seedlings.

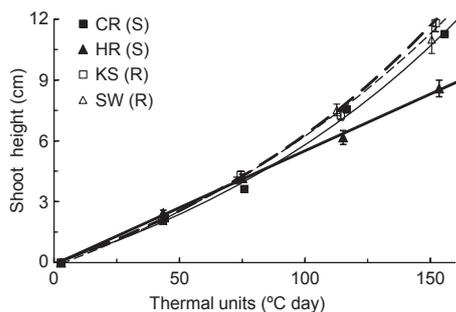


Fig. 4. Shoot length as a function of accumulated thermal units (TU). Fitted equations are: $y_{(CR)} = -5.64 + 5.62\exp(0.0071x)$; $y_{(HR)} = 0.07 + 18.10x$; $y_{(KS)} = -5.92 + 5.88\exp(0.0073x)$; and $y_{(SW)} = -7.66 + 7.47\exp(0.0062x)$, where y is maximum seedling height (cm) and x is $TU = \Sigma(T_a - T_b)$, where T_a is ambient temperature on a given day and T_b is the base temperature for growth (from Table 1); $R^2 > 0.99$, $P_{0.05} < 0.001$ for all regressions. Symbols are means \pm SE ($n = 10$).

Emerged seedling counts were expressed as a fraction of mean final emergence in each treatment and subjected to ANOVA, as described above. We evaluated model predictions using three common indices: root-mean-square error (RMSE), where units are the same as for observations; the modelling efficiency coefficient (EF), where 1 is the optimal value and a score < 0 indicates the observed mean is a better predictor than the model; and the index of agreement (d), which varies from 0.0 to 1.0 with higher values indicating greater agreement between predicted and observed values (Mayer & Butler 1993; Spokas & Forcella 2006).

Results

Average final germination across temperatures up to 35 °C was 97 ± 1 ($n = 16$), 96 ± 1 , 96 ± 1 and $97 \pm 1\%$ for populations CR, HR, KS and SW, respectively; there was no germination at 40 °C except in KS. Predictions based on a suboptimal temperature range of 15–31 °C for all populations fit observed germination data well (Fig. 5). T_b values for all populations ranged from 9.1 to 9.4 °C, and R and S populations did not differ in T_b , θ_{T50} , or synchronicity of germination ($\sigma_{\theta T}$) (Table 1). Average final germination across Ψ treatments was 91 ± 2 ($n = 24$), 97 ± 2 , 96 ± 1 and $98 \pm 1\%$ for CR, HR, KS and SW, respectively. Germination rates were affected by reduced moisture availability and time to 50% germination was more than doubled at -0.7 MPa, which is not even half as dry as the common plant wilting point (-1.5 MPa) (Fig. 6); however, Ψ_b was below -1.0 MPa for all populations (Table 2). Average final germination across oxygen treatments was 94 ± 2 ($n = 30$), 98 ± 1 , 96 ± 2 and $98 \pm 1\%$ for CR, HR, KS and SW, respectively. Failure of convergence in attempting to fit the oxygen-time model to the data (not shown) revealed the lack of germination sensitivity to oxygen availability (Fig. 7) across all populations. Thus, germination was driven by thermal and hydrotime accumulation, regardless of oxygen level, and moisture stress substantially delayed the process. There was no correlation between herbicide resistance and germination responses to temperature, moisture or oxygen.

