

ALS inhibitor-resistant smallflower umbrella sedge (*Cyperus difformis*) seed germination requires fewer growing degree days and lower soil moisture

Research Article

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Abstract

The repetitive use of ALS inhibitors for smallflower umbrella sedge (*Cyperus difformis* L.) control has selected for herbicide-resistant (R) populations that threaten the sustainability of rice (*Oryza sativa* L.) production and demand alternative control measures be developed. A better understanding of seedling recruitment patterns at the field level is required to optimize the timing and efficacy of control measures. Therefore, a population-based threshold model was developed for optimizing germination prediction in multiple acetolactate synthase (ALS)-R and ALS-susceptible (ALS-S) *C. difformis* biotypes and applied to field-level emergence predictions. Estimated base temperatures (T_b) ranged from 16.5 to 17.6 °C with no clear pattern between biotypes; such values are higher than T_b values of other important rice weeds, as well as for rice. Germination rates increased linearly from 16 to 33.7 °C. ALS-R seeds germinate faster due to smaller median thermal times to germination ($\theta_{T(50)}$) while also displaying lower germination synchronicity across water potentials. Interestingly, ALS-R biotypes were capable of germinating under lower moisture availability, as indicated by their lower (more negative) base water potential values ($\Psi_{b(50)}$) for seed germination; $\Psi_{b(50)}$ values ranged from -0.24 to -1.13 MPa. In-field soil germination measurements found thermal times to emergence varied across three water regimes (daily water, flooded, or saturated). Seedling emergence under the daily water treatment was fastest; however, total seedling density was lower than for the other water regimes. In order to optimize springtime *C. difformis* seedling emergence, soil moisture should be kept around field capacity, as germination is hindered at lower moisture contents. By predicting when most of the seed population germinates, the thermal-time model can address issues regarding the optimal timing for herbicide applications, thereby allowing for improved *C. difformis* management in rice fields.

Introduction

Smallflower umbrella sedge (*Cyperus difformis* L., CYPDI) is a troublesome annual weed that is widely distributed and constitutes a major deterrent to rice (*Oryza sativa* L.) productivity in 47 countries throughout tropical to warm temperate regions (Chauhan and Johnson 2009). Several traits have enabled this weedy Cyperaceae to persist and disperse as a weed of rice, primarily copious production of small seeds, early maturation, annual habit, and the ability to grow under submerged conditions (Sanders 1994).

Rice agriculture in California and in many regions worldwide relies on herbicides for proper weed management, and among these the acetolactate synthase (ALS, also called acetohydroxyacid synthase or AHAS) inhibitors are frequently used in rice (Merotto et al. 2010). ALS-resistant *C. difformis* populations were detected almost three decades ago and greatly complicated *C. difformis* management in Californian rice fields. Currently, herbicide-resistant (R) *C. difformis* is well documented in rice fields of eight countries (Heap 2019), threatening the sustainability of rice production. Furthermore, resistance to the photosystem II-inhibiting herbicide propanil has also been confirmed in several *C. difformis* biotypes from California (Pedroso et al. 2016), the only such case in rice agriculture worldwide. As a result, the number of efficient chemical tools for *C. difformis* control that growers have at their disposal has steadily decreased over the past decades. Therefore, innovations such as accurate estimations of the required time for weed seed

germination and seedling growth are needed to develop adequate weed management options against this troublesome sedge.

Knowledge of weed seed germination biology and crop–weed interactions have enabled improved effectiveness of weed control (Chauhan and Johnson 2009; Masin et al. 2010). Prediction of *C. diffiformis* emergence would be especially important in this regard, because the relative timing of seasonal weed and crop emergence is critical for the outcome of competition and a major factor affecting crop yield losses due to weed interference (Cousens et al. 1987).

Predicting *C. diffiformis* emergence would also be desirable for optimizing the use of the stale seedbed technique, which is an alternative rice establishment system designed to address the increasing problem of herbicide-resistant weeds in California rice fields (Fischer et al. 2009; Linquist et al. 2008). These systems take advantage of spring irrigation to encourage germination and emergence of a wide range of weed species, which are then controlled before rice sowing by either a broadcast nonselective herbicide application (such as glyphosate) or shallow tillage. The success of the technique largely depends on achieving maximum weed emergence before the preplant weed control. The extra time required for weed seed germination and growth is often a cause of concern, as it may entail a certain delay in rice seeding compared with a conventional system. In California, early planting places harvest operations away from fall rain and can also lead to air-quality improvements due to reduced dust (Linquist et al. 2008). Prediction of the time required for *C. diffiformis* germination and emergence would eliminate incertitude about possible delays in rice seeding in conjunction with the stale seedbed technique and enable optimization of weed control timing (Fischer et al. 2009); nonetheless, quantifying variation in germination behavior among seeds through physiologically and ecologically meaningful methods remains a difficult task (Bradford 2002).

Several studies have reported on the usefulness of mathematical models to predict weed germination and describe the variation that occurs in germination times among individual seeds in a population (Bradford 2002; Forcella et al. 2000; Masin et al. 2010; Roman et al. 1999). Weed seed germination has been successfully predicted using population-based threshold models that assume germination is driven by thermal time, which is the accumulation of temperature (T) in excess of a threshold or base temperature (T_b) below which phenological development ceases, multiplied by the time (t_g) required to reach a given germination fraction or percentage (g ; Covell et al. 1986; Ellis and Butcher 1988; Ritchie and NeSmith 1991). In addition to T_b , two other cardinal temperatures define the suitable range for germination of a given species: optimum temperature (T_o) and ceiling temperature (T_c). T_o is defined as the T that allows for germination to take place most rapidly, that is, the germination rate for a given percentage or fraction g (GR_g) is the greatest, whereas T_c is the highest T at which germination can occur for a given species (Alvarado and Bradford 2002; Forcella et al. 2000; Masin et al. 2010).

Germination under suboptimal temperatures (from T_b to T_o) can thus be described on the basis of heat units or growing degree days (GDD), yielding the thermal time constant, $\theta_T(g)$:

$$\theta_T(g) = (T - T_b) t_g \quad [1]$$

and

$$GR_g = 1/t_g = (T - T_b)/\theta_T(g) \quad [2]$$

where GR_g is the inverse of time to germination (t_g) for a given fraction g and constitutes a linear function of T above T_b (Alvarado and Bradford 2002). Thermal time quantifies degree days or hours above the minimum temperature (T_b) that have been accumulated in a certain amount of time by a fraction g within the seed population (Dahal et al. 1990); in the present work, the thermal time constant ($\theta_T(g)$) is described in terms of GDD.

As predicted by Equation 1, seeds of a population within a given species are assumed to have a relatively constant, numerically similar T_b , although some exceptions have been found when dormancy is present (Covell et al. 1986; Ellis and Butcher 1988). The thermal time model is based on the combination of temperature and time, which is considered more appropriate for estimating plant development than time alone (Ritchie and NeSmith 1991).

Other factors besides temperature are known to play an important role in controlling germination processes. A linear relationship was found between seed water potential and germination rates when these are described in a hydrotime scale analogous to the thermal time scale used for temperature responses (Forcella et al. 2000). The hydrotime constant (θ_H) is defined by Gummerson (1986) as:

$$\theta_H = [\Psi - \Psi_b(g)] t_g \quad [3]$$

in which θ_H is the hydrotime (MPa h) required for germination, Ψ is the water potential of the imbibition medium (MPa), $\Psi_b(g)$ is the base water potential above which germination can commence for a fraction g , and t_g is the germination time (h) of the corresponding fraction g . The hydrotime model assumes θ_H is constant for a seed population, whereas Ψ_b varies among fractions of a seed population according to a normal distribution with its mean, $\Psi_b(50)$, and standard deviation, σ_{Ψ_b} (Dahal and Bradford 1990).

