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Germination attributes of metsulfuron-resistant and metsulfuron-susceptible tropical ageratum (*Ageratum conyzoides*) populations under various environmental conditions

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Research Article

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Abstract

Tropical ageratum (*Ageratum conyzoides* L.) is a problematic weed frequently observed in association with commercially important crops in Australian agroecosystems. Knowledge of the germination response of *A. conyzoides* is crucial for proactively managing this weed species, especially when herbicide resistance is involved. Herbicide screening and metsulfuron dose–response experiments were conducted on two separate populations of *A. conyzoides* (referred to as Sugarcane and Roadside) in an open environment to identify a metsulfuron-resistant population. Based on the survival percentage in the metsulfuron dose–response experiment, the Sugarcane population was found to be 54 times more resistant compared with the metsulfuron-susceptible population (Roadside). Subsequent laboratory experiments were performed to investigate the differential germination response of the two populations. No germination or emergence difference was observed between the Sugarcane and Roadside populations under various thermal regimes (15/5 to 35/25 C with a 12/12-h photoperiod), salinity levels (0 to 320 mM), osmotic potentials (0 to −1.6 MPa), and burial depths (1 to 4 cm). However, different environmental conditions significantly impacted the germination and emergence of *A. conyzoides*. *Ageratum conyzoides* germinated over a wide range of temperatures, with the highest germination rate (>90%) occurring at 30/20 C. With increasing levels of salinity, osmotic potential, and burial depth, the germination/emergence of *A. conyzoides* declined and was completely inhibited at 300 mM salinity, −0.8 MPa osmotic potential, and a 1-cm burial depth. The data generated from this study will be useful in developing a model-based approach to predict the occurrence of this weed species and thus aid in designing ecologically sustainable integrated weed management protocols.

Introduction

Tropical ageratum (*Ageratum conyzoides* L.) is an annual herb belonging to the Asteraceae family and is considered a troublesome weed in tropical and subtropical regions across the world. The name *Ageratum* is derived from the Greek word *ageras*, which translates to "non-aging," referring to the longevity of the entire plant (Kaur et al. 2012). *Ageratum conyzoides* is commonly referred to as "billy goat weed" in Australia due to its peculiar smell, resembling that of a male goat (Okunade 2002). *Ageratum conyzoides* is known to have originated from Central America and the Caribbean (Ming 1999). However, it has become widely established in warm regions across the globe, potentially due to its highly invasive nature. This weed poses a problem in agroecosystems, as it interferes with 36 crops in 46 different countries (Kaur et al. 2023).

In Europe, the commercial cultivation of *A. conyzoides* began as an ornamental plant, and it was known to be cultivated in Bosnia, Bulgaria, France, and Sweden until the early 1970s (Johnson 1971). Although *A. conyzoides* was initially cultivated in Fiji and Lord Howe Island, Australia, it has now become a problematic weed species in the Australian farming systems (Kaur et al. 2012). It is widely naturalized in humid parts of Northern Australia and has been identified as a major invasive weed in crops, pastures, and disturbed sites in northern Queensland and the Northern Territory (Holm et al. 1977). While growing in association with desired vegetation, it can act as a host for various pathogens in numerous crops, including yellow leaf curl in tomato (*Solanum lycopersicum* L.), leaf curl in cotton (*Gossypium hirsutum* L.), and ringspot virus in papaya (*Carica papaya* L.) (Kaur et al. 2023). Furthermore, this weed species is responsible for acting as a host for the chlorosis virus in capsicum (*Capsicum annuum* L.) (≤92% infection) in eastern Queensland, Australia (Sharman et al. 2020).

Ageratum conyzoides can grow up to 1 m, displaying erect branching, trichome-covered stems, and a shallow taproot system (Kohli et al. 2006). Due to the ability of *A. conyzoides* to form dense stands and produce large leaves in a wide range of temperatures, moisture conditions, soil textures, and altitudinal ranges, it can exploit available ecological niches, leading to rapid establishment by displacement of native vegetation (Kaur et al. 2023). *Ageratum conyzoides* can produce a significant number of viable seeds, about 95,000 seeds plant^{-1}; therefore, it is regarded as a prolific seed producer, with an extended seed-shattering period lasting from 5 to 8 mo (Ekeleme et al. 2000; Rodriguez and Cepero 1984). Nonetheless, these seeds remain viable for ≤12 mo (Okunade 2002). Seeds of *A. conyzoides* are minuscule (3.4 by 0.3 mm), positively photoblastic (Kaur et al. 2012), and feature two prominent awns that facilitate anemochory (wind dispersal) and zoochory (animal dispersal). Additionally, the seeds can adhere to agricultural machinery and disperse within agroecosystems.

Seed germination and emergence are crucial events that determine a weed's survivability, growth, reproduction, and fecundity in agroecosystems. These processes are known to be regulated by various environmental factors, such as temperature, light, soil moisture, salinity, and burial depth (Baskin and Baskin 1977). Differential germination responses between herbicide-susceptible and herbicide-resistant weed populations have been extensively reported (Babineau et al. 2017; Desai and Chauhan 2021; Kumar et al. 2018; Vila-Aiub et al. 2005). These observed differences could be attributed to the resource trade-off in plant metabolism, enabling survival under both biotic (e.g., pest or disease) and abiotic (e.g., herbicide applications) stress (Dhanda et al. 2022). Babineau et al. (2017) reported distinctive germination between iodosulfuron-resistant (acetolactate synthase [ALS] inhibitors [Group 2]) and iodosulfuron-susceptible populations of common windgrass [*Apera spica-venti* (L.) P. Beauv.].

A comprehensive understanding of seed germination biology is essential for predicting cohort establishment during the growing season, which aids in developing sustainable management protocols for problematic weed species, such as *A. conyzoides*. The primary objective of the current study is to evaluate the germination response of metsulfuron-resistant (MR) and metsulfuron-susceptible (MS) populations of *A. conyzoides*. This study addresses two research questions: (1) What are the alternative herbicide options to manage MR *A. conyzoides*? (2) Do MR populations of *A. conyzoides* differ in their environment-mediated germination responses (e.g., temperature, moisture, soil pH, salinity, and burial depth)? This study aimed to contribute to the limited knowledge available about the germination behavior of *A. conyzoides*. The results from this study will assist weed researchers and agronomists in developing successful management strategies for *A. conyzoides*.

