

## Seed Germination Characteristics as Affected by Interaction of Moisture Stress and Temperature in Sethoxydim-Resistant Biotype of Goosegrass (*Eleusine indica* (L.) Gaertn.) from Malaysia

(Pencirian Percambahan Benih yang Dipengaruhi oleh Interaksi Tekanan dan Suhu Lembapan dalam Biotip Rumput Samba Rintangan Sethoxydim (*Eleusine indica* (L.) Gaertn.) dari Malaysia)

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### ABSTRACT

Understanding temperature and moisture stress that influence sethoxydim-resistant (R) goosegrass (*Eleusine indica*) germination is crucial for effective weed control, but little research has been done on the impact of these environmental factors on germination of the R goosegrass biotype. This research aims to confirm sethoxydim resistance in goosegrass and to examine interaction effects of different temperatures and water potentials on germination of the R goosegrass biotype. Dose–response tests showed that the R goosegrass biotype exhibited high resistance level to sethoxydim with 17-fold. In laboratory bioassays, the R goosegrass biotype germinated by 88-100% at 35 °C with water potentials ranging from 0 to -0.80 MPa, but no seed germination occurred at 10 °C and 40 °C under all water potential treatments. With rising water potentials from 0 to -0.80 MPa, seed germination at 15 to 30 °C decreased markedly. The time it took to achieve 50% seed germination ( $T_{50}$ ) increased drastically when decreasing temperature from 35 to 15 °C. The  $T_{50}$  at 20 to 30 °C also increased as the water potential increased from -0.20 to -0.80 MPa. At 35 °C, however, the water potential level had no impact on  $T_{50}$ , implying that the R goosegrass biotype seed is water stress tolerant at 35 °C. The base temperature and base water potentials estimated were 10.6 °C and -1.28 MPa, respectively. These findings can help in determining the optimal time to apply pre-emergent and early post-emergent controls when a large proportion of R goosegrass biotype have already germinated or emerged.

Keywords: Base temperature; base water potential; post-emergence control; pre-emergent control

### ABSTRAK

Pemahaman terhadap tekanan suhu dan kelembapan yang mempengaruhi percambahan rumput samba (*Eleusine indica*) yang rintang terhadap setoksidim (R) adalah penting untuk mengawal rumpai ini dengan berkesan, namun tidak sedikit kajian dilakukan mengenai kesan faktor persekitaran ini terhadap percambahan rumput samba biotip R. Penyelidikan ini bertujuan untuk mengesahkan kerintangan rumput samba terhadap setoksidim dan untuk mengkaji kesan interaksi suhu dan potensi air yang berbeza terhadap percambahan rumput samba biotip R. Ujian gerak balas dos mendedahkan bahawa rumput samba biotip R menunjukkan aras kerintangan yang tinggi terhadap setoksidim dengan 17 kali ganda. Pengasaian makmal menunjukkan bahawa rumput samba biotip R bercambah sebanyak 88 hingga 100% pada suhu 35 °C dengan potensi air antara 0 hingga -0.80 MPa, tetapi tiada percambahan berlaku pada suhu 10 dan 40 °C di bawah semua rawatan potensi air. Dengan potensi air yang meningkat daripada 0 hingga -0.80 MPa, percambahan biji benih pada 15 hingga 30 °C menurun dengan ketara. Masa yang diambil untuk mencapai 50% percambahan biji benih ( $T_{50}$ ) meningkat secara drastik apabila suhu menurun daripada 35 hingga 15 °C.  $T_{50}$  pada 20

hingga 30 °C juga meningkat apabila potensi air meningkat daripada -0.20 kepada -0.80 MPa. Walau bagaimanapun, aras potensi air tidak memberi kesan kepada  $T_{50}$  pada 35 °C. Ini membayangkan bahawa rumput sambau biotip R adalah toleran terhadap tekanan air pada 35 °C. Suhu asas dan potensi air asas dianggarkan masing-masing pada 10.6 °C dan -1.28 MPa. Penemuan ini dapat membantu dalam menentukan masa yang optimum untuk memberi kawalan pra-muncul dan pasca-muncul pada peringkat awal apabila sebahagian besar rumpai sambau biotip R telah bercambah atau muncul.

Kata kunci: Kawalan pasca-muncul; kawalan pra-muncul; potensi air asas; suhu asas

## INTRODUCTION

When the prevailing environmental conditions align with those needed for germination, germination and subsequent emergence in the field may occur. Two of the most significant factors that affect seed germination dynamics are temperature and moisture (Bakhshandeh & Gholamhossieni 2019). One way to understand how temperature affects seed germination dynamics is to define the base, optimum, and ceiling temperatures (Bewley et al. 2013; Bidgoly et al. 2018). Germination, on the other hand, is highly sensitive to moisture levels, and osmotic stress caused by a lack of water has been shown to prevent or delay seed germination (Tobe et al. 2001).

Goosegrass (*Eleusine indica*), one of the most troublesome weeds which has infested 46 crops in 60 countries (Ma et al. 2015), is widespread and found on almost every continent (Takano et al. 2016). In Malaysia, herbicide resistance has been evolved in goosegrass due to the extensive use of the same herbicides in agricultural areas. It has shown resistance to various herbicide groups including paraquat, glufosinate, fluazifop-butyl and/or glyphosate in rubber and oil palm (Dilipkumar et al. 2020a). Therefore, numerous studies have been done to minimize herbicide use for delaying the evolution of herbicide-resistant goosegrass using a combination of chemical and physical methods (Amirul, Diplikumar & Chuah 2019; Amirul, et al. 2019; Chuah & Lim 2021a, 2021b; Chuah, Lim & Ismail 2018; Dilipkumar et al. 2020b). Novel herbicides have been developed (Abdullah et al. 2021; Norhafizah et al. 2020) to provide another choice to increase the variety of available herbicides to slow down the development of herbicide resistance in goosegrass.