Accordingly, water availability and temperature, which are major climate drivers defining a species ecological niche, have been considered the most important determinants of seed germination timing in the beginning of growing seasons (Graziani and Steinmaus 2009). Combining both concepts, weed germination models have been developed based on the hydrothermal time concept, which integrates into one algorithm the thermal time (Equation 1) above T_b and the analogous hydrotime (Equation 3), accounted above $\Psi_b(g)$. The hydrothermal time model successfully describes seed germination patterns across suboptimum T and reduced Ψ , and has been defined as:

$$\theta_{HT} = [\Psi - \Psi_b(g)] (T - T_b) t_g \quad [4]$$

where θ_{HT} is the hydrothermal time constant (Alvarado and Bradford 2002; Boddy et al. 2012).

Recently, *C. diffiformis* germination at the population level was first described in terms of thermal time-model parameters, such as T_b , $\theta_{T(50)}$ and σ_{θ_T} (Pedroso et al. 2019). However, ALS-R and ALS-S *C. diffiformis* biotypes are known to exist in most California rice fields (Merotto et al. 2010). Differences in germination rates among ALS-R and ALS-S weed biotypes have been found in some species; in certain cases, such differences were associated with specific mutations in the ALS enzyme. Thus, ALS mutations at the Pro-197 residue were correlated with higher seed germination rates at relatively low temperatures, possibly related to the accumulation of branched-chain amino acids in tissues resulting from reduced feedback sensitivity of the enzyme

(Dyer et al. 1993; Eberlein et al. 1999; Park et al. 2004; Purrington and Bergelson 1999). However, to date, germination studies assessing differences among *C. diffiformis* biotypes with divergent responses to ALS inhibitors have not been conducted. Moreover, there is little information available in the literature regarding *C. diffiformis* germination and emergence as affected by moisture stress, nor are there any published hydrotume model parameters, such as Ψ_b (50) and σ_{Ψ_b} , which are needed for modeling plant growth and development.

Information on specific germination variables is required to parameterize models that can be used to predict germination and emergence as a means toward improving the management of this weed. Therefore, the specific objectives of this research were (1) to evaluate germination patterns of ALS-R and ALS-S biotypes in response to temperature and moisture gradients to determine cardinal temperatures and base water potential for seed germination, and (2) to parameterize and validate a population-based model capable of predicting *C. diffiformis* emergence in rice fields.

Materials and Methods

Seed Collection

Four genetically uniform *C. diffiformis* biotypes were employed in germination studies. Two ALS-S biotypes, namely AS (American susceptible) and IS (Italian susceptible), and two ALS-R biotypes (WA and IR [Italian resistant]) were selected. IR and IS were mass collected in rice farms located close by in northern Italy, whereas biotypes WA and AS were collected in rice fields located less than 10 km apart from each other in California's northern Sacramento Valley, USA. All biotypes except IS were part of earlier resistance gene-flow studies done by Merotto et al. (2009).

Twenty plants from each biotype were placed in individual greenhouses and selfed during the 2010 growing season. Mature seeds were hand harvested in September 2010 by gentle shaking into paper bags. Preliminary studies showed that seeds must be sterilized before germination to avoid fungal growth. Thus, immediately following harvest, seeds were soaked for 3 min in a solution containing sodium chloride at 3%, and then rinsed with de-ionized water (DI H₂O) for an extra 3 min. Seeds were then soaked in a solution containing 0.4% captan (N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide) fungicide (50% wettable powder) for 3 min, rinsed with DI H₂O for the same amount of time, and dry-stored at 7 C.

Germination Conditions

Germination tests were carried out on a one-dimensional thermogradient table, in which light was provided by four cool white fluorescent tubes with a 14-h photoperiod. Illuminance was measured using a light quantum meter (Spectrum Technologies, Plainfield, IL) and equaled a photosynthetic photon-flux density of 18 $\mu\text{mol m}^{-2} \text{s}^{-1}$, which is considered appropriate for germination studies (Baskin and Baskin 1998).

Starting in April 2011, seeds were subjected to a 3-mo-long cold, wet stratification period to simulate winter fallow conditions and overcome possible dormancy status, as stratification has been shown to be required for other weed species (Boddy et al. 2012). To this end, seeds of each biotype were placed into separate water-filled containers, which were further sealed to allow for dark conditions during stratification. Dark conditions were needed to

avoid germination during the stratification process, as *C. diffiformis* is a positively photoblastic species, that is, its seeds require a light treatment to germinate (Sanders 1994). Containers were then stored at 10 C and kept under these conditions until laboratory germination experiments were carried out in July and August 2011 for the elucidation of cardinal temperatures and base water potential, respectively.

Experimental units consisted of covered 3.5-cm-diameter petri dishes with two Whatman No. 1 filter paper disks. Dishes were randomized within isothermal lanes on the thermogradient table. DI H₂O was added until the paper was saturated (nearly 2.0 cm³). Afterward, the dish was held on its side to drain excess water and avoid formation of a film of water around the seeds, thus allowing for an aerobic environment for seed germination (Baskin and Baskin 1998). Dishes were sealed with tightly fitting covers to prevent evaporation.

Due to their minute size—*C. diffiformis* seeds measure only 300 by 600 μm (Sanders 1994)—germinating seeds were examined under a microscope (StereoZoom 6 Plus*, Leica Microsystems, Buffalo Grove, IL) at 10 \times magnification. In preliminary studies, around 5% of the germinating seed population showed abnormal germination, as they developed radicles but no further coleoptile development was noticed. Therefore, coleoptile protrusion of approximately 0.5 mm was taken as the end point of germination instead of radicle appearance in order to avoid scoring abnormal seeds as germinated ones.

Elucidation of Base and Optimum Temperatures

Seeds of all four *C. diffiformis* biotypes were allowed to germinate in July 2011, after removal from the cold, wet stratification period. Constant temperatures of 13.3, 16.4, 19.4, 22.3, 26.5, 28.1, 29.8, and 33.7 C were maintained on a thermogradient table, assuming that the stratification period allowed for dormancy breakage, therefore removing sensitivity to fluctuating temperatures (Allen et al. 2007). There were three replicates containing on average 100 seeds each per temperature range. Germination dishes were randomized in a split-plot design, in which temperature lanes constituted main plots. Each plot was then divided into three randomized subplots in which one dish per biotype was placed. Germination was monitored three to four times per day throughout the first week, and twice per day until 15 d had elapsed. Water in dishes was replenished whenever needed, using isothermal water kept within each temperature lane for this purpose. It was assumed that 15 d comprised enough time for nondormant seeds to germinate (Baskin and Baskin 1998). Only test temperatures that allowed for final germination percentages above 50% were used to obtain the parameters necessary for modeling (Boddy et al. 2012).

Germination Responses to Different Levels of Water Potential (Ψ)

Moisture stress effects upon germination were approximated through the imposition of gradients of water potential (Ψ) upon germinating seeds. Seed germination responses to osmotic stress were expressed and compared as thermal time courses using GDD (Covell et al. 1986; Dahal and Bradford 1994). Polyethylene glycol (PEG 8000, Sigma Chemical, St Louis, MO) solutions were prepared following Michel (1983), and Ψ values were measured using a WP4-T Dewpoint Potentiometer (Decagon Devices, Pullman, WA). Aqueous solutions with water potentials of 0, -0.25, -0.45, and -0.65 MPa were prepared to simulate a moisture gradient. PEG 8000 is known to be stable in aqueous

solutions, as it is neither absorbed by seeds nor does it evaporate, and hence pure DI H₂O was added to compensate for seed imbibition and evaporative losses.

