Crop Protection 31 (2012) 99–106

Contents lists available at SciVerse ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Herbicides to control poisonous *Pimelea* species (Thymelaeaceae)

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A R T I C L E I N F O

Article history:
Received 14 July 2010
Received in revised form
22 September 2011
Accepted 22 September 2011

Keywords:
Pimelea
Herbicides
2,4-D
Metsulfuron-methyl
2,4-DB, glyphosate

A B S T R A C T

Pimelea poisoning is an ongoing, periodically serious problem for cattle producers in inland Australia. The annual native plants of the Thymelaeaceae family that cause the problem are widespread and animal management is currently the main means of minimizing poisoning. However, there are situations in the higher rainfall parts of the natural distribution area of these plants where farming and quite intensive property development do occur and here the use of selective herbicides may be an option. This research looked for herbicides that could be considered for registration for *Pimelea* control, bearing in mind the large potential costs involved if used over large areas.

Group I hormone herbicides (for example 2,4-D) were quite effective as was metsulfuron-methyl and glyphosate at doses commonly registered for use on broad-leafed weeds. On the basis of minimizing costs and quickly suppressing seed-set, metsulfuron-methyl at 3.5–5 g a.i. ha^{-1} and 2,4-D at 375–500 g a.i. ha^{-1} were the most promising. Where medic (*Medicago* spp.) persistence is vital, 2,4-DB at 240–300 g a.i. ha^{-1} could be used and glyphosate at 1 kg a.i. ha^{-1} would be effective on fallowed ground if cost was not an overriding concern.

1. Introduction

Cattle in inland Australia are periodically poisoned by native plants of the genus *Pimelea* Banks & Sol. ex Gaertn. (Thymelaeaceae). The problem has been known for over 60 years (Maunder, 1947) and continues. The toxic plants involved were conclusively identified in 1971 (Clark, 1971). The *Pimelea* genus is native to Australia, Lord Howe Island, New Zealand and Timor (Merrett, 2007), with about 90 species now listed as endemic to inland Australia (APNI, 2010). Many are reported to be poisonous if eaten, such as *Pimelea altior* F. Muell., *Pimelea decora* Domin, *Pimelea elongata* Threlfall, *Pimelea flava* R. Br., *Pimelea glauca* R. Br., *Pimelea haematostachya* F. Muell., *Pimelea linifolia* Sm., *Pimelea microcephala* R. Br., *Pimelea pauciflora* R. Br., *Pimelea simplex* F. Muell., *Pimelea trichostachya* Lindl. (Everist, 1981), *Pimelea neo-anglica* Threlfall (Storie et al., 1986) and *Pimelea prostrata* Willd. (Zayad et al., 1982). Many species in this diverse genus contain the putative toxin simplexin, a diterpenoid orthoester (Freeman et al., 1979; Zayad et al., 1982; Chow et al., 2010) but three species cause most of the problems. They are *P. trichostachya*, *P. simplex* and *P. elongata*.

The means of poisoning still remains somewhat unclear (Silcock et al., 2008), but ingestion is now the most favoured route (Fletcher et al., 2009). Green *Pimelea* plants are distasteful to grazing stock, however dried stalks from a previous season's growth when grazed indiscriminately with other pasture species can lead to poisoning (Freeman et al., 1979). When animals first show signs of *Pimelea* poisoning, current recommendations are to remove them, if possible, to a paddock free of the plant (Collins and Scholz, 2006). This is sometimes not feasible because the plants are a common minor component of many native pastures in inland Australia. Hence some producers want to kill the plant cheaply with herbicide in a small paddock, enabling it to act as a hospital paddock for sick animals while they recover. Currently there are no herbicides registered for *Pimelea* control in Australia and very limited information about herbicide susceptibility of the Thymelaeaceae family. Matarczyk et al. (2002) found *Pimelea spicata* R. Br. was very sensitive to glyphosate and Washington State Noxious Weed Control Board (2006) in USA report swabbing cut stems of *Daphne laureola* L. with triclopyr to effectively kill the plant.

This paper reports on studies to identify effective post-emergence, applied herbicides that can be used to control *Pimelea* plants in both medic (*Medicago* spp.)- and grass-dominated

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doi:10.1016/j.cropro.2011.09.013

pastures. Glyphosate was also tested because it could have a role in controlling infestations in fallowed cropping paddocks.

2. Materials and methods

Two herbicide screening trials and one dose trial were undertaken on *Pimelea* spp. in the semi-arid rangelands (450–600 mm mean annual rain) of the Maranoa district of Queensland, Australia under AgVet Chemicals permit PER7250. Each of the trial sites was enclosed by an electric fence to exclude domestic stock.

