Investigating effects of temperature, humidity and photoperiod for efficacy of glufosinate on resistant and susceptible annual ryegrass

Peter Boutsalis[1], Sam Kleemann[1] and Rebekah Allen[2]

Plant Science Consulting[1] Hart Field-Site Group[2]

Key findings

- Results from pot experiments suggest that humidity within 24-hours of application of Liberty® was very important to glufosinate activity on annual ryegrass.
- It was also identified that under lower humidity, increasing the photoperiod from 1 to 8 hours was found to improve control with Liberty and suggests that applying in the morning may be better than late afternoon. This was observed in field trials where morning applications of sequential Liberty at 3 L/ha, or Liberty tank mixes at 2 L/ha with glyphosate or clethodim as the first application in the sequence still provided good control despite lower humidity conditions (60-80%).

Introduction

Active ingredient glufosinate-ammonium (200 g/L) registered as Liberty is a Group 10 herbicide which can now be applied in-crop to canola varieties with LibertyLink® technology (tolerance to Liberty herbicide). This registration provides a new herbicide mode of action (MOA) for use in broadacre cropping systems.

Glyphosate and glufosinate belong to two different MOA (Group 9 and 10, respectively), however their structural elements are similar in that they are both charged herbicides (Preston, 2024). This means they are unable to pass through wax layers of plant cuticles and are alternatively required to enter via pectin strands (intercellular plant tissue) within cuticles. As pectin strands contain negative charges, these herbicides are slow moving into the leaf and the rate of absorption is impacted by temperature and humidity (Preston, 2024). Previous research suggests that temperature plays only a small part when it comes to glufosinate uptake, whereas humidity is the more important factor (Preston, 2024). Low humidity can reduce the ability of glufosinate to move through the pectin strands, which need to remain hydrated for this to happen. However, it is suggested that humidity is not generally an issue in southern Australia during winter and that high humidity is only required for the first 24-hours after application for glufosinate uptake (Preston, 2024).

In addition to two field trials, a series of pot experiments exploring the effects of temperature and humidity on herbicide efficacy were implemented in 2023. In this article, data from two pot experiments conducted at Waite, SA are discussed.

Methodology

Two pot experiments were implemented at Waite, SA in 2023 (Table 1) to evaluate the influence of temperature and photoperiod on sequential applications of Liberty on three biotypes of annual ryegrass; Susceptible, DIM (Group A – Cyclohexanediones) resistant and DIM + glyphosate resistant (experiment 1). It also investigated the efficacy of Liberty on DIM-resistant ryegrass at two growth stages (2-3 leaf and 3-4 tiller) and three temperature regimes (warm, cold and outdoor temperatures) for 24-hours after spraying (experiment 2).

Table 1. Trial details for experiments at Waite, SA in 2023.

Experiment 1	**Plant density:**	5 plants/plot	**Pressure:**	2 bar
	Spray date:	Timing 1: August 10 Timing 2: August 24	**Nozzle:**	Teejet 110-01
	Growth stage:	2-tiller		
	Application:	Spray chamber		
	Spray volume:	100 L/ha		
	ARG biotypes:	1. Susceptible: Jeparit 2. DIM-resistant: 700.3-20 3. DIM + Glyphosate resistant: 896-20		
Experiment 2	**Plant density:**	5 plants/plot	**Pressure:**	2 bar
	Spray date:	Timing 1: November 14	**Nozzle:**	Teejet 110-01
	Growth stage:	2-3 leaf, 3-4 tiller		
	Application:	Spray chamber		
	Spray volume:	100 L/ha		
	ARG biotype:	DIM-resistant: 700.3-20		

Experiment 1

An initial pot study was undertaken in August to evaluate influence of temperature and photoperiod on sequential applications of Liberty on three biotypes of annual ryegrass: susceptible, DIM and DIM + glyphosate resistant (Table 2).

