Spraytopping reduces seed production in Chilean needle grass (*Nassella neesiana* (Trin. & Rupr.) Barkworth)

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Summary
Two field trials and one glasshouse trial were used to evaluate the effect of sub-lethal rates of glyphosate and 2,2-DPA on panicle and cleistogene seed production of Chilean needle grass (*Nassella neesiana* (Trin. & Rupr.) Barkworth). One field trial was conducted in each of the springs of 2003 and 2004 on established tussocks, while a glasshouse trial was done in spring 2004 using young tussocks raised from seedlings. Results in the first field experiment indicated that application of glyphosate at 510 g a.i. ha^{-1} from 3 September (vegetative) to 13 October (flag-leaf swelling) reduced panicle seed development and produced the minimum number of filled and germinable panicle seeds. 2,2-DPA proved to be ineffective. The second field experiment showed that application of glyphosate at ≥135 g a.i. ha^{-1} on 18 October (flag-leaf swelling) and 28 October (panicle emergence) prevented production of filled panicle seeds. However, this level of control could be achieved at 1 October (vegetative) only with glyphosate at ≥270 g a.i. ha^{-1}. The glasshouse trial showed that glyphosate at 216 and 270 g a.i. ha^{-1} applied in October prevented the production of filled panicle seeds. Increasing glyphosate rates decreased stem and panicle seed germination but did not influence basal seed germination. Glyphosate at ≥135 g a.i. ha^{-1} during November proved to be most effective in controlling stem seeds. We conclude that glyphosate spraytopping at rates of 250 g a.i. ha^{-1} between August and October may be a useful contribution to Chilean needle grass management, but timing is critical.

Introduction
Chilean needle grass (*Nassella neesiana* (Trin. & Rupr.) Barkworth) is a perennial exogenous stipoid grass that produces both panicle and cleistogene seeds. Chilean needle grass is widespread in pastures and natural ecosystems on the Northern Tablelands of New South Wales and in southern Victoria. It grows through the winter but provides a lower feed value than *Dactylis glomerata* L., which is considered as a moderate pasture grass when compared for protein, energy and digestibility (C. Grech personal communication). Under heavy infestations, pasture productivity decreases by as much as 60% and causes significant reduction in stock-carrying capacity during the summer (Anon. 2001, Gardener *et al*. 2003).

Gardener *et al.* (2003) measured potential panicle seed production of 22 203 seed m^{-2} depending upon the number of flowering heads per unit area. Spraytopping (sub-lethal herbicide application) has been widely employed as a tool in different cropping systems to reduce infestations of grasses, for the prevention of diseases and to enhance feed quality (Leys *et al*. 1991, Gatford *et al.* 1999). In particular, Hill *et al.* (1996) used spraytopping with glyphosate to prevent the formation of reproductive tillers in bent grass (*Agrostis castellana*). This suggests spraytopping with glyphosate might also be effective in preventing the development of viable seed in Chilean needle grass.

However, Chilean needle grass has a very versatile reproductive system (Connor *et al.* 1993). Like some other grasses, Chilean needle grass is an angiosperm that produces cleistogenes on the stem nodes and at the stem base (Dyksterhuis 1945). Panicle seeds mature and fall off in mid to late summer before the release of stem seeds. Together, these then form the bulk of the soil seedbank. The stem seeds are concealed under leaf sheaths and are released when the stem dries off and the leaf sheath ruptures. Each stem node, above the basal node, has the potential to produce a few seeds. Basal cleistogenes are single seeds produced at the very base of the stem. The newly formed basal seeds are dull light yellow colour and are also held under the leaf sheath. When stems senescence, they become brown and thin and are released in the soil after the leaf sheath ruptures. These different modes of seed production need to be considered when evaluating control/eradication measures.

This study was undertaken to evaluate the optimum time and rate of application during spring of glyphosate and 2,2-DPA to preclude panicle and cleistogene seed development in Chilean needle grass. These herbicides were chosen to compare a fast acting quickly degraded herbicide (glyphosate) to a slow acting residual herbicide (2,2-DPA) in reducing production of panicle seed.