Trials aimed at elucidation of moisture stress effects on ALS-R and ALS-S *C. diffiformis* seed germination were initiated in August 2011, immediately after seeds were removed from the cold, wet stratification period. Four replicates of approximately 100 seeds each were placed on a thermogradient table across the suboptimum constant-temperature ranges of 22.3, 26.5, and 29.8 °C. Germination was scored three to four times daily throughout the first week, and every 12 h until day 15. The experimental design was as described in the previous section.

Model Validation on Seedling Emergence from Field Soils

To ensure accuracy of emergence estimates, outdoor experiments were carried out in 2009 using soil from two rice fields typically planted with rice in California's northern Sacramento Valley, USA: HR (39°27'N, 121°43'W; 36% clay, 1.8% organic matter, Yolo clay loam soil, fine-silty, mixed, superactive, nonacid, thermic Mollisol Xerofluvents) and RD (39°34'N, 122°03'W; 28% clay, 2.8% organic matter; Castro clay, fine, thermic Typic Calciaquolls). Generalized lack of *C. diffiformis* control using ALS inhibitors had been reported in the rice fields sampled, hence the soil seedbank is expected to be composed mostly of R seeds, which are currently widespread throughout this region. There was no further addition of *C. diffiformis* seeds to the soil.

Both soils had received approximately 350 mm rainfall from October 1, 2008, through April 30, 2009. Soils were collected from fields in early May, mixed for uniform seed distribution and aggregate size, transferred to 26 by 26 by 5.5 cm plastic nursery flats (McConkey, Sumner, WA), and subjected to one of four alternative irrigation regimes: 1) flooding to 10 cm above the soil surface to impose hypoxia; 2) flooding to 5 cm above the base of flat, enough to prevent water from covering the soil surface inside the flats but allowing for continuous water flow into flats through side holes, resulting in a water-saturated treatment (henceforth referred to as "saturated soil treatment"); 3) daily application of 0.5 L of water to create minimum water stress conditions, approximating field capacity (hereinafter referred to as "daily water treatment"); or 4) application of 1 L of water every 3 d to provoke intermittent moisture stress (hereinafter referred to as 3-d flush treatment). Probes for soil temperature (WatchDog 450 data loggers, Spectrum Technologies, Plainfield, IL) buried 1 cm below the soil surface recorded temperatures at 15-min intervals, and daily averages were calculated from these data.

All four treatments received their initial application of water on the night before the first day of the trial to ensure all seeds began accumulating thermal time concurrently. Soil moisture probes (Hydrosense, Campbell Scientific, North Logan, UT) were inserted horizontally into the soil through two 0.5-cm perforations on the side of the flat. Daily soil moisture readings from each soil type were then converted to water potential using a calibration curve obtained by measuring a set of 12 soil samples with both the moisture probe and the water potential meter (Rundel and Jarrell 1989). Five 3 by 1.2 by 0.5 m outdoor basins (blocked replications) were split into four sections, each randomly assigned a water regime (main plots), within which soils (subplots) placed in nursery flats were randomly distributed. Seedlings were counted as emerged and carefully removed at 2 cm in height above the soil surface, which approximates the growth stage at which they would be subjected to chemical weed

control in the field as practiced for a stale seedbed technique using glyphosate (Fischer et al. 2009). Emerged seedlings in all treatments were counted and removed at 2- to 3-d intervals.

Data Analysis and Modeling

Thermal times for germination of the 50th percentile ($\theta_{T(50)}$) are reported and represent the median of the seed population. Biotypes' $\theta_{T(50)}$ values and final germination percentages, determined in the temperature-response experiment, were subjected to ANOVA using JMP v. 8.0 software (SAS Institute, Cary, NC), after Box-Cox transformation to meet assumptions.

Time (days) to median (50%) germination (t_{50}) was often not observed directly; hence it was estimated using the probit procedure in SAS (SAS Institute) as discussed by Steinmaus et al. (2000). Calculated t_{50} values were then converted into germination rates (GR_{50} , $1/t_{50}$, expressed in days) and plotted against temperature to estimate the optimum temperature at which the greatest GR was observed. Median values, that is, number of days until 50% of the seeds had germinated, were used because they typically exhibit less variation in developmental time than any other seed fraction (Dahal et al. 1990).

Parameters for the hydrotime and thermal time models were inferred from germination data using probit regression analysis (Dahal and Bradford 1990, 1994; Dahal et al. 1990). Base temperature was determined as follows:

$$\text{probit}(g) = \{\log(T - T_b) t_g\} - \log \theta_{T(50)}\} / \sigma_{\theta T} \quad [5]$$

where $\text{probit}(g)$ is the probit transformation of cumulative germination percentage g , $\theta_{T(50)}$ is median thermal time to germination, and $\sigma_{\theta T}$ is the standard deviation in log thermal times, calculated as the inverse of the probit regression line. Other variables were defined following Equation 1.

Original germination time courses were normalized on a thermal time basis and reproduced as cumulative normal curves of the function:

$$G = \{\log t_g - [\log \theta_{T(50)} - \log(T - T_b)]\} / \sigma_{\theta T} \quad [6]$$

where G represents cumulative percentage germination, as derived from Equation 1 (Dahal et al. 1990).

Parameter estimations for this function were obtained by using the Solver tool in Microsoft Excel® (2003–2010) to minimize the root mean-square error (RMSE) between observed and simulated germination data (Huarte and Benech-Arnold 2010), as follows:

$$\text{RMSE} = \sqrt{(1/n) \sum_{i=1}^n (\gamma_{\text{obs}} - \gamma_{\text{pred}})^2} \quad [7]$$

in which γ_{obs} and γ_{pred} are the observed and predicted germination values, respectively. For each biotype, three sets of model parameters were derived by replication. The average of the three sets of parameters per biotype is presented; protected LSD ($P < 0.05$) values were obtained from an ANOVA of each parameter.

The distribution of Ψ_b (g) and other parameters associated with the hydrotime model were determined using the following adjusted repeated probit analysis:

$$\text{probit}(g) = [\Psi - (\theta_H / t_g) - \Psi_{b(50)}] / \sigma_{\Psi b(50)} \quad [8]$$

Table 1. Final germination (G, %), base temperature for germination (T_b , expressed in C), thermal time constant to 50% germination ($\theta_{T(50)}$, GDD), and standard deviation in thermal time within the seed population ($\sigma_{\theta T(50)}$) of four *Cyperus difformis* biotypes, expressed in growing degree days (GDD).

Biotype ^a	Final G	T_b	$\theta_{T(50)}$	$\sigma_{\theta T(50)}$
	—%— ^b	—C \pm SE— ^c	—GDD \pm SE— ^c	—GDD \pm SE— ^c
AS (S)	94.1 \pm 1.2	17.01 \pm 0.4	21.01 \pm 0.7	0.180 \pm 0.01
IS (S)	99.7 \pm 0.1	16.49 \pm 0.4	21.31 \pm 1.2	0.136 \pm 0.01
IR (R)	92.4 \pm 0.9	17.62 \pm 0.2	15.27 \pm 0.6	0.283 \pm 0.01
WA (R)	94.5 \pm 1.0	16.75 \pm 0.8	16.11 \pm 1.7	0.256 \pm 0.01
LSD _{0.05} ^d	3.45	1.39	3.25	0.03

^aAS (American susceptible) and IS (Italian susceptible) are ALS-inhibitor susceptible smallflower umbrella sedge biotypes, whereas WA (Washington) and IR (Italian resistant) are ALS-inhibitor resistant biotypes.