Goosegrass is a C4 annual grassy weed which can produce up to 140,000 seeds in a single plant (Ma et al. 2015). The glyphosate-resistant biotype of goosegrass spreads primarily through seeds, with 79 % of seeds remaining viable after two years of soil burial at a depth of 20 cm in the field (Chuah, Salmijah & Ismail 2004). The majority of goosegrass emerged at a soil

depth of 0 to 5 cm (Ismail et al. 2002). Oxygen does not appear to be a limiting factor at these depths, but environmental factors such as soil temperature and moisture play a significant role in regulating goosegrass seed germination and dormancy. Although it has been demonstrated that goosegrass could germinate under a wide range of temperatures and moisture stress conditions (Shekoofa et al. 2020), little is known about how these two environmental factors interact to influence the seed germination.

In 2021, a field trial conducted by an agrochemical company complained that herbicides provided unsatisfactory control of mature goosegrass in an oil palm plantation, Pekan Nanas, Johor (Sim 2021). Hence, this study aims to 1) confirm the occurrence of herbicide resistance in goosegrass; 2) examine how seed germination in the sethoxydim-resistant (R) biotype of goosegrass reacted to various temperatures and water potentials.

## MATERIALS AND METHODS

### PLANT MATERIALS

The putative herbicide-resistant biotype of goosegrass seeds was sampled at an oil palm plantation owned by United Malaysian Pineapple Sdn. Bhd. in Pekan Nanas, Johor (1° 30' 36.00" N, 103° 30' 50.76" E), whereas the putative herbicide-susceptible resistant biotype was collected from a nearby roadside (1 km away) with no history of herbicide application. The seed coat was scarified with sandpaper to remove the seed coat. Seeds of putative herbicide-resistant and -susceptible biotypes were sown and germinated in seedling trays containing soil potting mixture. The seedlings were grown in a glasshouse at temperatures that ranged from 28 to 34 °C, with a 12-hour photoperiod and a light intensity of 1000  $\text{Em}^{-2}\text{s}^{-1}$ . The seedlings were transplanted into new seedling trays for herbicide resistance screening when they reached 3-to-4 leaf stage.

## HERBICIDES

Ten types of herbicides registered in oil palm were examined in this study. Table 1 shows the information of each herbicide.

## SCREENING FOR HERBICIDE-RESISTANT AND SUSCEPTIBLE BIOTYPES OF GOOSEGRASS

The seedlings were transplanted into new seedling trays for herbicide resistance screening when they reached 3-to-4 leaf stage. Two or three days after transplanting, the seedlings were sprayed with glyphosate, paraquat, glufosinate, fluazifop-p-butyl, diuron, MSMA, sethoxydim, oxyfluorfen, imazethapyr and topramezone at twice the recommended rates (Table 1) by using a compression sprayer (Matabi Style 7 model), delivering 450L ha<sup>-1</sup> at spray pressure of 200kPa. Plants that survived after herbicide treatment were grown for seed production to undergo subsequent dose-response experiments.

## DOSE RESPONSE TESTS

In the glasshouse, seeds of the sethoxydim-resistant (R) and susceptible (S) biotypes of goosegrass were scarified, germinated, and transplanted into seedling trays as mentioned previously. Seedlings of each biotype were treated with sethoxydim at a series of rates (0, 0.075, 0.3, 1.2, 4.8, and 19.2 kg a.i. ha<sup>-1</sup>) at the 3-to-4-leaf stage. A complete randomized block design with five replications was used in this experiment. The survival rate and shoot dry weight of seedlings was recorded three weeks after treatment. The seedlings were considered as killed when no new growth was observed.

## INTERACTION EFFECT OF TEMPERATURE AND WATER POTENTIAL ON GERMINATION OF R GOOSEGRASS BIOTYPE

The effect of seven constant temperatures and six water potentials on germination of R goosegrass biotype was tested using a completely randomized design in split plot arrangement with five replications, with temperature as the main plot and osmotic potential as a subplot. The temperatures studied were 10, 15, 20, 25, 30, 35, and

TABLE 1. Information of herbicides used for screening occurrence of resistance

Herbicides	Trade name	Application rate (kg/ha)	Formulation	Mode of action
Glyphosate	RoundupTransorb®	2.90	Soluble liquid (SL)	Inhibition of EPSP synthase
Paraquat	Gramoxone 100	1.50	Soluble liquid (SL)	Photosystem-I-electron diversion
Glufosinate	Basta®	0.90	Soluble liquid (SL)	Glutamine synthase inhibitors
Fluazifop-p-butyl	Fusilade	0.30	Emulsifiable concentrates (EC)	ACCase inhibitors
Diuron	Ancom Diuron F42	0.48	Soluble concentrates (SC)	Inhibition of photosynthesis at photosystem II
MSMA	Ansar® 550	3.96	Soluble liquid (SL)	Unknown
Sethoxydim	Expand	1.2	Emulsifiable concentrates (EC)	ACCase inhibitors
Oxyfluorfen	Boxy	0.28	Emulsifiable concentrates (EC)	Inhibition of protoporphyrinogen oxidase (PPO)
Imazethapyr	Imaz	0.48	Soluble liquid (SL)	Inhibition of branched-chain amino acid biosynthesis
Topramezone	Clio	0.05	Soluble concentrates (SC)	Inhibits the enzyme 4-Hydroxy-Phenyl-Pyruvate-dioxygenase (4-HPPD)

40 °C. The solution was made with polyethylene glycol (PEG) 8000 and water potentials of 0, -0.2, -0.4, -0.6, and -0.8 MPa (Michel 1983).

