The Impact of Herbicide Application and Defoliation on Barley Grass (*Hordeum murinum* subsp. *glaucum*) Management in Mixed Pasture Legumes

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Abstract: Barley grass (*Hordeum murinum* subsp. *glaucum*.) is an annual weed associated with grain revenue loss and sheep carcass damage in southern Australia. Increasing herbicide resistance led to a recent investigation into effective integrated weed management strategies for barley grass in southern Australia. Field studies in Wagga Wagga, New South Wales (NSW) during 2016 and 2017 examined the effect of post-emergent herbicide applications and strategic defoliation by mowing on barley grass survival and seed production in a mixed legume pasture. Statistically significant differences between herbicide-only treatments in both years showed propaquizafop to be more than 98% effective in reducing barley grass survival and seed production. Paraquat was not effective in controlling barley grass (58% efficacy), but led to a 36% and 63.5% decrease in clover and other weed biomass, respectively, after 12 months and increased lucerne biomass by over three-fold after 24 months. A single repeated mowing treatment resulted in a 46% decline in barley grass seedling emergence after 12 months and, when integrated with herbicide applications, reduced other weed biomass after 24 months by 95%. Resistance to acetyl-CoA carboxylase (ACCase)-inhibiting herbicides observed in local barley grass populations led to additional and more focused investigation comparing the efficacy of other pre- and post-emergent herbicides for barley grass management in legume pastures. Haloxyfop-R + simazine or paraquat, applied at early tillering stage, were most efficacious in reducing barley grass survival and fecundity. Impact of defoliation timing and frequency on barley grass seedlings was also evaluated at various population densities, highlighting the efficacy of repeated post-inflorescence defoliations in reducing plant survival and seed production. Results highlight the importance of optimal environmental conditions and application timing in achieving efficacious control of barley grass and improving pasture growth and biomass accumulation.

Keywords: defoliation; barley grass; mowing; herbicide; seed; integrated weed management

1. Introduction

Historically, lucerne (*Medicago sativa*) is the most widely grown perennial pasture legume in southern Australia [1], supplying a livestock feed source [2,3], facilitating the high growth rates and carcass weights desired by prime lamb markets [4], and often the only quality forage available in dry seasons.

Reduced fertiliser use [5–7], the prevalence of soil acidity [8], continuous grazing, and drought conditions over time led to a decline in legume productivity across southern Australia [1,9]. Thus, a cascading effect on pasture and soil fertility ensued, creating canopy gaps within pasture stands [10] that are exploited by annual grass weed species [11], which are often introduced from other sites via attachment of seeds to the fleece of grazing sheep. The propagules and seeds produced by some grasses are also problematic to livestock producers, due to their lodgement within animal tissue causing significant carcass damage and further welfare, production, and economic impacts at the farm and processing level [12]. Given the recent upward trend in Australian sheep meat prices [13], carcass damage due to grass seed penetration continues to pose significant challenges to the profitability of the Australian sheep industry.

Volunteer barley grass (*Hordeum murinum* L. spp. *glaucum* and *Hordeum murinum* L. subsp. *leporinum*) typically invades southern Australian cropland, pastures, and disturbed sites, especially on high-phosphorus [14,15] and -nitrogen [16] soils and competes successfully against common pasture legumes, such as lucerne [17], reducing the productive life of these pastures and of subsequent grain crops. Currently, barley grass is an important weed in Australian cropping regions, invading over 244,000 ha and incurring an annual Australian dollars (AUD) $1.7 million loss in grain crop revenue [18]. Its increased prevalence across southern Australia is attributed to climate variability [19,20], herbicide resistance [21,22], and variable seed dormancy patterns, which facilitate the escape of individual plants following herbicide treatment, allowing establishment in crop [23]. The recent appearance of biotypes exhibiting sporadic and/or later emergence results in greater reliance on post-emergent herbicide applications for management [24].

In legume crops and pastures, selective pre-emergent herbicides such as propyzamide (inhibitor of microtubule formation) [25], photosystem II (PS II) inhibitors [26], and post-emergent ACCase inhibitors ("fops" and "dims") are commonly effective against grass weeds [1,27], as are the non-selective bypiridyl photosystem I inhibitors (paraquat and diquat) and the acetolactate sythase (ALS)-inhibiting imidazolinones [24,27,28]. Recently, the development of bypiridyl photosystem I and ACCase inhibitor resistance in South Australian barley grass populations led to increased use of imidazolinones for barley grass management [21,24,29]. However, imidazolinone resistance is also emerging in some southern Australian *Hordeum murinum* L. subsp. *Leporinum* populations [22,30,31], highlighting the need for additional integrated approaches to weed management to further reduce *Hordeum* spp. infestations in pastures and seed contamination in sheep.

Defoliation by grazing or mowing is a weed control strategy historically used to control barley grass. The impacts of grazing on its survival are conflicting in the literature with control seemingly dependent on stocking rates [32], time of year [33], and the provision of adequate fencing. In pasture legumes, mowing for hay production as an alternative to grazing was shown to be highly effective [34], particularly when defoliation coincides with the onset of the reproductive phase of the target species, thereby reducing seed rain and altering botanical composition [35]. Defoliating plants at boot stage [36] resulted in decreased barley grass fecundity [37] and seed size [34] and subsequently reduced viability post-flowering [38]. The growth and survival of plants under defoliation is also dependent on the frequency and intensity of defoliation, plant size, and stress due to intra- and inter-species competition. This suggests that the impacts of defoliation may be exacerbated at high density given reduced resource allocation for reproduction [39] and limited carbohydrate reserves for regrowth [40,41]. Although previous modelling suggested that weed density influenced efficacy of control in other annual grasses [42], the impact of these interactions in barley grass is unknown.

The integration of defoliation by grazing with additional herbicide application, termed "spray-grazing", is a common method used for the control of broadleaf weeds in Australia [43], but is yet to be investigated for barley grass. Furthermore, the efficacy of integrating defoliation by mowing with herbicide application for effective barley grass management is currently unknown.

The objective of this research project was, therefore, (1) to investigate the interaction between herbicide application and defoliation by mowing performed at specific barley grass phenological

stages on barley grass survival and reproduction, and (2) to determine the impact of these treatments on dryland legume pasture production in southern New South Wales (NSW). A two-year field trial was undertaken in established lucerne pasture where the natural regeneration of barley grass (*Hordeum murinum* L. spp. *glaucum*) occurred; in addition, further investigative field studies compared the efficacy of a range of pre- and post-emergent herbicides for barley grass control within the same pasture. Greenhouse experimentation was also performed to assess the effects of defoliation frequency and timing on survival and seed production of barley grass (*Hordeum murinum* L. spp. *glaucum*) when placed and under various levels of intraspecific competition.

2. Materials and Methods

All experimentation was performed on the Wagga Wagga campus of Charles Sturt University (35.0578° south (S), 147.3544° east (E), elevation 215 m). Field studies were undertaken on a red silty loam kandosol (soil organic matter of 3.15%, pH of 5.9) sown to lucerne (*Medicago sativa* L.) and four other clover species (*Trifolium fragiferum* L., *Trifolium repens* L., *Trifolium subterraneum* L., and *Trifolium vesiculosum* Savi.) for the past five years. From 2012 to 2016, single superphosphate at the rate of 125 kg/ha was broadcast annually. In March 2016, 90 kg/ha of single superphosphate was broadcast, with 85 kg/ha applied in March the following year, in line with normal farm management. Available annual precipitation and mean monthly maximum and minimum temperatures for Wagga Wagga during the two years of the study are provided in Table 1.

Table 1. Total precipitation, maximum and minimum monthly temperatures, and the number of frost events experienced at Wagga Wagga during the 2016 and 2017 growing seasons (May to November) [44].

Climate Variable	2016	2017
[1] Precipitation (mm)	607	225
[1] Mean maximum temperature (°C)	18	19
[1] Mean minimum temperature (°C)	7	5
[2] Frost events (no. of days)	8	40

[1] Estimated mean values calculated from historical records obtained from the Bureau of Meteorology, Wagga Wagga, 1948–2003. [2] Frost events determined as the number of days where soil surface temperature fell below 0 °C [44].