Plants were grown outdoors and at 2-tiller stage (GS 22) sprayed with 2 L/ha Liberty in a spray chamber at either 9am or 4pm on August 10. After spraying, pots were transferred to one of two different locations, (1) a cold room or (2) shed (outdoor temperatures of 15-6^{o}C). At each location plants were exposed to the same light source (Arlec 20W) for either eight hours (9am-5pm) or one hour (4pm-5pm) after spraying. At 5pm lights were turned off exposing the plants to complete darkness until the next morning after which pots were returned outdoors to a common location (natural light plus fluctuating ambient temperature). This process was repeated on August 24, 14-days after the first application to mimic the sequential spray as per label directions.

An untreated control of each biotype was included for comparative purposes with assessments undertaken 25-days after the second application on September 18. Herbicide activity was measured using biomass reduction and control (mortality assessed as percent of the untreated). Data was analysed using Graph Pad Prism 6.0. Vertical bars represent the standard error of means for percentage biomass reduction and control.

Table 2. Treatment list for experiment 1 at Waite, SA. Outside refers to plants kept in a shed exposed to outside temperatures and 'Cold room' refers to a refrigerated cold room operating at 10^{o}C constant temperature. Light source: Arlec 20W light (1600 lumen, 5000K) producing light intensity of 30 µmol m^{-2} s^{-1}.

Trt	Timing 1 = 2-tiller	Timing 2 = 14 days after timing 1 on August 24, 2023	Spray time	Location
1	Untreated	Nil	9am	Outside
2	Liberty 2 L/ha	Liberty 2 L/ha	9am	Outside
3	Untreated	Nil	9am	Cold room
4	Liberty 2 L/ha	Liberty 2 L/ha	9am	Cold room
5	Untreated	Nil	4pm	Outside
6	Liberty 2 L/ha	Liberty 2 L/ha	4pm	Outside
7	Untreated	Nil	4pm	Cold room
8	Liberty 2 L/ha	Liberty 2 L/ha	4pm	Cold room

Table 3. Temperature and relative humidity at the two locations (cold room and shed) after spraying. Data collected from Tinytag Plus 2 data loggers.

Application	August 10				August 24			
	Cold room		Shed		Cold room		Shed	
	Temp (ºC)	Humidity (%)	Temp (ºC)	Humidity (%)	Temp (ºC)	Humidity (%)	Temp (ºC)	Humidity (%)
9am (8-hour light exposure)	10.5	90	15.6	64	10.3	86	12.9	67
4pm (1-hour light exposure)	10	95	15.2	67	9.8	94	15	88

Experiment 2

A secondary pot experiment was conducted in November of 2023 to investigate the efficacy of Liberty on DIM-resistant ryegrass at two growth stages (2-3 leaf and 3-4 tiller) and three temperature regimes (warm, cold and outdoor temperatures) for 24-hours after spraying.

Plants were grown outdoors during spring at the Waite Campus. At the 2-3 leaf (GS 12-13) and 3-4 tiller stage (GS 23-24) pots were sprayed with 2 L/ha Liberty in a spray chamber. After spraying, pots were transferred outdoors, or to growth rooms programmed to provide warm or cold temperatures (Table 4). After 24-hours, pots from both growth rooms (cold and warm) were relocated outdoors (i.e. outdoor location). The conditions for the 24-hour period were:

1. Cold growth room = 15ºC day/10ºC night, light intensity 250 µmol m-2 s-1, 14-hour night/ 10-hour day photoperiod; average relative humidity of 98% and an average temperature of 13.5ºC over the 24-hour period after spraying.
2. Warm growth room = 25ºC day/15ºC night, light intensity 250 µmol m-2 s-1, 14-hour night/ 10-hour day photoperiod; average relative humidity of 91% and an average temperature of 19.7ºC over the 24-hour period after spraying.
3. Outside outdoors in direct light = Overcast cloudy day with average light intensity of 400 µmol-2 s-1; average relative humidity of 76% and an average temperature of 16.1ºC over the 24-hour period after spraying.

Three days later all pots were transferred from direct sunlight to a shade house with 50% light reduction (white shade cloth). This resulted in three distinct groups where only conditions for the first 24-hours after spraying (i.e. most significant period of uptake and translocation) varied.