Materials and methods
Three experiments were conducted, two in the field and one in a glasshouse. The field trial sites were in *Phalaris* based pasture that was heavily infested with Chilean needle grass. The glasshouse trial was conducted on tussocks raised from panicle seeds. The field soil was a loam, whereas, in the glasshouse, steam-sterilized potting mix (1:1 sand:pine bark) in 15 cm diameter pots was used. In field trials no fertilizer was added. However, in the glasshouse trial Nutricote Standard Controlled Release Fertilizer; N16, P4.4, K8.3 (Orica Limited, Melbourne, Victoria) was applied at 6 g pot^{-1} at the beginning of spring. Herbicides in field trials were applied to 6 × 3 m plots with a hand held Azo-Dutch sprayer with a spray volume of 176 L of water ha^{-1}. In the glasshouse trial, herbicides were applied using a track-spray-unit with a spray volume 100 L ha^{-1}. Germination tests for cleistogene and panicle seeds were carried out for 50 filled seeds from each plot or pot (or maximum available if less than 50 seeds) in a germination cabinet at 25/15°C (alternating 12 h light/dark). Panicle and cleistogene seed germination was tested four and five months after panicle seed harvest, respectively. A sample of 100 panicle seeds per plot was examined for filled seeds by squeezing the seeds with tweezers.

Treatments in each experiment were set out in a randomized block design with four replicates except the first field experiment (three replicates). Except for the glasshouse trial (from mid December to the end of January) the panicles were harvested in mid December. In glasshouse trial, stems were harvested in the end of March but stem and basal cleistogenes were assessed in April. Stem and basal cleistogenes were not assessed in field trials.

First field experiment
A field trial was established in spring 2003 next to the Hamilton Highway, Inverleigh, Victoria. The site was selected in July 2003 and grazing was excluded until the end of the trial. The treatments were applied factorially and comprised six herbicide treatments (glyphosate (Roundup MAX) at 127.5, 255 and 510 g a.i. ha^{-1}; 2,2-DPA (Propon) at 2.22 and 3.7 kg a.i. ha^{-1}; and no herbicide control) by five times of application (3 and 22 September and 3, 13 and 27 October). Chilean needle grass tussocks

were vegetative (3 and 22 September and 3 October) and reproductive on 13 October (flag-leaf swelling) and 27 October (panicle emergence).

In each plot panicles were harvested from a centrally placed quadrat (50 × 50 cm). The panicle seeds were cleaned, sorted and weighed. A sub-sample of one hundred panicle seeds were randomly drawn from each plot, weighed and used to calculate the total number of seeds ha^{-1}. Appropriately transformed data were analysed as a six herbicide by four times of application (excluding 22 September as rain fell just after application) factorial analyses of variance, but with a residual error constructed from a randomized block analysis with all 6 × 5 = 30 treatments (Table 1).

Second field experiment

A second field experiment was designed as a five herbicide treatment (glyphosate (Roundup PowerMAX) at 0, 135, 270, 405 and 540 g a.i. ha^{-1}) by three application time (1, 18 and 28 October 2004) factorial, and conducted at the Roxby Estate, Inverleigh, Victoria on an ungrazed site. The tussocks were vegetative at 1 October, spiky stem (the stems incline toward the ground (45 to 60°) rather than upright, which is an early symptom of reproductive stage) at 18 October and at full panicle emergence at 28 October. The experimental site had old Chilean needle grass tussocks. These plants had 25–40 cm high tussocks with dead centres and green leaves growing from the margin. To get more uniform growth in early spring, the tussocks were slashed in the last week of July. The slashing promoted new growth, but prolonged dry weather caused thin tillers.