2.1. Herbicide screening trials on P. elongata and P. trichostachya

Herbicide screening trials were conducted on *P. elongata* and *P. trichostachya* in mid-August and mid-September 2007, respectively (late winter – early spring). Each trial used two doses each of 13 post-emergence herbicides (Table 1), plus extra unsprayed controls, on 4 × 2 m plots, in a randomized block design with three replications. Herbicides and doses chosen were based on recommendations for control of other herbs. The second dose for each herbicide was double the first (a commonly recommended dose for herbs), except for metsulfuron-methyl which was applied at four times the commonly used dose. Herbicides were applied using a 5 L Spraymaster handspray or an Ag-Murf 12-volt constant pressure spray unit (first trial only) at a spray volume of 1500 L ha^{-1}. All treatments included 0.25% (v/v) non-ionic surfactant (BS1000).

The first trial (Trial 1) was conducted 40 km west of Bollon (28.063°S, 146.839°E) on a dense stand of *P. elongata* (Queensland Herbarium, Brisbane acquisition number AQ751686) growing on a dry ephemeral lakebed with a grey, slightly acidic, cracking clay soil. Small ephemeral lakes and pans are common in arid inland Australia. At time of treatment, *P. elongata* plants ranged from single-stemmed seedlings <3 cm in height to large, densely branched bushes 25 cm in height and diameter with the majority 12–20 cm tall and 10–20 cm in diameter. Spraying was conducted under cloudy, mild conditions with variable, light winds and no rain the day before or after. Rain during the preceding month totalled only 3 mm but the surface water from May rain had only drained away in early June, 6 weeks prior to the trial. Other common vegetation were *Centipeda minima* (L.) A. Braun & Asch. (spreading sneezeweed), *Cullen cinereum* (Lindl.) J. W. Grimes (hoary scurf-pea), *Lobelia darlingensis* (E. Wimm.) Albr. (Darling River pratia), *Eryngium* L. sp. (blue devils) and *Cuphonotus humistratus* (F. Muell.) O. E. Schulz (mother-of-misery), but almost no grasses. A lack of grass is common at the end of a drought when *Pimelea* species grow abundantly, so the situation was not atypical and there were still legumes (*C. cinereum*) in the lakebed herbage. In late November 2007 (early summer), 100 days after spray application, 130 mm of rainfall flooded the site for months and the trial was terminated.

The second trial (Trial 2) was conducted 35 km south of Roma (26.935° S, 148.888° E) on pasture growing on a gently sloping, sandy red earth. The *P. trichostachya* there (Acquisition number AQ751764) was mostly young, unbranched plants, 2–20 cm high at pre-flowering or early flowering stage. At the time of spraying, soil moisture was high after good August rain (60 mm over 1 week). Weather conditions at spraying were warm, cloudless and free of significant winds. The grassy mixed pasture consisted of *Cenchrus ciliaris* L. (buffel grass); native grasses *Themeda triandra* Forssk. (kangaroo grass), *Aristida* L. spp. (wiregrasses), *Thyridolepis mitchelliana* (Nees) S. T. Blake (mulga Mitchell grass) and *Chrysopogon fallax* S. T. Blake (golden beard grass); a small amount of *Medicago laciniata* (L.) Mill. (cut-leaf medic); and an appreciable amount of *Sida* L. spp. (sida), *Vittadinia pustulata* N. T. Burb. (a fuzzweed), *Verbena aristigera* S. Moore (Mayne's pest), *Cheilanthes sieberi* Kunze (mulga fern) and *Erodium crinitum* Carolin (blue stork's-bill). Mixed grassy pastures like these occur in subtropical inland Australia wherever native woodlands on red earths have been cleared and buffel grass sown to boost stock carrying capacity.

Table 1
Herbicides used on *Pimelea* spp. in two screening trials at Bollon and Roma in spring 2007 (Trials 1 and 2).