Materials and Methods

Seed Collection

Seeds of two *A. conyzoides* populations were collected from a sugarcane field (18.664°S, 146.152°E) and a roadside area (18.590°S, 146.195°E) in Ingham, QLD, Australia, on March 23, 2021. Mature inflorescences were cut using a sickle and immediately placed in individual paper bags, sorted according to their respective populations. Seeds were collected from multiple plants (at least 50 plants) across the fields and were then bulked to ensure population representativeness. The collected seed bags were stored at room temperature (25 ± 2 C) at the Weed Science Laboratory (27.5551°S, 152.3343°E) of the University of Queensland, Gatton, until further use.

Herbicide Screening

A pot experiment was conducted in a randomized complete block design (RCBD) with four replications involving 14 herbicide treatments plus a control and two *A. conyzoides* (Sugarcane and Roadside) at Gatton Research Farm of the University of Queensland, from September 2021 to January 2022. Ten seeds of each population were placed on the surface of a pot filled with commercial potting mix (Centenary Landscaping, Mt Ommaney, QLD, Australia). The sieved potting mix was manually spread over seeds to achieve a consistent depth of 0.2 cm. Upon emergence, seedlings were thinned to 5 plants per pot. All pots were irrigated as needed.

Fourteen different herbicide treatments were applied at the 10- to 12-leaf stage (Table 1) using a stationary research track sprayer (manufactured by Woodlands Road Engineering, Gatton, QLD, Australia) calibrated to deliver a 108 L ha^{-1} spray volume through a flat-fan nozzle (TeeJet Spraying Systems Co., Wheaton, IL, USA). After spraying, all pots were kept away from any water supply for 24 h to account for the rainfast period of the herbicides.

The surviving seedlings per pot were recorded at 28 d after spraying (DAS) and converted into a survival percentage. At 28 DAS, surviving seedlings per pot were cut from the base, placed in paper bags, and then oven-dried at 70 C for 72 h. The oven-dried samples were weighed using a digital scale to record dry matter (g).

Metsulfuron Dose Response

The herbicide screening experiment revealed differential responses of the two populations to metsulfuron. Therefore, this pot experiment was conducted using RCBD with six replications and two *A. conyzoides* populations (Sugarcane and Roadside) from January to October 2022. Seed placement was carried out as described earlier. After emergence, plants were thinned to 4 plants per pot. Seven different doses of metsulfuron (0, 0.8, 1.5, 3, 6, 12, and 24 g ai ha^{-1}) were applied using a stationary research track sprayer, calibrated to deliver a 108 L ha^{-1} spray volume through flat-fan nozzles (TeeJet® XR 110015). Importantly, 3 g ai ha^{-1} is the recommended dose. Plants were sprayed at the 4- to 6-cm or 4- to 6-leaf stage. Sprayed plants were kept away from any water supply for 24 h to ensure the required rainfast period. Survival percentage and dry matter were recorded as described earlier.

Germination Ecology Experiments

Temperature-mediated Germination Response

An experiment was conducted in the Weed Science Laboratory, Gatton, QLD, Australia, by using RCBD with three replications from September to November 2022. Twenty-five seeds were evenly distributed in a petri dish (92-mm diameter by 16-mm height) with double filter papers (Macherey-Nagel, Duren, Germany). The petri dishes were moistened with 5 ml of ionized water using a micropipette (Boeco, Hamburg, Germany). Petri dishes were then placed in sealable transparent plastic bags, which were then placed in five different incubators (Labec Laboratory, Marrickville, Sydney, New South Wales, Australia) set to deliver distinct thermal conditions, 15/5, 20/10, 25/15, 30/20, and 35/25 C with a 12/12-h day/night photoperiod for 28 d. This experiment was repeated 10 d after completion of the first run.

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Table 1. Herbicides, their trade names, rates, and sites of actions and adjuvants used on two *Ageratum conyzoides* populations (Sugarcane and Roadside) collected from Ingham, QLD, Australia

Herbicide	Trade name	Rate	Site of action[a]	Group	Adjuvant
		—g ai or ae ha^{-1} —			
Control	—	—	—		—
2,4-D	Amicide	1,050	Growth regulator—T1R1 auxin receptors	4	—
2,4-D + picloram	Tordon75	112.5	Auxin receptors	4	—
Flumetsulam	Broadstrike	20	ALS inhibitors	2	0.5% Uptake®[b]
Glufosinate	Biffo	750	Inhibits glutamine synthase	10	—
Glyphosate	Roundup UltraMax®	570	Inhibits EPSPS	9	—
Imazapic	Impose	48	ALS inhibitors	2	1% Hasten®[c]
Imazethapyr	Spinnaker	25	ALS inhibitors	2	1% Hasten®[c]
Imazapyr + imazamox	Intervix	36	ALS inhibitors	2	1% Hasten®[c]
Ioxynil	Ioxynil	700	Inhibits photosynthesis	6	—
Metsulfuron	Associate	3	ALS inhibitors	2	—
Paraquat	Gramoxone®	600	Inhibits photosynthesis I	22	1% Hasten®[c]
Saflufenacil	Sharpen®	23.8	PPO inhibitors	14	1% Hasten®[c]
Saflufenacil + trifludimoxazin	Voraxor	37.5	PPO inhibitors	14	1% Hasten®[c]
Tiafenacil	Terrador	28	PPO inhibitors	14	1% CanDo™[d]

[a]ALS, acetolactate synthase; EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase; PPO, protoporphyrinogen oxidase.
[b]Uptake: Corteva Agriscience Australia Pty Ltd, Chatswood, New South Wales, Australia.
[c]Hasten: BASF Australia Ltd, Southbank, Victoria, Australia.
[d]Cando: Nufarm, Australia Ltd, Laverton North, Victoria, Australia.