One hundred panicles (or maximum available if less than 100 panicles) were harvested separately from the experimental area (excluding a 50 to 100 cm wide border area). The panicle seeds were cleaned and sorted for each plot to estimate the filled seeds and seed germination.

Data were analysed using general linear model analysis with effects for blocks and five specific combinations of treatments, using those plots where panicles were present (Table 3). There was no evidence of effects between individual treatment combinations within these groupings (P ≥0.1). Analyses were restricted to treatment combinations that had some non-zero values, and the residual error was constructed from deviations from all treatments present in the analysis (Payne *et al.* 2005).

Glasshouse experiment

A third five glyphosate (Roundup PowerMAX) rate (0, 135, 216, 270 and 405 g a.i. ha^{-1}) by five times of application (first week of July, August, September, October and November 2004) factorial experiment was conducted in a glasshouse at Frankston, Victoria. Except for the November application (reproductive), the plants were vegetative at the time of application. Chilean needle grass seedlings were raised from panicle seeds from the previous season. Four-week-old single seedling was placed in each jiffy pot and transplanted into 15 cm pot in April 2004. The tussocks were vegetative until October but were producing full panicles in November. The mature panicles were harvested regularly from mid December to the end of January and the seeds were cleaned and sorted for assessment. Watering was withdrawn at the end of February to terminate the experiment.

All the mature stems in each treatment were harvested 1–2 cm above the soil surface and dissected for stem cleistogenes. The total number of stem seeds was scaled to 100 stems per pot, because the number of reproductive stems differed between pots. The clumps were dug out from the pots and assessed for basal seeds. In each pot, 20 mature stem bases were examined for basal seeds and scaled to 100 stems per pot.

Measurements were analysed using different types of generalized linear models as presented in Table 1 (Payne *et al.* 2005). The linear component of each generalized linear model analysis included a block effect and the most parsimonious representation of application date and glyphosate rate combinations that fitted the data, using chi-square or F tests (depending on whether a dispersion parameter was estimated) from an appropriate analysis of deviance. Other than the analysis for

Table 1. Details of models fitted in statistical analyses in glasshouse experiment (Experiment 3). A fixed effect for blocks was also fitted in each model.

Variate (pots used in analysis)	Model type	Date and germination terms in fitted model
Presence of panicle seeds (All pots)	Logistic regression with Bernoulli errors	Common linear response (on logistic scale) to glyphosate rate for August, September and October; Presence = 1 in November and 0 g a.i. ha^{-1} glyphosate in July; Presence = 0 for glyphosate rate >0 in July
Total filled panicle seeds (Pots with panicle seeds present)	General linear regression	Parallel quadratic response to glyphosate rate for each month
Number of stem cleistogenes per 100 stems (Pots with stems present)	General linear regression after log(y + 50) transformation	Separate fitted means for (i) ≥270 g a.i. ha^{-1} glyphosate in August (ii) 405 g a.i. ha^{-1} glyphosate in September (iii) ≥216 g a.i. ha^{-1} glyphosate in October (iv) ≥135 g a.i. ha^{-1} glyphosate in November (v) All other date by glyphosate rate combinations combined
Number of basal cleistogenes per 100 stems (Pots with stems present)	General linear regression after log(y + 10) transformation	Separate means for ≤135 g a.i. ha^{-1} and ≥216 g a.i. ha^{-1} glyphosate rate
Germination rate (%) of filled panicle seeds (Pots with filled panicle seeds present)	Logistic regression with over-dispersed binomial errors on proportions	Common linear response to glyphosate rate for all months
Germination rate (%) of stem cleistogenes (Pots with stems present)	Logistic regression with over-dispersed binomial errors on proportions	Common linear response to glyphosate rate for all months
Germination rate (%) of basal cleistogenes (Pots with stems present)	Logistic regression with over-dispersed binomial errors on proportions	Constant for all dates and germination rates

presence of panicle seeds (that had a residual mean deviance less than 1), each measurement used a single over-dispersion parameter that was calculated assuming that the linear component of the model included a block effect and the full factorial combinations of application date and glyphosate rates.