A total of 25 goosegrass seeds with no seed coat were placed in 10-cm-diameter Petri dishes lined with two 9-cm-diameter filter papers for each treatment. The Petri dishes were covered with Parafilm and kept in a seed germinator under darkness after the PEG water potential solution was applied. For 14 days, germinated seeds were counted and removed every 24 h. When the radicle length is greater than 2 mm, the seeds are called germinated.

#### STATISTICAL ANALYSIS

The following logistic regression model was used to fit the shoot dry weight data (Kuk et al. 2002):

$$Y = d (1 + [x/x_0]^b) \quad (1)$$

where  $Y$  represents the dry weight of the harvested plants expressed as percentage of control;  $d$  represents the coefficients corresponding to the upper asymptotes;  $b$  represents the line's slope;  $x_0$  represents the herbicide rate needed to inhibit shoot growth by 50%; and  $x$  represents the herbicide dose. The herbicide rates that were needed to reduce the shoot dry weight by 50% ( $GR_{50}$ ) were calculated using regression equations. The resistance level was determined by dividing the R biotype's  $GR_{50}$  by the S biotype's  $GR_{50}$ .

The Chapman function is used to model germination rates and cumulative germination curves for each temperature and water potential (Garcia et al. 2013):

$$Y = K[1 - \exp(-b*t)]^c \quad (2)$$

where  $Y$  is the cumulative germination;  $K$  is the maximum germination;  $b$  is the rate of increase;  $t$  is the time from the start of germination period in days; and  $c$  is a shape parameter.

For each temperature and water potential, the rates for 50% germination ( $1/T_{50}$ ) were determined and regressed against temperature. The optimal temperature ( $T_{opt}$ ) was calculated as a point estimate from the intersection of these two regression lines (Dumur, Pilbeam & Craigon 1990) using the intercepts and slopes of the two regression lines as follows (Dumur, Pilbeam & Craigon 1990; Fyfield & Gregory 1989):

$$T_{opt} = (a_2 - a_1) / (b_1 - b_2) \quad (3)$$

where  $T_{opt}$  is the optimum temperature;  $a_1$  and  $a_2$  are the intercepts of Regressions 1 and 2, and  $b_1$  and  $b_2$  are the slopes of Regressions 1 and 2.

Probit analysis was used to estimate base water potential ( $\Psi_b$ ), in which hydrotime for 50% germination was calculated for the entire temperature range as:  $\theta_{H50} = (\Psi - \Psi_{b50}) t_{50}$  where  $\theta_{H50}$ ,  $t_{50}$  and  $\Psi_{b50}$  are the hydrotime, temperature, and base water potential for 50% germination (Ellis, Simon & Covell 1987). This procedure estimated constant values for cardinal temperatures and  $\Psi_b$ .

Germination ( $G$ , %) is a function of water potential ( $P$ , MPa) and temperature ( $T$ , deg C). We proposed the following to govern the relation,

$$G = G_{\max@T} + (1 - G_{\max@T}) [1 - \exp(-\beta_1 P - \beta_2 P^2)] \quad (4)$$

where  $G_{\max@T}$  is the maximum germination at temperature  $T$ ,  $\beta_1$  and  $\beta_2$  are fitting variables.

The  $G_{\max@T}$ ,  $\beta_1$ ,  $\beta_2$  variables, as a function of temperature, are predicted by a third order polynomial equation using,

$$V = aT^3 + bT^2 + cT + d \quad (5)$$

where  $V$  is a dependent variable that can be either  $G_{\max@T}$ ,  $\beta_1$ , or  $\beta_2$ . The  $a$ ,  $b$ ,  $c$  and  $d$  are curvefitting parameters for variable  $V$ .

## RESULTS

### CONFIRMATION OF SETHOXYDIM-RESISTANT GOOSEGRASS

The goosegrass seedlings died after treatment of various herbicides at double the recommended rates with the exception of sethoxydim. This finding suggests that the goosegrass biotype has evolved resistance to sethoxydim but it is susceptible to glyphosate, paraquat, glufosinate, fluazifop-p-butyl, diuron, MSMA, oxyfluorfen, imazethapyr and topramezone. Dose response tests further shown that all sethoxydim-susceptible (S) plants were completely killed when sprayed with sethoxydim at double the recommended rate of 1.2 kg ha<sup>-1</sup>, while the sethoxydim-resistant (R) plants were only fully killed when treated with sethoxydim at 32-fold the recommended rate (Figure 1). Figure 2 shows the dry weight of both biotypes of goosegrass after being sprayed with different sethoxydim rates. Shoot dry weight of both biotypes reduced as application rates rose, but the S biotype apparently exhibited a faster decline than the R biotype. The occurrence of R biotype of goosegrass has been confirmed with 17-fold resistance level as shown in Table 2.