Salinity-mediated (NaCl) Germination Response

An experiment was carried out using RCBD with three replications and two *A. conyzoides* populations (Sugarcane and Roadside), from October to December 2022. Seed placement in petri dishes was carried out as described previously. Petri dishes were treated with 0 (water only), 20-, 40-, 80-, 160-, and 320-mM sodium chloride (NaCl) solutions using a micropipette corresponding to the treatments. Petri dishes, including control (0 mM NaCl), were placed in the incubator set at 30/20 C (temperature conditions found optimum in the temperature experiment) and 12/12-h photoperiod for 28 d. This experiment was repeated 10 d after completion of the first run.

Osmotic Potential–mediated Germination Response

An experiment was undertaken using RCBD with three replications and two *A. conyzoides* populations (Sugarcane and Roadside) from October to December 2022. Seed placement in petri dishes was done as described earlier. All petri dishes were either treated with ionized water (control: 0 MPa osmotic potential) or −0.1, −0.2, −0.4, −0.8, and −1.6 MPa osmotic potential solutions corresponding to the treatment. The osmotic potential solutions were prepared by dissolving the appropriate quantity of polyethylene glycol (PEG) powder (Equation 1) in deionized water (Michel and Radcliffe 1995):

$$\Psi s = 0.13(\mathrm{PEG})^2\, T - 13.7(\mathrm{PEG})^2 \quad [1]$$

where ψ_s represents gravimetric component, "PEG" represents the quantity of polyethylene glycol (g PEG g^{-1} water) and T represents the temperature. The PEG-treated petri dishes plus control were incubated at 30/20 C temperature and 12/12 h-photoperiod for 28 d. This experiment was repeated 10 d after completion of the first run.

Response Variable Recorded in Germination Ecology Experiments

Once a clearly visible radicle emerged (≥1 mm), the seeds were considered germinated, and germination counts were recorded by removing seeds with forceps at 28 d after incubation. The germination counts were then converted into germination (%), which was used for further analysis.

Burial Depth–mediated Emergence Response

A pot experiment was conducted in RCBD with three replications and two *A. conyzoides* populations (Sugarcane and Roadside) from October 2022 to January 2023 at Gatton Research Farm of the University of Queensland. Thirty seeds of *A. conyzoides* were sown in individual pots (14-cm diameter) at a depth of 0 (on the soil surface), 0.5, 1, 2, and 4 cm for the emergence study. The soil used in this experiment was collected from the Gatton Research Farm and sieved through a 0.3-cm mesh. The soil is identified to contain 33:46:21 of sand:silt:clay with 2.6% organic matter and 7.1 pH. The pots were subirrigated to avoid damage caused by the sprinkler system. The seedlings were considered emerged once their coleoptiles became visible on the soil surface. The emergence counts were recorded after 28 d and converted into the emergence percentage before being subjected to data analysis. This experiment was repeated 21 d after completion of the first run.

Statistical Analyses

RStudio (v. 4.2.2, Posit Software, Boston, MA, USA) was the primary tool used for statistical model development. Two-way ANOVA models were developed for each experiment using the *aov* function to assess the variation between two runs. No significant difference was observed; therefore, data were pooled across the runs. Data from herbicide screening and temperature-mediated germination experiments were square-root transformed before being subjected to further analysis, as normality violations were detected in diagnostic plots developed using the DHARMa package. These data were fit to the linear mixed model using the LME4 package, and the effects of explanatory variables were compared based on the type II Wald χ^2 test using the *Anova* function of the CAR package. For the herbicide screening experiment, herbicide treatments, populations, and their interaction were fixed effects, and replication was a random effect. For the temperature-mediated germination experiment, temperatures, populations, and their interaction were fixed effects, and replication was a random effect. Means were separated by Fisher's

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protected LSD test ($P < 0.05$) using the EMMEANS and MULTCOMP packages.

Metsulfuron dose–response, NaCl, osmotic potential, and burial depth experimental data were fit to a three-parameter log-logistic model (Equation 2) using the *drm* function of the DRC package in R Studio (Ritz et al. 2015; Seefeldt et al. 1995):

$$Y = \{d/1 + \exp[b\ (\log X - E_{50})]\} \quad [2]$$

where Y is the response variables (survival [%], dry matter [% of control], germination [%], or emergence [%]); d represents the upper limit; b is the slope of each curve; X is the metsulfuron dose (g ai ha^{-1}), NaCl concentrations (mM), osmotic potential (MPa), or burial depth (cm); and E_{50} represents the metsulfuron dose (g ai ha^{-1}) required to cause 50% injury (referred to as LD_{50} and GR_{50} for survival percentage and dry matter, respectively), NaCl concentration (mM) or osmotic potential (MPa) required to inhibit 50% germination (referred to as GI_{50}), or burial depth (cm) required to inhibit 50% emergence (referred to as EI_{50}).

In addition, the metsulfuron dose required to cause 10% and 90% injury (referred to as LD_{10}/GR_{10} and LD_{90}/GR_{90}, respectively), NaCl concentration (mM) and osmotic potential (MPa) required to inhibit 10% and 90% of germination (referred to as GI_{10} and GI_{90}, respectively), and burial depth (cm) required to inhibit 10% and 90% emergence (referred to as EI_{10} and EI_{90}, respectively) were calculated using the *ED* function of the DRC package. For the metsulfuron dose–response experiment, estimated parameters were compared by approximate *t*-test using the *CompParm* function of the DRC package. The resistant index (R:S) was calculated by dividing LD_{50} and GR_{50} of two *A. conyzoides* populations.