We applied the PBTM approach to predict germination in field soils subjected to different irrigation regimes, under their respective temperature and moisture characteristics (Fig. 2). Under the daily irrigation regime, soils were within the general range of field capacity with Ψ from 0.0 to -0.1 MPa (Singer & Munns 2002). As different fractions of the seed population successively achieved their θ_{HT} requirements, germination was predicted to occur in a series of flushes 2–5 days and 2–3 days after irrigation was initiated in soils of populations HR and RD, respectively (Fig. 3). Meanwhile, in the intermittent irrigation regime drier conditions, as evidenced by two nonconsecutive days below -2.0 MPa and multiple days below -0.2 MPa (Fig. 2), delayed the completion of θ_{HT} accrual and distributed germination into a greater number of flushes over a longer period (3–10 and 3–7 days after irrigation initia-

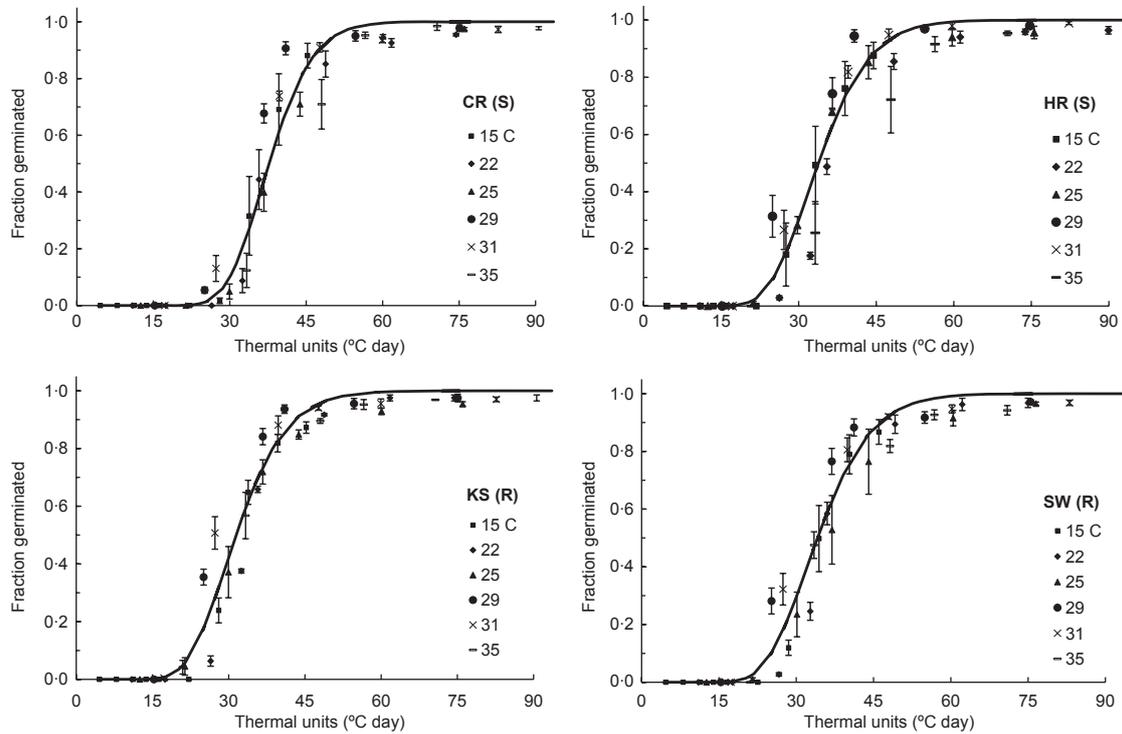


Fig. 5. Cumulative germination across temperature treatments on a thermal time basis. Symbols are averages of three replicates (55 seeds each) \pm SE; prediction curves were calculated for each population with eqn 3 using parameters in Table 1.