The number of filled panicle seeds was not analysed directly because the large number of plants without any panicle seeds (42%) implied many zero observations. This precluded standard statistical analysis assumptions. Instead (i) the presence of panicles, and (ii) filled panicle seeds when panicle seeds were present, were analysed separately.

Results

Panicle seed development and germination

In experiment 1, the medium and high rates of glyphosate (255 and 510 g a.i. ha^{-1}) produced the minimum number of panicle seeds at all times of application (from 3 September to 27 October) along with the high rate of 2,2-DPA (3700 g a.i. ha^{-1}) at 3 September. No panicle seeds were present with the highest rate of glyphosate when applied at any time from 3 September to 13 October (Table 2). Glyphosate was the more effective herbicide in reducing the number of filled seeds, percent seed germination and total number of germinable seeds. The lowest rate of glyphosate (127.5 g a.i. ha^{-1}) had less filled and germinable seed than the higher rate of 2,2-DPA (3700 g a.i. ha^{-1}) (Table 2). There was no evidence (P >0.1) that these herbicide effects in Table 2 differed with application time.

In experiment 2, some of the plots with 135 g a.i. ha^{-1} or higher glyphosate applied on 18 October and one higher rate glyphosate plot with application on 1 October did not have panicle seeds present (P = 0.0018 for comparing five treatment groupings using analysis of deviance from logistic model with Bernoulli errors). On 18 and 28 October the lowest rate was sufficient to prevent the occurrence of filled and germinable seeds. However, this level of control was observed on 1 October only with higher rates (≥270 g a.i. ha^{-1}) (Table 4).

In the glasshouse trial, all the herbicide rates (135, 216, 270 and 405 g a.i. ha^{-1}) at the July application killed the plants whereas at the November application even the highest rate (405 g a.i. ha^{-1}) did not prevent panicle seeds developing. In August, September and October applications, the probability of occurrence of panicle seeds decreased with increasing glyphosate rate (Figure 1a). For those plants producing seed, the total filled panicle seed production decreased with increasing glyphosate rate irrespective of application time (August, September, October and November) (Figure 1b). November application had more filled seeds in plants producing panicle seed, at any glyphosate application rate, than other times of application.

Cleistogene development and germination

In the glasshouse trial, lower rates of glyphosate (135 and 216 g a.i. ha^{-1}) had less impact on stem cleistogene development

Table 2. Effect of herbicides and application time on total panicle seed production ha^{-1} (× 10^6) (Experiment 1).

Herbicide	Rate (g a.i. ha^{-1})	Times of application			
		3 Sep	3 Oct	13 Oct	27 Oct
Glyphosate	127.5	21 (1.4)[A]	9 (1.1)	13 (1.2)	6 (1.0)
Glyphosate	255	2 (0.8)	1 (0.7)	2 (0.7)	4 (0.9)
Glyphosate	510	0 (0.6)	0 (0.6)	0 (0.6)	2 (0.8)
2,2-DPA	2220	42 (1.7)	18 (1.3)	14 (1.3)	29 (1.5)
2,2-DPA	3700	2 (0.8)	41 (1.7)	15 (1.3)	21 (1.4)
Untreated	–	39 (1.6)	50 (1.7)	41 (1.7)	66 (1.8)
LSD (P = 0.05)			(0.38)		

[A] Transformed data in parenthesis: $\log_{10}$(panicle seed + 4).

Table 3. Effect of herbicides, averaged over four times of application, in spring on panicle filled seed, seed germination and germinable seed (Experiment 1).