Results and Discussion

Herbicide Screening

The results showed that the interaction of herbicides and populations had a significant impact ($P = 0.001$) on both dry matter (g) and survival percentage (Table 2). Paraquat, saflufenacil, saflufenacil + trifludimoxazin, glufosinate, and tiafenacil were found to be effective in controlling both populations of *A. conyzoides* (100% dry matter reduction and 0% survival at 28 DAS) (Table 2). The application of 2,4-D, 2,4-D + picloram, and glyphosate provided >95% dry matter reduction of both populations at 28 DAS; however, the survival percentage ranged from 10% to 38% (Table 2).

Metsulfuron provided varied degrees of control on two populations, as the Sugarcane population displayed 98% survival as opposed to 0% survival of the Roadside population (Table 2). These results suggest that the Sugarcane population could be resistant to metsulfuron; therefore, the dose–response study was conducted on both populations to confirm the level of resistance.

Metsulfuron Dose Response

The survival and dry matter responses of Sugarcane and Roadside populations were accurately described ($P > 0.05$) by a three-parameter log-logistic model (Equation 2) (Figure 1A and 1B). The Sugarcane population exhibited a 100% survival rate until the metsulfuron dose increased to 12 g ai ha^{-1}. To achieve complete control of the Sugarcane population, 48 g ai ha^{-1} metsulfuron was required, 16 times more than the recommended rate. In contrast, a mere 1.5 g ai ha^{-1} of metsulfuron was sufficient to control the Roadside population (Figure 1A and 1B). The LD_{50} of the Sugarcane population (26.9 g ai ha^{-1}) was significantly higher ($P < 0.001$ based on an approximate *t*-test) compared with the LD_{50} of the Roadside population (0.5 g ai ha^{-1}) (Table 3). Based on LD_{50} values of both populations, the Sugarcane population exhibited an average 54-fold (R/S ratio) higher resistance to metsulfuron compared with the Roadside population (Table 3). Similarly, the GR_{50} of the Sugarcane population (3 g ai ha^{-1}) was significantly higher ($P < 0.001$ based on an approximate *t*-test) than that of the Roadside population (0.1 g ai ha^{-1}), indicating that the Sugarcane population was 30-fold (R/S ratio) more resistant to metsulfuron compared with the Roadside population (Table 3). Additionally, a consistent trend was observed in LD_{10}/GR_{10} and LD_{90}/GR_{90} values (Table 3). The metsulfuron resistance in the Sugarcane population may be attributed to the extensive use of metsulfuron in agroecosystems, as the Roadside population was found to be susceptible. To our knowledge, this is the first report of MR *A. conyzoides* in Australia, as no other cases have been reported (Heap 2023).

The evolutionary process of resistance development results in functional changes in weeds (Baucom and Mauricio 2004) that aid in their survival and reproduction under abiotic stress (e.g., herbicide application). However, these spontaneous functional changes could reduce the overall fitness of weeds (Roux et al. 2004; Tardif et al. 2006), termed "fitness penalties." As an illustrative example from another system, early germination has been observed in sulfonylurea-resistant kochia [*Bassia scoparia* (L.) A.J. Scott] when compared with a sulfonylurea-susceptible population (Thompson et al. 1994). Therefore, in the present study, Sugarcane (MR) and Roadside (MS) populations were further employed to evaluate the germination and emergence responses in different environmental conditions.

Germination Ecology Experiments

Temperature-mediated Germination Response

The temperature regimes significantly impacted ($P < 0.001$) the germination of *A. conyzoides*; however, no significant difference was observed ($P = 0.844$) in germination between Sugarcane (MR) and Roadside (MS) populations (Figure 2). Therefore, the population variable was removed from the fitted model to select the parsimonious model. Seeds of both populations germinated at a wide range of temperatures (from 15/5 to 35/25 C) (Figure 2), indicating the potential for *A. conyzoides* to germinate throughout the year in the Queensland area. Similar year-round germination abilities were found in redflower ragleaf [*Crassocephalum crepidioides* (Benth.) S. Moore], horseweed [*Conyza canadensis* (L.) Cronquist], and *A. conyzoides* (Yuan and Wen 2018). The highest germination (>90%) was recorded at 30/20 C, which could be considered an ideal temperature for optimum germination. Moreover, *A. conyzoides* exhibited >80% germination at 20/10, 25/15, and 35/25 C (Figure 2). These results suggest that *A. conyzoides* could germinate throughout the spring and summer months and may possibly emerge in the early autumn in the northern part of Australia. The interference of this weed species in summer months with vegetable crops, such as *S. lycopersicum*, potato (*Solanum tuberosum* L.), *C. annuum*, and eggplant (*Solanum melongena* L.), has been observed (Pegg and Moffett 1971). *Ageratum conyzoides* also germinated (25% to 30%) at low temperatures (15/5 C); however, winter frost may damage seedlings in the early vegetative phase. We also acknowledge that the photoperiod that was utilized in the experiment does not reflect the photoperiod when

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Table 2. Survival and dry matter of two populations of *Ageratum conyzoides* (Sugarcane and Roadside) 28 d after herbicide treatments at the University of Queensland, Gatton, Australia

Herbicide(s)	Survival[a]		Dry matter[a,b]	
	Sugarcane	Roadside	Sugarcane	Roadside
	%		g plot^{-1}	
Control	100 a	100 a	4.9 a	4.64 a
2,4-D	38 d	28 de	0.1 (97.9) g	0 (100) g
2,4-D + picloram	15 efg	10 fgh	0.1 (98.6) g	0.1 (98.6) g
Flumetsulam	100 a	92.5 a	2.7 (46.3) ed	2.8 (40.1) cd
Glufosinate	0 h	2.5 gh	0 (100) g	0 (100) g
Glyphosate	18 ef	15 efg	0.1 (98.6) g	0 (100) g
Imazapic	100 a	100 a	3.6 (27.7) b	3.2 (31.9) bc
Imazethapyr	100 a	100 a	2.4 (52.7) de	2.4 (47.4) de
Imazapyr + imazamox	75 b	55 c	1.1 (77.9) f	0.2 (95.9) g
Ioxynil	18 ef	70 b	0.4 (92.7) hi	1.8 (60.6) g
Metsulfuron	98 a	0 h	3.5 (30.3) b	0.00 (100) g
Paraquat	0 h	0 h	0 (100) g	0.00 (100) g
Saflufenacil	0 h	0 h	0 (100) g	0.00 (100) g
Saflufenacil + trifludimoxazin	0 h	0 h	0 (100) g	0.00 (100) g
Tiafenacil	0 h	0 h	0 (100) g	0.00 (100) g

[a]Means followed by similar letters within the same response variable are nonsignificant differences based on Fisher's protected LSD test ($P < 0.05$). The interaction of herbicide and population were significant ($P < 0.001$) for both survival (%) and dry matter; therefore, Fisher's protected LSD test was conducted for interactive (herbicide:population) variable.
[b]Values in parentheses are dry matter reduction percentages compared with the control.

