

Article

Potential Use of Herbicide Seed Safener and Pre-Emergent Residual Herbicides When Establishing Tropical Perennial Grasses—A Preliminary Study

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Abstract: Annual grass weeds can provide significant competition to an establishing sown tropical perennial grass pasture. At least two years of grass weed control prior to sowing is required to reduce the weed seed bank. Pre-emergent herbicides used in summer cereals, such as atrazine or s-metolachlor with metcamifen seed safener, may reduce this preparation time. Two controlled-environment experiments were conducted to assess the potential for these pre-emergent herbicides to be used with several tropical perennial grasses. Experiment 1 tested the effect of metcamifen (400 g L⁻¹ a.i. at 0–2× label rate) on the emergence and vigor of *Chloris gayana*, *Dichanthium aristatum*, *Digitaria eriantha* and *Panicum coloratum*, with *Sorghum bicolor* as the control. Experiment 2 tested the effect of s-metolachlor (960 g ha⁻¹ a.i.) with metcamifen-treated or untreated seed, and atrazine (1800 g ha⁻¹ a.i.) on the emergence and early growth of the grasses. Metcamifen did not inhibit emergence or vigor of the grasses. Without metcamifen seed treatment, s-metolachlor reduced the growth of the tropical perennial grasses by 47–100%, while it had no such effect on *S. bicolor*. In contrast, there was no effect of atrazine on shoot yields of the grasses, nor of s-metolachlor when *D. aristatum*, *D. eriantha* and *P. coloratum* seed had been treated with metcamifen. The collective results indicate that the herbicide safener metcamifen does not reduce the viability of tropical perennial grass seed and provides some protection against s-metolachlor, albeit not complete protection at the rates used in our study. Atrazine did not affect emergence or early growth of the grasses.

Keywords: C₄ grasses; paddock preparation; field preparation; pasture establishment; seed treatment; weed control



Received: 14 February 2025

Revised: 24 March 2025

Accepted: 28 March 2025

Published: 2 April 2025

Citation: Boschma, S.P.; McDouall, H.W.; McLachlan, J.W. Potential Use of Herbicide Seed Safener and Pre-Emergent Residual Herbicides When Establishing Tropical Perennial Grasses—A Preliminary Study. *Seeds* **2025**, *4*, 18. <https://doi.org/10.3390/seeds4020018>

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1. Introduction

Sown tropical perennial grasses are a major component of livestock production systems in tropical and subtropical environments worldwide [1,2], including the extensive grazing systems of northern Australia [3]. In the last two decades, the distribution of these grasses in Australia has extended beyond the traditional zones. These include regions of summer dominant rainfall that receive significant frost (e.g., northern New South Wales; [4,5]) or have a Mediterranean environment (e.g., Western Australia; [6,7]), where they have become an important component of the feedbase. Most recently, these grasses have been trialed in non-seasonal/winter dominant rainfall environments so as to utilize the increased summer

rainfall that these areas have been experiencing and to help fill the traditional summer–early autumn feed gap [8,9].

Successful pasture establishment is key to ensuring an adequate density of desirable species and faster productive capacity for the grazing enterprise [10]. Soil moisture conditions need to be conducive for establishment [11], but other factors important for successful establishment include sowing high-quality seed with shallow seed placement when soil temperatures are favorable [10,12,13]. Good field preparation, to reduce weed burdens and the weed seed bank, is also key to successful establishment because weeds provide significant competition to the establishing sown grass [10]. This is particularly important for summer-growing annual grass weeds such as *Echinochloa glabrescens* Munro ex Hook.f. and *Urochloa panicoides* P. Beauv. as they cannot easily be controlled after the sown grass pasture has emerged.

Tropical perennial grasses are relatively slow to establish and are not competitive against summer weeds at the high seed numbers that can occur in weed seed banks [10]. It can take at least two years to reduce a weed seed bank to levels that allow successful tropical grass pasture establishment [10]. The length of time required to reduce the seed bank is associated with the size of the seed bank and the number of summer rainfall events that result in weed emergence [10,14–16]. While this ‘cleanup’ phase can be incorporated into a farming and/or grazing system by sowing winter crops (grain and/or forage) and maintaining a weed-free fallow over the summer period [17,18], the 2+ year timeframe is not favorable for producers [18]. It would be advantageous to incorporate other strategies such as pre-emergent herbicides.

Pre-emergent herbicides such as atrazine and s-metolachlor are widely used in summer cereal crops such as *Sorghum bicolor* (L.) Moench. and *Zea mays* L.; however, neither herbicide is commonly used in pastures. A range of tolerance levels among tropical perennial grass species to atrazine applied pre-emergence have been reported. For example, *Bothriochloa insculpta* (Hochst. ex A. Rich.) A. Camus, *Urochloa decumbens* (Stapf) R.D. Webster (syn. *Brachiaria decumbens*), and *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs (syn. *Panicum maximum*) have been reported as tolerant [19–21]. Conversely, *Cenchrus ciliaris* L., *Chloris gayana* Kunth, *Panicum coloratum* L., *Paspalum plicatulum* Michx and *Setaria sphacelata* (Schumach.) Stapf & C.E. Hubb. (syn. *Setaria anceps*) have been reported as susceptible to atrazine [19–23]. *Cenchrus clandestinus* (Hochst. ex Chiov.) Morrone (syn. *Pennisetum clandestinum*) is considered intermediate as it is tolerant to lower rates of atrazine [22]. Currently atrazine is registered for use in *M. maximus* and *U. decumbens* seed crops [24]. The herbicide s-metolachlor is registered to control common summer-growing grass weeds in crops such as *Z. mays* and *Saccharum officinarum* L. Among perennial pasture grasses, *C. gayana* and *C. clandestinus* have low tolerance to s-metolachlor [25], while *P. coloratum* is tolerant of low rates (0.28 kg ha^{−1} a.i.) [23]. Also, s-metolachlor is registered to control summer grasses in *S. bicolor* that has been treated with a seed safener.

Herbicide seed safeners are a group of chemicals that protect grass crops against herbicide without reducing the effectiveness of the herbicide to control the target weed species, thereby providing a level of herbicide selectivity [26,27]. A herbicide seed safener such as metcamifen (2-methoxy-N-[[4-(3-methylureido)phenyl]sulfonyl]benzamide, [28]) can be used with s-metolachlor to protect emerging crop seedlings while the herbicide is still effective on weeds. This is because metcamifen stimulates production of glutathione-S-transferase enzymes within cells of the germinating treated crop seed. These enzymes increase the ability of cells to detoxify s-metolachlor, thus providing tolerance to the herbicide [29,30]. This seed safener technology is used to protect emerging seedlings of *S. bicolor* from s-metolachlor damage [29,31]; however, there are no known studies testing the potential of metcamifen to protect the seed of tropical perennial grasses against s-

metolachlor. Therefore, this technology could provide a novel option to use pre-emergent herbicides when establishing tropical grass pastures.