Table 4. Treatment list for experiment 2 at Waite, SA.

Trt	Treatment	Location	Light	Time at this location	Time outdoors
1	Untreated	Outside	400 µmol m-2 s-1	-	-
2	Liberty 2 L/ha	Outside	400 µmol m-2 s-1	-	-
3	Untreated	Cold room	250 µmol m-2 s-1	24 hours	3 weeks
4	Liberty 2 L/ha	Cold room	250 µmol m-2 s-1	24 hours	3 weeks
5	Untreated	Warm room	250 µmol m-2 s-1	24 hours	3 weeks
6	Liberty 2 L/ha	Warm room	250 µmol m-2 s-1	24 hours	3 weeks

Annual ryegrass was assessed for herbicide damage (%) and survival (%). Data was analysed using Graph Pad Prism 10.0. Vertical bars represent the standard error of means for herbicide damage and control (%). Means were analysed using ANOVA and separated with use Tukey's multiple comparisons test at p ≤0.05. Data logger measurements of temperature (ºC) and relative humidity (%) were taken at each of the three locations for the 24-hour period directly after spraying (Appendix 1). These measurements were also taken for the 7-day period from the common location (e.g. 'outside') for which pots from both growth chambers (cold and warm) were relocated (Appendix 2).

Results

Experiment 1

Irrespective of biotype (resistance status), significantly greater control resulted when plants were exposed to colder temperatures (Figure 1) in the cold room (10ºC) relative to those housed in the shed under warmer outdoor temperatures (+5ºC). This result is contrary to some reports in literature indicating that control with glufosinate is greater at warmer temperatures (Kumaratilake and Preston 2005).

Apart from temperature, another key factor that has been identified as important in glufosinate activity is humidity. At both locations (cold room vs shed) humidity varied but was significantly higher in the cold room than shed location (91% vs 72%). Humidity is a key driver of glufosinate activity with higher humidity levels enhancing control, and most likely contributed to the stronger activity observed following exposure to the cold room despite the lower temperatures. High humidity within 24-hours of application can assist glufosinate passage through the leaf and therefore the overall amount absorbed (Preston 2024).

Timing of application and consequently the photoperiod (light exposure) can also influence glufosinate performance (Takano and Dayan 2021). Like humidity, light has been shown to increase absorption of glufosinate (Preston 2024). In this study where humidity was highest (cold room), photoperiod after spraying (1 vs 8 hours) appeared to be less influential on glufosinate.

The effect of photoperiod was much more pronounced when humidity was low after spraying (shed), with Liberty providing significantly greater control and biomass reduction of all three biotypes exposed to the 8-hour photoperiod compared to 1-hour. This finding tends to indicate that application timing and therefore photoperiod is perhaps more important when humidity is suboptimal.