Herbicide (active ingredient)	Trade name	Rates applied (g a.i. ha^{-1})[a]	Herbicide group[b]
2,4-D amine	Amicide 625	500, 1000	I
2,4-DB amine	Buticide	320, 640	I
2,4-D/picloram	Tordon 75-D	450/112.5, 900/225	I
Aminopyralid/fluroxypyr	Hotshot	5/70, 10/140	I
Diflufenican	Brodal Options	125, 250	F
Flumetsulam	Broadstrike	10, 20	B
Fluroxypyr	Starane 200	150, 300	I
Glyphosate	Roundup	1080, 2160	M
Imazamox	Raptor WG	17.5, 35	B
MCPA/dicamba	Kamba M	1020/240, 2040/480	I
MCPA/diflufenican	Tigrex	62.5/6.25, 125/12.5	I and F
Metsulfuron-methyl	Brushoff	6, 24	B
Triclopyr/picloram	Grazon DS	300/100, 600/200	I

[a] All treatments included a non-ionic surfactant (BS1000) at 0.25% v/v.
[b] These are the standardised mode-of-action herbicide groups used in Australia. See www.croplifeaustralia.org.au/default.asp?V_DOC_ID=1954.

2.2. Herbicide dose trial on P. trichostachya

The herbicide dose trial (Trial 3) began in June 2008 on *P. trichostachya* (AQ783675) growing in pasture 35 km south of Mungallala (26.613° S, 147.552° E). Experimental design was a randomised block with three replications. Plot size was 20 m^2. Treatments were four doses of each of the three herbicides (2,4-D, 2,4-DB and metsulfuron-methyl) shown to be most cost effective from the previous two herbicide screening trials, and unsprayed control plots. As there was little difference in efficacy between the lower and higher doses used in the screening trials, the doses chosen (Table 2) were 50, 75, 100 and 125% of the lower dose used in those trials. Treatments were applied using a 5 L Spraymaster spray unit at a spray volume of 1500 L ha^{-1}. All treatments included 0.1% (v/v) non-ionic surfactant (BS1000). For each herbicide, two extra plots were sprayed at the 100% dose to provide *P. trichostachya* plants over time for chemical analysis, comparable samples from unsprayed plants being taken from around the perimeter of the trial site on the allotted sampling dates. Conditions at spraying were good with air temperatures between 18 and 27 °C, a broken cloud cover, no rain and very little wind. The soil was visibly wet below 3 cm depth.

The soil was a sandy red earth and *Pimelea* plants ranged from seedlings to mature plants 30 cm in height. The other dominant species were *Bothriochloa decipiens* (Hack.) C. E. Hubb. (pitted bluegrass), *Aristida* spp. (wiregrasses), *Panicum effusum* R. Br. (hairy panic), *Sclerolaena birchii* (F. Muell.) Domin (galvanised burr) and *Vittadinia* spp. with seedlings of *Glossocardia bidens* (Retz.) Veldkamp (native cobbler's-pegs), *Medicago* spp. (medic), *Oxalis corniculata* L. (yellow wood sorrel) and *Calotis* R. Br. spp. (burr daisy).

Table 2
Herbicides and doses used on *P. trichostachya* in a herbicide dose trial near Mungallala, Queensland in June 2008 (Trial 3).

Active ingredient	Trade name	Rates applied (g a.i. ha^{-1})[a]	Group
2,4-D amine	Amicide® 625	250, 375, 500, 625	I
2,4-DB amine	Buticide®	160, 240, 320, 400	I
Metsulfuron-methyl	Brush-Off®	3, 4.5, 6, 7.5	B

[a] All treatments included a non-ionic surfactant (BS1000) at 0.1% v/v.

2.3. Data collection

Preliminary data about each plot, including a photograph, were collected within a day of spraying. Three 0.25 m^2 quadrats (five in Trial 3) were randomly placed in each plot away from the borders at each sampling time. In the last recording of Trial 3, almost no live *Pimelea* plants remained, so counts were done of the entire plot except for the extreme edges where some spray overlap was likely. Data recorded included –

1. number of live adult *Pimelea* plants
2. number of *Pimelea* seedlings (<3 cm tall)
3. % green *Pimelea* ground cover (Trial 1 and 3) and/or % green pasture cover (Trial 2 and 3)
4. the average vigour of flowering of *Pimelea* plants on a 1 (not flowering) to 5 scale where 4 was equivalent to that of unsprayed plants and 5 was for enhanced flowering vigour, if that seemed to be happening.
5. the degree of *Pimelea* foliage damage by herbicide on a 1 (no visible damage) to 5 (all tops dead) scale. A rating of dead was made if the lower stems snapped off as the plant was bent over. A rating of 2 indicated foliage yellowing or obvious distortion, 3 was for all leaves dead but branches still green while 4 meant all leaf was lost, many branches were apparently dead, but the stem base was green and the lower branches were still flexible.
6. notes about the effect of herbicides on companion species, and
7. notes on recent seasonal conditions.