The aims of this study were to (1) test the effects of the seed safener metcamifen on the emergence and vigor of several commonly sown tropical perennial grasses, and (2) assess the tolerance of these grasses to atrazine and s-metolachlor plus metcamifen. This is pioneering work that will provide an insight into the potential for metcamifen to be used in conjunction with s-metolachlor when establishing tropical perennial grass pastures, and will enhance our understanding of the appropriateness of atrazine as a pre-emergent herbicide for several untested tropical perennial grasses.

2. Materials and Methods

Two controlled-environment experiments were conducted to assess the potential for pre-emergent herbicides to be used when establishing several commonly sown tropical perennial grasses. Each experiment was conducted once using the same growing conditions. The first experiment investigated the effect of the herbicide seed safener metcamifen on seedling emergence and vigor. Previous findings indicated that the viability of tropical perennial grass seed is reduced when treated with a seed safener [S.P Boschma, Pers. Comm.]. The second experiment investigated the effect of atrazine and s-metolachlor with and without metcamifen seed treatment on the emergence and early growth of the grasses.

2.1. Experiment 1: Assessing the Effect of the Herbicide Seed Safener Metcamifen on the Emergence and Vigour of Several Tropical Perennial Grasses

A clay soil was collected from the 0–15 cm layer of a field at ‘Laureldale Research Station’ at the University of New England, Armidale, NSW (30°28′24.9″ S 151°39′06.4″ E). The soil was crushed and sieved to <5 mm. Basal nutrients were applied to the soil by spraying a solution that contained 176 mg kg^{−1} soil KH₂PO₄, 34 mg kg^{−1} MgSO₄, 36 mg kg^{−1} CaSO₄·2H₂O, 141 mg kg^{−1} KNO₃, 23 mg kg^{−1} (NH₄)₂SO₄, 14 mg kg^{−1} NH₄NO₃, 99 µg kg^{−1} H₃BO₃, 635 µg kg^{−1} MnCl₂·4H₂O, 301 µg kg^{−1} ZnSO₄·7H₂O, 28 µg kg^{−1} CuSO₄·5H₂O, 60 µg kg^{−1} (NH₄)₂MoO₄, 17 µg kg^{−1} CoCl₂·6H₂O and 1283 µg kg^{−1} FeNa-EDTA while mixing the soil. After being amended with basal nutrients, the soil contained 18 mg kg^{−1} ammonium nitrogen (N), 95 mg kg^{−1} nitrate N, 487 mg kg^{−1} Colwell phosphorus (P), 660 mg kg^{−1} Colwell potassium (K), 29 mg kg^{−1} KCl-40 sulfur (S), and 3.2% organic carbon, and had a pH_{CaCl2} of 5.5. Seedling trays (335 mm length, 280 mm width, 50 mm depth) were filled with 3 kg (oven-dry equivalent) of the amended soil and placed in a glasshouse at the University of New England, Armidale, NSW, during January and February 2023. The glasshouse was naturally lit and the temperature was set at 30 °C during the day and 22 °C overnight for the duration of the experiment. The average day and night temperatures fluctuated no more than 0.5 °C from the set temperatures, while humidity averaged 51% during the day and 70% overnight.

Twenty grams of commercially available pre-coated seed of *C. gayana*, *Dichanthium aristatum* (Poir.) C.E. Hubb., *Digitaria eriantha* Steud., *P. coloratum* and *S. bicolor* was weighed into petri dishes and treated by spraying a suspension of metcamifen (400 g L^{−1} a.i., Syngenta Epivio® C) on the seed of each species at 0×, 0.25×, 0.5×, 1× and 2× the label rate (250 mL per 100 kg seed) for *S. bicolor*. The petri dishes were large enough that a single layer of seed was treated, meaning that there was thorough coverage of metcamifen. Then, the treated seed was air-dried and mixed before 200 randomly selected seeds were sown to a depth of 10 mm in the seedling trays described above. There were four replicates for each species at each application rate of herbicide seed safener. The seedling trays were arranged in a randomized complete block design in the glasshouse bay. Shade cloth was placed over the trays for four days following sowing to reduce soil evaporation and was removed once

seedlings started to emerge. Adequate soil moisture was maintained by watering the trays twice daily using rainwater for the duration of the experiment.

Seedling emergence and average sward height were recorded daily, commencing three days after sowing. Seedling emergence was assessed until 14 days after sowing and average sward height recorded until the plants were harvested. Nine days after sowing, all *S. bicolor* plants were harvested due to the high growth rates of this crop species. Fifteen days after sowing, the four tropical perennial grasses were thinned to 20 plants per tray by randomly removing seedlings. This was done so that any effect the seed safener had on seedling emergence did not confound the effect it had on the subsequent growth of the grass microwards. Twenty-four days after sowing, shoots of the tropical perennial grasses were harvested at the soil surface. Harvested plant samples were dried at 70 °C for 72 h and then weighed.

The time to seedling emergence was determined by fitting a self-starting Weibull growth function ($y = a - b \times \exp(-\exp(c) \times x^d)$, where x is days and y is emergence). The model and its parameterization are described by Crawley [32], with the parameters of the fitted growth functions provided in the Supplementary Materials (Table S1). Time to 90% emergence was calculated as the number of days taken to achieve 90% of maximum emergence, based on the fitted Weibull growth functions. The 95% confidence intervals of the time to emergence were determined by bootstrapping residuals, as described by Crawley [32]. Shoot yield, used as an indicator of seedling vigor, was presented as shoot dry matter on a per plant basis. Statistical tests were performed in R Version 4.0.2 [33] at a significance level of $\alpha = 0.05$. Measured parameters of the four perennial grasses were analyzed by fitting linear models and using an analysis of variance (ANOVA) with ‘species’ and ‘treatment’ as predictor variables. When appropriate, the effect of ‘replicate’ was included in the most parsimonious model. *Sorghum bicolor* was not included in the analysis because of its significantly higher growth rate and earlier harvest date compared to the tropical perennial grasses. Normal quantile–quantile plots and Shapiro–Wilk tests were used to test the normality of the residuals for all fitted models. Fisher’s least significant difference (LSD) method was used to determine the difference between treatments (R package: agricolae) [34].