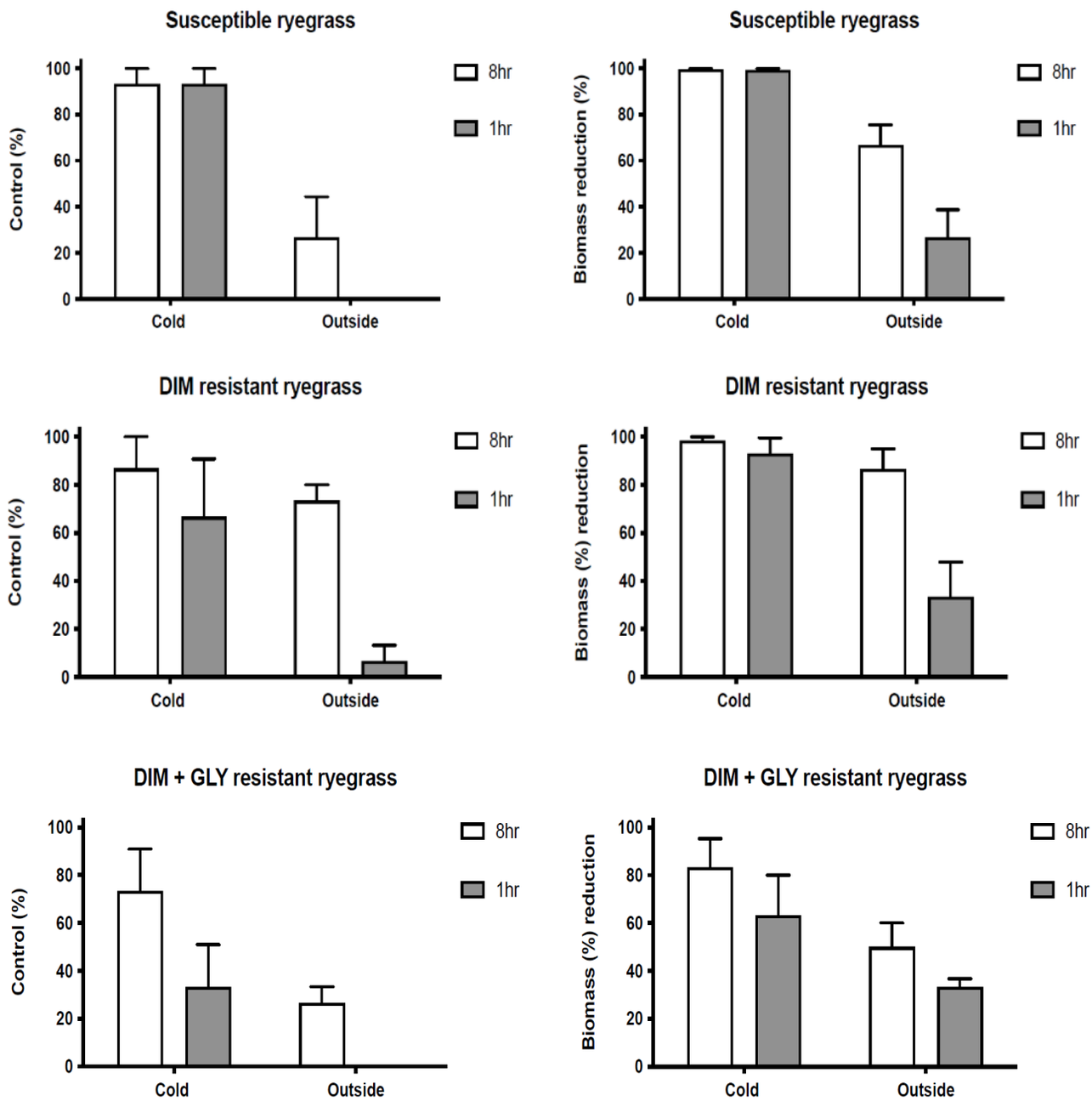


Figure 1. Percent control and biomass reduction (%) of three annual ryegrass biotypes treated to sequential applications of 2 L/ha Liberty 14 days apart.

Experiment 2

On 2-3 leaf and tillering ryegrass there was improved efficacy following treatment to 'warm' and 'cold' conditions compared to 'outside' (27ºC day maximum/15ºC minimum) (Figures 2 and 3). The temperature range between both growth rooms and the outside was relatively similar, however humidity was the key difference, with humidity levels higher for both growth rooms (cold = average 98%, range 82.1–100%; warm = average 91%, range 72.5–100%) relative to outside (average 76%, range 43–100%; Figure 4).

Of other potential influences of activity, light intensity was found to be within similar range between both growth rooms (average = 250 µmol m^{-2} s^{-1}) and outdoors (average = 400 µmol m^{-2} s^{-1}).