Subsequent data was collected 2, 4 and 8 weeks after spray application in Trial 1; 2, 4, 8 and 12 weeks after in Trial 2; and 3, 8, 13 and 19 weeks later in Trial 3. Trial 1 terminated prematurely before conclusive death data was collected because very heavy rain flooded the dry lake in over 1 m of water 14 weeks after spraying and it remained flooded for 6 months.

2.4. Chemical analysis for simplexin and its analogues

Samples of sprayed *Pimelea* plants from the extra treated plots described earlier in Trial 3 plus nearby unsprayed *Pimelea* plants were collected just prior to spraying and at 1, 3, 8 and 13 weeks after spraying, for analysis of their simplexin content. Later sampled plants were taken from those still partly alive in the plot rather than dead ones. Three to five mature-sized plants were pulled out entire and bagged from each plot with as much root, almost exclusively taproot, as would remain attached. They were air-dried and then dissected before analysis into three components for each bag, roots, main stems, and remaining foliage plus seeds. Simplexin concentration of milled material was measured by Liquid Chromatography-Mass Spectrometry/Mass Spectrometry using the method of Chow et al. (2010). Mostly there were two samples for each treatment at each sample date but complete death of all sprayed plants in some replicates left only a single treatment sample available 13 weeks after spraying.

2.5. Statistical analysis of data

Results were tested for statistical significance by analysis of variance using Genstat 8 (Genstat, 2007) after allowing for initial variations in plant numbers and plant cover, which were used as covariates (Cochran and Cox, 1957). Testing for residual plots justified the analysis of untransformed data, except for plant counts which were square root transformed. An Arcsin transformation was used on the percentage dead values in Trial 2 (Table 4) because residual error plots were not random. The potential lack of independence between successive sampling times was accounted for by using an analysis of variance of repeated measures (Rowell and Walters, 1976). This included the Greenhouse-Geisser adjustment to correct the degrees of freedom and significance level tests.

3. Results

3.1. Trial 1 on P. elongata

3.1.1. Growing conditions post-spraying

Little rain fell after spraying, the biggest fall of 17 mm being 7 weeks later and having minimal benefit to existing plants that were growing on subsoil moisture remaining from the May flooding. On average, about 75 mm of rain falls in that period of the year. However some seedling recruitment was recorded after that rain as well as some natural death of older *Pimelea* plants in the unsprayed control plots. Over 130 mm of rain then fell in the last week of November causing the lake to flood to over 1 m depth and that killed all plants and terminated the trial.

3.1.2. Initial P. elongata condition

There were no appreciable differences associated with treatments in the initial canopy cover or density of *P. elongata* plants at the time of spraying. However individual plots ranged in *Pimelea* cover from 13 to 32% and *Pimelea* numbers from 4.1 to 9.9 m^{-2}. The double strength MCPA/dicamba plots had significantly greater numbers of *Pimelea* plants (9.9 m^{-2}) than nine of the other treatments (<6.6 m^{-2}, data not shown).

3.1.3. Herbicide impacts

Two weeks after spraying (4 Sept), almost all herbicides had significantly ($P < 0.05$) reduced flowering intensity of *Pimelea* with the greatest impact from glyphosate and high doses of 2,4-DB amine and MCPA/dicamba while least impact resulted from low doses of flumetsulam, imazamox and fluroxypyr. After a month (17th September), the extent of foliage damage was greatest from glyphosate, MCPA/dicamba, triclopyr/picloram and 2,4-DB amine (Table 3) and least from flumetsulam and imazamox.

After 8 weeks (18th October), glyphosate, MCPA/dicamba and 2,4-D amine had the greatest damage rating but the double dose of diflufenican had been highly effective too. Use of a double dose only resulted in a significantly better kill for glyphosate and diflufenican while flumetsulam at double the dose was significantly less effective. The relative cost effectiveness of the sprays is highlighted in Table 3 when, for similar levels of final damage by 2,4-D amine, triclopyr/picloram and MCPA/dicamba, chemical costs per hectare were $\$^{AUS}$ 4.22, $\$^{AUS}$ 36.00 and $\$^{AUS}$ 36.00 respectively at the lower dose.

3.2. Trial 2 on P. trichostachya

3.2.1. Growing conditions post-spraying

No significant rain fell in the first 48 h after spraying. However from early October onwards (week 3) a series of significant falls of rain were received (240 mm in 7 rainfall events over 3 months) as the weather warmed up and this promoted vigorous grass and herbage growth. The long term average rain received there in this period is only 170 mm. By December (early summer) a dense grass pasture existed in most plots except the non-selective glyphosate treatments. The exceptions were the unsprayed Controls and non-effective herbicide treatments where *V. pustulata* developed a major presence. Thus the *Pimelea* plants were battling against increasing plant competition as well as the herbicides.