2.2. Experiment 2: Assessing the Effect of Atrazine and S-Metolachlor Plus Metcamifen on the Emergence and Early Growth of Several Tropical Perennial Grasses

The same clay soil was collected and amended with basal nutrients as described in Experiment 1. After amending with basal nutrients, the soil had 15 mg kg^{−1} ammonium N, 38 mg kg^{−1} nitrate N, 426 mg kg^{−1} Cowell P, 758 mg kg^{−1} Colwell K, 26 mg kg^{−1} KCl-40 S, 4.0% organic carbon, and a pH_{CaCl2} of 5.7. Cylindrical PVC pots (200 mm depth, 87 mm diameter) were filled with 1 kg (oven-dry equivalent) of the amended soil. The pots were placed in a glasshouse under the same conditions as described in Experiment 1 during July and August 2023. Again, the average day and night temperatures fluctuated no more than 0.5 °C from the set temperatures, while humidity averaged 25 and 33% during the day and night, respectively. The five species investigated in Experiment 1, *C. gayana*, *D. aristatum*, *D. eriantha*, *P. coloratum*, and *S. bicolor* (as the control), were again used.

Four treatments were prepared to investigate the effect of two pre-emergent herbicides on the grasses: (1) no herbicide or seed safener (the control), (2) atrazine (900 g kg^{−1} a.i., Syngenta Gesaprim® Granules) applied at 2 kg ha^{−1}, (3) s-metolachlor (960 g L^{−1} a.i., Syngenta Dual Gold®) applied at 1 L ha^{−1} without herbicide seed safener, and (4) s-metolachlor with metcamifen (400 g L^{−1} a.i.) seed safener applied at 250 mL per 100 kg seed. The herbicide rates were at the lower end of the recommended range for *S. bicolor* as it was expected higher rates would cause excessive damage to the less vigorous seedlings of the tropical perennial grasses.

Untreated seed was sown for treatments 1, 2 and 3, while seed treated with metcamifen (as described in Experiment 1) was sown for treatment 4. There were 100 seeds of the four tropical perennial grasses sown per pot and 50 seeds of *S. bicolor* sown per pot. *Sorghum bicolor* was sown at a lower rate due to its significantly higher germination percentage than the other grasses. All seeds were sown at a depth of 10 mm and the pots were watered using rainwater.

The following day, atrazine and s-metolachlor were applied by spraying solutions of the herbicides onto the soil surface. Then, all pots were covered with shade cloth, which was removed after three days once seedlings started to emerge. Each of the pots was placed in an individual saucer so that soil moisture could be maintained near field capacity for the duration of the experiment, which reduced the need for rainwater to be applied to the soil surface and minimized leaching of the applied herbicides. Seedling emergence and average sward height were recorded twice weekly from three days post sowing until the pots were thinned, after which only average sward height was recorded until harvest. Seventeen days after sowing, the pots were thinned to six plants per pot by randomly removing seedlings. This was to ensure that any effect the herbicide treatments had on emergence did not confound the effect on early shoot growth. Then, each of the species was harvested 28 days after sowing. The harvested plant samples were dried and weighed as described in Experiment 1.

Statistical tests were again performed using R Studio Version 4.0.2. Measured parameters were analyzed by fitting linear models and using an ANOVA with ‘species’ and ‘treatment’ as predictor variables. The effect of ‘replicate’ was included as required in the most parsimonious model. *Sorghum bicolor* was not included in the analysis because it had a significantly higher growth rate than the tropical perennial grasses and responded similarly to the four herbicide treatments. Normal quantile–quantile plots and Shapiro–Wilk tests were used to test the normality of the residuals for all fitted models. Fisher’s LSD method used to determine the difference between treatments (R package: agricolae).

3. Results

3.1. Experiment 1

3.1.1. Seedling Emergence

Seedlings started to emerge ~3 days after sowing and continued emerging until day 14 (Figure 1). There were differences in the rate of seedling emergence among the tropical perennial grasses, with time to 90% emergence occurring between 6 and 12 days (Table 1), depending on the species and treatment combination. On average, *C. gayana* was the fastest of the four perennial grasses to emerge (avg. 6.6 days), whereas *D. aristatum* was the slowest (avg. 10.2 days). There was no effect of metcamifen on the time to 90% emergence when the four perennial grasses were treated with rates between 0 and 1× the label rate for *S. bicolor*. Similarly, the 2× rate had no effect on the time to 90% emergence of *D. eriantha* or *P. coloratum*, but the emergence time was extended 32% and 20% for *C. gayana* and *D. aristatum*, respectively, compared to the 1× rate. Although the highest rate of metcamifen slowed the emergence of these two species, there was no effect of metcamifen on the final emergence of the four perennial grasses ($p > 0.05$), but there were differences among the grasses ($p < 0.05$). On average, final emergence was highest for *D. eriantha* (43%), followed by *P. coloratum* (40%), *D. aristatum* (31%) and *C. gayana* (30%). *Sorghum bicolor* seedlings emerged quickly and were unaffected by the metcamifen.

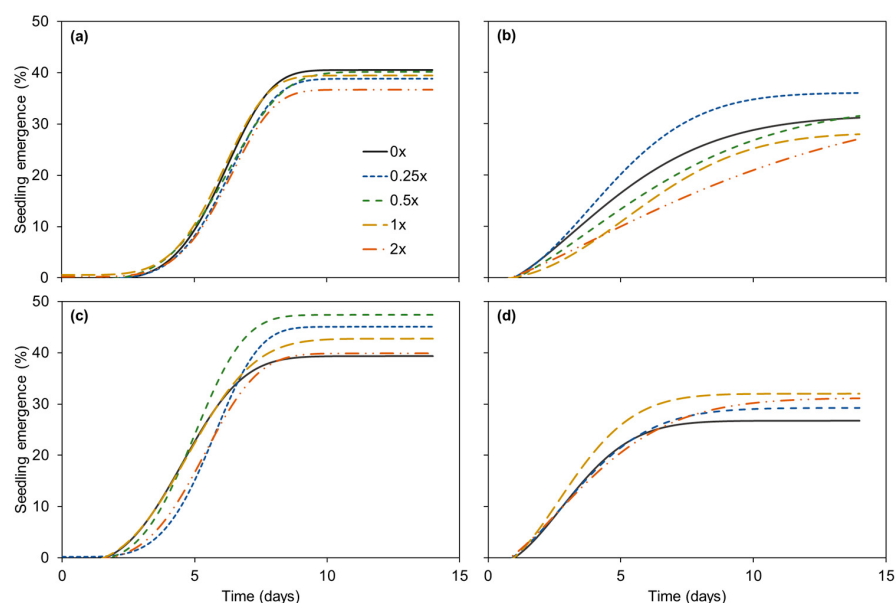


Figure 1. Cumulative seedling emergence (%) of (a) *P. coloratum*, (b) *D. aristatum*, (c) *D. eriantha*, and (d) *C. gayana* grown in response to metcamifen (400 g L^{-1} a.i.) seed safener applied at $0\times$, $0.25\times$, $0.5\times$, $1\times$ and $2\times$ the label rate for *S. bicolor* (250 mL per 100 kg of seed). The lines show Weibull growth functions. Model parameters for each of the growth functions are provided in the Supplementary Materials (Table S1).