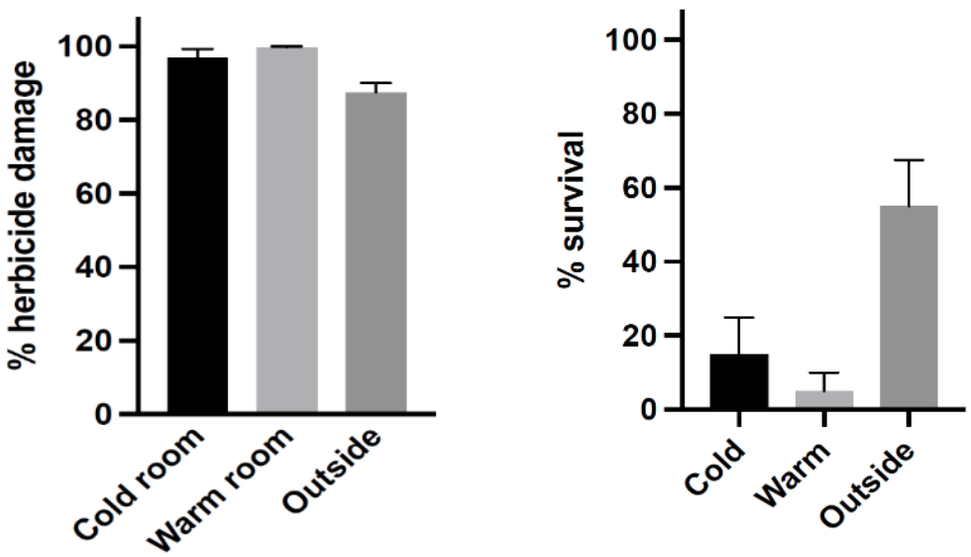


Figure 2. Herbicide damage and survival (%) of a susceptible ryegrass biotype three weeks after treatment at the 2-3 leaf stage with 2 L/ha Liberty. Vertical bars represent the standard error of means.

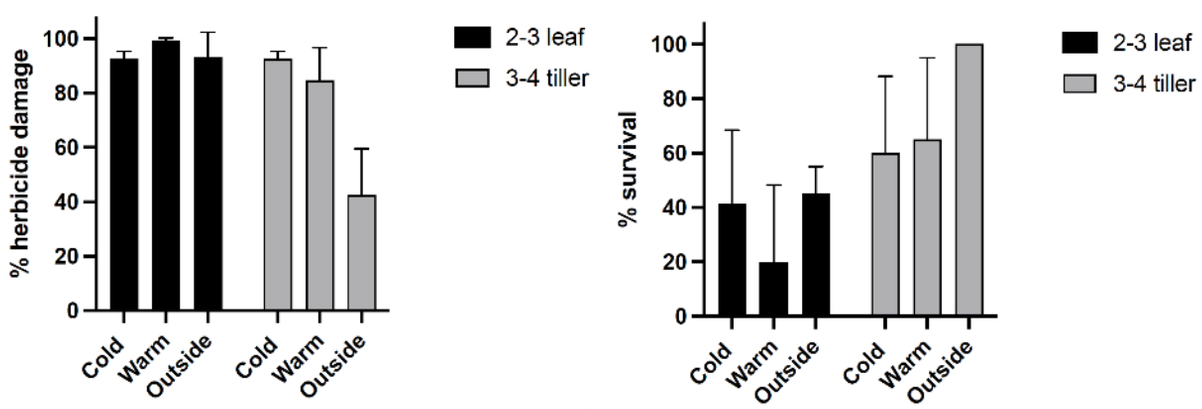


Figure 3. Herbicide damage and survival (%) of a Group 1 DIM resistant ryegrass biotype ('96-22') 3 weeks after treatment at the 2-3 leaf and 3-4 tiller stage with 2 L/ha Liberty. Vertical bars represent the standard error of means.