Herbicide	Rate (g a.i. ha^{-1})	Filled seed ha^{-1} (× 10^6)	Seed germination (%)	Germinable seed ha^{-1} (× 10^6)
Glyphosate	127.5	4.2 (1.28)	23 (29)	1.3 (0.80)[A]
Glyphosate	255	0.4 (1.19)	7 (16)	0.1 (0.71)
Glyphosate	510	0.0 (1.18)	0.4 (4)	0.0 (0.70)
2,2-DPA	2220	18.8 (1.53)	47 (43)	8.8 (1.14)
2,2-DPA	3700	12.6 (1.44)	27 (31)	5.5 (1.12)
Untreated	–	34.7 (1.70)	39 (39)	11.7 (1.22)
LSD (P = 0.05)		(0.12)	(12.0)	(0.16)

[A] Transformed data in parenthesis: $\log_{10}$(filled seed + 15), % seed germination (angular), $\log_{10}$ (germinable seed + 5).

Table 4. Glyphosate rates and time of application effect on Chilean needle grass panicle seed production (Experiment 2).

Variates	Mean					SED	
	Untreated (A)	Oct 1 at 135 g ha^{-1} (B)	Oct 1 at ≥270 g ha^{-1} (A)	Oct 18 at ≥135 g ha^{-1} (A)	Oct 28 at ≥135 g ha^{-1} (A)	A v/s A Column	A v/s B Column
No. of plots with panicles present	12 out of 12	4 out of 4	11 out of 12	10 out of 16	16 out of 16		
Germinable seeds per 100 panicles (1)	913	124	0	0	0	–	129.3[C]
Seeds per 100 panicles (2)	1572	1015	1104	332	750	79.1–111.5	122.9–143.2
Per cent filled seeds (3)	81	24	0	0	0	–	6.5[C]
Germination per cent (4)	70	46	–	–	–	–	6.4[C]

[C] Only applicable for treatments with a value greater than 0.

In rows (1), (3) and (4) LSDs are obtained by multiplying SED by 2.262. In row (2) LSD is obtained by multiplying SED by 2.030.

during the August and September applications compared with higher rates (270 and 405 g a.i. ha^{-1}). During the October application all glyphosate rates except 135 g a.i. ha^{-1} gave the same level of control. However, in the November application glyphosate at ≥135 g a.i. ha^{-1} stopped stem seed development (Figure 2). The lowest rate of glyphosate (135 g a.i. ha^{-1}) was found to be ineffective in preventing basal seed development but higher rates (≥216 g a.i. ha^{-1}) showed similar levels of basal seed control (Figure 3).

Basal seeds exhibited a higher percent germination compared with panicle and stem seeds and they were unaffected by glyphosate treatment at any rate. However, panicle and stem seeds decreased with increasing glyphosate rate (Figure 4).

Discussion

In this investigation glyphosate was a more effective herbicide for preventing viable Chilean needle grass panicle seed production than 2,2-DPA. In particular, no panicle seed was present when the highest rate (510 g a.i. ha^{-1}) was applied between 3 September and 13 October. This effectiveness of glyphosate may be attributed to the non-selective and translocated action, which quickly disables the plant from performing physiological processes needed for seed development and results in unfilled seeds or complete loss of panicles. The poor performance of 2,2-DPA may be attributed to the mismatch of the slow absorption of 2,2 DPA with the short period of seed formation and maturation in Chilean needle grass. This explanation of the effect of 2,2-DPA is supported by the early September application at 3.7 kg a.i. ha^{-1}, where its effect was comparable to medium glyphosate rates (255 g a.i. ha^{-1}).

In the second field experiment, a lower rate of glyphosate was not effective in preventing the production of germinable seeds when applied in early October, but was comparable to medium and high rates in mid and late October applications. To deal with anticipated poor tiller development, the old tussocks were slashed in August to promote new tiller growth in the early spring. Though slashing promoted new tillers, dry weather conditions in spring lead to thin stems. This may be the reason why glyphosate performed well at lower rates in mid and late applications.