Table 1. Time to 90% emergence (days), with lower and upper 95% confidence intervals (CI) in parentheses, and final emergence (%), with standard errors (SE) in parentheses, of four tropical perennial grasses grown in response to metcamifen (400 g L^{-1} a.i.) seed safener applied at $0\times$, $0.25\times$, $0.5\times$, $1\times$ and $2\times$ the label rate for *S. bicolor* (250 mL per 100 kg of seed). Time to 90% emergence was calculated after fitting Weibull growth functions to the emergence data. Asterisks (*) indicate where the confidence intervals could not be determined due to variability in the data.

Treatment	Time to 90% Emergence (Days)	Final Emergence (%)
<i>C. gayana</i>		
$0\times$	5.9 (4.9–7.0)	27.3 (1.8)
$0.25\times$	#	29.2 (1.2)
$0.5\times$	6.7 (5.7–7.9)	29.3 (1.5)
$1\times$	5.9 (4.9–7.5)	32.6 (1.9)
$2\times$	7.8 (6.8–9.0)	31.3 (1.5)
<i>D. aristatum</i>		
$0\times$	9.5 (7.7–*)	32.0 (3.7)
$0.25\times$	8.6 (7.3–9.7)	36.6 (1.9)
$0.5\times$	11.0 (9.3–*)	31.7 (4.5)
$1\times$	10.0 (8.4–*)	27.6 (3.4)
$2\times$	12.0 (10.3–*)	26.2 (4.2)
<i>D. eriantha</i>		
$0\times$	6.9 (6.2–7.9)	40.8 (3.3)
$0.25\times$	7.5 (6.8–8.3)	44.1 (3.9)
$0.5\times$	6.9 (6.4–7.8)	47.9 (4.8)
$1\times$	7.4 (6.8–8.5)	43.2 (0.8)
$2\times$	7.5 (6.8–8.0)	39.7 (3.1)
<i>P. coloratum</i>		
$0\times$	7.9 (7.4–8.7)	40.6 (3.4)
$0.25\times$	8.0 (7.5–8.8)	38.8 (4.5)
$0.5\times$	8.4 (7.8–8.9)	40.8 (1.4)
$1\times$	7.8 (6.9–8.8)	39.8 (5.5)
$2\times$	8.0 (7.5–8.7)	37.6 (3.6)

Time to 90% emergence was not calculated for *C. gayana* in the $0.25\times$ treatment because the Weibull growth function could not be fitted to the data.

3.1.2. Seedling Vigor

Shoot dry mass per plant was used as an indicator of seedling vigor in response to the pre-sowing treatment of seed with metcamifen (Figure 2). On average, there was no effect of the application rate of metcamifen on the shoot dry mass of each of the tropical grasses ($p > 0.05$). Nevertheless, there were significant differences in shoot yield among the four perennial grasses ($p < 0.05$). The largest shoot yields were achieved by *C. gayana*, followed by *D. eriantha*, *D. aristatum* and *P. coloratum*.

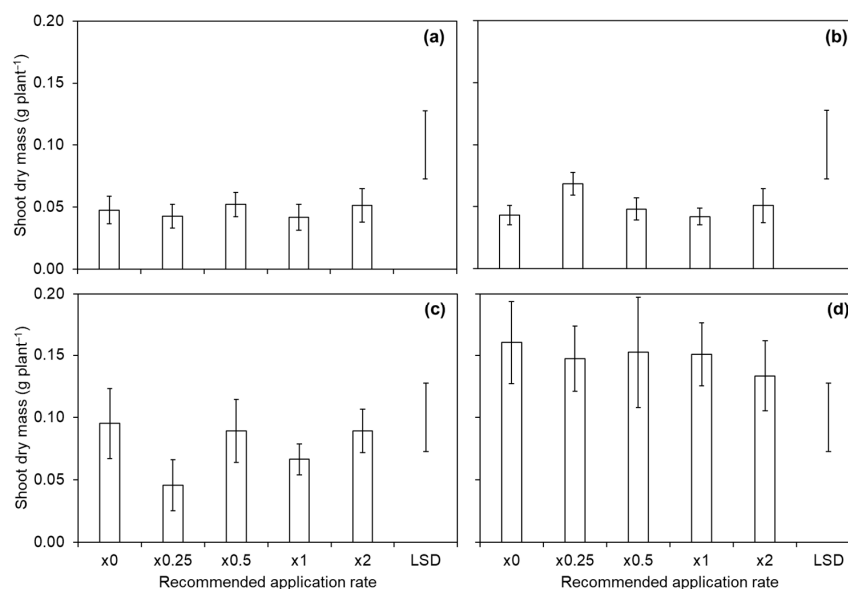


Figure 2. Shoot dry mass per plant of (a) *P. coloratum*, (b) *D. aristatum*, (c) *D. eriantha* and (d) *C. gayana* grown in response to metcamifen (400 g L^{-1} a.i.) seed safener applied at $0\times$, $0.25\times$, $0.5\times$, $1\times$ and $2\times$ the label rate for *S. bicolor* (250 mL per 100 kg of seed). The standard error is shown for individual means ($n = 4$). The bar shows the LSD ($p = 0.05$) for the species $\times$ treatment interaction. ANOVA results: species $p < 0.05$, treatment $p > 0.05$, species $\times$ treatment interaction $p > 0.05$.

3.2. Experiment 2

3.2.1. Seedling Emergence

Sorghum bicolor seedlings emerged quickly and were unaffected by any of the herbicide treatments. In contrast, the emergence response of the four perennial grasses varied. Compared to the control, seedling emergence among the perennial grasses was not reduced by the application of atrazine (Figure 3). In contrast, there were different responses to the pre-emergent application of s-metolachlor, both with and without the seed safener metcamifen. Metcamifen provided protection to seedlings of *D. eriantha* and *P. coloratum*, with emergence higher when seed was protected. However, for *D. eriantha*, the s-metolachlor without metcamifen treatment had similar emergence to the control, indicating that of the four perennial grasses, it was least affected by the rate of s-metolachlor used in the experiment. Seedling emergence of *D. aristatum* was reduced compared to the control with no benefit of the seed safener metcamifen on emergence. *Chloris gayana* was most severely affected as very few seedlings emerged when treated with s-metolachlor. Treating *C. gayana* seed with metcamifen prior to sowing did not prevent herbicide damage (Figure 3).