^bAverage percent of total germinated seeds placed at temperatures ≥ 22.3 C.

^cParameters are derived from the equation $\text{probit}(g) = [(\log(T - T_b)t_g) - \log_{10}50]/\sigma_{\theta T}$ and $\theta_{T(50)}$ is calculated as $10^{0.50}$.

^d $n = 15$.

where $\sigma_{\Psi b(50)}$ is the standard deviation of the base water potential within a seed population, and Ψ is the actual water potential of the imbibition medium. The model assumes that $\sigma_{\Psi b}$, $\Psi_{b(50)}$ (base water potential for the median cohort), and θ_H are constants (Dahal and Bradford 1990). Germination data from a range of water potentials were combined, and Equation 8 was then used to predict germination as a function of thermal time. The Solver tool was used as described earlier, yielding four sets of model parameters by replication. Parameter averages were obtained from an ANOVA of each parameter, and means were separated by Student's *t*-test at the 95% confidence level.

To test our thermal time unit model on field-level seedling emergence, time to 50% emergence expressed in GDD at each water regime and soil was calculated using PROC PROBIT in SAS across observed values (SAS Institute) (Steinmaus et al. 2000). The base temperature for growth was assumed to be the same as the base temperature for germination (Bradford 2002). Parameters necessary for developing such model ($\theta_{T(50)}$, T_b , and $\sigma_{\theta T(50)}$) were chosen from the ALS-R biotype WA, given that ALS-inhibitor resistance is widespread throughout rice fields from which soils were collected. Emergence prediction of ALS-R biotypes, moreover, is necessary for optimizing stale seedbed systems for *C. difformis* management, given that this system is designed to address the increasing issue of herbicide resistance in California. Time to 50% emergence values ($\theta_{T(50)E}$, expressed in GDD) were subtracted from time to 50% germination ($\theta_{T(50)G}$) of the WA biotype. The remaining GDD values were then added to the germination prediction line to yield an emergence prediction model in each water regime and soil in order to explain differences in emergence patterns as a function of differences in germination times among the seed population.

Results and Discussion

Seed Germination Experiments

Seed viability was high across all *C. difformis* biotypes (Table 1), as also reported for *C. difformis* at the population level (Pedroso et al. 2019). Final percentage germination averaged above 92% for all biotypes when the test temperature was ≥ 22.3 C (Figure 1), values slightly larger than those reported by Chauhan and Johnson (2009) and Ismail et al. (2007).

Characterizing Germination Responses to Temperature

Differences in germination among biotypes were noticed at cold test temperatures (i.e., those ≤ 19.3 C) (Figure 1), as the ALS-R biotypes IR and WA germinated earlier than all S biotypes under these conditions. At a constant temperature of 13.25 C, neither S nor R biotypes were able to germinate throughout the course of the experiment.

Final germination percentages (Table 1) varied significantly among biotypes, with the ALS-S biotype IS having the greatest final germination among all biotypes. There was no clear pattern between ALS-R and ALS-S biotypes in this regard; base temperatures (T_b) also did not vary across *C. difformis* biotypes (Table 1). Estimated T_b values averaged 16.96 C, similar to those obtained by Pedroso et al. (2019). However, this value is 2.15 C larger than that reported by Derakhshan and Gherekhloo (2013), which could be related to the model presented in this work being developed using a wider range of test temperatures (Bradford 2002). Variations within a species due to genetic diversity of ecotypes from separate geographic regions can also be expected (Baskin and Baskin 1998).

ALS-R biotypes had significantly lower thermal time to 50% germination ($\theta_{T(50)}$) values relative to ALS-S (Table 1), and hence need to accumulate fewer thermal units to complete their germination process, allowing them to germinate faster than S biotypes given similar T_b values. Interestingly, enhanced germination under cold temperatures and ability to germinate faster than S biotypes have been linked with mutations in the *ALS* gene (Dyer et al. 1993; Eberlein et al. 1999; Park et al. 2004; Purrington and Bergelson 1999). The standard deviation in thermal time within the seed population ($\sigma_{\theta T(50)}$) averaged 0.21 GDD and was larger in ALS-R relative to ALS-S biotypes.

ALS-R biotypes (WA and IR) had higher median germination rates (GR_{50} , 1/ t_{50}) and thus completed the germination processes more rapidly than their S counterparts (Figure 2). GR_{50} increased linearly with test temperature, and therefore no thermoinhibition effect was noticed (i.e., no decline in germinability was found as test temperatures increased). Regardless of biotype, GR_{50} was thus greatest at the warmest temperature tested (33.7 C), indicating that T_0 was ≥ 33.7 C. Original germination time courses (Figure 1) were normalized on a thermal-time basis and reproduced as cumulative normal curves (Figure 3). Prediction lines fit observed germination well, although slight deviations from the model were found at 19.3 C, which can be attributed to the proximity to the species' estimated T_b (Steinmaus et al. 2000).

Germination and Osmotic Stress

Figure 4 shows observed germination time courses at a range of constant Ψ levels and test temperatures. A delay in germination was observed when seeds were placed at reduced Ψ relative to the time course in water, and germination of all biotypes was greatly affected by water stress. Temperature affected model parameters (Table 2) differently across biotypes, and a statistically significant interaction ($P < 0.0109$) was found between test temperature and median base water potential ($\Psi_{b(50)}$) estimations. Similar results have been reported by Kebreab and Murdoch (1999), in which Ψ_b values of *Orobanche* spp. seeds varied significantly with temperature. Importantly, unlike the present work, several other studies have reported relatively constant $\Psi_{b(50)}$ values in the suboptimal range of T for a given plant species, that is, base water potential values do not commonly vary across a range of suboptimal test temperatures (Alvarado and Bradford 2002; Masin et al. 2010; Roman et al. 1999).

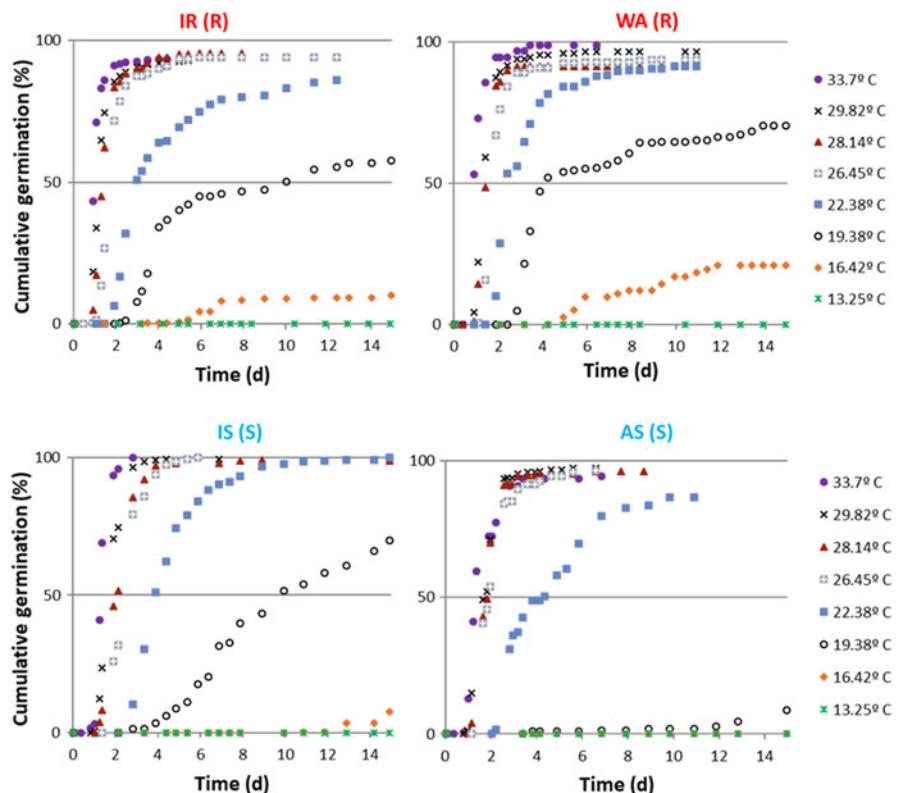


Figure 1. Cumulative germination percentages of two acetolactate synthase-resistant (ALS-R; WA and IR [Italian resistant]) and two acetolactate synthase-susceptible (ALS-S; AS [American susceptible] and IS [Italian susceptible]) *Cyperus difformis* biotypes, plotted over time (days after seeding). Data points (symbols) are averages based on three replicates of ~100 seeds each.