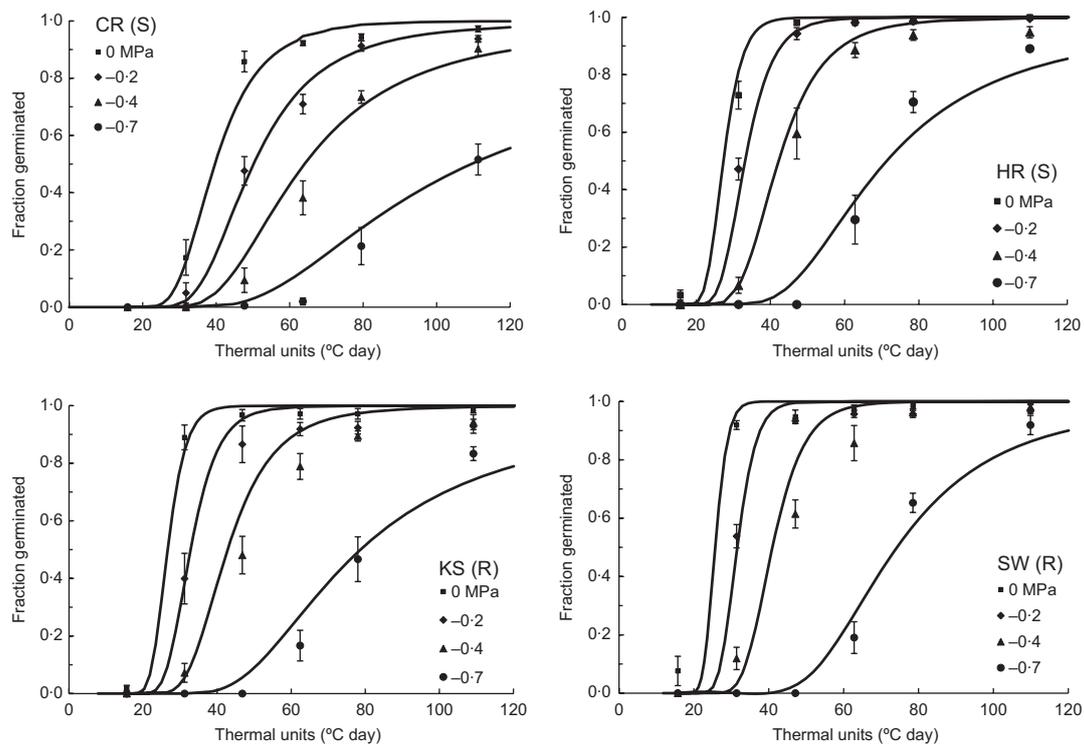


Fig. 6. Cumulative germination across moisture regimes on a thermal time basis. Symbols are averages of six replicates (35 seeds each) \pm SE; prediction curves were calculated for each population with eqn 5 using parameters in Table 2.

tion for HR and RD, respectively) (Fig. 3). Although Ψ in the daily irrigation and flooded regimes were very similar (Fig. 2), flooding concentrated predicted germination in

both soils into a single flush, whereby 90% of seeds germinated on day 2 (Fig. 3), effectively neutralising differences between populations in time to germination.