Table 3. Three-parameter log-logistic regression ($Y = \{d/1 + \exp[b(\log X - E_{50})]\}$) parameters for survival frequency and dry matter of metsulfuron-susceptible (Roadside) and metsulfuron-resistant (Sugarcane) populations of *Ageratum conyzoides* mentioned in Figure 1

	Regression parameter (±SE)[a]				
Population(s)	*b*	LD_{10} or GR_{10}	LD_{50} or GR_{50}	LD_{90} or GR_{90}	R/S[b]
Based on survival %					
Sugarcane	6.3 (1.7)	19.0 (1.4) a	26.9 (0.8) a	38.1 (4.5) a	54
Roadside	2.1 (0.5)	0.2 (0.1) b	0.5 (0.1) b	1.3 (0.2) b	—
Based on dry weight (% of control)					
Sugarcane	0.9 (0.02)	0.3 (0.01) a	3.0 (0.1) a	34.3 (1.5) a	30
Roadside	1.8 (1.3)	0.02 (0.1) b	0.1 (0.1) b	0.3 (0.2) b	—

[a]Regression parameters: *b*, slope; LD_{10} and GR_{10}, lethal dose of metsulfuron (g ai ha^{-1}) required for 10% control and dry matter reduction compared with control, respectively; LD_{50} and GR_{50}, lethal dose (g ai ha^{-1}) of metsulfuron required for 50% control and dry matter reduction compared with control, respectively; LD_{90} and GR_{90}, lethal dose (g ai ha^{-1}) of metsulfuron required for 90% control and dry matter reduction compared with control, respectively.
[b]Resistance/susceptible ratio is calculated based on LD_{50} and GR_{50} of Sugarcane and Roadside populations.

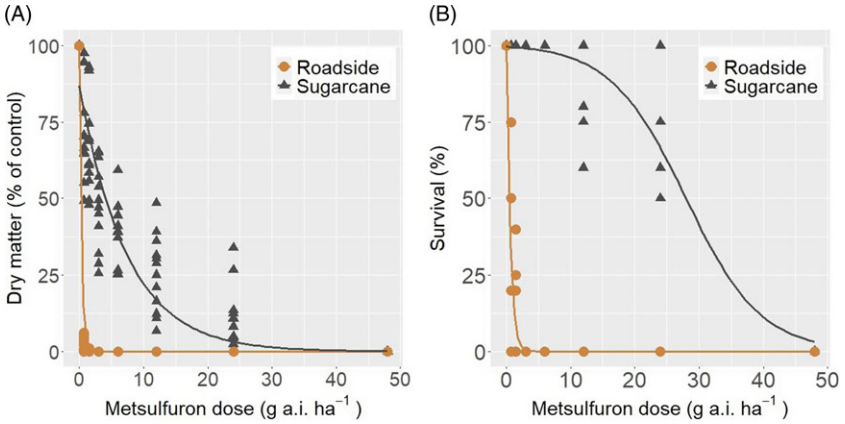

Figure 1. Dry matter (A) and survival frequency (B) of metsulfuron-susceptible (Roadside) and metsulfuron-resistant (Sugarcane) populations of *Ageratum conyzoides* at 28 d after the application of increasing doses of metsulfuron, modeled by a three-parameter log-logistic regression ($Y = \{d/1 + \exp[b(\log X - E_{50})]\}$) model. The parameters of the fitted model are presented in Table 3.

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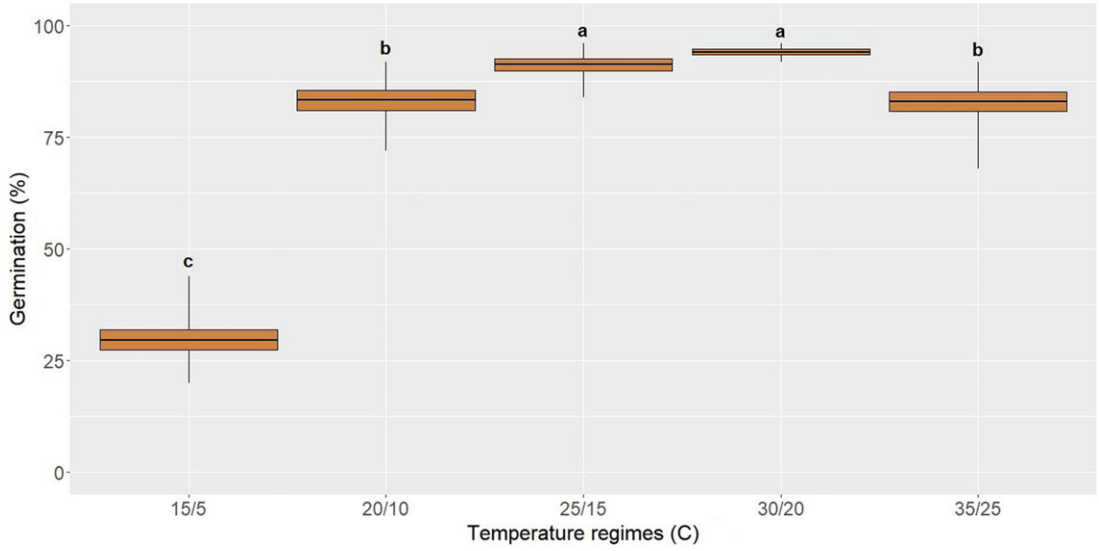

Figure 2. Germination response of *Ageratum conyzoides* when incubated in five alternating temperature regimes with 12/12-h light/dark photoperiod. The interaction of alternating temperature regimes and populations was nonsignificant; hence, two populations were combined to perform the mean separation test. Similar letters denote nonsignificant difference based on Fisher's protected LSD test ($\alpha = 0.05$). The boxes, center lines, and vertical lines represent the standard error of mean, mean, and whole data range, respectively.

temperatures decrease. Therefore, a phenology study on *A. conyzoides* should be conducted in the future, wherein different populations can be planted every month throughout the year to explore the survivability, growth, and fecundity of this weed species.