3.2.2. Initial P. trichostachya condition

Despite an uneven distribution of litter, established plants and density of *Pimelea* plants, there were no statistically significant differences in initial *Pimelea* numbers, flowering vigour and total pasture cover linked to herbicide treatment. On average, imazamox dose1 plots had the greatest *Pimelea* density at 10.8 plants m^{-2} while there were as few as 2.6 plants m^{-2} in the MCPA/dicamba dose1 plots initially. This reduces the confidence that we can have in the statistical significance of the mean effects.

Plants were not flowering as vigorously here as at the *P. elongata* site but they were much younger and *P. trichostachya* is less demonstrative in its flowering than *P. elongata*. Plant number per square metre was slightly less than at the Bollon lakebed site.

3.2.3. Herbicide impacts

A month after spraying (16th October), almost all herbicides had significantly reduced the degree of flowering and seed set of *P. trichostachya*. The most obvious damage was due to glyphosate, metsulfuron-methyl and triclopyr/picloram and least effective were aminopyralid/fluroxypyr and flumetsulam (Table 4). There were no significant differences due to herbicide application dose at this stage.

After two months (13th November), most sprays had produced a significant degree of foliar damage on *Pimelea*, except MCPA/diflufenican. The greatest damage recorded at this time was from glyphosate and MCPA/dicamba, with 2,4-D amine having a large impact also (Table 4). However a notable proportion of *Pimelea* plants were still alive in many plots at this time. The effect of herbicide dose was sometimes apparent with 62% of plants sprayed with quadruple strength metsulfuron-methyl rated dead while none at the lower dose were yet rated as completely dead. At this time 2,4-D amine, 2,4-D/picloram, MCPA/dicamba and glyphosate had all produced a similar degree of kill at both application doses (Table 4). In contrast, fluroxypyr, imazamox and aminopyralid/fluroxypyr had not killed any plants after two months despite damaging them and noticeably reducing flowering.

By mid-January there remained a small population of living *Pimelea* plants in many plots but they were weak and struggling against vigorous grass growth. Flumetsulam and fluroxypyr plots were noticeable for the much greater number of plants still alive at that time, 4 months after spraying and in mid-summer. Most surviving plants had a few flowers on them but poor embryo development in seeds was noted for plants previously sprayed with 2,4-DB amine, fluroxypyr and flumetsulam. Some unsprayed *Pimelea* plants in Control plots were still alive amongst the dense grass.

3.3. Trial 3 (dose trial on P. trichostachya)

3.3.1. Growing conditions post-spraying

Cold weather followed the mid-June herbicide application and only 23 mm of rain fell in the next 10 weeks. A third of the mornings experienced grass frosts over that time before warmer weather arrived. Thereafter, isolated falls of between 7 and 17 mm in spring did not revive *Pimelea* until 70 mm fell in early October, prior to the final recording. Such weather is not uncommon during winter in many areas where annual *Pimelea* species grow. That October rain produced no more *Pimelea* seedlings and there was no big growth flush from unsprayed plants. By early December almost no live *Pimelea* plants existed at the site as hot summer weather took hold. General pasture cover at the site was not high, estimated at 9% initially, and did not increase much as summer advanced but parts were well grassed with over 30% ground cover. *Pimelea* biomass was inversely proportional to grass biomass but some areas were devoid of all vegetation. Contrary to the experience at

Table 3
Effect of different herbicides applied 22nd August at 2 doses on flowering and foliage damage of *P. elongata* swards on a dry ephemeral lakebed in spring (Trial 1).