2.1. Experimental Design and Treatments

The first of three experiments ("*defoliation/herbicide experiment*") was carried out in 2016 and 2017, with the aim of examining the efficacy of single-herbicide application and defoliation by mowing in comparison to combined application on barley grass stands and fecundity under typical field conditions. The second experiment was performed in 2017 ("*herbicide experiment*"), to evaluate the efficacy of alternative selected post-emergent herbicides on barley grass survival and fecundity within a legume pasture. An additional greenhouse experiment ("*defoliation/density experiment*") was performed under controlled conditions in 2018 to further elucidate the direct impact of defoliation frequency and timing on barley grass survival and seed production.

2.1.1. Defoliation/Herbicide Experiment

The defoliation/herbicide experiment was performed as a randomised strip plot, with five replicates and 6 × 4 m plots. Treatments imposed in 2016 were repeated in 2017, without removing the previous year's biomass. There were nine treatments, consisting of an untreated control, two herbicide treatments, two mowing treatments, and all combinations of both herbicides and mowing (T1 to T9, Table 2). The order of herbicide and mowing applications within each treatment was determined by plant growth stage. The herbicide 1 treatment was propaquizafop (100 g·L^{-1} Shogun®, Adama Agricultural Solutions, St Leonards, Australia), applied at a rate of 250 mL/ha in autumn of both years (T2, T5, and T8, Table 2) during early tillering, in line with label directions. A non-ionic surfactant, alcohol alkoxylate (1000 g·L^{-1} Chemwet 1000®, Nufarm Australia Limited, Laverton, North

Victoria, Australia), was added at the rate of 250 mL/ha during 2016 (0.25% *v/v*) to ensure better leaf coverage under the dry conditions present at the time of application and at 200 mL/ha under adequate environmental conditions operating at spraying in 2017 (0.2% *v/v*). The herbicide 2 treatment was paraquat (250 g·L^{-1}, Sinmosa 250®, Sinon Australia, Golden Square, Victoria, Australia) applied to plots at a rate of 1.6 L/ha during August (T3), September (T6), and October (T9) in 2016 and June (T3), August (T6), and September (T9) during 2017 (Table 2), in line with label directions. Paraquat applications were timed as per label directions for legume pastures (to growing plants), but were delayed during 2016 due to frequent rainfall events. Herbicide treatments were applied using an all-terrain vehicle fitted with a 6-m spray boom at 600 mm height. Two alleyways were placed between replications, and induction Teejet 11002 nozzles delivered a volume of 100 L·ha^{-1} using a ground speed of approximately 9 km/hr. The two defoliation regimes consisted of a single mowing to 7.5 cm average plant height (T4, T5, and T6, Table 2), achieved with a rotary mower, once 50% of barley grass plants in plots reached full boot stage (Zadok's growth stage 49) [36]. The second defoliation regime consisted of a repeated mowing once 50% barley grass regrowth also reached growth stage 49 (T7, T8, and T9, Table 2). Since barley grass matured rapidly to boot stage during both years, lucerne plants were noted to be at late vegetative stage during all defoliations, prior to bud formation. Mowing was performed in designated plots where barley grass establishment was observed in more than 10% of the plot. Windrows created by mown herbage were not removed.

Table 2. Experimental scheme for barley grass defoliation and herbicide treatments including respective treatment rates.

Treatment	Barley Grass Treatment Descriptions and Application Rates	Date of Herbicide Application		Date of Mowing Application	
		2016	2017	2016	2017
T1	No mow/no herbicide (control)	-	-	-	-
T2	No mow/herbicide 1 (100 g·L^{-1} propaquizafop, 250 mL/ha)	13 June	16 May	-	-
T3	No mow/herbicide 2 (250 g·L^{-1} paraquat, 1.6 L/ha)	26 August	13 June	-	-
T4	One mow/no herbicide	-	-	10 August	11 August
T5	One mow/herbicide 1 (100 g·L^{-1} propaquizafop, 250 mL/ha)	13 June	16 May	[1] ***	***
T6	One mow/herbicide 2 (250 g·L^{-1} paraquat, 1.6 L/ha)	8 September	30 August	10 August	11 August
T7	Repeat mow/no herbicide	-	-	10 August, 7 September	11 August, 10 September
T8	Repeat mow/herbicide 1 (100 g·L^{-1} propaquizafop, 250 mL/ha)	13 June	16 May	***	***
T9	Repeat mow/herbicide 2 (250 g·L^{-1} paraquat, 1.6 L/ha)	10 October	20 September	10 August, 7 September	11 August, 10 September

[1] *** No mowing occurred in these plots during the experiment if prior removal of the barley grass population by propaquizafop occurred or if barley grass occupied less than 10% of plot biomass. Herbicide treatments applied after mowing were conducted in accordance with label directions and performed when barley grass was actively growing and possessed sufficient leaf area after regrowth to facilitate uptake. T = treatment.

2.1.2. Herbicide Experiment

The "*herbicide experiment*" consisted of 6 m × 2 m plots in close proximity to the defoliation/herbicide experiment, where significant and uniform barley grass infestation previously occurred. Six herbicide treatments and an untreated control were arranged in a randomised complete block design with four replicates, as shown in Table 3. All herbicides were applied post-emergent to plots on 2 June 2017, when barley grass in 50% of plots was estimated to have reached the three-leaf stage (growth stage 20), using an all-terrain vehicle fitted with a spray boom. Wind speed at the time of application was less than 1 km/h, and the temperature was 13 °C.

Table 3. Herbicide treatments and additives applied during the 2017 herbicide trial.

		Herbicide Group					
Treatments	**Mode of Action**	**[1] APVMA**	**[2] HRAC**	**[3] WSSA**	**Rate g ai·ha^{-1}**	**Timing of Application**	**Additive Rate (Rate/100 L H$_2$O)**
Control	_	_	_	_	_	_	_
imazamox	ALS and acetohydroxyacid synthase (AHAS) inhibitor	B	B	2	50	Post	Hasten$^®$ 500 mL
propyzamide	Inhibitor of microtubule assembly	D	K1	3	750	Post	_
propaquizafop	ACCase inhibitor ("fop")	A	A	1	25	Post	Chemwet 1000 $^®$ 200 mL
fluazifop-P + butroxydim	ACCase inhibitor ("fop" + "dim")	A	A	1	320	Post	Supercharge $^®$ 1 L
paraquat	Inhibitor of photosynthesis at photosystem I (PS I)	L	D	22	400	Post	_
haloxyfop-R +simazine	ACCase inhibitor ("fop") + PS II inhibitor.	A + C	A + C1	1 + 5	52 + 550	Post	Chemwet 1000 $^®$ 200 mL

[1] APVMA = Australian Pesticides and Veterinary Medicines Authority, [2] HRAC = Herbicide Resistance Action Committee, [3] WSSA = Weed Science Society of America.

2.1.3. Defoliation/Density Experiment

The defoliation/density experiment was conducted from May to December 2018 in a greenhouse at Charles Sturt University, Wagga Wagga, NSW. The experiment consisted of two factors (comprising five plant densities and three separate defoliation regimes) and was arranged in a randomised complete block design with four replicates. Barley grass seed was previously collected at plant maturity in 2017 from the same field experimental site. Following harvest, seed was stored at room temperature until mid-March 2018 and subjected to cold stratification at 4 °C for five weeks [23] to encourage optimal germination. Seeds were later sown to a 1-cm depth in trays (width 28 cm × breadth 33 cm × depth 6 cm) filled with a 1:1 mixture of peat moss and river wash sand on 16 and 17 April 2018. Trays were initially watered daily until seedlings emerged to a height of 5 cm, at which time they were fertilised with a water-soluble fertiliser.

On 21 and 22 April 2018, seedlings were transplanted from germination trays into plastic containers (measuring 39 cm × 28 cm × 14 cm) filled with a similar peat moss and river wash sand mixture, and they were maintained as above for the duration of the experiment. Maximum and minimum temperatures reached were 37.6 and −3.6 °C, respectively, with variable relative humidity. Plant densities consisted of 1, 6, 36, 64, and 117 plants, (adjusted to provide 1, 54, 288, 576 and 1053 seedlings·m^{-2}) to reflect the range of densities found adjacent to the original field experimental site.