3.2.2. Shoot Yields

Sorghum bicolor was highly productive and its shoot yields were unaffected by any of the herbicide treatments (avg. $0.47 \text{ g plant}^{-1}$). However, shoot yields varied among the four perennial grasses (Figure 4). In the control treatment, the most productive species was *C. gayana*, followed by *D. aristatum*, *D. eriantha* and then *P. coloratum*. On average across the

species, there was no difference in shoot dry mass per plant between the control and atrazine treatments. However, shoot yields of each of the perennial grasses were lower in response to the s-metolachlor-only treatment ($p < 0.05$). Compared to the control, the decrease in shoot yield ranged between 47 and 65% for *P. coloratum*, *D. aristatum* and *D. eriantha*. Moreover, the few *C. gayana* seedlings that had originally emerged in that treatment were dead by the time of harvest, which meant that there was no shoot yield. When seed of the four tropical perennial grasses were treated with the seed safener metcamifen, the shoot dry mass of *P. coloratum*, *D. aristatum* and *D. eriantha* was equivalent to that of the control. Treating the *C. gayana* seed with metcamifen did not prevent the effect of s-metolachlor on seedling survival.

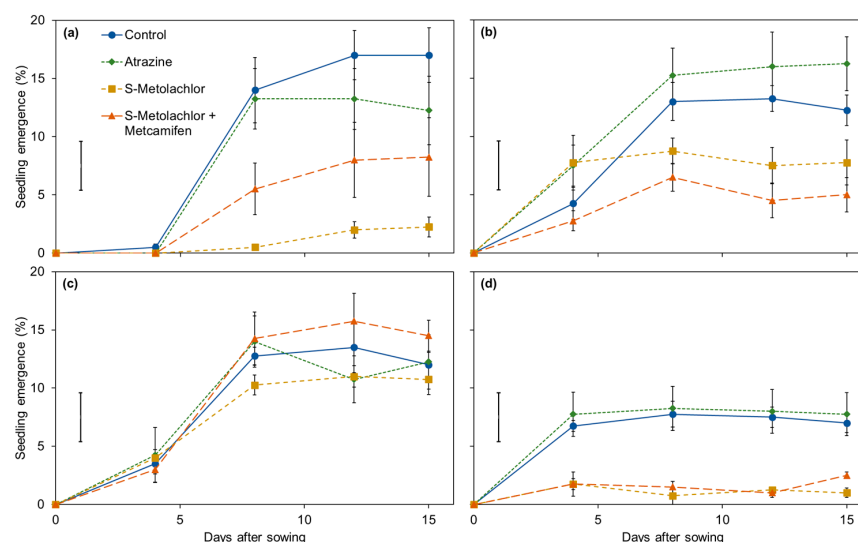


Figure 3. Seedling emergence (%) for the two weeks following sowing of (a) *P. coloratum*, (b) *D. aristatum*, (c) *D. eriantha*, and (d) *C. gayana* grown in response to (1) no treatment, (2) atrazine (900 g kg^{-1} a.i.) applied at 2 kg ha^{-1} , (3) s-metolachlor (960 g L^{-1} a.i.) applied at 1 L ha^{-1} , or (4) s-metolachlor plus metcamifen (400 g L^{-1} a.i.) seed safener applied at 250 mL per 100 kg of seed. The data points show the mean ($n = 4$) $\pm$ standard error. The bars show the LSD ($p = 0.05$) for the species $\times$ treatment $\times$ time interaction. ANOVA results: species $p < 0.05$, treatment $p < 0.05$, time $p < 0.05$.

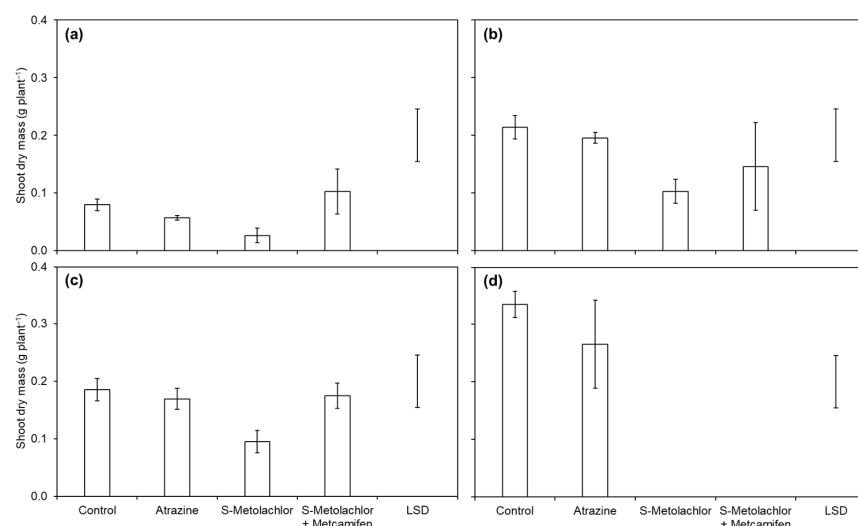


Figure 4. Shoot dry mass per plant of (a) *P. coloratum*, (b) *D. aristatum*, (c) *D. eriantha*, and (d) *C. gayana* grown in response to (1) no treatment (control), (2) atrazine (900 g kg^{-1} a.i.) applied at 2 kg ha^{-1} , (3) s-metolachlor (960 g L^{-1} a.i.) applied at 1 L ha^{-1} , or (4) s-metolachlor plus metcamifen (400 g L^{-1} a.i.) seed safener applied at 250 mL per 100 kg of seed. The standard errors for individual means ($n = 4$) are shown. The bars show the LSD ($p = 0.05$) for the species $\times$ treatment interaction. ANOVA results: species $p < 0.05$, treatment $p < 0.05$, species $\times$ treatment interaction $p < 0.05$.

4. Discussion

Seedling emergence and early growth varied among the four tropical perennial grasses, and there were clear responses to the seed safener and pre-emergent herbicides. We found that atrazine did not affect the emergence or early growth of any of the grasses tested and could be an option for pre-emergent weed control when establishing tropical perennial grass pastures. Similarly, the seed safener metcamifen, when applied at label rates (for *S. bicolor*), did not affect either the seed viability or seedling vigor of the tropical perennial grasses tested. However, it did not provide adequate protection for each perennial grass against s-metolachlor either. S-metolachlor, applied at the lower end of the recommended range for *S. bicolor*, resulted in varied plant responses, ranging from *C. gayana*, which had few seedlings emerge and none which survived, to *D. eriantha* whose emergence was unaffected, although its seedling growth was reduced. This is the first report of pre-emergent herbicide application to *D. eriantha* and *D. aristatum*. Both were unaffected by atrazine, while seedling emergence of *D. aristatum* was reduced by s-metolachlor, even when it had been treated with metcamifen.