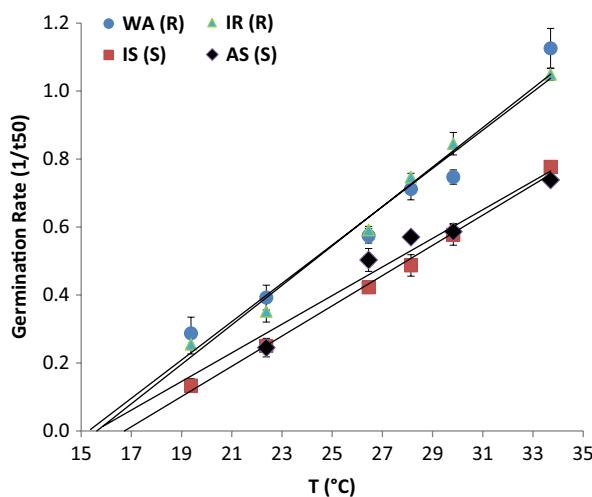


Figure 2. Median germination rates (symbols) of four *Cyperus difformis* biotypes across a temperature range from 19 to 33.7 C. Bars represent standard errors based on three replicates of ~100 seeds. Coefficient of determination (R^2) is >0.95 for all lines. See Figure 1 for biotype descriptions.

The ALS-R biotype WA had consistently lower (more negative) $\Psi_{b(50)}$ values regardless of the test temperature, whereas IR had a significantly lower base water potential value in comparison with ALS-S biotypes at 26.5 and 29.8 C, suggesting greater capacity of ALS-R biotypes for germinating under drier conditions. General trends indicate *C. difformis* biotypes had a more negative $\Psi_{b(50)}$ at 26.4 C, indicating greater ability to take up

water and hence germinate at drier conditions around such a temperature. High temperatures are known to reduce water viscosity and therefore increase its diffusion, which could account for lower (more negative) Ψ_b values at 26.5 C rather than at 22.3 C.

The standard deviation in Ψ_b among seeds (σ_{Ψ_b}) was relatively constant with T (Table 2). Interestingly, ALS-R biotypes had significantly higher σ_{Ψ_b} values at 22.3 and 26.5 C, indicating greater variability in base water potential within the seed population, as was also found in regard to $\sigma_{\theta T(50)}$. Both findings indicate less synchronous germination of ALS-R seeds. This constitutes an undesirable trait from a weed control point of view, because it is correlated with less uniform seedling emergence (Forcella et al. 2000), which could hamper control using POST herbicides.

Dormancy Levels

Germination of *C. difformis* seeds subjected to the cold, wet stratification process—designed to simulate overwintering conditions in California—reached values close to 100% (Figure 1). Furthermore, *C. difformis* seeds placed to germinate right after harvest were also able to reach germination percentages of 100% (data not shown), indicating absence of primary dormancy in this species, as also reported by Chauhan and Johnson (2009) and Pedroso et al. (2019); such results, however, contrast with previous work done by Ismail et al. (2007) and Kuk et al. (2004). Conflicting results suggest that *C. difformis* seed dormancy may be imposed according to environmental conditions during seed development, therefore being a site-specific phenomenon; alternatively, such variation could be also related to

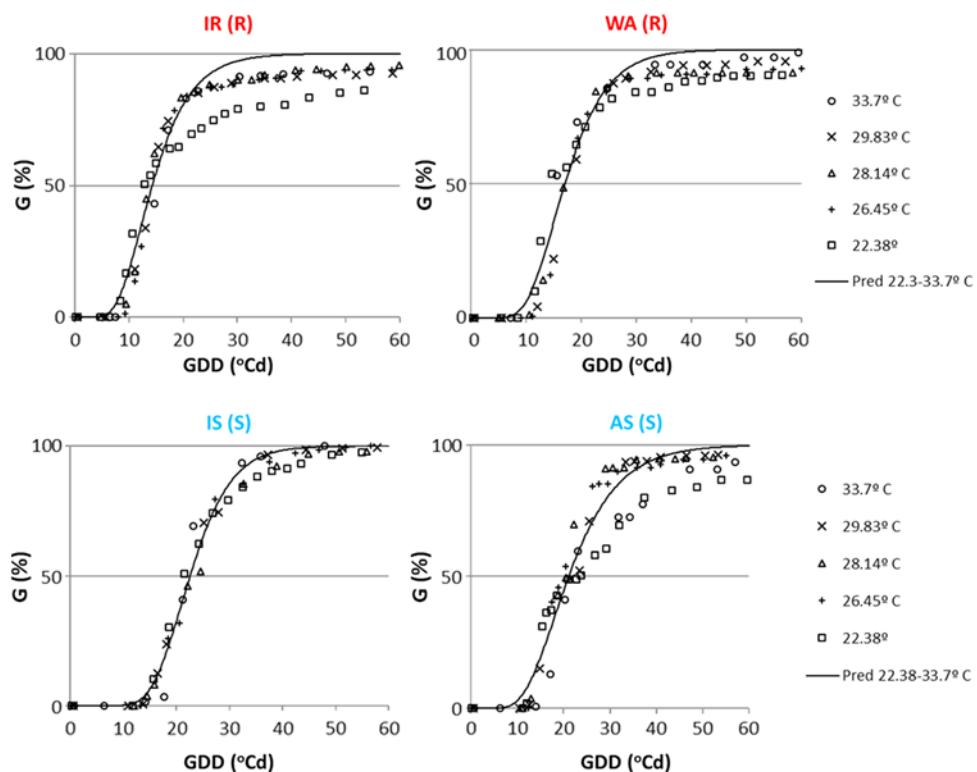


Figure 3. Thermal-time model germination curves of four *Cyperus difformis* biotypes across six constant temperature regimes at 0 MPa. Cumulative observed (symbols) and predicted (lines) germination are plotted over growing degree days calculated for each biotype according to parameters in Table 1, and using the equation $G = \{\log t_g - [\log \theta_{T(50)} - \log(T - T_b)]/\sigma_{\theta T(50)}\}$. Bars represent SEs based on three replicates of ~100 seeds. See Figure 1 for biotype descriptions.