Spray	Rate (g a.i. ha^{-1})	Orig. flowrng rating[c]	Flowrng rating (4 Sep)	Damage rating[d] (17 Sep)	Damage rating (18 Oct)	Cost ha^{-1} chemical only ($)
2,4-D amine	500	3.4	2.2	3.4	3.4	4.22
	1000	3.6	2.2	3.8	3.4	
2,4-DB amine	320	3.2	2.4	3.6	2.7	11.96
	640	3.0	1.6	3.8	3.1	
2,4-D/picloram	450/112.5	3.1	2.2	3.6	2.9	80.70
	900/225	3.0	2.8	3.2	3.1	
Aminopyralid/fluroxypyr	5/70	3.1	3.0	2.9	2.3	10.25
	10/140	3.2	2.9	3.4	2.2	
Control		3.3	3.6	1.0	1.5	0.00
Diflufenican	125	3.3	2.3	3.0	2.8	36.58
	250	3.6	2.8	2.7	4.1	
Flumetsulam	10	3.1	3.3	2.0	1.9	10.13
	20	3.2	2.7	1.7	1.2	
Fluroxypyr	150	3.3	3.0	3.1	2.2	27.00
	300	3.0	2.2	3.4	2.8	
Glyphosate	1080	3.1	1.9	4.2	2.9	35.25
	2160	3.0	1.2	4.8	4.1	
Imazamox	17.5	3.2	3.2	2.2	1.5	23.50
	35	3.2	3.1	2.3	1.7	
MCPA/dicamba	1020/240	3.2	2.2	3.8	3.5	36.00
	2040/480	3.1	1.8	4.0	3.5	
MCPA/diflufenican	62.5/6.25	3.2	2.9	3.0	2.6	8.50
	125/12.5	3.1	2.8	3.3	2.4	
Metsulfuron-methyl	6	3.2	2.1	2.6	2.7	1.88
	24	3.2	2.4	2.9	2.6	
Triclopyr/picloram	300/100	3.3	2.3	3.7	3.4	36.00
	600/200	3.3	2.2	3.6	3.2	
lsd trtmts[b] ($P < 0.05$)			0.60	0.72	0.72	
lsd con-trt[a] ($P < 0.05$)		n.s.	0.47	0.56	0.56	

4th Sept was 13 days after spraying and 18th Oct was 57 days after spraying.
[a] For comparisons between controls and a herbicide.
[b] For comparisons amongst herbicides.
[c] Flowering assessed on a 1 (not flowering) to 5 (greater than mean control profusion) scale.
[d] Herbicide damage rated on a 1 (healthy) to 5 (all shoots completely dead) scale. A value of 3 meant that all leaves were dead but stem and main branches were still green.

Table 4
Initial flowering vigour rating of *P. trichostachya* and then later measures of herbicide damage levels for all treatments sprayed at the Roma site on 12th September (Trial 2).

Spray	Rate (g a.i. ha^{-1})	Orig. flowrng rating	Flowrng rating (16 Oct)	Damage rating (13 Nov)	% plants dead (13 Nov)[e]	Final live nbrs m^{-2} (23 Jan)[e]
2,4-D amine	500	2.4	1.2	3.5	76.4[b,i]	0.4
	1000	2.2	1.0	3.2	61.6[bc]	0.1
2,4-DB amine	320	2.3	1.1	2.9	21.4[cd]	0.8
	640	2.1	1.5	2.9	9.8[cd]	0.1
2,4-D/picloram	450/112.5	2.2	1.1	3.5	64.1[bc]	0.1
	900/225	2.0	0.9	2.3	88.1[ab]	0.0
Aminopyralid/fluroxypyr	5/70	2.3	1.8	2.7	0.0[d]	0.4
	10/140	2.4	2.0	2.5	0.4[cd]	0.5
Control		2.2	2.3	1.5	0.0[cd]	0.9
Diflufenican	125	2.0	1.0	2.3	98.2[a]	0.1
	250	2.3	1.1	2.3	14.7[cd]	0.3
Flumetsulam	10	2.6	1.2	1.9	25.0[c]	2.3
	20	2.0	1.5	2.3	3.6[cd]	1.3
Fluroxypyr	150	2.3	1.9	2.3	0.0[d]	1.4
	300	2.3	1.9	2.4	0.0[d]	2.6
Glyphosate	1080	2.0	0.9	3.8	99.5[a]	0.0
	2160	2.0	0.9	5.0	90.1[ab]	0.1
Imazamox	17.5	2.0	0.9	2.4	0.0[d]	0.2
	35	2.2	1.2	2.4	0.0[d]	1.3
MCPA/dicamba	1020/240	2.4	1.1	3.7	67.2[bc]	0.1
	2040/480	2.3	1.0	4.0	56.5[bc]	0.0
MCPA/diflufenican	62.5/6.25	2.1	1.0	1.7	6.7[cd]	0.5
	125/12.5	2.2	1.1	1.8	0.0[d]	1.1
Metsulfuron-methyl	6	2.1	1.0	2.9	0.0[cd]	0.9
	24	2.0	0.9	3.6	62.2[bc]	0.0
Triclopyr/picloram	300/100	2.1	1.0	2.9	9.4[cd]	1.0
	600/200	2.2	1.0	2.4	47.7[bc]	0.0
lsd trtmt[g] ($P < 0.05$)			0.76	1.25		1.38[h]
lsd trt-con[f] ($P < 0.05$)		n.s.	0.59	0.97		1.07[h]

Superscript letters had to be used to show significant differences for the % dead results because lsd values can only be used against the arcsin transformed data.
[e] Backtransformed means.
[f] For comparisons between the unsprayed Controls and a herbicide.
[g] For comparisons amongst herbicides.
[h] These lsd values must be used against the square root of the means presented above.
[i] Values for % dead followed by the same letter are not significantly different ($P < 0.05$).

the Roma trial site, *Pimelea* density was greater in barer places rather than where litter may have caught seed previously.