TABLE 2. Resistance level of goosegrass after sethoxydim

Herbicide	*GR <sub>50</sub> value (kg ai ha <sup>-1</sup> )		Resistance level
	R biotype	S biotype	
Sethoxydim	10.6 (1.4)	0.6 (0.2)	17.3

\*GR<sub>50</sub> value represents the herbicide rates to inhibit seedling growth by 50%, data represent the mean whereas value in parentheses is the standard error

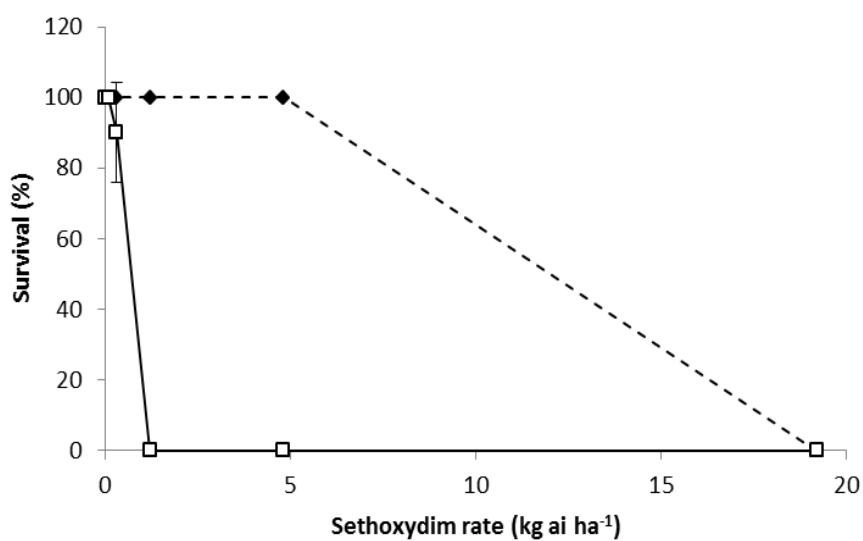


FIGURE 1. Survival of the susceptible (—) and resistant (- -) biotypes of goosegrass, as affected by sethoxydim in the whole-plant bioassay three weeks after treatment under glasshouse conditions. Every point is a mean of five replicates, each containing 10 plants. The vertical bars represent the standard deviation of the mean

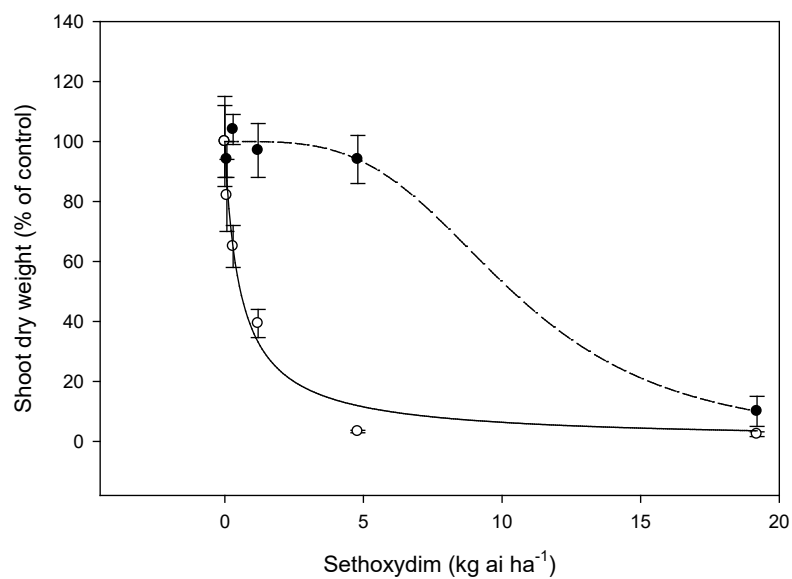


FIGURE 2. Shoot dry weight of resistant (- -) and susceptible (—) biotypes of goosegrass as affected by sethoxydim in whole-plant assay. The herbicide was applied at 3 to 4-leaf stage and harvested three weeks after treatment. Every point is a mean of five replicates, each containing 10 plants. The vertical bars represent the standard deviation of the mean

TEMPERATURE AND WATER POTENTIAL EFFECTS ON FINAL GERMINATION

The optimum temperatures and water potentials for germination of the R goosegrass biotype ranged from 25 to 35 °C and 0 to -0.20 MPa. At all temperatures measured, a decrease in germination was observed with an increase in osmotic potential (Figure 3). With the exception of temperature 35 °C, the percent seed

germination decreased rapidly as the water potential increased to more than -0.20 MPa. It is noted that no germination occurred at temperatures of 10 or 40 °C at any degree of water potential. Germination occurred at temperatures of 20, 25, 30, and 35 °C at -0.80 MPa, with the exception of 15 °C. When the temperature was raised above 15 °C and up to 35 °C, the percentage of germination increased (Figure 4).