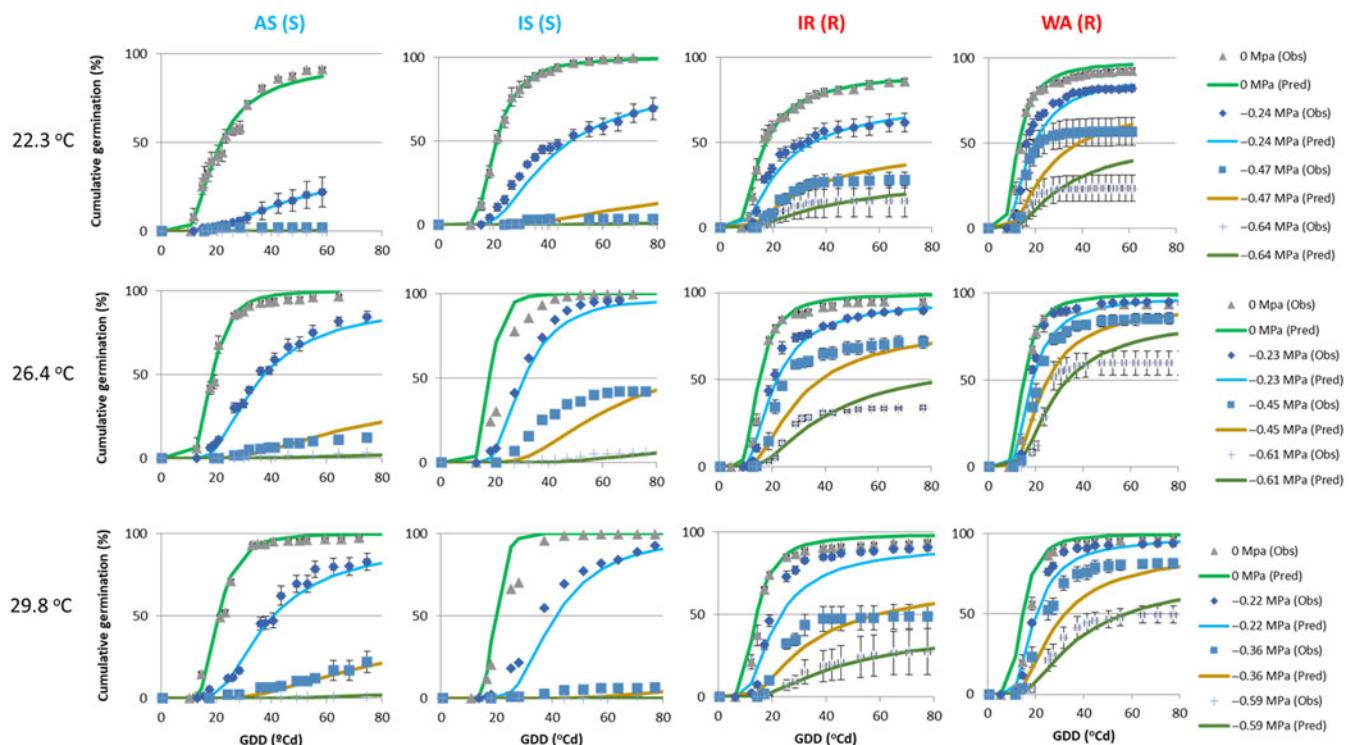


Figure 4. Hydrotime-model germination curves of four *Cyperus difformis* biotypes across four constant moisture regimes at three test temperatures: 22.3 C, 26.45 C, and 29.8 C. Time courses of observed (symbols) and predicted germination (lines); predicted lines were obtained according to parameters in Table 2 and the population-based threshold model: $\text{probit}(g) = [\Psi - \theta_H/t_g(\Psi) - \Psi_b]/\sigma_{\Psi b}$, which predicts the germination time courses at each Ψ based upon a normal distribution of $\Psi_b (g)$, and the hydrotime constant θ_H . Bars represent SEs based on four replicates of 100 seeds. See Figure 1 for biotype descriptions.

Table 2. Base water potential ($\Psi_{b(50)}$, MPa), hydrotime constant (θ_H , days), and standard deviation in base water potential within the seed population (σ_{Ψ_b} , MPa) of four *Cyperus diffiformis* biotypes, estimated using the probit method.

Biotype ^a	22.3 C			26.5 C			29.8 C		
	$\Psi_{b(50)}$ ^b	θ_H ^b	σ_{Ψ_b} ^b	$\Psi_{b(50)}$ ^b	θ_H ^b	σ_{Ψ_b} ^b	$\Psi_{b(50)}$ ^b	θ_H ^b	σ_{Ψ_b} ^b
AS (S)	-0.24cB	0.98a	0.12a	-0.45dA	0.86a	0.12a	-0.45cA	0.74ab	0.12b
IS (S)	-0.42bB	1.59bC	0.13aB	-0.57cA	1.13bB	0.12aB	-0.42cB	0.64aA	0.07aA
IR (R)	-0.46bB	1.41abC	0.30b	-0.74bA	1.14bB	0.27b	-0.61bA	0.66aA	0.22c
WA (R)	-0.76a	1.76b	0.34b	-1.13a	1.72c	0.39c	-0.83a	0.93b	0.27c

^aAS (American susceptible) and IS (Italian susceptible) are ALS-inhibitor susceptible smallflower umbrella sedge biotypes, whereas WA (Washington) and IR (Italian resistant) are ALS-inhibitor resistant biotypes.

^bParameters derived from the equation $\text{probit}(g) = [\Psi - \theta_H/t_g - \Psi_{b(50)}]/\sigma_{\Psi_b(50)}$. The same lowercase letters within a column indicate means are not statistically different at $P < 0.05$. The same capital letters within a row indicate means for a given parameter are not statistically different at $P < 0.05$. Data were log transformed.

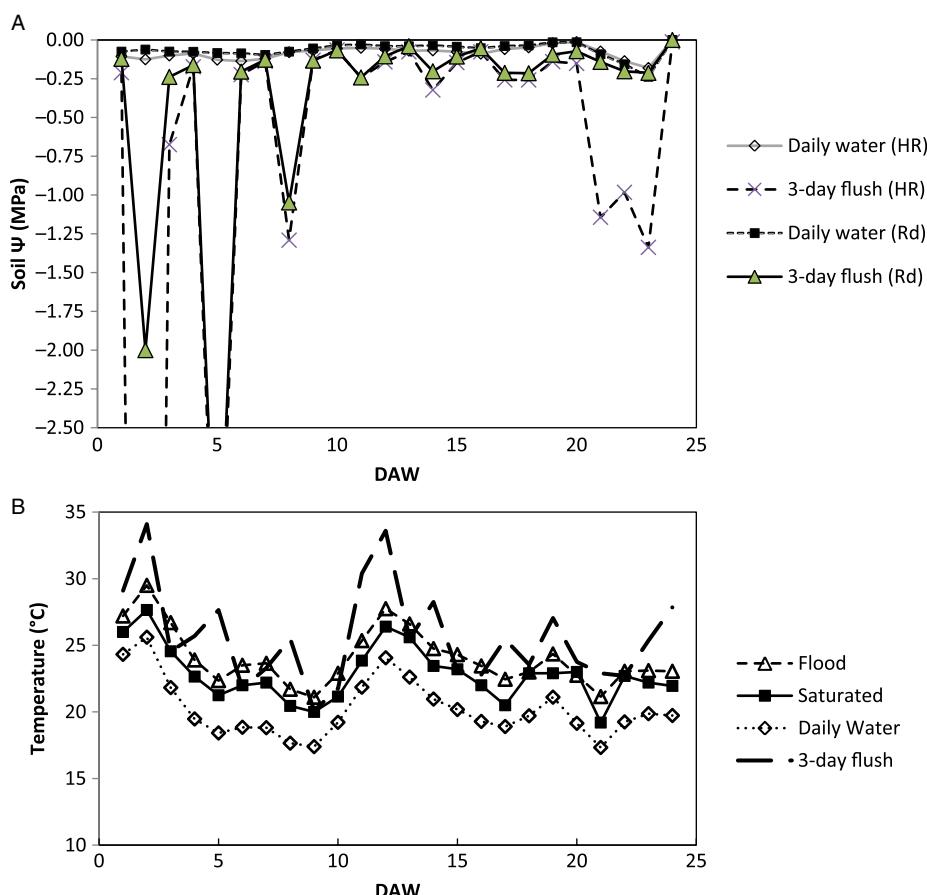


Figure 5. Environmental conditions during field soil emergence experiments expressed in days after watering (DAW). Water potential values (A) calculated as the average of moisture readings taken at 8 AM and 8 PM daily at the daily and 3-d flush treatments at each soil. Temperatures (C; chart B) represent 24-h averages of five replicates pooled for both soils, as they did not vary significantly between soils.

differences among *C. diffiformis* ecotypes across geographic regions (Baskin and Baskin 1998).