3.3.2. Initial Pimelea condition

Initial *Pimelea* populations at spraying time are shown in Table 5. Total pasture cover, the cover of *Pimelea* foliage, the number of adult *Pimelea* plants and the number of *Pimelea* seedlings in a 0.25 m^2 quadrat varied greatly. Adult plants numbered nearly 40 plants m^{-2} in some patches and virtually none in others. However, there was no strong correlation between total pasture cover and *Pimelea* plant density nor between density of adult *Pimelea* plants and density of seedlings emerging. Without follow-up rain, many June seedlings died especially if they had been sprayed with herbicide.

3.3.3. Herbicide impacts

The cold, dry conditions immediately after spraying inhibited any major changes in the sprayed plants until over a month after application. By then (early August) metsulfuron-methyl had caused a significant reduction in flowering activity to effectively zero (rating 1) (Table 5). Metsulfuron-methyl also had a greater mean damage rating than 2,4-D amine or 2,4-DB amine, except at the 50% application dose. However by mid-September, 13 weeks after spraying, all herbicides had either killed or seriously damaged the sprayed *Pimelea*. By mid-October most *Pimelea* that had been sprayed was dead and had set very little, if any, seed since spraying. However, the 50% herbicide doses were not fully effective for any of the herbicides and the 125% doses did not show significant improvement ($P > 0.05$) over the standard (100%) dose (Table 5).

Under the conditions, metsulfuron-methyl was the most consistently effective herbicide and 2,4-D amine performed less effectively than in the previous two trials. Any Control plant that had survived until the good October rain fell, then had a vigorous burst of flowering and those that survived the lowest doses of herbicide also had a flush of flowering. Many of those new flowers produced a seed with a plump, white embryo but we cannot say whether the embryo may still have been malformed or damaged in some way by the herbicide. Despite the good kill by most herbicide doses, seedling recruitment after spraying, between mid-June and October, saw a few live *P. trichostachya* plants in sprayed plots late in October (Table 5). However overall populations then were very low but highest in the Control plots where *Pimelea* had died back noticeably as is common in grassy pastures as summer approaches.

3.3.4. Herbicide effect on simplexin concentration in plants

The concentration of simplexin measured in all plants generally remained between 60 and 270 mg kg^{-1} throughout the trial and did not show any marked trend to increase or decrease following spraying at the 100% dose. Appreciable variation in simplexin levels between some replicates could have been due to variability in plant size, age or degree of upper foliage death at sampling time. Thus the changes associated with herbicide application were not statistically significant ($P = 0.061$) and some error values are high (Table 6).

Over the 3 months after spraying, the mean simplexin concentration of the foliage of unsprayed plants ranged from 131 mg kg^{-1} to 184 mg kg^{-1} of plant dry weight and was significantly higher ($P < 0.05$) than that of stems and the upper root in general, apart from one anomalously high root value. Many structurally related analogues of simplexin were also found but we do not know whether they are precursors, degradation products or closely related metabolites.

Table 5
P. trichostachya population density m^{-2} at time of herbicide application at Mungallala (12th June) and effects of the herbicide application dose at various times thereafter (Trial 3).