We found no significant detrimental effects of atrazine applied pre-emergent at 1800 g ha⁻¹ a.i. to either *P. coloratum* or *C. gayana*. These findings are consistent with Scattini [21] who reported no effect of atrazine at 1000 g ha⁻¹ a.i. on the emergence and seedling survival of either grass species. Additionally, Scattini [21] reported no effect on either root or shoot dry mass of *P. coloratum*; however, both components declined in *C. gayana*. In our study, shoot dry mass of *C. gayana* also declined, but not significantly. Atrazine is effective for control of weeds in seedling *M. maximus* and *U. decumbens* stands [19,21], and atrazine is currently registered for use in seed crops of both grasses in Australia [24]. However, warnings are provided on the herbicide label that damage can occur under certain conditions. This is because environmental conditions can alter a species susceptibility to atrazine [19,35]. If a species has limited tolerance and is subjected to unfavorable environmental conditions as well, the combination of factors may result in seedling losses and failed establishment due to the herbicide rather than weed competition.

The ability of a species to detoxify atrazine determines its tolerance to the chemical, and there are multiple pathways used by plants. Jensen et al. [36] tested a range of grasses for the effect of atrazine on their net carbon dioxide exchange (NCE), including the initial impact following root uptake of atrazine, and then the subsequent recovery rate. They identified a range of mechanisms used by the grasses and a relationship between the rate of NCE recovery and formation of the glutathione–atrazine conjugate, where conjugation was the major detoxification pathway in grasses exhibiting atrazine tolerance. They found all grasses tested from the Festucoideae subfamily (C₃ grasses) showed poor recovery (low NCE recovery rate) and were susceptible to atrazine. Grasses from the Eragrostideae subfamily were either susceptible or had low tolerance, while several species from the Panicoideae subfamily were tolerant to atrazine with high NCE recovery rates [36]. While there was a relationship between field tolerance of some annual grass weeds and the NCE recovery rates, Jensen et al. [36] noted that there are many factors which influence a plant's response to herbicides under field conditions. Therefore, the NCE recovery rate cannot be used to predict how a species would respond to atrazine under field conditions [36]. Nevertheless, there are a range of tropical perennial grasses that are tolerant of atrazine, and we have demonstrated this among four commonly sown species in the present experiment.

The seed safener metcamifen had no effect on the seedling emergence or vigor of the four tropical perennial grasses. Albeit conducted in a controlled environment, our tests suggest that metcamifen is safe for use on small-seeded tropical perennial grasses. Additionally, we suspect that there is a relatively high safety margin as there was no effect of rates applied between zero and two times the recommended rate for *S. bicolor*. However,

at the label rate for *S. bicolor*, metcamifen provided at best limited protection against the low rate of s-metolachlor applied. Rushing et al. [37] evaluated two methods of applying the seed safener: controlled hydration where only sufficient water was added with the safener to completely wet the seed [38], and seed coating where the seed was coated with a mix of safener, seed coating polymer and conditioning powder. They found that, generally, controlled hydration was equally or more effective than the seed-coating method. We applied our seed safener using a method similar to controlled hydration, using the same volume of water for all of the seeds. However, the volume was likely insufficient to provide complete coverage of the seeds. Tropical perennial pasture seeds in Australia are usually coated with a mixture commonly comprising lime and a sticker. This coating covers the whole floret (of some species), improving the ballistics of the seed unit which is otherwise light, fluffy and prone to binding together, blocking seeders during sowing. The coating sometimes includes additives such as insecticides and fungicides to further support seedling establishment. The pasture seeds used in our study were pre-coated with lime and an insecticide. While there may have been an interaction between the insecticide and metcamifen, it is industry practice to coat *S. bicolor* seed with insecticide prior to sowing. Furthermore, there are no warnings on the herbicide seed safener label indicating a potential interaction with other seed coatings, which suggests that an interaction is not expected [31]. The proportion of seed coat to seed is variable but can be 500% or more. In contrast, the *S. bicolor* seed was bare, with the additives applied without lime. We propose that the high proportion of lime coating on the perennial grasses may have prevented optimum coverage of the seed safener and more water may be required to better cover seed when applying metcamifen. Complete seed coverage may provide better protection to seedlings against s-metolachlor.

The low emergence of the tropical perennial grass controls in Experiment 1 highlight the low viability of these species. Of the grasses used in our study, *D. eriantha* and *P. coloratum* had the highest proportion of viable seed (41% and 39% seedling emergence, respectively) and *C. gayana* the lowest (28% emergence). In contrast, the *S. bicolor* emergence was 77%. The germination of tropical perennial grasses is usually lower than that of other crop and pasture species. This is, in part, due to the indeterminate flowering and, therefore, seed ripening of the grasses. While the quality of the *C. gayana* seed was below average, the quality of *D. eriantha* and *P. coloratum* was fair [17]. In our study, metcamifen did not impact seed viability or the emergence rate, but it did have a positive effect on seedling vigor. This is in contrast to previous safeners (e.g., oxabetrinil) which cause reduced seed viability, resulting in recommendations that they are only applied to high quality seed [17,37].

The relative seedling vigor of the perennial grasses was variable, although all were less vigorous than *S. bicolor*. Of the perennial pasture grasses tested, we found that *C. gayana* was vigorous and produced the largest shoot yields, whereas *P. coloratum* produced lower shoot yields. *Chloris gayana* is recognized for its high seedling vigor and for being relatively competitive against weeds and other sown perennial grasses [39]. Additionally, *C. gayana* emerges over a wide temperature range, commencing when soil temperatures are greater than 11 °C, with 50% of maximum emergence occurring when temperature of the warming soil is 17 °C [13]. In contrast, *P. coloratum* has a higher temperature requirement of 20 °C and, generally, is slower to establish. Less vigorous species may benefit more from the use of seed safener herbicide technologies at sowing to improve their competitive advantage. The growth of the species may also have been influenced by soil characteristics because there are known differences in their preferred soil type. For example, the pH was slightly too acidic for *D. aristatum*, which prefers neutral to alkaline soils, while the clay texture was heavier than ideal for *D. eriantha* [40]. However, we do not anticipate these differences to have negatively affected the seedling emergence or early growth of the species because

the soil had been crushed and sieved, and adequate soil moisture was maintained for the duration of the experiments. Although not the ideal soil for each of the species, it was appropriate for testing the different treatments applied in our experiments, treatments which are prone to leaching in coarser-textured soils.

The shoot yields of the control and the treatments, atrazine and s-metolachlor plus metcamifen, were the same for most of the grasses in Experiment 2. We propose that these two pre-emergent herbicides are potentially viable options for these species. However, more work is required to determine if the rates we used are high enough to control problematic weeds in pasture situations. If higher rates are required to control significant weed issues, there may be a detrimental effect on the emergence and growth of the tropical perennial grasses. Nevertheless, an initial yield penalty may be acceptable if weed control is successful and the seedlings grow through the damage to establish well with an adequate population to be an effective pasture.