Salinity-mediated (NaCl) Germination Response

The germination of *A. conyzoides* was significantly affected ($P < 0.001$) by salinity but not by population ($P = 0.233$). Therefore, the population variable was removed from the fitted model to select the parsimonious model. The three-parameter log-logistic model adequately described ($P > 0.05$) the germination of *A. conyzoides* in response to salinity levels (Figure 3A). The maximum germination (88% to 100%) was observed in 0 mM NaCl concentration (control), which declined gradually as the salinity level increased (Figure 3A). Germination was completely inhibited at the 320 mM NaCl concentration (Figure 3A). The GI_{10}, GI_{50}, and GI_{90} were 96.45, 168.84, and 295.8 mM, respectively (Table 4).

Differential germination responses to salinity levels have been reported in two Asteraceae weeds: eclipta [*Eclipta prostrata* (L.) L.] and siamweed [*Chromolaena odorata* (L.) R.M. King & H. Rob.]. The GI_{50} values of *E. prostrata* and *C. odorata* were 194 mM (Chauhan and Johnson 2008b) and 171.6 mM (Chauhan and Johnson 2008a), respectively. Due to the high salinity tolerance of these Asteraceae weeds, crop cultivation might be impacted not only by salinity but also by weed competition (Chauhan and Johnson 2008a). A similar study on beggar's tick (*Bidens pilosa* L.) in Queensland reported that 120 mM NaCl inhibited 50% germination, while no seeds germinated at 250 mM NaCl (Chauhan et al. 2019). These results indicated that *A. conyzoides* might be more immune to salinity compared with other Asteraceae weed species. According to the Australian Bureau of Statistics, 107,000 ha of land in Queensland is considered saline (>100 mM NaCl) (Queensland Government 2013). Due to the higher salinity tolerance, these saline conditions could provide a germination niche for the establishment of this weed species.

Osmotic Potential–mediated Germination Response

The osmotic potential significantly affected ($P < 0.001$) the germination of *A. conyzoides*; however, no significant difference ($P = 0.716$) was found in germination between MR and MS populations. Therefore, the population variable was excluded from the fitted to select the parsimonious model. Germination of *A. conyzoides* under various levels of water stress was modeled using a three-parameter log-logistic model ($P > 0.05$) (Figure 3B). Maximum germination (92% to 100 %) was recorded under 0 MPa osmotic potential (Figure 3B). Consistent with the salinity-mediated germination response, germination declined as osmotic potential decreased (Figure 3B). The osmotic potential of −0.8 MPa was required to completely inhibit the germination of *A. conyzoides* (Figure 3B; Table 4).

At −0.3 MPa osmotic potential, the germination of *A. conyzoides* was 50% inhibited (GI_{50}) (Table 4), which is lower than the GI_{50} of blue weed (*Ageratum houstonianum* Mill.) (−0.7 MPa) (Lamsal et al. 2019). In other Asteraceae weed species, such as *C. odorata*, the osmotic potential required to inhibit 50% germination was recorded at −0.6 MPa, which is also higher than the GI_{50} of *A. conyzoides*. These results suggest that *A. conyzoides* is less tolerant to water stress compared with other Asteraceae weed species. Moreover, in the present study, the germination of *A. conyzoides* drastically declined beyond the −0.4 MPa osmotic potential, suggesting that a moist environment is needed for the germination of this weed species. Lal (2022) mentioned that the occurrence of *A. conyzoides* is more common in tropical and subtropical regions with higher precipitation, which supports the aforementioned claim of the less water stress–tolerant characteristic of this weed species.

Burial depth–mediated Emergence Response

The burial depth had a significant effect ($P < 0.001$) on the germination of *A. conyzoides*. However, there was no significant difference ($P = 0.840$) in germination between the MR and MS populations. Therefore, the population variable was removed from

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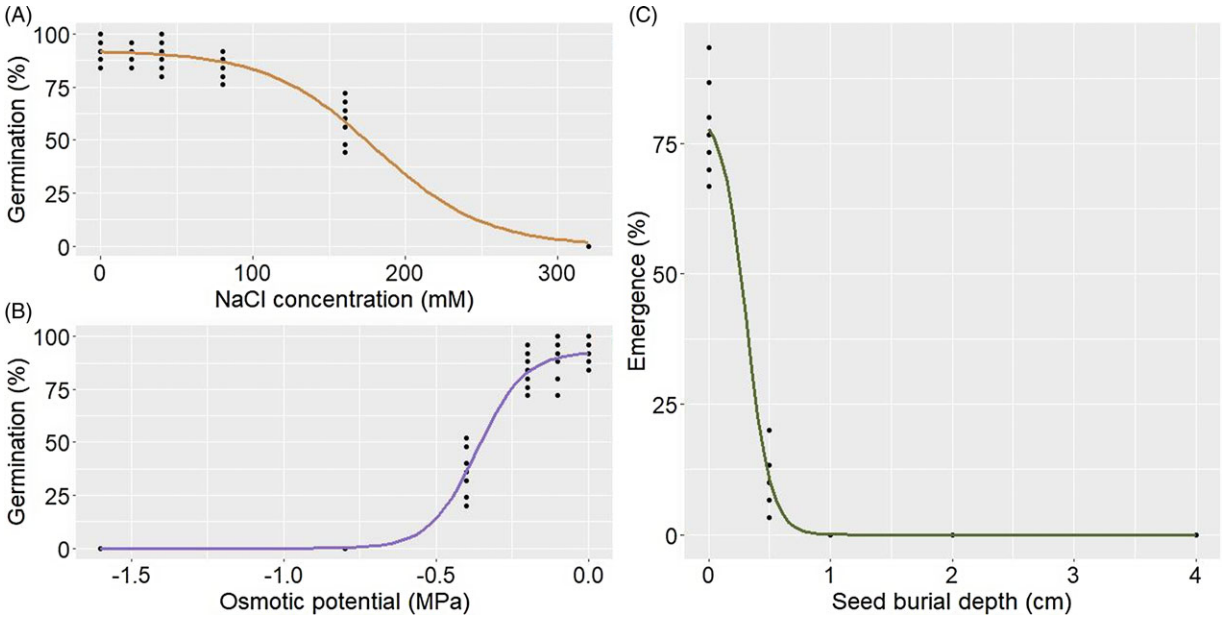