To ensure consistent and uniform seedling placement per replicate, planting was performed using a 1 cm × 1 cm wire grid laid upon the soil surface. Seedlings were later removed from germination trays with tweezers and transplanted into their respective treatments at the associated grid positions for each density treatment. The treatments were sown in a similar manner twice more per replicate in separate containers in order to facilitate three individual destructive harvests to further investigate the impact of defoliation on seed production. The three separate defoliation regimes consisted of cutting plants to 5-cm height with garden shears at (1) seedling stage (2–3 leaf) (entitled "SDL"), (2) post-inflorescence emergence (entitled "head cut 1" or "HC1"), or (3) a second defoliation performed on the regrowth of plants which were previously defoliated in line with the HC1 regime, conducted at post-inflorescence emergence (entitled "head cut 2" or "HC2") (Table 4). A defoliation height of 5 cm was chosen to mimic the height achieved by field machinery during field experimentation, a height reported to reduce barley grass seed production [37]. Replicates were re-randomised fortnightly to avoid greenhouse variation. Plants were subjected to fluctuating light and temperature conditions in the greenhouse simulating natural conditions due to open air circulation. Fertiliser applications and watering to field capacity occurred weekly from sowing until experiment termination on 8 November 2018. No plant loss was noted due to the transplanting process.

Table 4. The defoliation regimes of three individual defoliations of barley grass plants planted at five different seedling densities and at two stages of growth under greenhouse conditions at Wagga Wagga, New South Wales (NSW).

Defoliation Treatment	Barley Grass Stage of Growth at Time of Defoliation	Date
SDL	2–3 leaf seedling stage	22 May 2018
HC1 "Head cut 1"	Post-inflorescence emergence—once all inflorescences emerged	28 September 2018
HC2 "Head cut 2"	Post-inflorescence emergence on previously defoliated plants—once all inflorescences emerged	3 October 2018

2.2. Measurements

2.2.1. Defoliation/Herbicide Experiment

Evaluations were performed in the defoliation/herbicide experiment following each treatment application. Repeated rainfall events slightly delayed field paraquat application in 2016. The experiment concluded in November 2016 and October 2017, when lodgement of barley grass plants occurred.

Biomass. Two quadrats (25 cm × 25 cm) were randomly selected away from plot boundaries for biomass collection in each plot, and all foliage was cut at ground level with hand shears. Samples were collected monthly following application of herbicide/mowing treatments until barley grass seed fall. Plant material within each quadrat was pooled per plot and separated manually into barley grass, lucerne, clover, and other weed species (OWS) such as *Vulpia* spp., capeweed (*Arctotheca calendula* (L.) Levyns), and annual ryegrass (*Lolium rigidum* Gaud.). Samples were dried in a plant dehydrator at 70 °C for 24 h and weighed when dry. All data are presented as $g \cdot m^{-2}$.

Barley grass plant density. Due to the extremely high densities of barley grass seedlings in field plots, barley grass plants were collected from smaller 10 cm × 10 cm quadrats taken from within three separate, diagonally positioned $0.25\text{-}m^{-2}$ quadrats, to estimate species composition. Data are presented as plant density$\cdot m^{-2}$. Plant density counts were taken during November 2016 and August 2017.

Inflorescence number. Barley grass inflorescence numbers were recorded from the final biomass sampling at plant maturity in 2016 (21 November) and 2017 (25 September). Inflorescences counted from the two biomass quadrat samples/treatment plot were combined, and values are expressed as the number of inflorescences$\cdot m^{-2}$.

Fecundity. At plant maturity in 2016 and 2017, 20 inflorescences were collected at random from each plot, and seeds within each inflorescence were counted individually, from which the mean number of seeds per inflorescence was determined. Total seed number$\cdot m^{-2}$ was subsequently determined by multiplying the number of inflorescences$\cdot m^{-2}$ by the average seed number per inflorescence.

Seedling emergence counts. Ten soil cores (5 cm × 5.5 cm) were collected per plot using a standard metal soil corer in mid-December each year. Soil cores were stored at ambient temperature for three months to allow any dormancy to break down. In March of the following year, cores were broken, mixed separately, and uniformly spread in 29 × 34 cm trays containing moistened potting mix within a greenhouse, to record the number of subsequently germinating barley grass seedlings. Barley grass seedlings emerging more than 2 cm above the soil surface were counted and removed weekly until no new plants appeared for three consecutive weeks (early July 2017 and late June 2018). Seedling counts were assumed to represent the number of germinable seeds$\cdot m^{-2}$ that would emerge in the following year.

2.2.2. Herbicide Experiment

Plant density. Plant density was assessed after plants reached flowering stage to facilitate identification. Barley grass was counted in five quadrats (10 cm × 10 cm) placed diagonally across plots, averaged, and converted to mean number of plants$\cdot m^{-2}$ in each plot.

Inflorescence number. Inflorescence number was assessed in two quadrats (25 cm × 25 cm) randomly collected across each plot once all inflorescences emerged, averaged across the plot. Values were converted to the number of inflorescences$\cdot m^{-2}$.

Fecundity. Due to environmental conditions resulting in acceleration of the 2017 growing season, the collection of inflorescences for fecundity measurements risked seed detachment from inflorescences as barley grass seeds are not retained in the seed head. This would have made counting seeds per inflorescence impossible, as per methods in the defoliation/herbicide experiment. Fecundity (total seed production) was, thus, estimated using methods similar to those described by Shergill, Fleet, Preston, and Gill [24]. Twenty-five plants were randomly collected from each plot, and total inflorescence length and seed number were measured per plant. In plots with fewer than 25 plants, all plants were collected. A linear model based on total inflorescence length and seed number was used to fit the data as follows, as shown in Figure 1a:

$$Y = Y_0 + bX, \tag{1}$$

where Y is the number of seeds produced from total inflorescence length, b is the slope of the regression line (i.e., an average of 3.4 seeds per mm of inflorescence), and Y_0 is the intercept (set at zero). Although other models were also noted to suitably fit the data ($R^2 > 0.96$), a linear model was deemed to most realistically represent this relationship since increasing length of inflorescences did not result in decreasing numbers of seeds at any point. Fecundity$\cdot m^{-2}$ was then estimated based on barley

grass plant density (as obtained in the field) and seed number per plant (as generated from the linear relationship described between total inflorescence length, and seed production per plant) [22]. A linear model was fitted to these data, where the dependent variable was fecundity·m^{-2} (estimated from plant density *x*), b was the slope of the regression line (an average of 80.8 seeds per plant), and the intercept was set at zero, as shown in Figure 1b.
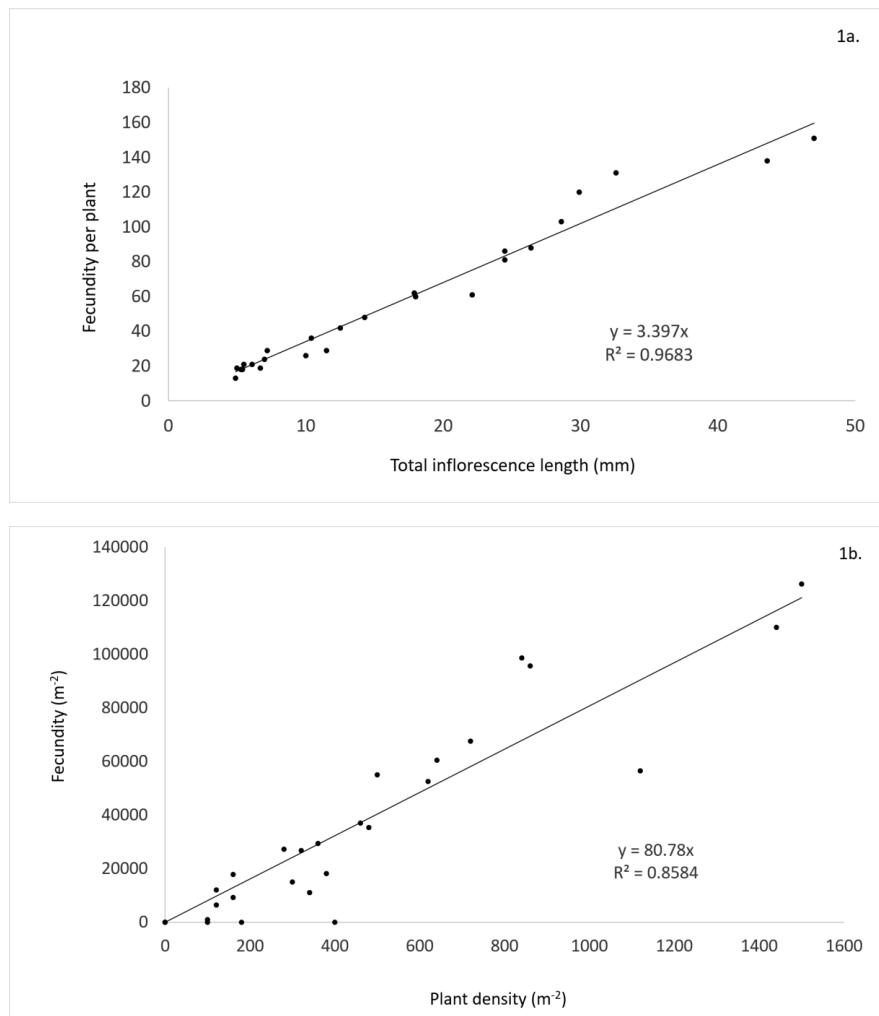