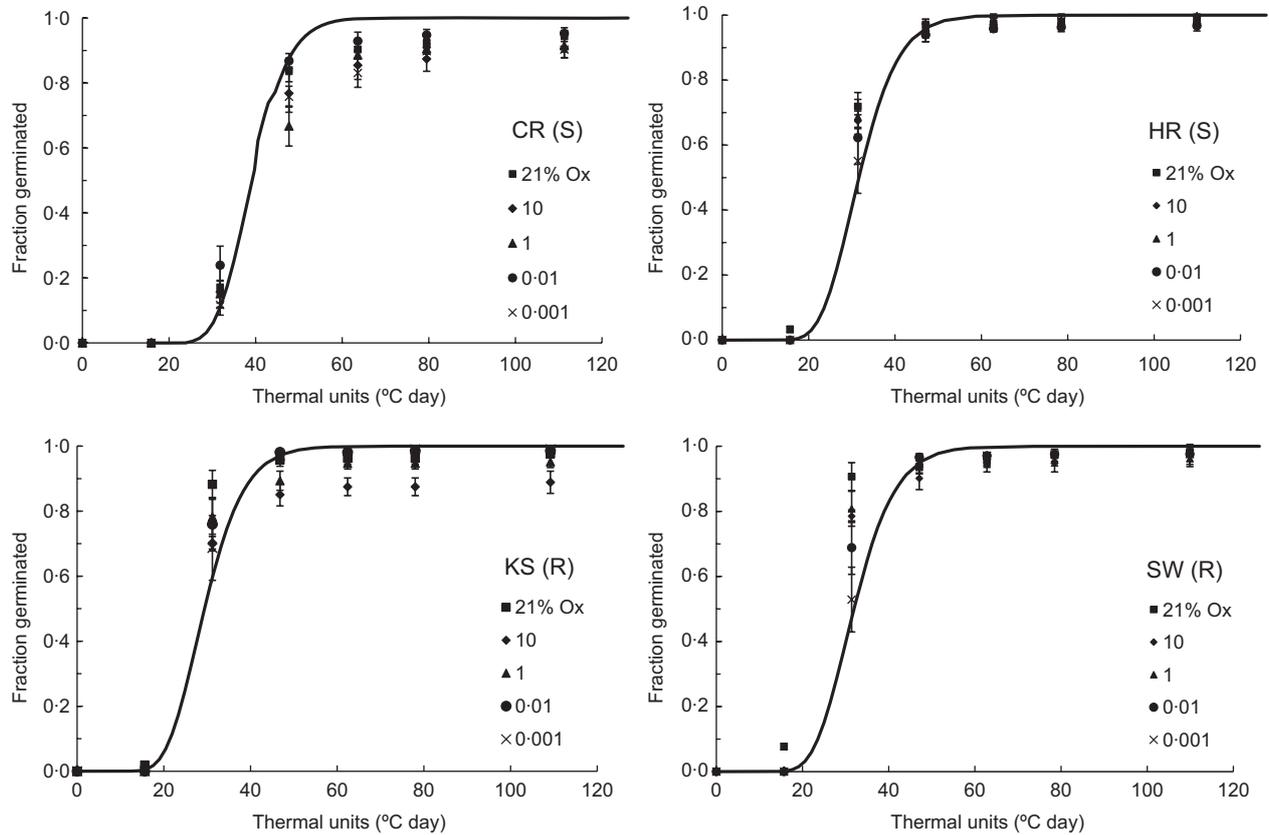


Fig. 7. Cumulative germination across oxygen regimes on a thermal time basis. Symbols are averages of six replicates (35 seeds each) ±SE; prediction curves were calculated for each population according to the thermal model using parameters in Table 1.

Table 3. Average final *Echinochloa phyllopogon* seedling emergence after 24 days of exposure to three irrigation regimes

Soil	Daily irrigation	Intermittent irrigation	Flooding	LSD _{0.05} *
	No. of seedlings ± SE			
HR	19 ± 3	14 ± 3	12 ± 2	6
RD	25 ± 3	5 ± 2	8 ± 1	

*LSD for the interaction of irrigation regime by population; d.f. = 30.

Of the three moisture regimes tested, daily irrigation produced the greatest number of emerged seedlings in both soils (Table 3). Emergence time course predictions (Fig. 8a,b) generally matched observed data in both soils under daily irrigation, as corroborated by the model evaluation indices (Table 4). This modelling approach does not account for water stress or flooding-induced inhibitions of seedling growth; thus, we applied it to different irrigation regimes to explore possible effects of soil moisture availability and flooding upon early growth. Compared to the daily irrigation regime, final seedling emergence and emergence rates were reduced by the moisture stress under intermittent irrigation, especially in soil RD (Table 3, Fig. 8c,d). Similarly, in soils exposed to

hypoxic stress via flooding, both final emergence and emergence rates were inhibited (Table 3, Fig. 8e,f). The accuracy of model predictions declined with both moisture stress and flooding (Table 4).