Of the grasses tested in our study, *C. gayana* appeared to be the most sensitive to s-metolachlor, with few seedlings emerging and none surviving the 28 days to harvest. This sensitivity could, in part, be associated with its small seed size as *C. gayana* was the smallest seeded of the grasses tested [17]. Tolerance to both atrazine and metolachlor are higher in some grasses when the herbicides are applied post-emergence (at about 14 days after emergence) [21,23]. Therefore, changing to post-emergent herbicide application may be a strategy to increase application rates or increase the range of species for which these herbicides could be effective in weed control.

Our studies, which were conducted under controlled environmental conditions, have shown that pre-emergent herbicides have potential when establishing tropical perennial grass pastures. However, to have confidence in these results and to understand their relevance, field studies are required with assessments conducted for at least 12 months to understand any long-term effects of the pre-emergent herbicides on plant persistence, ability to over-winter and subsequent recruitment (species dependent). The following areas warrant investigation:

1. Testing with higher rates of metcamifen with different rates of water when treating coated seed. Given there was no effect of metcamifen on seedling emergence and vigor, and considering its variable effect as a safener when s-metolachlor was applied (albeit not statistically significant nor commercially viable), rates higher than those recommended for *S. bicolor* may provide better protection to emerging tropical perennial grasses. Also, higher rates of water to provide complete surface coverage when treating the seed with metcamifen may provide better protection.
2. Field testing of both herbicides is required across a range of soil types, environmental conditions and sowing times. Inconsistent results due to interactions between seed safeners and herbicides or environmental factors have been reported (e.g., [21,23,35]). Further, sowing time (e.g., spring v. late summer–early autumn) may provide an opportunity to manipulate the relative competitiveness of the sown perennial grasses and weeds. Additionally, applying the herbicides after the sown grasses have emerged may be an option and warrants investigation.

5. Conclusions

Our study showed that atrazine has potential as a pre-emergent herbicide when establishing tropical perennial grass pastures as it did not affect the emergence or seedling growth of any of the grasses tested. Metcamifen had no effect on the seedling emergence or vigor of the four perennial grasses, suggesting that it is safe to use on small-seeded tropical perennial grasses. However, at the rate applied, it did not provide adequate protection of the grasses against s-metolachlor either. S-metolachlor, at the rate applied, resulted in

varied plant responses, ranging from *C. gayana* failing to emerge to no effect on emergence but reduced seedling growth of *D. eriantha*.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/seeds4020018/s1>. Table S1: Model parameters for the self-starting Weibull growth functions ($y = a - b \times \exp(-\exp(c) \times x^d)$, where x is days and y is emergence) that were used to determine time to 90% emergence of four tropical perennial grasses grown in response to metcamifen (400 g L⁻¹ a.i.) seed safener applied at 0×, 0.25×, 0.5×, 1× and 2× the label rate for *S. bicolor* (250 mL per 100 kg of seed). As outlined by Crawley [31], a is the horizontal asymptote on the right, b is the difference between the asymptote and the intercept (the value of y at $x = 0$), c is the natural logarithm of the rate constant, and d is the power to which x is raised.

Author Contributions: Conceptualization, S.P.B. and J.W.M.; methodology, J.W.M. and S.P.B.; formal analysis, J.W.M.; investigation, H.W.M., J.W.M. and S.P.B.; data curation, H.W.M. and J.W.M.; writing—original draft preparation, S.P.B. and J.W.M.; writing—review and editing, S.P.B., J.W.M. and H.W.M.; supervision, J.W.M. and S.P.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors acknowledge the support provided by the University of New England and NSW Department of Primary Industries and Regional Development. The authors thank Calista McLachlan for technical assistance. Bernie Dominiak and Sean Murphy reviewed a presubmission version of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Vendramini, J.M.B.; Silveira, M.L.; Moriel, P. Resilience of warm-season (C4) perennial grasses under challenging environmental and management conditions. *Anim. Front.* **2023**, *13*, 16–22. [CrossRef] [PubMed]
2. Moser, L.E.; Burson, B.L.; Sollenberger, L.E.E. *Warm-Season (C4) Grasses*; Moser, L.E., Burson, B.L., Sollenberger, L.E., Eds.; American Society of Agronomy, Inc.: Madison, WI, USA; Crop Science Society of America, Inc.: Madison, WI, USA; Soil Science Society of America, Inc.: Madison, WI, USA, 2004.
3. Quirk, M.; McIvor, J.G. *Grazing Land Management Technical Manual*; Meat and Livestock Australia Limited: Sydney, Australia, 2005.
4. Schwenke, G.D.; McLeod, M.K.; Murphy, S.R.; Harden, S.; Cowie, A.L.; Lonergan, V.E. The potential for sown tropical perennial grass pastures to improve soil organic carbon in the North-West Slopes and Plains of New South Wales. *Soil Res.* **2013**, *51*, 726–737. [CrossRef]
5. Boschma, S.P.; Murphy, S.R.; Harden, S. Growth rate and nutritive value of sown tropical perennial grasses in a variable summer-dominant rainfall environment, Australia. *Grass Forage Sci.* **2017**, *72*, 234–247. [CrossRef]
6. Moore, G.A.; Albertsen, T.O.; Ramankutty, P.; Nichols, P.G.H.; Titterton, J.W.; Barrett-Lennard, P. Production and persistence of subtropical grasses in environments with Mediterranean climates. *Crop Pasture Sci.* **2014**, *65*, 798–816. [CrossRef]
7. Moore, G.A.; Sanford, P.; Dolling, P.J.; Real, D. The challenges of developing resilient perennial pastures for a Mediterranean environment—A review for Western Australia. *Crop Pasture Sci.* **2021**, *72*, 613–633. [CrossRef]
8. Badgery, W.; Broadfoot, K.; Millar, G.; Harris, C.; Boschma, S. Tropical pastures add value to grazing systems in temperate environments of Eastern Australia. *Field Crops Res.* **2024**, *312*, 109402. [CrossRef]
9. Descheemaeker, K.; Llewellyn, R.; Moore, A.; Whitbread, A. Summer-growing perennial grasses are a potential new feed source in the low rainfall environment of southern Australia. *Crop Pasture Sci.* **2014**, *65*, 1033–1043.
10. Lodge, G.M.; Brennan, M.A.; Harden, S. Field studies of the effects of pre-sowing weed control and time of sowing on tropical perennial grass establishment, North-West Slopes, New South Wales. *Crop Pasture Sci.* **2010**, *61*, 182–191. [CrossRef]