Figure 1. (**a**) Relationship between total inflorescence length and total seed production per barley grass plant. (**b**) Relationship between plant density and fecundity during 2017 within a legume pasture at Charles Sturt University campus, Wagga Wagga, NSW. Data are presented as functional two-parameter linear models fitted to total inflorescence length and total seeds produced per plant (Figure 1a, *n* = 25) and plant density and fecundity per metre (Figure 1b, *n* = 28).

2.2.3. Defoliation/Density Experiment

Plant density counts were collected at the seedling stage and at inflorescence emergence, and inflorescence counts and seed data were collected at seed maturity. Due to the limited growth of barley grass seedlings in the two heaviest populations, measurements were taken from a sample of 36 equally spaced and centrally located plants within these treatments in order to facilitate accurate measurements, in a sample equivalent to that of the third highest density treatment.

Plant density. Surviving mature plants were counted at flowering stage in the non-defoliated treatments, as well as after each defoliation in order to investigate plant density, determined as the number of plants surviving from sowing to maturity.

Inflorescence number per plant. Inflorescence numbers per plant were counted and averaged across each treatment once all inflorescences emerged.

Fecundity. Three plants were randomly collected from each density treatment, and total inflorescence length and seed number were measured per plant and averaged per treatment. A linear fecundity model based on total average inflorescence length and fecundity was again found to closely fit the data as follows (R^2 = 0.9924, root-mean-squared error (RMSE) = 14.98, F (1, 18) = 2347, $p < 0.001$, Figure 2):

$$Y = Y_0 + bX, \tag{2}$$

where Y is the number of seeds produced from the total length of inflorescences (x), b is the slope of the regression line, and Y_0 is the intercept set at zero.
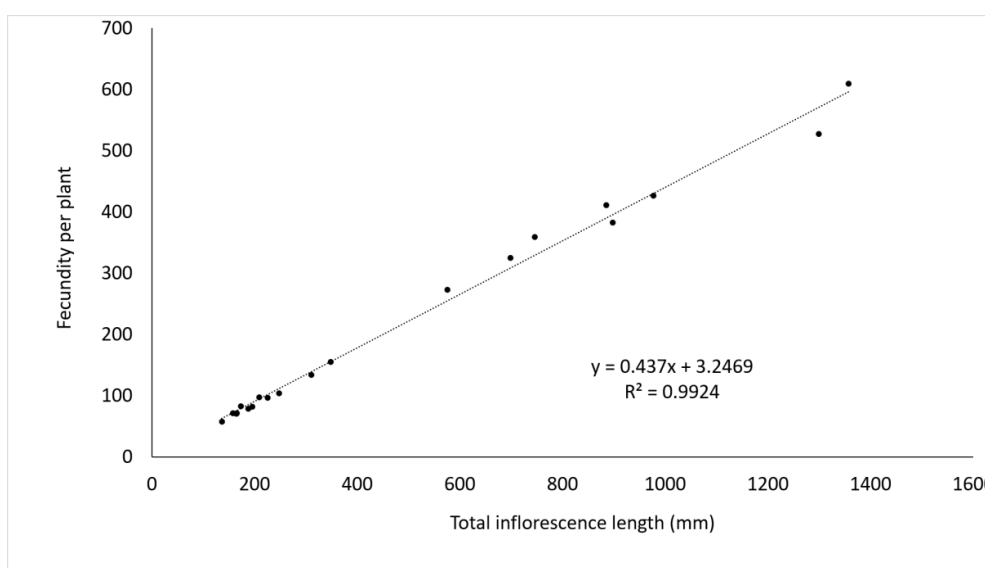
Figure 2. Relationship between total inflorescence length and fecundity per barley grass plant grown under greenhouse conditions at Wagga Wagga, NSW. Data are presented as a functional two-parameter linear model fitted to total inflorescence length and fecundity per plant (Figure 2, *n* = 20).

Total inflorescence length was measured in mm per plant in each treatment. Fecundity per plant in each treatment was, thus, generated from the linear relationship described between measured total inflorescence length and seed number per plant. Total fecundity was then determined by multiplying fecundity per plant by final mature plant density within each treatment.

2.3. Statistical Analyses

All data collected during the defoliation/herbicide experiment were subjected to factorial ANOVA for a strip plot experimental design. Assumptions of normality and homoscedasticity were verified using visual analysis of residuals and, when assumptions were not met, data were square-root- or log-transformed to normalise residuals. Significant effects ($p < 0.05$) were tested by comparing the least squares means of the transformed data using Scheffe's multiple comparison tests, appropriate for unplanned a posteriori comparisons [45].

When a significant interaction was noted, Scheffe's test was applied and means were transformed; then, they were back-transformed for presentation with standard errors. As preliminary analysis of barley grass biomass in 2017 revealed significant differences in botanical composition occurring between treatments, separate analyses were conducted for data collected after 12 and 24 months.

Single-factor ANOVA was used to compare the means among the seven herbicides applied during the herbicide experiment. Assumptions of normality and homoscedasticity were verified after using square-root transformations. As this experiment included planned (a priori) comparisons between

the treatments and the control, pairwise differences were adjusted using Tukey's honestly significant difference (HSD) test (type I error $\alpha = 0.05$ as the threshold value for testing statistical significance) and estimated means and standard errors that, on the original measurement scales, are presented for reporting the analysis outcomes. All ANOVAs for the first two experiments were done using IBM SPSS software, version 20 [46], and regression analyses were done using MS Excel ®.

Data collected during the defoliation/density study were analysed using three statistical regression models. The effect of predictor variables (barley grass seed density and defoliation timing) on total fecundity data was determined using a linear model, while generalised linear models (glm function in R package MASS) within R software [47] were identified as most appropriate for the analysis of both predictor variables on mature plant density and the inflorescences per plant data, since the response variables in both cases were not normally continuously distributed. Final models for each variable were chosen based on the Akaike information criterion (AIC) for model selection, where the model with the lowest AIC score was considered as the optimal model among all candidate models examined. Resulting models are listed in Table 5, including error structures and link functions. All differences between means in each dataset were adjusted using Tukey's honestly significant difference (HSD) test in R software [47], using Tukey's HSD function for total fecundity data and the emmeans () function in R package, "emmeans", for mature plant density and inflorescences per plant data. A level of statistical significance of $p < 0.05$ was assumed for all analyses. Model estimated means and 95% confidence intervals are presented for all datasets.

Table 5. The resulting optimal models, their error structures, and link functions used in the statistical analysis of seed density and defoliation on mature barley grass plant density and seed production under greenhouse conditions at Wagga Wagga, NSW.