Discussion

GERMINATION ASSAYS

High final germination across experiments suggests dormancy was minimal in both seed lots used (Baskin & Baskin 2001). Values of T_b were lower than those reported for *E. crus-galli* and many other California summer annual weeds (Steinmaus, Prather & Holt 2000), averaged 3 °C less than reported for *indica* type rice genotypes (Ali, Naylor & Matthews 2006), and were about the same as for *japonica* type rice cultivars (Lee 2001). This is consistent with the ability of *E. phyllopogon* to germinate under the cool environment of rice fields in early spring (Williams 2009).

Echinochloa phyllopogon is typically identified with flooded rice fields (Yamasue 2001), but $\Psi_b(50)$ values (Table 2) were comparable to those of many nonaquatic weeds (Grundy *et al.* 2000; Roman, Murphy & Swanton 2000; Batlla & Benech-Arnold 2006), suggesting adaptation to also establish under moderately dry conditions. This explains why *E. phyllopogon* is commonly found in both flooded and dry-seeded rice fields of California. The

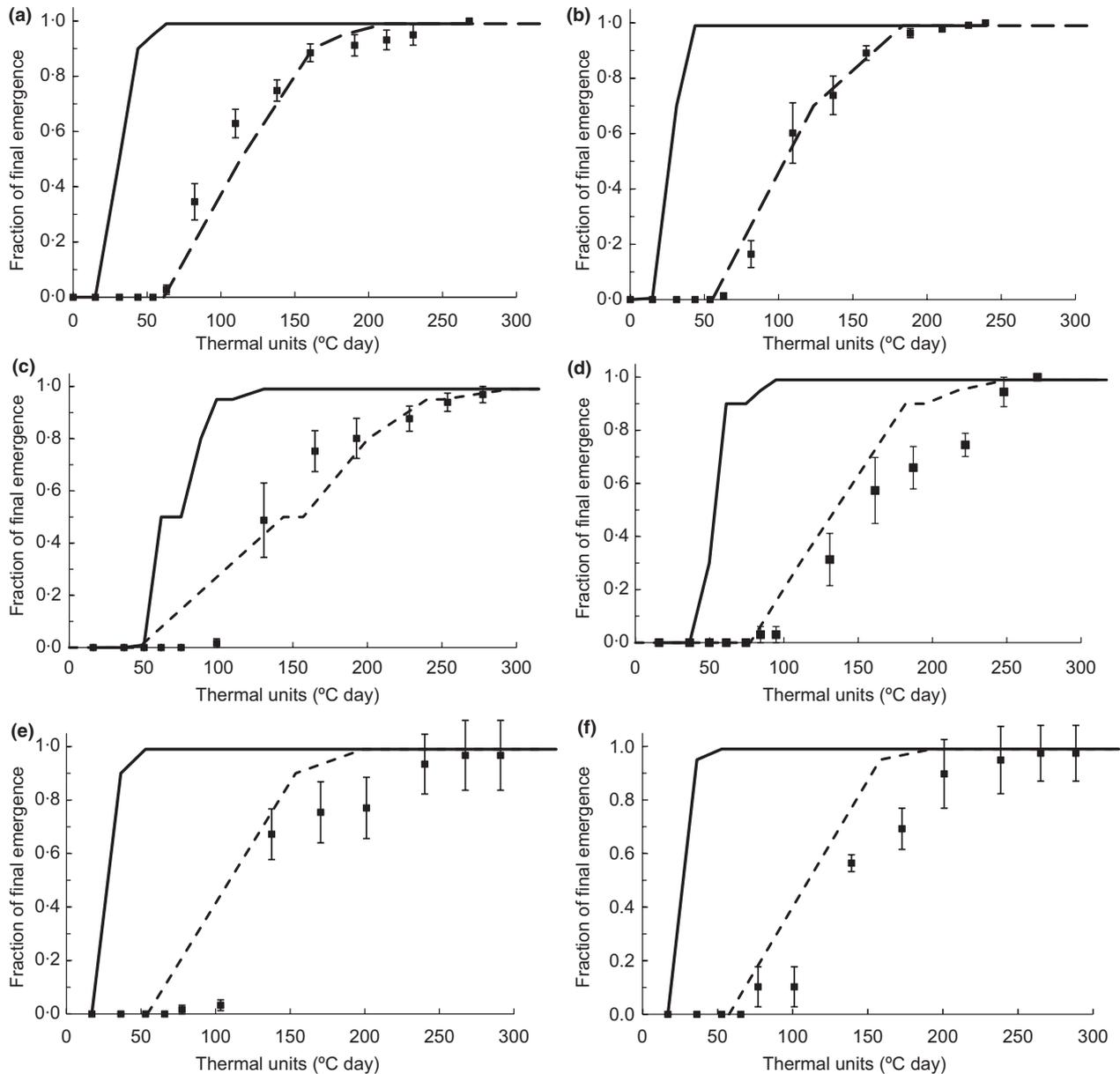


Fig. 8. Cumulative emergence and germination from rice field soils RD and HR, harbouring multiple herbicide-resistant (R) and -susceptible (S) *Echinochloa phyllopogon* seed populations, respectively, and subjected to different irrigation regimes: daily irrigation (a, b), intermittent irrigation (c, d) and flooding (e, f). Germination is predicted (solid lines) using eqns 7–9 with parameters from Tables 1 and 2 and Fig. 2; emergence is predicted (dashed lines) by adding the thermal units (TU, °C day) for growth to 4.5 cm above the soil surface (Fig. 4) to the TU for germination (eqn 10) and adjusting for seed burial depth (eqn 11). Symbols are averages ($n = 5$) of observed values \pm SE.

large differences in θ_H and σ_{ψ_b} between CR and HR agree with the greater genetic and phenotypic variability found among S compared with R populations (Tsuji *et al.* 2003).