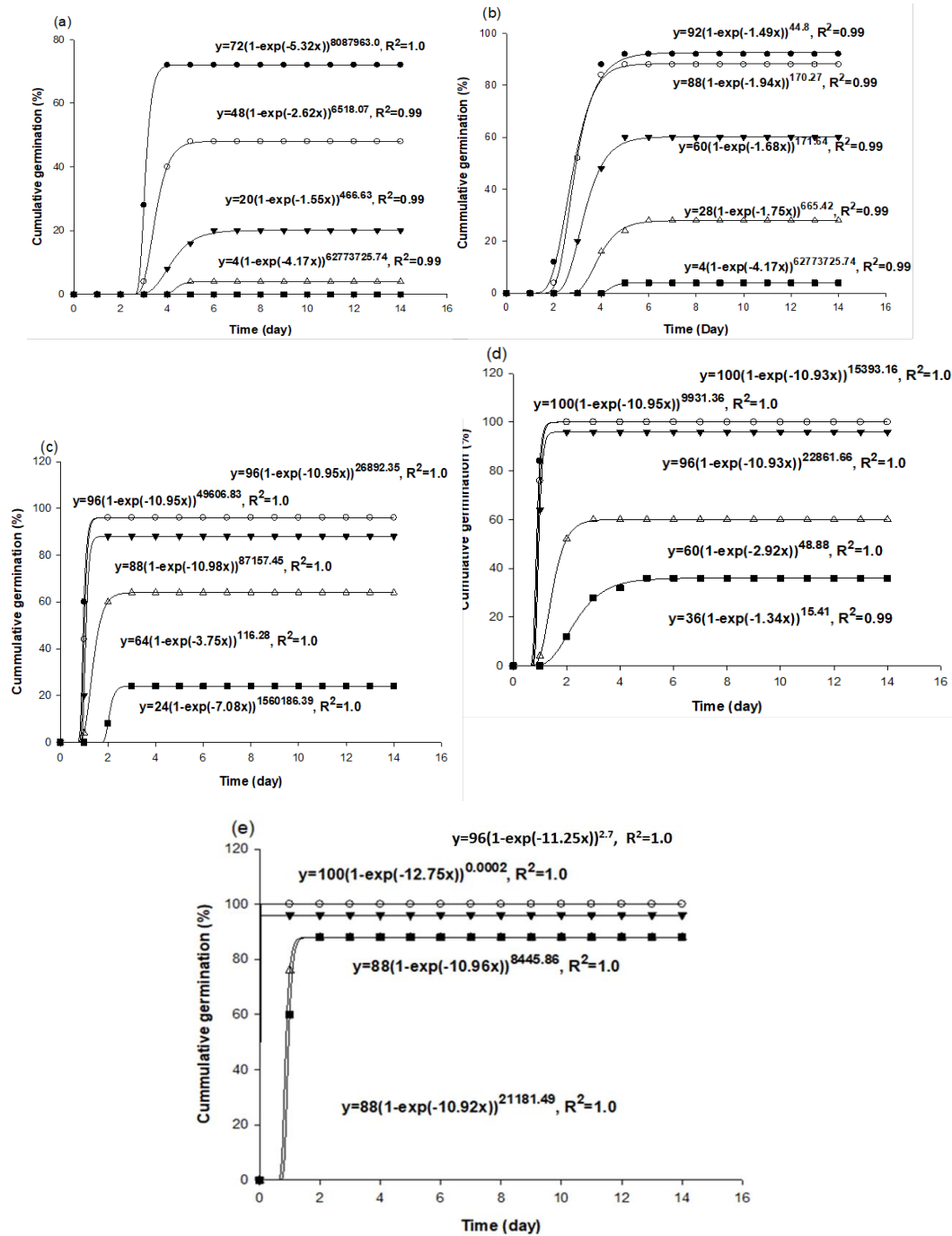


FIGURE 3. Cumulative germination pattern of the sethoxydim-resistant goosegrass biotype with different osmotic potentials of (○) 0MPa, (●) -0.2MPa, (▼) -0.4MPa, (Δ) -0.6MPa, and (■) -0.8MPa at constant temperature regimes of (a) 15, (b) 20, (c) 25, (d) 30, and (e) 35 °C under darkness