Figure 3. Effect of increasing levels of sodium chloride (NaCl) concentration (A), osmotic potential (B), and seed burial depth (C) on germination or emergence of *Ageratum conyzoides*, modeled by a three-parameter log-logistic regression model ($Y = \{d/1 + \exp[b(\log X - E_{50})]\}$). The parameters of the fitted model are presented in Table 4.

Table 4. Three-parameter log-logistic regression ($Y = \{d/1 + \exp[b(\log X - E_{50})]\}$) parameters for germination in sodium chloride (NaCl) concentrations and osmotic potential and emergence in seed burial depth of *Ageratum conyzoides* mentioned in Figure 3

	Regression parameter (±SE)[a]				
Population(s)	*b*	GI_{10} or EI_{10}	GI_{50} or EI_{50}	GI_{90} or E_{90}	R^2 [b]
NaCl concentration (mM)	3.9 (0.6)	96.45 (10.27)	168.84 (4.99)	295.58 (25.55)	0.98
Osmotic potential (MPa)	3.2 (0.2)	−0.16 (0.01)	−0.32 (0.01)	−0.65 (0.03)	0.99
Burial depth (cm)	7.3 (11.9)	0.29 (0.26)	0.38 (0.16)	0.52 (0.04)	0.99

[a]*b*, slope; GI_{10}, NaCl concentration and osmotic potential required for 10% germination inhibition; GI_{50}, NaCl concentration and osmotic potential required for 50% germination inhibition; GI_{90}, NaCl concentration and osmotic potential required for 90% germination inhibition. Similarly, EI_{10}, burial depth required for 10% emergence inhibition; EI_{50}, burial depth required for 50% emergence inhibition; EI_{90}, burial depth required for 90% emergence inhibition.
[b]R^2, coefficient of determination averaged over two populations within the same ecological factors.

the fitted model to select the parsimonious model. The three-parameter log-logistic model accurately described ($P > 0.05$) the emergence response of *A. conyzoides* under increasing burial depths (Figure 3C). The maximum emergence (66.7% to 93.3%) was observed for surface seeds (Figure 3C). However, with increasing burial depth, the emergence of *A. conyzoides* drastically declined and was completely inhibited at 1-cm depth (Figure 3C). The burial depth values required to inhibit 10% (EI_{10}), 50% (EI_{50}), and 90% (EI_{90}) emergence were 0.3, 0.4, and 0.5 cm, respectively (Table 4).

As *A. conyzoides* populations failed to emerge from 1-cm or greater burial depth (Figure 3C), this species is categorized as a surface-emerging weed. A similar emergence pattern has been reported for other Asteraceae weed species, such as *A. houstonianum* (Lamsal et al. 2019) and *C. canadensis* (Nandula et al. 2006). These results suggest that the seeds of *A. conyzoides* are photoblastic in nature. Due the absence of sunlight in a deeper soil profile, seeds were not able to emerge from the 1-cm burial depth. In conventional tillage systems, *A. conyzoides* seeds can be buried below 1 cm through tillage to manage this weed species. *Ageratum conyzoides* seeds can remain viable for up to 12 mo in the soil seedbank (Marks and Nwachuku 1986). Therefore, tillage operations should be carried out at a shallow depth to avoid the reintroduction of seeds from a deeper soil profile (>1 cm) to the soil surface.

The present study discovered the evolution of metsulfuron resistance in an *A. conyzoides* population (MR) collected from a sugarcane field in Ingham, QLD, Australia. However, the germination and emergence studies revealed a similar response between the MR and MS populations to various environmental factors. The germination ability of *A. conyzoides* under a wide range of alternating temperature regimes indicates the potential year-round occurrence of this weed species in the Queensland region. *Ageratum conyzoides* is well adapted to a broad range of salinity levels; however, its water-stress adaptability is lower compared with other Asteraceae weed species. *Ageratum conyzoides* could not emerge when buried at 1 cm or deeper in the soil, suggesting the potential utilization of shallow tillage to manage this weed species. This study identifies important research gaps, such as a lack of phenology studies, to acquire comprehensive knowledge of the year-round growth, reproduction, and fecundity of this weed species. The evolution of metsulfuron resistance impacted

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neither the salinity and water-stress tolerance nor the emergence adaptability to different burial depths of *A. conyzoides*. However, we acknowledge that these results cannot be generalized, as we used only one population of each category and our findings may not apply to other weed species. Results from the current study could be useful for weed scientists and agronomists to proactively design an effective management protocol to control this weed species.