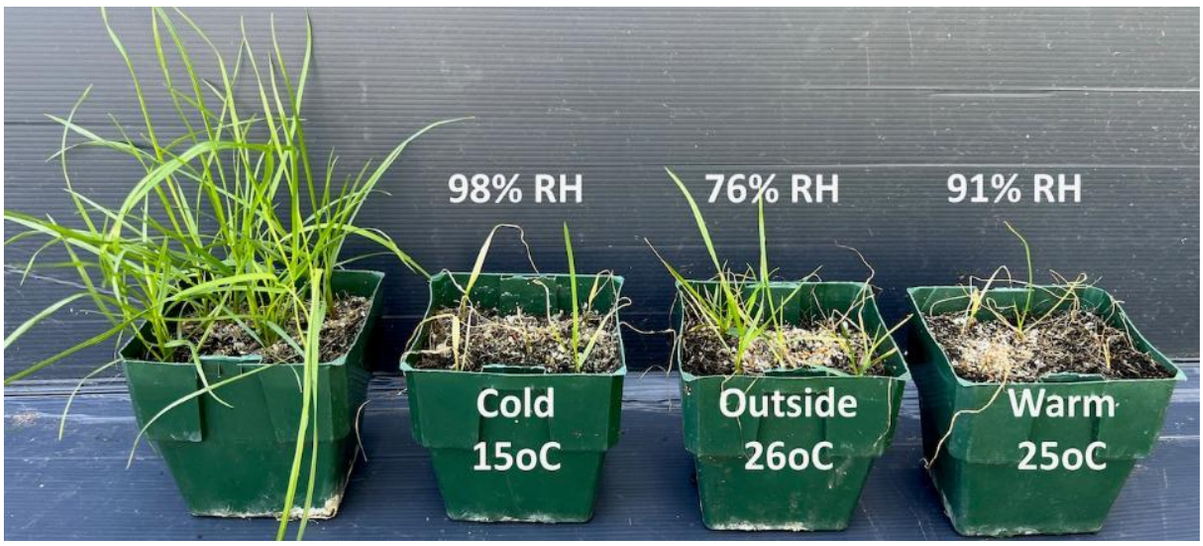


Figure 4. Visual symptoms in response to relative humidity (%) conditions after applying Liberty at 2 L/ha to susceptible ryegrass (2-3 leaf).

There was no significant difference between biotype, location or their interaction in regard to damage, however, survival was significantly affected by location (P<0.05) (Figure 5).

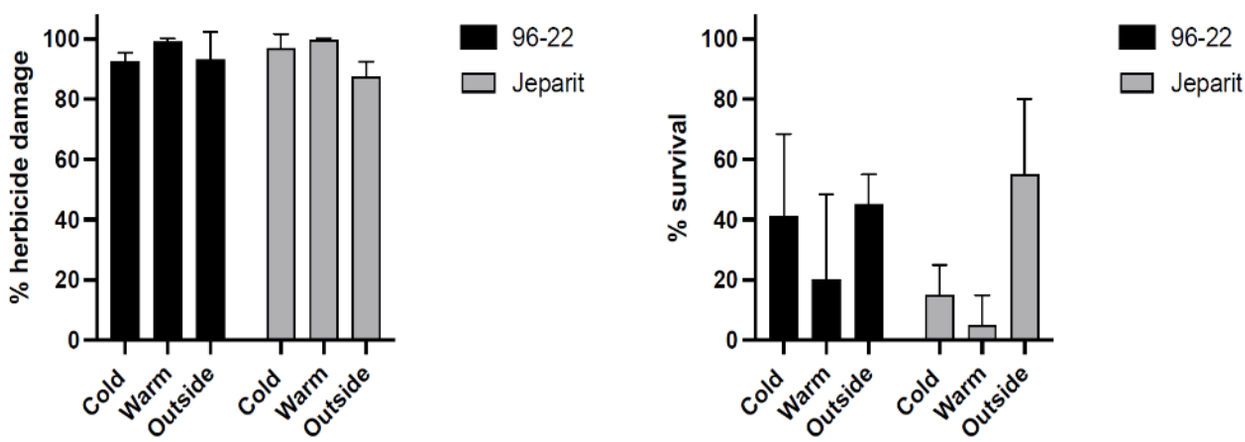


Figure 5. Herbicide damage and survival (%) of a Group 1 DIM resistant ('96-22') and susceptible ryegrass biotype ('Jeparit') 3 weeks after treatment at the 2-3 leaf stage with 2 L/ha Liberty. Vertical bars represent the standard error of means.