The lower oxygen concentrations used in our experiments can be assumed to be lower than those actually found in flooded rice fields (Patrick & Mikkelsen 1971). Thus, we exposed seed to a broad and realistic spectrum of oxygen concentrations. Most seeds will not germinate at oxygen concentrations below 1%, or below 0.016% in the case of rice (cv Cigalon) (Bradford, Côme &

Corbineau 2007). But the *E. phyllopogon* populations studied here were able to germinate regardless of the oxygen concentration, explaining why flooding is an ineffective means of suppressing nondormant *E. phyllopogon* seed germination (Yamasue 2001). This response is consistent with previous results for the species and has been attributed to a broad array of metabolic adaptations (Kennedy *et al.* 1980; Kennedy, Rumpho & Fox 1992; Yamasue 2001). For example, anoxia and chilling induce production of hexokinases, which regulate glucose entry

Table 4. Predictive accuracy of an *Echinochloa phyllopogon* seedling emergence model applied to two soils (HR and RD) after 24 days of exposure to three irrigations regimes (Fig. 8)

Evaluation parameter	HR			RD		
	Daily irrigation	Intermittent irrigation	Flooding	Daily irrigation	Intermittent irrigation	Flooding
Number of observations	50	36	45	45	24	45
Mean of observed values \pm SE	0.64 \pm 0.12	0.65 \pm 0.12	0.64 \pm 0.13	0.63 \pm 0.13	0.53 \pm 0.14	0.53 \pm 0.13
Mean of simulated values \pm SE	0.62 \pm 0.13	0.65 \pm 0.10	0.78 \pm 0.08	0.65 \pm 0.13	0.65 \pm 0.14	0.64 \pm 0.11
Root-mean-square error (RMSE)	0.077	0.111	0.181	0.046	0.144	0.162
Modelling efficiency (EF)	0.958	0.910	0.827	0.987	0.839	0.834
Modelling index (<i>d</i>)	0.990	0.959	0.944	0.996	0.961	0.953

into glycolysis and increase pyruvate supply for fermentation and ATP production under anoxia (Fox *et al.* 1998; Lasanthi-Kudahettige *et al.* 2007). Our pre-germination chilling of seeds in water to remove dormancy could therefore have also rendered them less sensitive to oxygen deficiency (Fox *et al.* 1998).