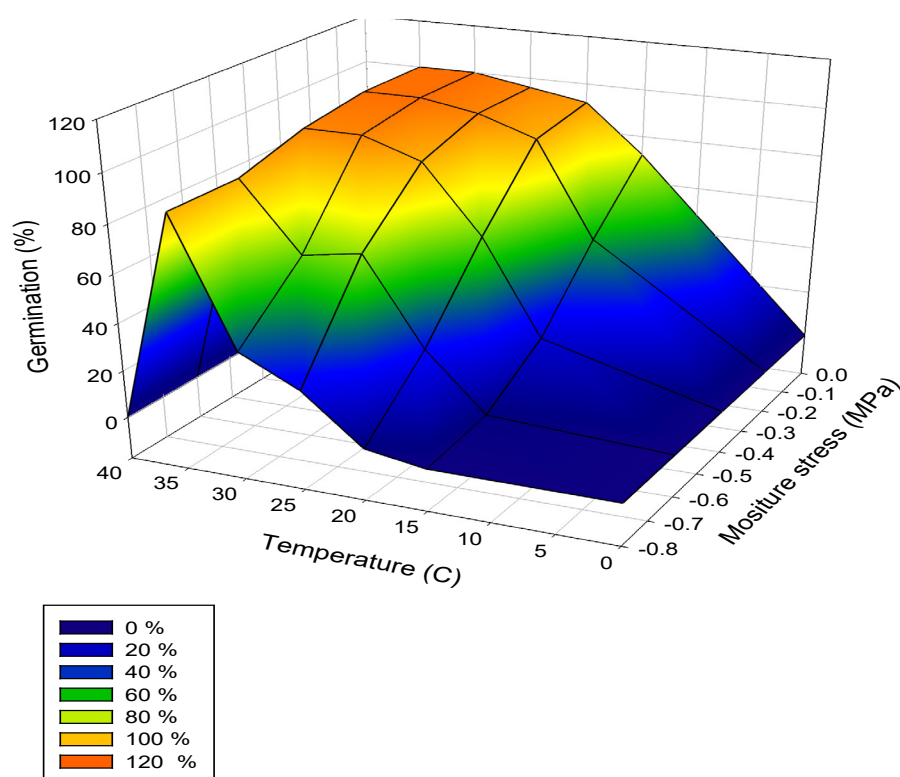


FIGURE 4. Interaction effect of water potential and temperature on seed germination of the sethoxydim-resistant goosegrass biotype

The percentage germination of goosegrass fell from 72% at 0MPa to 48% at -0.2MPa at 15 °C. At -0.40MPa and -0.6MPa, there was a significant drop in percentage germination. At a water potential of -0.8MPa, no germination was observed. The percentage germination of goosegrass dropped dramatically from 92% at 0MPa to 4% at -0.8MPa at 20 °C. The percentage germination of goosegrass was constant at 0MPa and -0.2MPa at temperatures of 25, 30, and 35 °C. Temperatures of 25 and 30 °C at -0.6MPa and -0.8MPa resulted in a decline in percentage germination. The percentage germination decreased marginally at temperature 35 °C, from 96% at -0.4MPa to 88% at -0.6MPa (Figure 4).

#### TEMPERATURE AND WATER POTENTIAL EFFECTS ON GERMINATION RATE

For all temperatures, the time required to attain 50% germination ( $T_{50}$ ) of the R biotype goosegrass was delayed with decreasing water potential (Figure 3). At 0MPa water potential and a temperature of 15 °C,

the goosegrass took about 3 days to achieve  $T_{50}$ . The goosegrass took 68 h, 70 h, and 4 days at 20 °C to attain  $T_{50}$  at 0, -0.2, and -0.4MPa water potential, respectively. At 25 °C, the goosegrass took 23 h, 1 day, 26 h, and 39 h to achieve  $T_{50}$  at 0, -0.2, -0.4, and -0.6MPa water potential, respectively. At 0, -0.2, -0.4, and -0.6MPa water potential, the goosegrass took approximately 21, 21, 23 and 46 h to achieve  $T_{50}$  at 30 °C, respectively. At a constant temperature of 35 °C, it took 20 to 23 h for goosegrass to achieve  $T_{50}$  at 0 to -0.8MPa water potential, respectively (Table 3).

**CARDINAL TEMPERATURE AND BASE WATER POTENTIAL**  
The cardinal temperature for germination of the R biotype goosegrass was determined using linear regressions (Figure 5). Cardinal temperature was estimated to be 10.6, 34.6 and 40 °C for base temperature ( $T_b$ ), optimum temperature ( $T_o$ ) and maximum temperature ( $T_{max}$ ). The base water potential varied with temperature. At temperature 10 and 40 °C, no germination occurred, and the base water potential

cannot be estimated. At 15 °C, there was insufficient data input for estimation of base temperature with linear regression. As compared with 20, 25 and 30 °C, the estimated base water potential at 35 °C was -4.80 MPa which is apparently higher (Table 4). The median base water potential was estimated at -1.28 MPa.

GERMINATION AS A FUNCTION OF TEMPERATURE AND WATER POTENTIAL

To determine the germination percentage ( $G$ ) at a specific water potential, in Equation (4), the  $G_{\max@T}$ ,  $\beta_1$

, and  $\beta_2$  at a particular temperature must be calculated using parameters  $a$ ,  $b$ ,  $c$  and  $d$  given in Table 5. Figure 6 depicts the comparison of seed germination between experimental data and curve-fitted data. The regression on 25 experimental data points gives an R-squared of 0.993. To determine the maximum germination ( $G_{\max@T}$ ), only Equation (5) is needed, and the water potential is assumed at zero, that is at saturated soil water content.

TABLE 3. Estimated time for 50% germination and germination rate of sethoxydim-resistant goosegrass biotype for different temperatures and water potential combinations

Temperature (°C)	Water potential $\Psi$ (-MPa)	50% germination time $T_{50}$ (hr)	Rate of germination $1/T_{50}$ (hr <sup>-1</sup> )
10	0	*NE	NE
	-0.2	NE	NE
	-0.4	NE	NE
	-0.6	NE	NE
	-0.8	NE	NE
15	0	76.3	0.013108
	-0.2	NE	NE
	-0.4	NE	NE
	-0.6	NE	NE
	-0.8	NE	NE
20	0	68.9	0.01451
	-0.2	70.7	0.01415
	-0.4	97.7	0.01024
	-0.6	NE	NE
	-0.8	NE	NE
25	0	23.3	0.04295
	-0.2	24.4	0.04099
	-0.4	26.1	0.0383
	-0.6	39.4	0.02537
	-0.8	NE	NE
30	0	21.0	0.04769
	-0.2	21.0	0.04552
	-0.4	23.0	0.04355
	-0.6	46.0	0.02175
	-0.8	NE	NE
35	0	20.0	0.05
	-0.2	20.0	0.05
	-0.4	20.0	0.05
	-0.6	21.0	0.04753
	-0.8	23.1	0.0432
40	0	NE	NE
	-0.2	NE	NE
	-0.4	NE	NE
	-0.6	NE	NE
	-0.8	NE	NE