Model Validation and Emergence in Soils

Experimental temperature and moisture conditions recorded throughout the course of outdoor experiments are presented in Figures 5 and 6. Even though calculated $\Psi_{b(50)}$ values for germination (Table 2) indicate seed germination can take place in somewhat dry conditions of up to -1 MPa, emergence of seedlings in both soils (HR and RD) was poor across treatments

subjected to moisture stress (Figure 7). Across both soils, total *C. diffiformis* seedling density was higher at the flooded and saturated water regimes, which represents useful information from a *C. diffiformis* management point of view, because the goal of the stale seedbed technique is to maximize weed emergence before rice sowing.

Cyperus diffiformis seedling emergence under the daily water treatment was inconsistent across soils, indicating low tolerance to water stress, as expected for an aquatic species. Such results cannot be explained by differences in soil water potential values, which were similar between both soils throughout the course

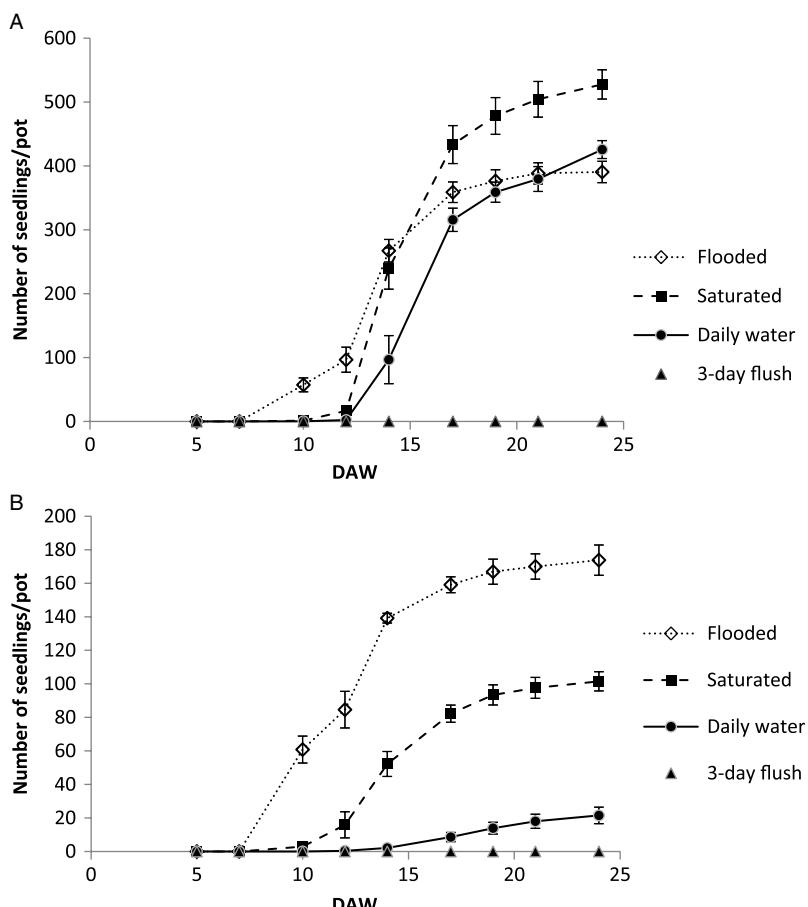


Figure 6. Observed cumulative emergence (symbols) of *Cyperus difformis* seedlings expressed in days after watering (DAW), as affected by four water regimes in soil HR (A) and soil RD (B). Bars represent SEs based on five replicates. Soil HR = fine-silty, mixed, superactive, nonacid, thermic Mollisol Xerofluvents with 36% clay and 1.8% organic matter; soil RD = Castro clay, fine, thermic Typic Calcicaquolls with 28% clay and 2.8% organic matter.

of the experiment (Figure 5A). Temperatures were also not significantly different between soils (Figure 5B). Furthermore, there was no *C. difformis* emergence at the 3-d flush treatment regardless of soil (Figure 6), because it produced dry conditions that prevented seed germination and emergence, as indicated by solid lines dropping well below -2 MPa on Figure 5.

Field test results (Figure 6A and B) indicated *C. difformis* seedling emergence is affected by water potential at a much greater magnitude than seed germination itself, thus not allowing for the use of the hydrotime model, because variations in soil Ψ greatly affected emergence of this species. Therefore, only data from water regimes not affected by or under low water stress (daily water, saturated, and flooded) were used to develop and validate a thermal time model capable of predicting *C. difformis* emergence in soils, given these allowed for successful emergence and establishment of this species. Flooded and water-saturated treatments were not subjected to any moisture stress whatsoever; under the daily water regime, water potential in both soils remained within 0.1 MPa of field capacity (Figure 5A), creating low moisture stress conditions (Singer and Munns 2002).

In the absence of soil moisture stress, early growth phases of emergence are dependent on thermal time exclusive of hydrotime (Roman et al. 1999). Figure 7 shows cumulative emergence recorded throughout the course of 25 d from May to June 2009. Water regimes significantly differed in GDD until 50% emergence of seedlings (Table 3), and a significant interaction between water regime and soil was determined by ANOVA

($P < 0.001$). There was an average lapse of 64.5, 93.5, and 100.3 GDD from the start of the experiment until emergence (2.0-cm seedling height) for the water regimes: daily water, saturated, and flooded, respectively (Figure 7). Differences in emergence patterns and thermal times between anaerobic and aerobic water regimes were also found by Boddy et al. (2012) while studying herbicide-resistant biotypes of the rice weed rice barnyardgrass [*Echinochloa phyllospadix* (Stapf) Koso-Pol.]. Emergence of *C. difformis* from soils subjected to anaerobic stress imposed by flooding required a greater number of GDD to take place relative to nonflooded, aerobic soils (Figure 7; Table 3). Indeed, previous studies found a significant reduction in shoot elongation in *E. phyllospadix* in response to anoxia due to reduced growth rates (Fox et al. 1994); in turn, the daily water regime allowed for the lowest thermal time to emergence across all water regimes, which is expected, due to aerobic conditions for germination that favor growth. Moreover, differences in germination times due to the flooded condition could also play a role in delaying seedling emergence, which our germination experiments in the laboratory could not account for, because germination was performed under aerobic conditions.

Model predictions scored well against observed emergence data in all moisture treatments (Figure 7; Table 4), although a better fit was observed for daily water and saturated treatments, which bear a closer resemblance to the aerobic conditions used for developing the germination model in the laboratory. Our models were more successful at predicting emergence in the

Table 3. Time to 50% emergence (growing degree days [GDD]) of *Cyperus difformis* seedlings across three water regimes, observed for soils HR and RD.

Water regime	$\theta_{T(50)}E^a \pm SE$	
	HR ^b	RD ^b
Daily water	61 \pm 0.8aA	68 \pm 1.6aB
Saturated	95 \pm 1.6b	92 \pm 3.3b
Flooded	105 \pm 1.4cB	95 \pm 4.9bA

^aThe same lowercase letters within a column indicate means are not statistically different at $P < 0.05$. The same capital letters within a row indicate means are not statistically different at $P < 0.05$.