Model	Selected Model Description	Model Type (Error Structure)	Link Function
1	Total fecundity = seed density + defoliation + seed density × defoliation	Ordinary Linear Model (normal)	Identity
2	Mature plant density = seed density + defoliation	Generalised Linear Model (Poisson)	Log
3	Inflorescences per plant = seed density + defoliation + seed density × defoliation	Generalised Linear Model (gamma)	Inverse

3. Results

3.1. Defoliation/Herbicide Experiment

Significant differences between herbicide treatments were observed in mean barley grass, clover and OWS biomass, barley grass inflorescence number, density, fecundity, and seedling emergence counts after 12 and 24 months. Significant differences in barley grass seedling emergence counts (F = 8.2, df = 2, 8; $p < 0.001$) due to mowing alone were noted after 12 months (Figure 2). After 24 months, there was a significant interaction between herbicide and mowing, resulting in significant differences in lucerne biomass (F = 14.7; df = 4, 16; $p < 0.001$) and other weed biomass (F = 4.0, df = 4, 16; p= 0.02) only (Figures 3 and 4, respectively).

3.1.1. Herbicide-Only Effects

In comparison to the untreated control, propaquizafop significantly reduced mean barley grass biomass, inflorescence number, fecundity, and density by over 99% after 12 months and increased OWS biomass by 128%, while reducing the number of viable barley grass seeds by 95% (Table 6). In contrast to the control, the application of paraquat resulted in a 37% decline in OWS biomass, a 35% decline in clover biomass, and a 162% increase in the number of viable barley grass seeds, despite there being no statistically significant differences in barley grass biomass, inflorescence number, fecundity, or plant density.

Table 6. Mean responses to herbicide alone on barley grass, clover and other weed species (OWS) biomass, barley grass density, inflorescence number, and seed production within a standing legume crop invaded by barley grass 12 and 24 months after application ($p < 0.05$).

Treatment	2016							2017					
	BG Biomass g·m⁻¹	Clover Biomass g·m⁻¹	OWS Biomass g·m⁻¹	BG Inflorescence Number·m⁻²	BG Density·m⁻²	BG Fecundity·m⁻²	BG Seedling Emergence·m⁻²	BG Biomass g·m⁻¹	Clover Biomass g·m⁻¹	BG Inflorescence Number·m⁻²	BG Density·m⁻²	BG Fecundity·m⁻²	BG Seedling Emergence·m⁻²
No herbicide	396. a [1]	173.8 a [2]	277.6 b	1296.5 a	1396.7 a	33602.6 a	1635.2 b	158.04 x [2]	15.63 y	552.5 x	680.0 x	6212.60 x	1929.2 x
Propaquizafop	1.0 b	311.8 a	632.8 a	5.3 b	6.7 b	0.0 b	89.6 c	3.63 y	53.26 x	1.07 y	5.0 y	59.4 z	50.4 y
Paraquat	326.1 a	112.6 b	101.5 c	1264.0 a	1596.7 a	31474.2 a	2662.8 a	123.49 x	14.84 y	348.3 x	444.4 x	3580.6 y	1251.6 x
Pooled standard error	33.2	33.7	32.5	183.2	109.9	4851.8	128.9	14.7	6.4	32.4	71.5	309.1	215.6

[1] Back-transformed means and pooled standard errors (SE) are shown. [2] Sets of symbols indicate separate analyses. Means within each column with the same letter are not significantly different ($p < 0.05$) after the Scheffe correction for type I error. OWS = other weed species. BG = barley grass. All data other than BG fecundity data were transformed prior to analyses, and significant differences are shown for transformed data.

After 24 months, propaquizafop significantly reduced mean barley grass biomass, inflorescence number, fecundity, and density by over 98%, tripled clover biomass, and reduced the number of viable barley grass seeds by 97%. Although applications of paraquat reduced barley grass fecundity by 58%, no significant differences in any other parameters were observed with paraquat applications in comparison to the control ($p > 0.05$).

3.1.2. Mowing-Only Effects

Seedling emergence counts. A second mowing at growth stage 40 [34] resulted in a 54% reduction in mean emerging barley grass seedlings in contrast to the control (adjusted $p < 0.05$) after 12 months (Figure 3). In comparison, no significant differences were observed under a single mowing regime.
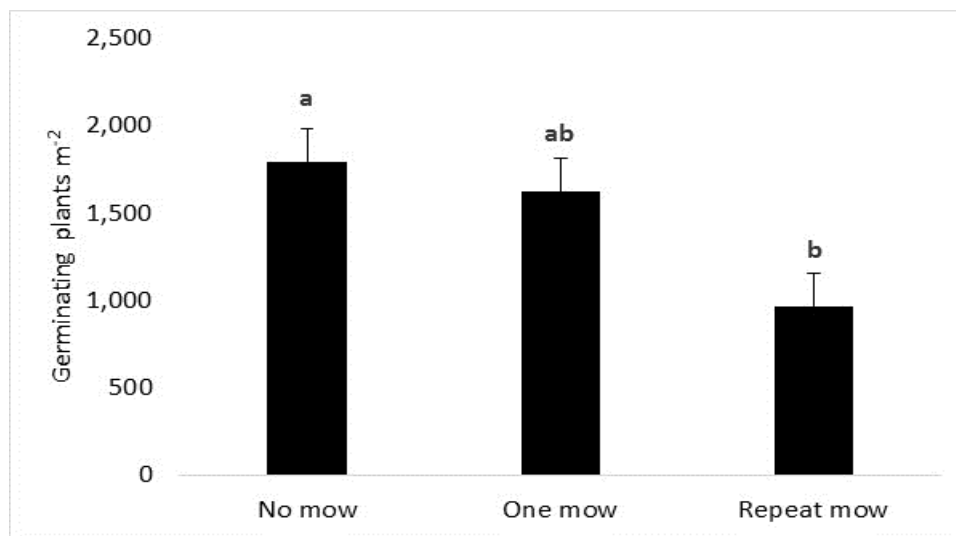
Figure 3. Mean (± pooled SE) number of emerging barley grass seedlings·m^{-2} in response to the mowing of a barley grass infested legume crop after 12 months (2016). Bars with the same letter are not significantly different ($p < 0.05$) after the Scheffe correction for type I error.

3.1.3. Herbicide × Mowing Interaction

Lucerne biomass. Lucerne biomass was significantly influenced by the interaction between herbicide and mowing after 24 months (Figure 4). Paraquat applied alone (treatment H2M0) resulted in the most significant increase in lucerne biomass in comparison to all other treatments (adjusted $p < 0.05$). A declining trend in lucerne biomass was observed with increasing mowing frequency, regardless of herbicide level.

Other weed species biomass. The interaction between herbicide and mowing also significantly influenced OWS biomass after 24 months, as shown in Figure 5. At each mowing frequency, treatments that included propaquizafop (H1) generally resulted in higher OWS biomass than those consisting of no herbicide, and they also resulted in significantly higher OWS biomass than those that included paraquat (H2) (adjusted $p < 0.05$).
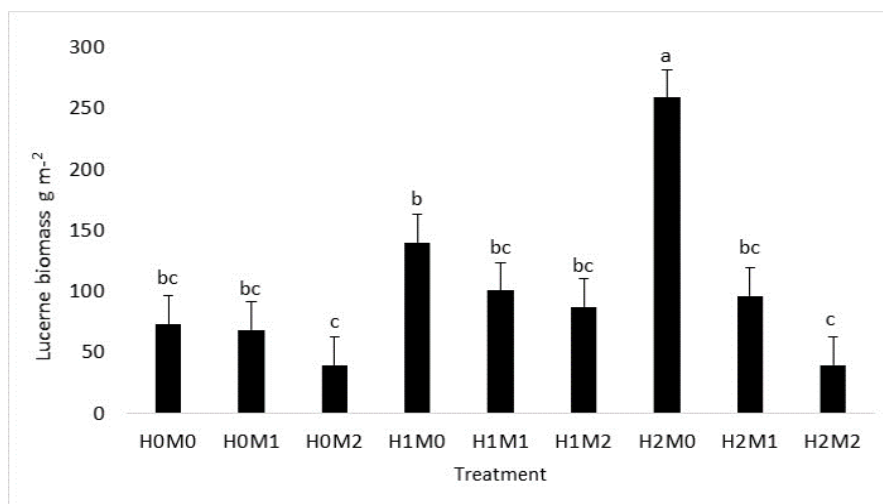
Figure 4. Mean (± pooled SE) lucerne biomass (g·m^{-2}) present as a result of the interaction between herbicide and mowing after 24 months (2017) in a barley grass-infested mixed pasture stand. Bars with the same letter are not significantly different ($p < 0.05$) after the Scheffe correction for type I error. H0, H1, H2: no herbicide, herbicide 1 (propaquizafop), herbicide 2 (paraquat), respectively. M0, M1, M2: No mow, one mow, repeat mow, respectively.
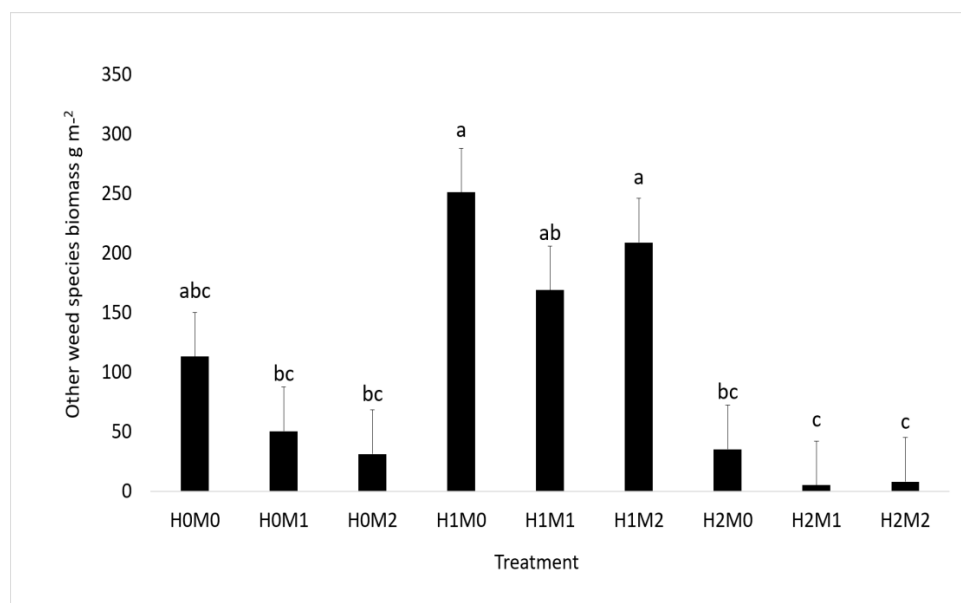