Control irrespective of growth stage (2-3 leaf and 3-4 tiller) was similar between both growth rooms despite the difference in temperature ('Cold' = 15ºC day/10ºC night; 'Warm' = 25ºC day/15ºC night) and appeared to be more strongly correlated to exposure to higher humidity levels at both locations (91% and 98%). This indicates that temperature is perhaps not as important as humidity for activity of Liberty on ryegrass.

Although temperature has been found to be important for glufosinate activity (Kumaratilake and Preston 2005) the current study suggests that humidity is the key factor in determining glufosinate activity with higher humidity enhancing control, irrespective of the temperature. Coetzer et. al. 2001 concluded that humidity was more important than temperature for increasing glufosinate control of *Amaranthus* spp.

There was no significant influence of biotype on herbicide damage (biomass reduction) with activity similar on both DIM-resistant and susceptible ryegrass (2-3 leaf) from the same location. However, survival of the susceptible was more strongly influenced by location, with significantly ($p \leq 0.05$) better control from both growth rooms (cold and warm) relative to outside.

Conclusion

Results from these trials suggest that humidity following application of Liberty is very important to glufosinate activity on ryegrass. In addition, it also identified that under lower humidity, increasing the photoperiod from 1 to 8 hours was more conducive to ryegrass control with Liberty. The experiments have shown that Liberty can be effective across a range of temperatures (cold and warm) provided relative humidity is high soon after application (>90%) and ryegrass is targeted at younger growth stages. Control (herbicide damage and survival) was clearly correlated to growth stage with 2-3 leaf ryegrass more effectively controlled than later growth stages. Growth stage is therefore an important factor to consider with Liberty application. The current Liberty label recommends applications be made to ryegrass between 2-4 leaf to start of tillering.

Data from the two Mid North field trials showed that sequential applications of 3 L/ha Liberty, or Liberty tank mixes at 2 L/ha with glyphosate or clethodim as the first application in the sequence can still provide good control despite lower humidity conditions (60-80%). Often the best results with Liberty are observed when using higher rates, applying to small weeds, and when conditions of high humidity, modest temperature and adequate light intensity prevail.

Acknowledgements

The authors would like to gratefully acknowledge the South Australian Grains Industry Trust (SAGIT) for their financial contribution to support this project.

References

Brent E. Tharp, Oliver Schabenberger, James J. Kells (1999). Response of Annual Weed Species to Glufosinate and Glyphosate. Weed Technology, Vol. 13, No. 3, pp. 542-547

Elmé Coetzer, Al-Khatib, K., & Loughin, T. M. (2001). Glufosinate Efficacy, Absorption, and Translocation in Amaranth as Affected by Relative Humidity and Temperature. Weed Science, 49(1), 8–13. http://www.jstor.org/stable/4046378