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References

Babineau M, Mathiassen SK, Kristensen M, Kudsk P (2017) Fitness of ALS-inhibitors herbicide resistant population of loose silky bentgrass (*Apera spica-venti*). Front Plant Sci 8:1660

Baskin JM, Baskin CC (1977) Role of temperature in the germination ecology of three summer annual weeds. Oecologia 30:377–382

Baucom RS, Mauricio R (2004) Fitness costs and benefits of novel herbicide tolerance in a noxious weed. Proc Natl Acad Sci USA 101:13386–13390

Chauhan BS, Ali HH, Florentine S (2019) Seed germination ecology of *Bidens pilosa* and its implications for weed management. Sci Rep 9:1–9

Chauhan BS, Johnson DE (2008a) Germination ecology of two troublesome Asteraceae species of rainfed rice: Siam weed (*Chromolaena odorata*) and coat buttons (*Tridax procumbens*). Weed Sci 56:567–573

Chauhan BS, Johnson DE (2008b) Influence of environmental factors on seed germination and seedling emergence of eclipta (*Eclipta prostrata*) in a tropical environment. Weed Sci 56:383–388

Desai HS, Chauhan BS (2021) Differential germination characteristics of glyphosate-resistant and glyphosate-susceptible *Chloris virgata* populations under different temperature and moisture stress regimes. PLoS ONE 16: e0253346

Dhanda S, Kaur S, Chaudhary A, Jugulam M, Hunjan MS, Sangha MK, Bhullar MS (2022) Characterization and management of metsulfuron-resistant *Rumex dentatus* biotypes in northwest India. Agron J 114:366–378

Ekeleme F, Akobundu IO, Isichei AO, Chikoye D (2000) Influence of fallow type and land-use intensity on weed seed rain in a forest/savanna transition zone. Weed Sci 48:604–612

Heap I (2023) The International Herbicide-Resistant Weed Database. https://www.weedscience.org/Home.aspx. Accessed: November 22, 2023

Holm LG, Plucknett DL, Pancho JV, Herberger JP (1977) The World's Worst Weeds: Distribution and Biology. Honolulu: University Press of Hawaii. 609 p

Johnson MF (1971) A monograph of the genus *Ageratum* L. (Compositae-Eupatorieae). Ann Missouri Bot Gard 58:6–88

Kaur A, Kaur S, Singh HP, Datta A, Chauhan BS, Ullah H, Kohli RK, Batish DR (2023) Ecology, biology, environmental impacts, and management of an agro-environmental weed *Ageratum conyzoides*. Plants 12:2329

Kaur S, Batish DR, Kohli R, Singh H (2012) *Ageratum conyzoides*: an alien invasive weed in India. Pages 57–76 *in* Bhatt JR, Singh JS, Singh SP, Tripathi RS, Kohli RK, eds. Invasive Alien Plants: An Ecological Appraisal for the Indian Subcontinent. Wallingford, UK: CABI

Kohli RK, Batish DR, Singh H, Dogra KS (2006) Status, invasiveness and environmental threats of three tropical American invasive weeds (*Parthenium hysterophorus* L., *Ageratum conyzoides* L., *Lantana camara* L.) in India. Biol Invasions 8:1501–1510

Kumar V, Jha P, Lim CA, Stahlman PW (2018) Differential germination characteristics of dicamba-resistant kochia (*Bassia scoparia*) populations in response to temperature. Weed Sci 66:721–728

Lal M (2022) Floristic diversity and Ethnobotany of family Asteraceae of district Bhiwani (Haryana), India

Lamsal A, Devkota MP, Shrestha DS, Joshi S, Shrestha A (2019) Seed germination ecology of *Ageratum houstonianum*: a major invasive weed in Nepal. PLoS ONE 14:e0225430

Marks M, Nwachuku A (1986) Seed-bank characteristics in a group of tropical weeds. Weed Res 26:151–158

Michel BE, Radcliffe D (1995) A computer program relating solute potential to solution composition for five solutes. Agron J 87:126–130

Ming LC (1999) *Ageratum conyzoides*: A tropical source of medicinal and agricultural products. Pages 469–473 *in* Janick J, ed. Perspectives on New Crops and New Uses. Alexandria, VA: ASHS Press

Nandula VK, Eubank TW, Poston DH, Koger CH, Reddy KN (2006) Factors affecting germination of horseweed (*Conyza canadensis*). Weed Sci 54: 898–902

Okunade AL (2002) *Ageratum conyzoides* L. (Asteraceae). Fitoterapia 73:1–16

Pegg K, Moffett ML (1971) Host range of the ginger strain of *Pseudomonas solanacearum* in Queensland. Aust J Exp Agric 11:696–698

Queensland Government (2013) Types of Salinity. https://www.qld.gov.au/environment/land/management/soil/salinity/types. Accessed: November 22, 2023

Ritz C, Baty F, Streibig JC, Gerhard D (2015) Dose-response analysis using R. PLoS ONE 10:e0146021

Rodriguez GS and Cepero GS (1984) Number of seeds produced by some weed species. Cent. Agric 11:45–50

Roux F, Gasquez J, Reboud X (2004) The dominance of the herbicide resistance cost in several *Arabidopsis thaliana* mutant lines. Genetics 166:449–460

Seefeldt SS, Jensen JE, Fuerst EP (1995) Log-logistic analysis of herbicide dose-response relationships. Weed Technol 9:218–227

Sharman M, Thomas J, Tree D, Persley D (2020) Natural host range and thrips transmission of capsicum chlorosis virus in Australia. Australas Plant Pathol 49:45–51

Tardif FJ, Rajcan I, Costea M (2006) A mutation in the herbicide target site acetohydroxyacid synthase produces morphological and structural alterations and reduces fitness in *Amaranthus powellii*. New Phytol 169:251–264

Thompson CR, Thill DC, Shafii B (1994) Germination characteristics of sulfonylurea-resistant and-susceptible kochia (*Kochia scoparia*). Weed Sci 42:50–56

Vila-Aiub M, Neve P, Steadman K, Powles S (2005) Ecological fitness of a multiple herbicide-resistant *Lolium rigidum* population: dynamics of seed germination and seedling emergence of resistant and susceptible phenotypes. J Appl Ecol 42:288–298

Yuan X, Wen B (2018) Seed germination response to high temperature and water stress in three invasive Asteraceae weeds from Xishuangbanna, SW China. PLoS ONE 13:e0191710

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