11. Leslie, J.K. Factors responsible for failures in the establishment of summer grasses on the black earths of the Darling Downs, Queensland. *Qld. J. Agric. Anim. Sci.* **1965**, *22*, 17–38.
12. Lodge, G.M.; Harden, S. Effects of depth and time of sowing and over-wintering on tropical perennial grass seedling emergence in northern New South Wales. *Crop Pasture Sci.* **2009**, *60*, 954–962. [[CrossRef](#)]
13. Simpson, M.; Boschma, S.; Alemseged, Y.; Newell, M.; Norton, M.; Smith, W.; Brennan, M.; Munday, N.; Harden, S.; Price, A. Seedling emergence of tropical perennial pasture species in response to temperature used to determine sowing time recommendations. *Trop. Grassl.-Forrajes Trop.* **2023**, *11*, 199–209. [[CrossRef](#)]
14. Cullen, B.R.; Johnson, I.R.; Eckard, R.J.; Lodge, G.M.; Walker, R.G.; Rawnsley, R.P.; McCaskill, M.R. Climate change effects on pasture systems in south-eastern Australia. *Crop Pasture Sci.* **2009**, *60*, 933–942. [[CrossRef](#)]
15. Lodge, G.M.; McCormick, L.H. Ten tips for better establishment of sown tropical perennial grasses in northern New South Wales. In Proceedings of the 25th Annual Conference of The Grassland Society of NSW, Dubbo, NSW, Australia, 28–29 July 2010; pp. 135–136.
16. Widderick, M.J.; Walker, S.R.; McLean, A.R. Manipulating the seed bank to manage herbicide-resistant weeds. In Proceedings of the Seventeenth Australasian Weeds Conference, Christchurch, New Zealand, 26–30 September 2010; pp. 262–265.
17. Harris, C.A.; Boschma, S.P.; Murphy, S.R.; McCormick, L.H. *Tropical Perennial Grasses for Northern Inland NSW*, 2nd ed.; Future Farm Industries Cooperative Research Centre: Perth, Australia, 2014.
18. Sinclair, K.; Curtis, A.L.; Baker, S.J.; Boschma, S.P. Investigating producer interest in and experience with tropical perennial grasses in inland NSW. In Proceedings of the 32nd Conference of the Grassland Society of NSW Inc., Virtual, 20–21 July 2021; pp. 99–102.
19. Hawton, D. The effectiveness of some herbicides for weed control in *Panicum maximum* and *Brachiaria decumbens* and some factors affecting the atrazine tolerance of these species. *Trop. Grassl.* **1980**, *14*, 34–39.
20. Hawton, D. Atrazine for weed control in the establishment of *Brachiaria decumbens* and *Panicum maximum*. *J. Aust. Inst. Agric. Sci.* **1976**, *42*, 189–191.
21. Scattini, W. Atrazine tolerance in five tropical pasture grasses. *Trop. Grassl.* **1978**, *12*, 35–39.
22. Cook, B.G.; O’Grady, R. Atrazine in kikuyu grass establishment: A preliminary study. *Trop. Grassl.* **1978**, *12*, 184–187.
23. Bovey, R.W.; Baur, J.R.; Merkle, M.G.; Bashaw, E.C. Response of Kleingrass and Buffelgrass to Herbicides. *Agron. J.* **1980**, *72*, 53–55. [[CrossRef](#)]
24. Moore, C.B.; Moore, J.H. *HerbiGuide—The Pesticide Expert on a Disk V37.0*; HerbiGuide Pty. Ltd.: Albany, Australia, 2023.
25. Moore, J.H.; Ryder, A.; Master, R. Herbicide tolerance of five young perennial grasses. In Proceedings of the 17th Australasian Weeds Conference “New Frontiers in New Zealand: Together We Can Beat the Weeds”, Christchurch Convention Centre, Christchurch, New Zealand, 26–30 September 2010; pp. 402–405.
26. Jablonkai, I. Herbicide safeners: Effective tools to improve herbicide selectivity. In *Herbicides*; Price, A.J., Kelton, J.A., Eds.; IntechOpen: Rijeka, Croatia, 2013; pp. 589–620.
27. Rosinger, C. Herbicide Safeners: An overview. *Julius-Kühn-Archiv* **2014**, *443*, 516–525. [[CrossRef](#)]
28. Kim, S.; Chen, J.; Cheng, T.; Gindulyte, A.; He, J.; He, S.; Li, Q.; Shoemaker, B.A.; Thiessen, P.A.; Yu, B.; et al. PubChem 2025 update. *Nucleic Acids Res.* **2025**, *53*, D1516–D1525. [[CrossRef](#)]
29. Australian Pesticides and Veterinary Medicines Authority. *Public Release Summary on the Evaluation of the New Active Metcamifen in the Product EPIVIO C Sorghum Seed Safener*; APVMA Product Number 87317; Australian Pesticides and Veterinary Medicines Authority: Sydney, Australia, 2020; ISSN 1443-1335.
30. Watson, P.A. *Herbicide Safening in Maize (Zea mays)*; Newcastle University: Newcastle, UK, 2019.
31. Syngenta Australia. Epivio(R) C Sorghum Seed Safener. Available online: <https://www.syngenta.com.au/products/seed-treatments/epivio-c> (accessed on 21 March 2025).
32. Crawley, M.J. *The R Book*; John Wiley & Sons Ltd.: Chichester, UK, 2013.
33. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2020.
34. de Mendiburu, F. *Agricolae: Statistical Procedures for Agricultural Research*. R Package Version 1.3-3. 2020. Available online: <https://CRAN.R-project.org/package=agricolae> (accessed on 21 March 2025).
35. Nel, P.C.; Reinhardt, C.F. Factors affecting the activity of atrazine in plants and soil. *S. Afr. J. Plant Soil* **1984**, *1*, 67–72. [[CrossRef](#)]
36. Jensen, K.I.N.; Stephenson, G.R.; Hunt, L.A. Detoxification of atrazine in three Gramineae Subfamilies. *Weed Sci.* **1977**, *25*, 212–220. [[CrossRef](#)]
37. Rushing, J.B.; Baldwin, B.S.; Taylor, A.G.; Owens, V.N.; Fike, J.H.; Moore, K.J. Seed safening from herbicidal injury in switchgrass establishment. *Crop Sci.* **2013**, *53*, 1650–1657. [[CrossRef](#)]
38. Finch-Savage, W.E.; Dent, K.C.; Clark, L.J. Soak conditions and temperature following sowing influence the response of maize (*Zea mays* L.) seeds to on-farm priming (pre-sowing seed soak). *Field Crops Res.* **2004**, *90*, 361–374.

39. Lodge, G.M.; Boschma, S.P.; Harden, S. Replacement series studies of competition between tropical perennial and annual grasses and perennial grass mixtures in northern New South Wales. *Crop Pasture Sci.* **2009**, *60*, 526–531. [[CrossRef](#)]
40. Cook, B.G.; Pengelly, B.C.; Schultze-Kraft, R.; Taylor, M.; Burkart, S.; Cardoso Arango, J.A.; González Guzmán, J.J.; Cox, K.; Jones, C.; Peters, M. Tropical Forages: An Interactive Selection Tool. Available online: <https://tropicalforages.info/text/intro/index.html> (accessed on 21 March 2025).

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