On seedling-raised tussocks in the glasshouse, it was observed that increasing rates of glyphosate decreased the occurrence of panicle seeds during August, September and October applications (vegetative stage) but after panicle emergence, glyphosate was not able to prevent the production of panicle seeds. Since the basal seeds are at an advanced stage in spring (Dyksterhuis 1945) they can largely escape the effect of glyphosate but panicle and stem seeds are developing

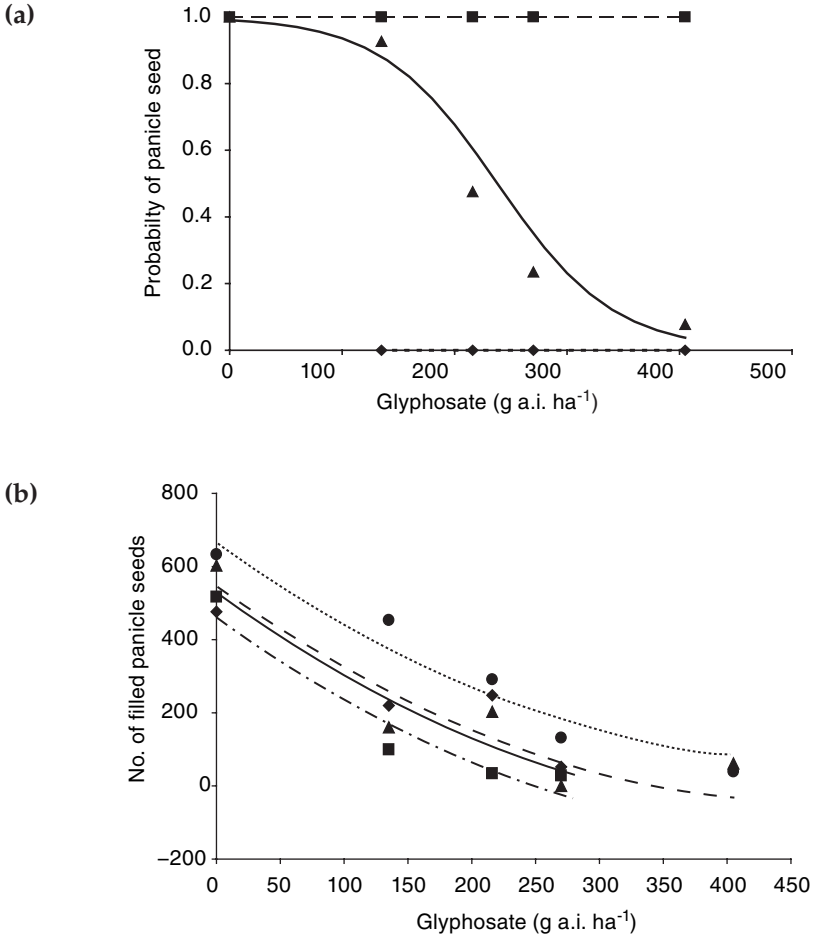

Figure 1. (a) The effect of glyphosate rate on panicle seed occurrence in July (♦), August to October (▲) and November (■), in the glasshouse experiment (Experiment 3). (b) The effect of glyphosate rate on number of filled seeds, for those pots with panicle seed present, in August (♦), September (▲), October (■) and November (●). All fitted curves are adjusted for the block effect.

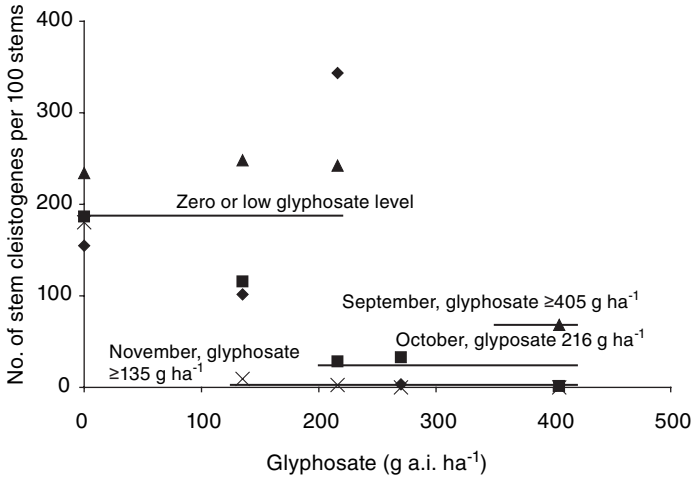

Figure 2. The effect of glyphosate rate on number of stem cleistogenes per 100 stems in August (♦), September (▲), October (■) and November (×), in the glasshouse experiment (Experiment 3). The fitted lines are adjusted for block on the log(y + 50) transformed scale.