Figure 5. Mean (± pooled SE) OWS biomass (g·m^{-2}) present as a result of the interaction between herbicide and mowing after 24 months in a barley grass-infested mixed pasture stand. Bars with the same letter are not significantly different ($p < 0.05$) after the Scheffe correction for type I error. H0, H1, H2: no herbicide, herbicide 1 (propaquizafop), herbicide 2 (paraquat), respectively. M0, M1, M2: no mow, one mow, repeat mow, respectively.

3.2. Herbicide Experiment

Significant differences due to herbicide treatment were observed in barley grass plant density (F = 9.4, df = 6, $p < 0.001$), inflorescence number (F = 11.714, df = 6, $p < 0.001$), and fecundity (F = 24.6, df = 6, $p < 0.001$) (Table 7).

In comparison to the untreated control, the haloxyfop-R + simazine and paraquat treatments resulted in a 95% and 93% decline, respectively, in plant density and a 97% and 98% decline in inflorescence number, respectively, 10 weeks after treatment application (Table 7). Differences observed

due to herbicide treatment were also reflected in the respective 97% and 99% reduction in barley grass fecundity. Imazamox and propaquizafop were not effective in significantly reducing the number of barley grass inflorescences or fecundity in comparison to the untreated control. The application of fluazifop-P + butroxydim and propyzamide also produced a significant 68% and 82% reduction in inflorescence number, respectively, and a 74% and 85% respective decline in fecundity.

Table 7. Mean responses to herbicide treatments on barley grass plant density, inflorescence number, and fecundity during August 2017 within a barley grass-infested mixed pasture at Charles Sturt University in Wagga Wagga, NSW.

Herbicide Treatment	BG Plant Density·m^{-2} (SE = 141.9) [2]	BG Inflorescence Number·m^{-2} (SE = 58.9)	BG Fecundity·m^{-2} (SE = 10,228.6)
Untreated control	842 ab [1]	427 m	81650 v
Imazamox	950 a	324 mn	75828 v
Propyzamide	333 abc	79 no	12652 xy
Propaquizafop	492 ab	253 mn	48635 vw
fluazifop-P + butroxydim	192 bc	135 no	20959 wx
Paraquat	67 c	11 o	225 z
Haloxyfop-R + simazine	50 c	14 o	2303 yz

[1] Means within the same column followed by the same letters are not significantly different according to Tukey's honestly significant difference (HSD) test at α = 0.05. Data were square-root-transformed prior to ANOVA. Back-transformed means and pooled standard errors (SE). [2] are presented. All data were collected 10 weeks post-treatment application. BG = barley grass.

3.3. Defoliation/Density Experiment

Highly significant differences due to individual effects of density and defoliation timing were found for mean mature barley grass density (χ^2 = 2921.5, df = 4, $p < 0.001$ and χ^2 = 89.03, df = 3, $p < 0.001$, respectively) when grown in the greenhouse. The interaction between density and defoliation produced significant differences in the number of inflorescences per plant (F = 3.8, df = 12, 60, $p < 0.001$) and total fecundity (F = 10.4, df = 12, 60, $p < 0.001$).

Survival of plants to maturity was consistent across all density treatments at 83.85% (Figure 6a). Defoliation at seedling stage (SDL) or at initial inflorescence emergence (HC1) was not effective in significantly reducing plant density, but density was reduced by 35% when plants were subjected to a repeat defoliation of regrowth (HC2) after inflorescences re-emerged (Figure 6b).
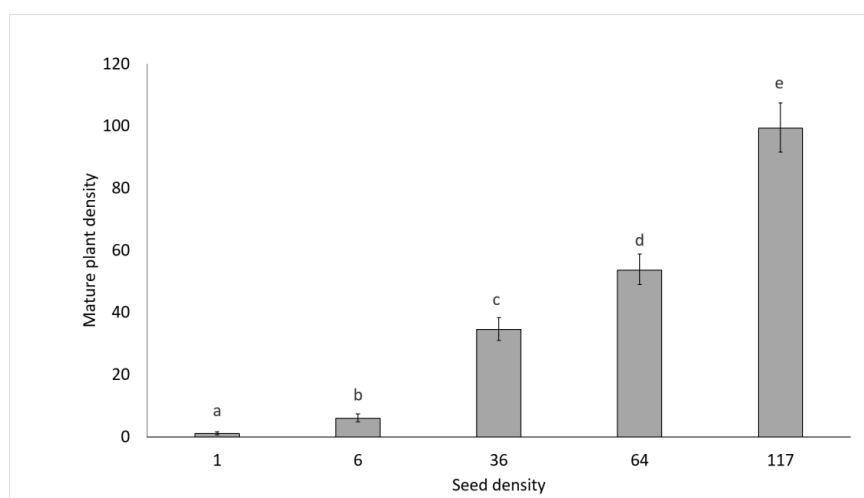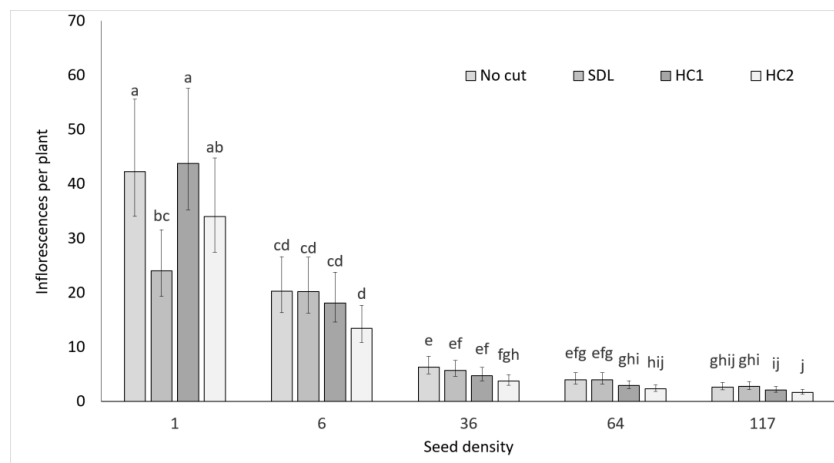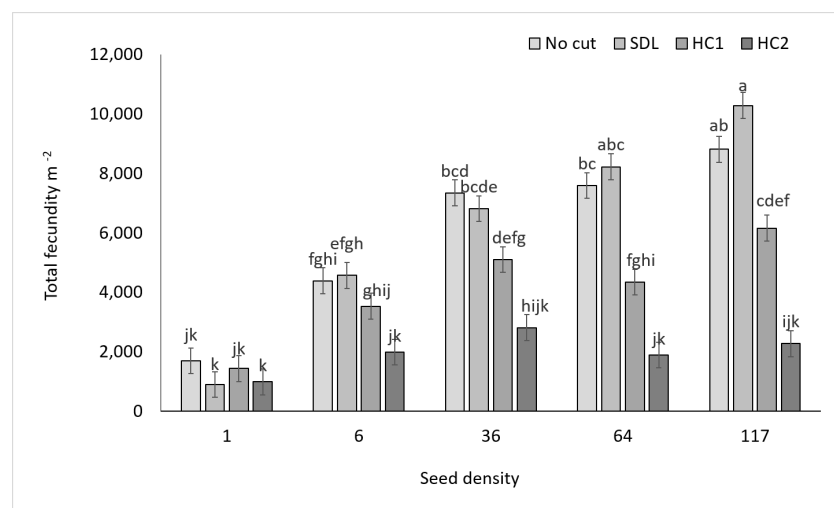