^bSoil HR: fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents with 36% clay and 1.8% organic matter; soil RD: Castro clay, fine, thermic Typic Calciqaquolls with 28% clay and 2.8% organic matter.

upper fractions of the seed population at saturated and flooded treatments (Figure 7).

Overall, results indicate a greater ability of ALS-R *C. difformis* biotypes used in this study to successfully complete seed germination under drier conditions, as shown by their more negative base water potential values relative to ALS-S seeds (Table 2; Figure 4). In addition, ALS-R *C. difformis* was also shown to germinate more quickly at colder test temperatures, as indicated by greater germination rates (Figure 2), while also displaying significantly lower $\theta_{T(50)}$, therefore germinating faster than ALS-S biotypes due to the need for fewer GDD. Such findings indicate that the repeated use of ALS-inhibiting herbicides has selected for more aggressive and noxious ecotypes of *C. difformis* that bear no fitness penalty in this regard.

Table 4. Validation measurements of predicted *Cyperus difformis* emergence in two field soils (HR and Rd) subjected to three irrigation regimes.

Evaluation parameter	HR ^a			RD ^a		
	Daily Water	Saturated	Flooded	Daily Water	Saturated	Flooded
Number of observations	45	45	45	45	45	45
Means of observed values $\pm SE$	0.41 \pm 0.16	0.46 \pm 0.16	0.55 \pm 0.15	0.33 \pm 0.14	0.49 \pm 0.15	0.61 \pm 0.13
Means of simulated values $\pm SE$	0.39 \pm 0.15	0.46 \pm 0.17	0.52 \pm 0.17	0.29 \pm 0.13	0.48 \pm 0.16	0.59 \pm 0.16
Mean absolute percent error	0.067	0.176	0.147	0.869	0.180	0.227
Root mean-square error (RMSE) ^b	0.043	0.083	0.090	0.065	0.060	0.131
Modeling efficiency ^c	0.99	0.93	0.95	0.98	0.98	0.86
Modeling index (d) ^d	0.99	0.99	0.99	0.99	0.99	0.97

^aSoil HR: fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents with 36% clay and 1.8% organic matter; soil RD: Castro clay, fine, thermic Typic Calciqaquolls with 28% clay and 2.8% organic matter.

^bRMSE is expressed in the same units as the observed quantities, and is calculated as $\{[\sum(O_i - P_i)^2]/n\}^{0.5}$, where O_i is an observation and P_i the corresponding prediction (Mayer and Butler 1993; Spokas and Forcella 2006).

^cFor the validation measurement EF, 1.0 is the optimal value, while a score below 0 indicates the observed mean is a better predictor than the model; calculated as $1 - \sum(O_i - P_i)^2 / \sum(O_i - \bar{O})^2$, where \bar{O} is the mean of observed values (Mayer and Butler 1993; Legates and McCabe 1999; Spokas and Forcella 2006).

^dThis metric varies from 0.0 to 1.0, with higher values indicating greater agreement between predicted and observed values; calculated as $1 - \sum(O_i - P_i)^2 / \sum[(P_i - \bar{O}) + (O_i - \bar{O})]^2$ (Legates and McCabe 1999; Spokas and Forcella 2006).

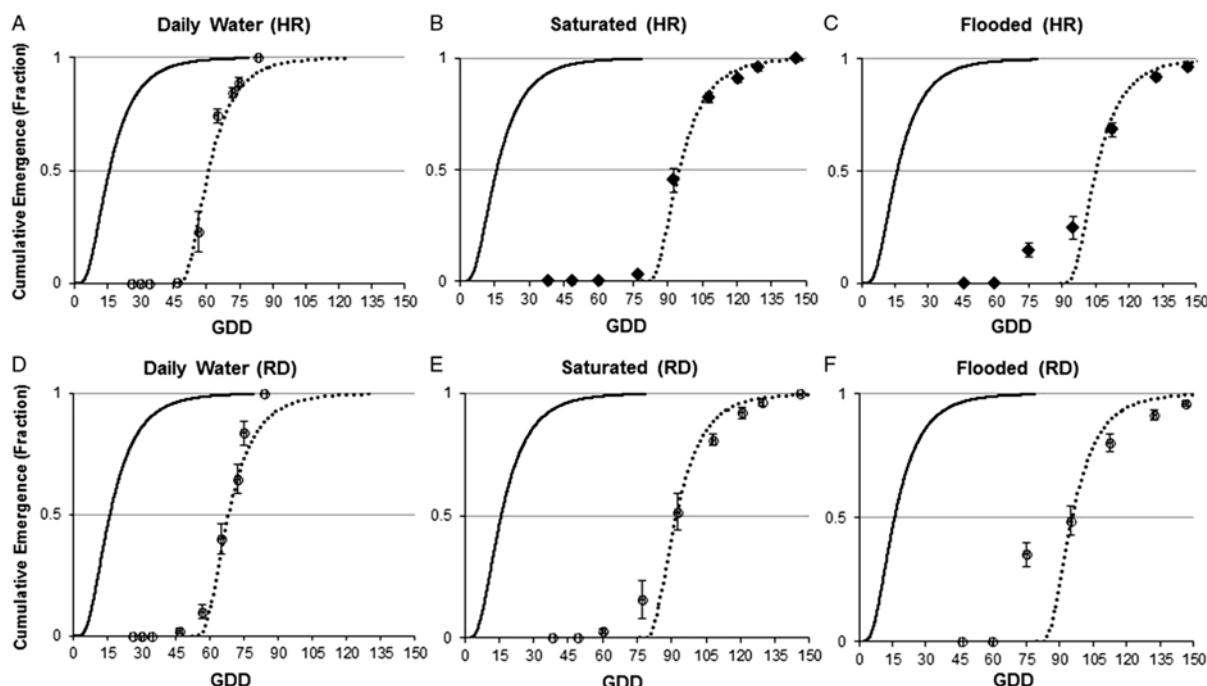


Figure 7. Cumulative observed emergence of *Cyperus difformis* seedlings, expressed in growing degree days for three water regimes in two soils: HR (A-C) and RD (D-F). Symbols indicate observed emergence; solid lines are the predicted germination; and dashed lines are predicted emergence. Emergence expressed as a fraction of final emergence within the treatment to better observe differences in emergence timing. Bars represent SEs based on five replicates. Soil HR = fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents with 36% clay and 1.8% organic matter; soil RD = Castro clay, fine, thermic Typic Calciqaquolls with 28% clay and 2.8% organic matter.

Although specific mutations of the *ALS* gene are yet to be determined for the ALS-R *C. difformis* biotypes employed in the present study, alterations at their ALS-inhibitor target site have been positively confirmed (Pedroso et al. 2017). Field experiments showed poor and inconsistent *C. difformis* emergence under water-deficit irrigation, suggesting that to optimize springtime seedling emergence for this species, soil moisture should be kept around field capacity. Furthermore, the values of modeling efficiency coefficients obtained are good indicators of the model's goodness of fit, and suggest that growers could rely on the proposed mathematical model for decision-making guidance regarding the best moment for *C. difformis* control in rice fields through a better understanding of the dynamics of its germination and emergence.

In conclusion, this study is the first to report clear differences in base water potential values for germination between ALS-R and ALS-S biotypes and the germination process being completed more rapidly in ALS-R biotypes—a fact attributed mainly to their lower thermal-time requirements and greater germination rates relative to ALS-S *C. difformis* biotypes. This has important implications for developing management strategies to control this weed.

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