\*NE, not estimated due to insufficient germination data



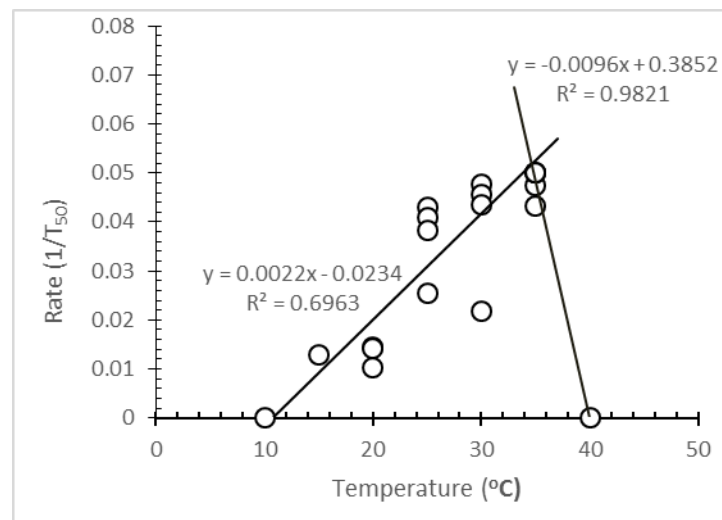


FIGURE 5. Effect of temperature on the germination rate of sethoxydim-resistant goosegrass biotype

TABLE 4. Estimated base water potential ( $\Psi_b$ ) and  $R^2$  at different temperatures

Temperature °C	$\Psi_b$ MPa	$R^2$
10	*NE	NE
15	NE	NE
20	-1.18	0.81
25	-1.39	0.82
30	-1.03	0.73
35	-4.80	0.73
40	NE	NE

\*NE, not estimated due to insufficient germination data

TABLE 5. Parameters  $G_{\max@T}$ ,  $\beta_1$ , and  $\beta_2$  as function curve-fitted using Eq. (5)

$V$	Parameters				R-squared
	$a$	$b$	$c$	$d$	
$G_{\max@T}$	0.010104	-0.845621	23.776354	-128.197739	0.982
$\beta_1$	0.000402	-0.045232	1.492894	-14.174435	0.983
$\beta_2$	-0.001430	0.108152	-2.927134	31.445681	0.984

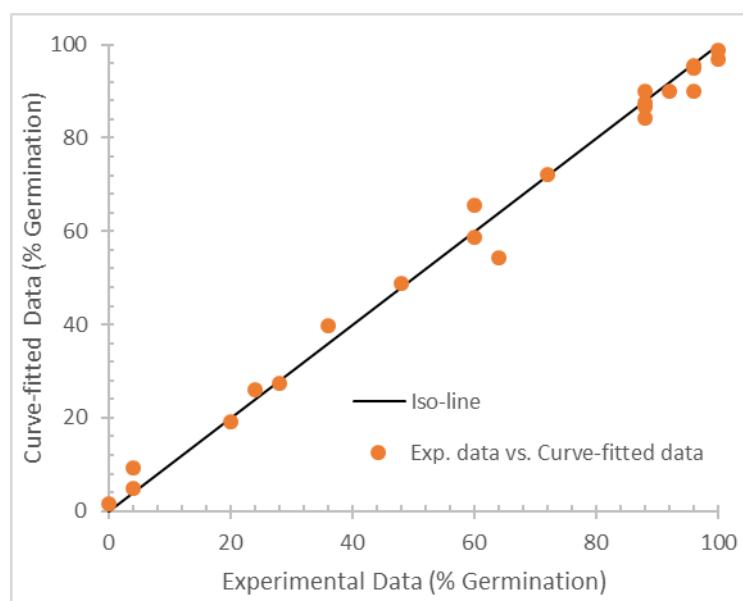


FIGURE 6. Comparing seed germination of sethoxydim-resistant goosegrass biotype between experimental data and curve-fitted data using Equations (4) and (5)

#### DISCUSSIONS

High dependency on herbicides causes intense selection pressure, which leads to the evolution of herbicide resistance in many weed species (Heap 2023). The current research has demonstrated the first instance of a sethoxydim-resistant (R) goosegrass biotype infesting an oil palm plantation in Malaysia. The first case of the R goosegrass biotype was discovered in soybean farms in Brazil in 2003 (Heap 2023). Later on, the R biotype of goosegrass was discovered in turf grass fields in the United States (McCullough et al. 2016). Both of the R biotypes were fluzifop cross resistant, and amino acid substitutions in the CT domain of plastidic acetyl-CoA carboxylase (ACCase) conferred resistance to the R biotypes (Heap 2023; McCullough et al. 2016). However, the R biotype in the present study does not evolve resistance to fluzifop, which has a similar mechanism of action to sethoxydim, an ACCase inhibitor herbicide. In Malaysia, fluzifop resistance in goosegrass was also caused by a point mutation in ACCase (Cha et al. 2014), but the point mutation probably does not contribute to sethoxydim resistance in goosegrass because the R biotype plants were fully killed after fluzifop treatment in this study. The current study's results also imply that enhanced metabolism is unlikely

to be the resistance mechanism, since a wide variety of herbicides, including photosystem I and II inhibitors, EPSPS inhibitors, ALS inhibitors, HPPD inhibitors, PPO inhibitors, and glutamine synthase inhibitors, can be employed to control the R biotype of goosegrass.