a

Figure 6. *Cont.*
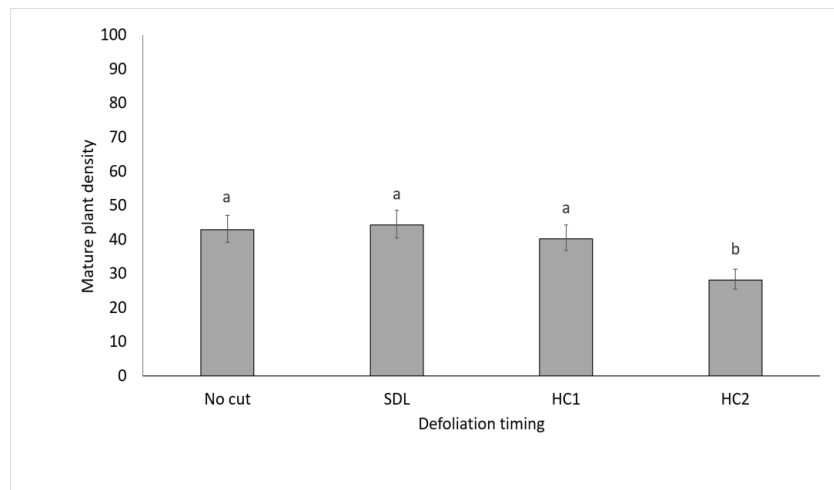
b

c

d

Figure 6. (**a**) Mean number of barley grass plants surviving to flowering when sown at five population densities in the greenhouse. (**b**) Mean number of mature barley grass plants in response to defoliation timing and frequency. (**c**) Mean number of barley grass inflorescences per plant. (**d**) Mean total fecundity, as a result of the interaction between density and defoliation timing, all conducted under greenhouse conditions in Wagga Wagga, NSW. Model estimated means and 95% confidence intervals

shown in all figures. Bars with the same letter are not significantly different according to Tukey's honestly significant difference (HSD) test at α = 0.05. No cut = no defoliation, SDL = defoliation at seedling stage, HC1 = defoliation at inflorescence emergence, HC2 = defoliation of regrowth at inflorescence emergence.

Reduced inflorescence number per plant was observed in those plants subjected to a repeat defoliation (HC2), and this was observed at all densities over a single plant grown alone (Figure 6c). At intermediate densities (36 and 64 plants), differences were significant (59% and 58%, respectively). At the lowest density only, defoliation at seedling stage (SDL) also produced a significant reduction in inflorescence density (57%).

At plant densities of six plants and above, total fecundity was found to decline after plants were subjected to a single (HC1) and also repeated defoliation at inflorescence emergence, although impact due to repeated defoliations (HC2) was greater (Figure 6d). The greatest reduction in total fecundity was observed when plants were subjected to a repeated defoliation (HC2) at the two highest densities (64 and 117 plants) (75% and 74%, respectively).

4. Discussion

The use of conservation tillage practices, continuing climate variability, and the exclusive use of certain herbicides for annual grass control are all likely to facilitate current barley grass invasion across southern Australia, enabling the recent proliferation of highly competitive biotypes [12]. Despite the utilisation of barley grass for livestock fodder during its vegetative phase, efficacious control is crucial to reduce potential for seed contamination in sheep and improve pasture productivity later in the season. However, the co-occurrence of seed dormancy and herbicide resistance in numerous barley grass populations is likely to complicate management due to limited herbicide options available [31], while prevalence in steep or rocky sites can make herbicide application impractical. Combining chemical with cultural control methods including defoliation may, thus, be valuable for reducing annual grass weed populations and consequently facilitating growth and competitive attributes of desirable pasture species.

4.1. Defoliation/Herbicide Experiment

Herbicide efficacy was negatively impacted by seasonal conditions (i.e., drought or frequent rain) and timing of application (i.e., late application to larger plants) resulted in poor control. A single application of propaquizafop timed optimally at early tillering (Zadok's growth stage 21) [36] was most successful in reducing barley grass survival and fecundity during 2016 and 2017, similar to the findings of others regarding ACCase-inhibiting herbicides used against barley grass in mixed pastures [48,49].

Despite high propaquizafop efficacy in 2016, a small number of barley grass plants survived in 2017 when low rainfall and temperatures likely facilitated rapid maturation of barley grass and reduced propaquizafop activity due to limited translocation, an effect commonly observed in plants treated with ACCase inhibitors during cold and dry conditions [50–56]. Low subsequent barley grass seedling emergence counts the following year may have also reflected the limited seedbank from barley grass survivors as opposed to low seed viability per se [48,49]. The subsequent invasion of *Vulpia* spp. and other weeds and the consequent increase in clover biomass following barley grass removal are consistent with findings of previous studies investigating annual weed management in pasture legumes [25]. Such shifts in botanical composition were also likely supported by previous annual superphosphate applications [14] and the limited competition afforded by the sparse legume population. Successful pasture production encompasses practices which assist the maintenance of an established, competitive legume pasture following herbicide application to limit future annual grass invasion and prevent their occupation in niches created by barley grass control earlier in the season [25,57].

The timing of herbicide application was also critical in determining the success of paraquat against barley grass in both years. Efficacy was reduced in 2016 due to frequent rainfall events resulting in delayed applications, leading to more mature plants with reduced susceptibility. As a common contact herbicide, paraquat should typically be applied when plants are no taller than 12 to 20 cm, facilitating adequate canopy penetration [58]. Previous studies examining the effect of paraquat timing in annual weed control [59] showed ill-timed applications, which resulted in reduced legume biomass and exploitation of canopy gaps by later-germinating annual grasses such as *Vulpia* spp., a result observed during 2016 in this experiment. Application of paraquat, an effective desiccant [60], may have expedited seed maturity resulting in seed rain drying on the soil surface in gaps created by prior eradication of *Vulpia* spp. An extended period of time ensued between the point of seed maturity and subsequent germination the following year, a characteristic noted by others to be associated with higher seed germinability in *Hordeum* populations [23]. In contrast, plant residues from 2016 that remained on the soil surface may have reduced contact between paraquat and barley grass, contributing to poor efficacy observed in 2017. However, well-timed applications at earlier phenological stages in 2017 led to greater pasture clover content and lower annual weed fecundity, results consistent with previous studies [59,61]. It is also important to note that the lack of available soil moisture due to limited rainfall during flowering may have also contributed to lower weed fecundity in 2017 [62].

Mowing alone and the interaction between mowing and herbicide did not significantly impact barley grass survival or fecundity under field conditions. These results were unexpected, given previous success of mowing at boot-stage (Zadok's growth stage 40) [36] in reducing barley grass fecundity [37]. This could have been due to sub-optimal timing of defoliation, which was implemented when 50% of plots reached boot stage (growth stage 40) and remaining plants were likely still tillering (growth stage 20–29), a stage of growth less conducive to reducing fecundity under defoliations [37]. This conclusion supports findings of the third study, where the timing of defoliation (at post-inflorescence emergence) was found to be more effective in reducing plant survival and seed production, particularly when subsequently repeated at the same stage. Under field conditions, repeated defoliation by mowing also proved valuable in significantly reducing the number of emerging barley grass seedlings during the following year, likely due to limited rainfall, thereby impacting seed maturation [63].