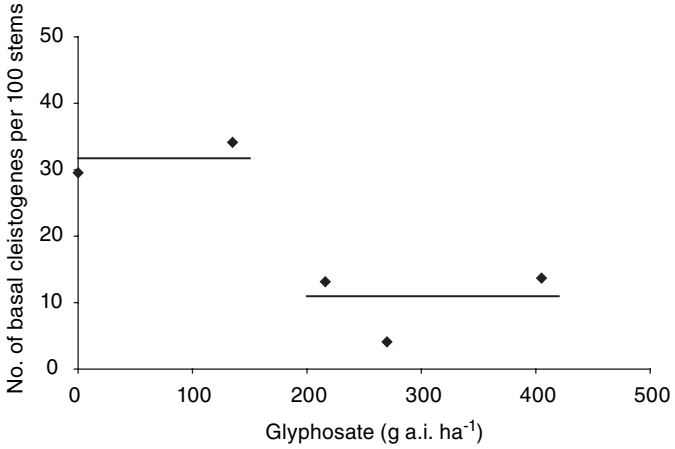

Figure 3. The effect of glyphosate rate on basal cleistogene development, in the glasshouse experiment (Experiment 3). The fitted lines are adjusted for block and date on the log(y + 10) transformed scale.

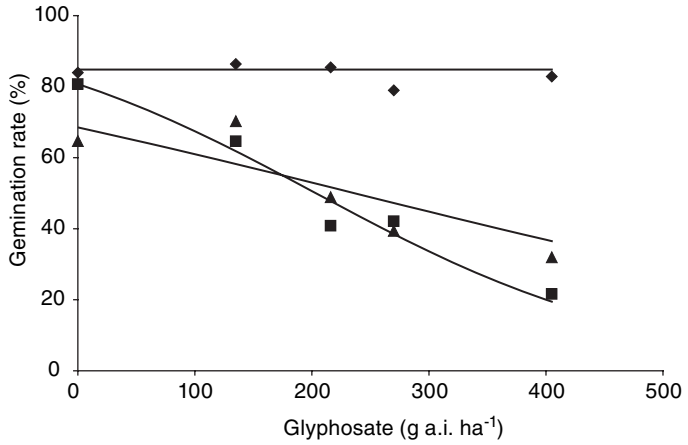

Figure 4. The effect of glyphosate rate on panicle (■), stem (▲) and basal (♦) seed germination, in the glasshouse experiment (Experiment 3). The fitted lines are adjusted for block and date on a logistic transformed scale.

during this period and thus their production is influenced by glyphosate. The reason for basal seeds reduction by medium and high rates of glyphosate to a certain degree may be attributed to the killing of some of the young stems in tussocks before they became reproductive. However, once the stem becomes aerially reproductive, it is likely that basal seed are already mature (Gardener *et al* 2003), and will not be affected by glyphosate. Reduction of stem seeds in November applications in all the glyphosate rates might be due to stem death or very restricted stem growth. The overall conclusion from these experiments is that the application of glyphosate at rates of 250 g a.i. ha^{-1} between August and October is likely to very substantially reduce production of viable Chilean needle grass seeds and may be a useful contribution to Chilean needle grass management. However 2,2-DPA is ineffective for this purpose unless applied early and at high rates. For spraytopping of glyphosate to be effective in preventing seed development, application must be concluded before panicle emergence. Thus the timing is critical.

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