According to Chauhan and Johnson (2008), the germination of goosegrass sampled from in Los Baños and Batangas, Philippines decreased drastically as the osmotic potential decreased from 0 to -0.6 MPa and then ceased at -0.8 MPa. Similarly, Ismail et al. (2002) demonstrated that the germination of goosegrass seeds collected from the central region of Peninsular Malaysia was completely inhibited at a water potential of -0.80 MPa. In the present study, the R biotype of goosegrass seeds sampled from southern region of Peninsular Malaysia, did, however, germinate under -0.8 MPa osmotic potential. On the other hand, the base water potential estimated in this study was -1.28 MPa. This finding is in agreement with Masin et al. (2005) who discovered that goosegrass had a base water potential of -1.21 MPa. By contrast, the base water potential of different *Digitaria sanguinalis* populations were -0.50 and -0.83 MPa as reported by Forcella et al. (2000) and Masin et al. (2005), respectively. These discrepancies could be attributable to maternal and environmental

influences on seed physiology during maturation of the seeds, varying genetic structure of the seed accessions, different seed burial depths in soils in the studied populations, and different soil and management characteristics (Forcella et al. 2000).

At 35 °C, the R biotype of goosegrass seeds germinated by more than 85%, with water potentials varying from 0 to -0.80 MPa. This result is in line with the finding of Ma et al. (2012) who documented that goosegrass emergence occurred from April to September, with the peak of seedling emergence between early June and mid-July at high temperature of 32 °C. On the other hand, seed germination of goosegrass at 15 to 30 °C decreased dramatically as water potentials rose from 0 to -0.80 MPa. However, a previous study by Ismail et al. (2002) documented that hydration after experiencing moisture stress at -0.80MPa could break seed dormancy of goosegrass. When the temperature was decreased from 35 to 15 °C, the time it took to reach 50% seed germination ( $T_{50}$ ) increased. Similarly, as the water potential increased from 0 to -0.80 MPa, the  $T_{50}$  at 20 to 30 °C increased. However, at 35 °C, the water potential level had no effect on  $T_{50}$ . The results clearly showed that the R biotype of goosegrass seed is water stress tolerant at 35 °C. These competitive characteristics might explain why goosegrass is distributed almost throughout the tropical world and extends significantly into the sub-tropics, especially in South Asia, Southeast Asia, the Pacific, eastern and southern Africa, and tropical North America (Holm et al. 1977). In addition, it is also one of the most common rice weeds, particularly in rain-fed (Chauhan & Johnson 2009) and aerobic rice fields in tropical regions (Jaya Suria et al. 2011).

The goosegrass seeds were able to germinate at constant temperature under dark conditions in a previous study by Ismail et al. (2002), despite the germination percent being lower than alternating temperature. The current study found a similar pattern in goosegrass seed germination to that reported by Ismail et al. (2002) for temperature regimes of 20, 25, and 30 °C in the dark. Chauhan and Johnson (2008) demonstrated some populations of goosegrass could germinate regardless of light conditions. They discovered that after a certain amount of time, seeds lose their sensitivity to light, resulting in a similar germination pattern between alternating temperature and constant temperature. Kanzler and Staden (1984) showed that no germination of goosegrass seed occurred at a constant temperature of 10 °C in the dark, which is in line with the findings of the

present study. However, the estimated base temperature of goosegrass (10.6 °C) in this study was marginally lower than base temperature (12.6 °C) reported by Masin et al. (2005).

Temperature lower than base temperature can slow down the germination process. Cold temperatures delay the process of diffusion that interrupts imbibition and solutes escape from the seeds. This is considered as the most sensitive phase as the cold stress effect is very noticeable at the imbibing phase. Similar to this study, 10 °C is considered as minimum critical temperature for rice, where rice seeds cannot germinate (Bewley et al. 2013). However, goosegrass seeds did not germinate at 40 °C may be due to thermoinhibition. Endospermic phyB (phytochrome) can sense high temperature which increases its reversion into the inactive Pr state from the active signaling Pfr state. This causes thermoinhibition mediated by phytochrome-interacting factors (PIFs), mostly by PIF1, PIF3 and PIF5. Endospermic ABA catabolic gene CYP707A1 expression stops endospermic PIF3 and promotes the accumulation of endospermic ABA which released towards the embryo to block its growth. Moreover, endospermic ABA stops the accumulation of embryonic PIF3. Thus, PIF3 exerts opposite growth responses in the endosperm and embryo under high temperature (Piskurewicz et al. 2023).

#### CONCLUSIONS

The first instance of sethoxydim-resistant (R) goosegrass biotype without cross resistance to fluzifop has evolved in the field and confirmed in this study. At 35 °C, the R biotype of goosegrass seed was tolerant to water stress. However, the seed germination could be reduced drastically at 15 to 30 °C under water stress conditions. Knowing how temperature and moisture stress interact to affect germination in the R biotype of goosegrass allows us to determine the best time to implement early post and pre-emergent control practices after a large proportion of the R biotype of goosegrass seedlings have emerged, thus preventing seedling escapes from control practices. The base temperature and water potential obtained in this study could be used to build a mathematical model that explains the R biotype of goosegrass seed germination in terms of hydrothermal time in future research. The developed model will assist us in predicting the emergence of R goosegrass in the field for effective weed management.

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