The interaction between herbicide and mowing influenced both lucerne and OWS biomass after 24 months, a result likely associated with interspecies competition. Thus, eradication of barley grass by early propaquizafop applications evidently enabled exploitation of resources by both lucerne and OWS. The marked increase in lucerne biomass during 2017 as a result of the single paraquat application treatment (H2M0) may also reflect this mechanism, highlighting the value of optimal timing of herbicide applications in creating opportunities for later competition by desirable species. In treatments combining paraquat with mowing, mowing significantly reduced lucerne and OWS biomass, while reduced soil moisture availability resulted in limited re-growth. Furthermore, since barley grass is a rapidly maturing weed [19], the timing of defoliations likely coincided with earlier vegetative growth in lucerne, which may have resulted in damage to the crown in some lucerne plants [64]. The defoliation regime may also have hindered the regeneration capacity of lucerne plants in twice-mown plots due to reduced root reserves and direct impact upon crown buds [65]. This treatment combination limited competition with the mature (and more tolerant) barley grass population, which may have re-tillered after defoliation [66] and subsequently outcompeted the other species as a result of being poorly impacted by paraquat. Low OWS biomass in paraquat-treated plots likely points to the efficacy of paraquat for controlling later-emerging *Vulpia* spp., applied at an optimal growth stage for effective control.

4.2. Herbicide Experiment

Differences in herbicide efficacy were observed during 2017 as low temperatures and drought prevailed throughout the growing season. Paraquat and haloxyfop-R + simazine proved most efficacious in reducing barley grass density and seed production, consistent with results from previous

studies in annual pastures [27]. Paraquat is most effective when applied in full sunlight as it disrupts photosynthesis by rapid cell lysis, photo-bleaching [67], and necrosis, resulting in plant death. Low moisture and air temperatures were proven to be problematic for translocation of systemic herbicides, but evidently did not hamper paraquat efficacy, as contact herbicides are typically less impacted by such atmospheric conditions [68]. The optimal timing and relatively high rate of paraquat applied also likely contributed to increased efficacy and may largely explain the superior performance of paraquat between both experiments.

The haloxyfop-R + simazine treatment also resulted in efficacious barley grass control and may be associated with reported synergistic activity. Post-emergent systemic herbicides such as haloxyfop-R are typically adversely impacted by low moisture and air temperature conditions. In contrast, simazine exhibits limited foliar activity, but residual soil activity can result in uptake and transport following application [68]. Similarly, propyzamide (inhibitor of microtubule formation) also exhibits activity on established grass weeds [69] via root uptake, requiring higher levels of soil moisture as a result of low solubility [68]. The efficacy of propyzamide and haloxyfop-R + simazine treatments may be attributed to the considerable residual activity of propyzamide and simazine on later-emerging barley grass seedlings [24,68], with uptake facilitated by successive rainfall events. Residual control of later emerging seedlings may also help to explain the lower seed production observed in these treatments in contrast to both propaquizafop and fluazifop-P + butroxydim treatments, where later emerging seedlings set considerable seed. Despite the cold and dry environmental conditions experienced by barley grass plants in both field experiments during 2017, the reduced efficacy of propaquizafop may largely be the result of the later timing of applications to more mature plants and their greater tolerance of herbicide applications.

The imazamox treatment resulted in limited control at best, which was unexpected because of its propensity for effective foliar penetration and translocation under optimal conditions [58] and its residual activity resulting in control of later emerging weeds [69]. Therefore, ALS resistance in this population of barley grass is suspected, given the frequency of resistance to imidazolinone herbicides observed in southern Australia [22,30,31,70]. Further evaluation is required to confirm this possibility.

4.3. Defoliation/Density Experiment

The results of the greenhouse defoliation study revealed that plant survival and seed production were both significantly impacted by repeated defoliation, especially when defoliation was applied during reproductive development, after inflorescence emergence. This was not surprising, since plants are typically more sensitive to defoliation upon entering the reproductive phase [37]. The success of repeated defoliation may have been enhanced by the limited recovery period for regrowth between the first and successive defoliations. Short recovery intervals between defoliation frequently impact the ability of plants to replenish adequate water-soluble carbohydrate reserves required for regrowth and seed development [71]. This effect was intensified as density increased, as plants were already competing heavily for light and resources.

Similar findings observed in defoliation studies performed with other annual plants (*Abutilon theophrasti*) showed defoliation at higher population density reduced seed production by 50%, potentially as a result of increased light competition [72]. Despite the lower inflorescence numbers observed per plant in the high density populations, a phenomenon typically associated with competitive stress [72], our findings highlight the capacity of barley grass populations to ensure continuing survival via high community level seed production. Although the repeated defoliation did not completely eliminate seed output, there may be value in using well-timed and more frequent defoliation to limit barley grass seed production, since significant costs and herbicide resistance risks are associated with heavy reliance on herbicides. Further investigation over an extended time frame would better substantiate the value of defoliation and/or grazing as an effective control method for barley grass management, thereby reducing weed seedbanks over time.

5. Conclusions

Despite emerging resistance issues, post-emergent herbicides continue to be particularly useful for managing annual grass weeds in less competitive mixed legume pastures in southern Australia, due to their efficacy and ease of use, if rainfall is sufficient to facilitate translocation.

Regions characterised by significant levels of seed contamination in grazing sheep would likely benefit from the application of post-emergent herbicides such as the ACCase inhibitors, PS I inhibitors, and PS II inhibitors for control of susceptible populations of *Hordeum* spp., particularly in legume pastures. In arid regions or during dry seasons, contact herbicides and/or systemic combinations applied to young plants may be of greater value, a scenario of increasing consequence to southern Australia. The selection of herbicides with improved residual activity will be increasingly important for control of grass weeds expressing variable seed dormancy patterns. Following barley grass removal, the appropriate management of other weed species, such as *Vulpia* spp., is also important, particularly as their remaining seedbank may present similar risks to grazing sheep. Careful consideration of timing of herbicide application in relation to plant growth stage and the prevailing environmental conditions will be imperative to achieving effective barley grass control, given the capacity for prolific seed production.

Defoliation by mowing may be effective at a later stage of the season, after inflorescence emergence, to control seed set from individuals escaping herbicide application, thereby reducing barley grass numbers in the seedbank the following year, or reducing infestations of barley grass on fence lines, along roadsides and in areas of livestock congregation to the degree where eradication by herbicides is possible. Tactical grazing may also be a possible alternative to mowing in less accessible areas if high stocking rates ensure close grazing to remove seed heads. However, the random nature of grazing and reluctance of animals to eat barley grass once inflorescences emerge may limit utility of this practice later in the season.

Defoliation by mowing at late maturity and prior to seed desiccation may also be valuable in limiting barley grass germination during dry years, since limited moisture may hamper seed development and maturity prior to defoliation. However, in degraded legume pastures, defoliation by mowing may also limit regrowth of pasture legumes and result in crown damage if defoliation is timed too early during vegetative growth of lucerne plants. Therefore, it is important to consider the timing of defoliation in relation to lucerne growth stage.

If legume pastures remain competitive, particularly in late winter and early spring, barley grass growth and seed set will be limited. Therefore, pasture regeneration by over-seeding of drought-tolerant legume and grass species, improving soil nutrient availability, and manipulating grazing frequency and intensity will be important considerations for influencing botanical composition as a means of maintaining a competitive pasture stand.

This study highlights the value of herbicide application and repeated defoliations in legume pastures for reducing barley grass fecundity and seedbank deposition over time, resulting in improved control. However, under southern Australian conditions and an increasingly dry climate, integrated approaches for barley grass management will likely become increasingly important. The combination of herbicide application and defoliations, thus, shows significant promise as an integrated weed management (IWM) strategy against barley grass, provided applications are timed accurately. Currently, modelling is underway to examine the long-term effect of defoliation and herbicides on both barley grass survival and fecundity, in order to better predict the utility of a systems-based approach for management under variable Australian conditions.

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