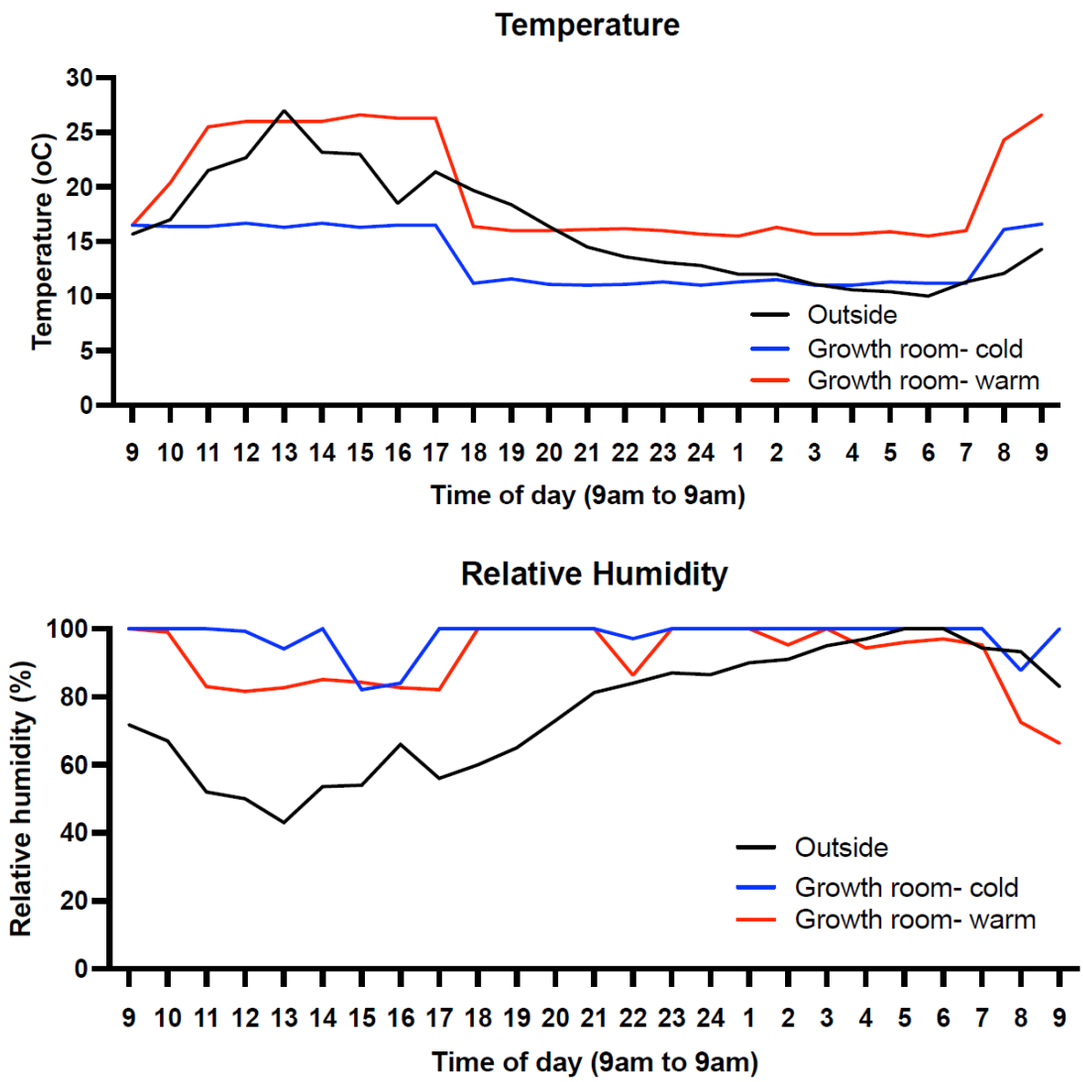
Gregory J. Steckel, Loyd M. Wax, F. William Simmons, William H. Phillips (1997). Response of Annual Weed Species to Glufosinate and Glyphosate. II Weed Technology, Vol. 11, No. 3 pp. 484-488.

Anuja R. Kumaratilake, Christopher Preston (2005). Low Temperature Reduces Glufosinate Activity and Translocation in Wild Radish (Raphanus raphanistrum). Weed Science, Vol. 53, No. 1. pp. 10-16.

Hudson K. Takano, Franck E. Dayan (2021), Biochemical basis for the time-of-day effect of glufosinate efficacy against Amaranthus palmeri. Plants, Vol 10, Issue 10

Preston C. (2024). Strategies for optimising glufosinate and tackling efficacy challenges. Available online: https://grdc.com.au/resources-and-publications/grdc-update-papers/tab-content/grdc-update-papers/2024/02/strategies-for-optimising-glufosinate-and-tackling-efficacy-challenges

Appendix 1. *Temperature (ºC) and relative humidity (%) at each of the three locations for the 24-hour period directly after spraying.*



Appendix 2. *Temperature (ºC) and relative humidity (%) for the 7-day period from the common location (i.e. 'outside') for which pots from both growth chambers (cold and warm) were relocated.*