EMERGENCE MODELLING

The accuracy in predicting emergence under the daily irrigation regime (Table 4) lends support to the approach of combining thermal and hydrotime accumulation to predict germination of nondormant seeds (Bradford 2002) and to our assumption of a normal distribution of seed germination across burial depths used to predict seedling emergence. Reduced model accuracy in the intermittent irrigation regime (Table 4) suggests moisture stress may have affected early shoot elongation. Other factors can also contribute to the loss of significant portions of germinated seed prior to emergence (Grundy 2003), such as drought-induced secondary dormancy (Hegarty 1978) or the crusting of dry soils (Forcella *et al.* 2000).

There was no effect of oxygen deficiency on nondormant seed germination (Fig. 7); therefore, reduced emergence under flooding (Table 3) might be attributed to the inhibiting action of anoxia on germination of dormant fractions of the seed bank or on early seedling growth. Because *E. phyllopogon* under complete anoxia does not produce roots until its coleoptiles are able to reach the water surface and access ambient oxygen (Kennedy *et al.* 1980), some seedlings may have perished during this phase. Although *E. phyllopogon* is a weed in aquatic environments, anoxia reduces shoot elongation rates (Fox, Kennedy & Rumpho 1994) and flooding is used as a weed control technique to suppress its growth (Williams *et al.* 1991). Therefore, although flooding may have been the optimal irrigation treatment to enhance the uniformity and speed of germination in our experiment (Fig. 3), it may ultimately delay

E. phyllopogon emergence and the timing of a subsequent control measure.

Conclusions

Using a PBTM approach, we delimited the environmental framework for *E. phyllopogon* germination, confirmed the plasticity of this aquatic species as regards oxygen availability, and demonstrated its ability to germinate across a wide range of suboptimal temperatures and under drier than expected conditions. We found minimal differences in germination between nondormant R and S populations, but large reductions in R seedling emergence from moisture-stressed soils, suggesting lower drought tolerance compared with S seedlings.

Elimination of this multiple herbicide-resistant weed requires the use of herbicides or tools that cannot be selectively employed once the crop is established. Thus, weed control must be implemented prior to seeding the crop and once weed seedlings have emerged to prevent interfere with the crop (Fischer *et al.* 2009). But, the timing of weed control must not be delayed beyond what is necessary for full weed emergence to avoid yield losses due to a shorter growing season. The application of PBTM to field soils, using readily obtainable environmental data, revealed a potential means of forecasting field germination as a series of consecutive flushes, determined by soil water status, population moisture thresholds and temperature accumulation, which could be used to predict the timing of seedling emergence. Given the absence of negative effects of hypoxia on germination, the greater uniformity of germination under flooding, and the inhibitory effects of hypoxia and moisture stress during emergence, optimisation of *E. phyllopogon* field emergence to enable timely weed control would initially involve flooding followed by field draining for adequate seedling growth to emergence under near field capacity conditions. However, beyond the optimisation of a weed control tactic, this approach could also help define habitat suitability for potential invasions, and thus, provides a tool for containing weed spread (Panetta & Cacho 2012). It could be similarly useful in restoration ecol-

ogy, where moisture levels influence seedling emergence patterns and subsequent species establishment (Ambrose & Wilson 2003). Future improvements upon this approach may result from improved knowledge of the effects of seed burial depth on emergence, germination dynamics in dormant seed and of anoxia and moisture stress effects on emerging seedling growth.

Acknowledgements

We are grateful to the California Rice Research Board for financial support. Thanks also to Drs. Mikal Saltveit, Arnold Bloom and John Krochta for generously providing facilities, advice and equipment.

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Received 19 April 2012; accepted 22 August 2012
Handling Editor: Jennifer Finn

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Population origin and herbicide resistance characterization.

Appendix S2. Determination of temperature ranges for modeling.

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