Herbicide	Dose (g a.i. ha^{-1})	Orig. *Pimelea* nbr m^{-2}	Flowrng rating (1 July)	Flowrng rating (7 Aug)	Damge rating (7 Aug)	Flowrng rating (16 Sep)	Damge rating (16 Sep)	*Pimelea* nbr m^{-2} (23 Oct)[d]
2,4-D amine	250	6.9	2.3	1.6	1.0	1.0	4.1	0.13[bc,g]
	375	15.5	2.8	1.7	0.9	1.0	4.5	0.15[bc]
	500	12.8	1.7	1.4	1.6	1.0	4.3	0.21[bc]
	625	16.0	2.1	1.5	1.0	1.0	4.2	0.13[bc]
2,4-DB amine	160	20.5	2.3	1.7	1.0	1.1	3.9	0.06[ab]
	240	5.6	2.1	1.6	1.8	1.0	4.7	0.12[b]
	320	7.5	2.8	1.7	0.9	1.0	4.5	0.04[ab]
	400	8.8	1.9	1.3	1.4	1.0	4.5	0.06[ab]
Metsulfuron-methyl	3	4.8	1.3	1.0	1.6	1.0	4.1	0.16[bc]
	4.5	12.8	1.6	1.0	2.1	1.0	5.0	0.00[a]
	6	16.3	1.7	1.0	2.2	1.0	4.8	0.00[a]
	7.5	7.7	1.3	1.0	2.7	1.0	5.0	0.02[ab]
Control	0	7.1	2.1	1.8	0.6	1.8	1.2	0.25[c]
lsd trtmt[f]	($P < 0.05$)		0.79	0.79	0.75	0.79	0.75	
lsd trt-con[e]	($P < 0.05$)	n.s.	0.69	0.69	0.65	0.69	0.65	

Superscript letters had to be used to show significant differences for the plant number results because lsd values can only be used against the square root transformed data.
[d] Backtransformed means.
[e] For comparisons between the unsprayed Control and a herbicide.
[f] For comparisons amongst herbicides.
[g] Values for *Pimelea* numbers on 23rd October that are followed by the same letter are not significantly different ($P < 0.05$).

After spraying with 2,4-DB amine, the simplexin levels of foliage were generally appreciable higher (but not significantly so) than in the unsprayed plants on the same date while the reverse was true for those sprayed with metsulfuron-methyl (Table 6). Main stems and upper taproot levels did not have the same consistency of difference between treatments but variability (as measured by the standard deviation) was also greater.

4. Discussion

4.1. Effective and selective herbicides

Two of the *Pimelea* species that cause problems for sections of the cattle industry have been shown to be susceptible to a range of commercial herbicides. Many effective ones are based on Group I 'hormone' chemicals (2,4-D amine, 2,4-DB amine, aminopyralid, dicamba, fluroxypyr, MCPA, picloram and triclopyr) that vary in their cost depending on the formulation and the other active herbicides incorporated. Glyphosate was also very effective, as reported for *P. spicata* (Matarczyk et al., 2002), but its cost is currently about $ ^{AUS} 36 per hectare at normal doses and it kills almost all plants, which is often not desired. It, like all the effective herbicides apart from 2,4-DB, is also toxic to highly-valued annual medics (Sandral and Dear, 2005) and so would appreciably damage whatever medic was in the sprayed pastures. Some less desirable herbage species are relatively resistant to the hormone weedkillers, so use of those sprays may encourage a rapid build-up of them in pasture, as happened at the Roma site with *V. pustulata*.

Table 6
Mean simplexin concentration (mg kg^{-1}) in the combined upper foliage plus inflorescences of *P. trichostachya* plants sprayed with 3 herbicides compared to their stems and upper taproot and to unsprayed control plants. Results are means from 4 sampling times over 3 months after spraying with one rate of chemical and for unsprayed Control plants (Trial 3).

Herbicide	Simplexin (mg kg^{-1} DWt)		
	Upper foliage	Main stem	Upper taproot
2,4-D amine	153 (48.8)[a]	132 (40.3)	121 (95.7)
2,4-DB amine	215 (60.1)	149 (70.6)	90 (25.1)
Metsulfuron-methyl	116 (40.3)	109 (42.6)	140 (40.6)
Control	159 (45.7)	101 (32.9)	162 (142.3)

[a] Standard deviation in brackets.

The slightly different order of efficacy of the chemicals at different sites and on different species is not unexpected. Growing conditions after spraying can have a marked impact on results even though commercial herbicides are formulated so that the most broadly reliable products are put into the market place (Kudsk and Mathiassen, 2007). Growth stage of the plants at spraying is also important and the Bollon *P. elongata* plants were generally much more mature than were the Roma *P. trichostachya* at spraying time. Soil moisture was good initially at both sites but the degree of competition from other pasture plants was minimal at Bollon compared to Roma. Thus *P. elongata* may appear to be less sensitive to metsulfuron-methyl than *P. trichostachya* but, without trials under identical conditions with plants of the same age, that cannot be stated categorically. The *P. trichostachya* plants at Roma were mostly of a single recruitment cohort but general impressions were that seedlings of both species were more susceptible to herbicides than well established, flowering plants. Nonetheless, at Bollon the large *P. elongata* bushes protected small *Pimelea* seedlings growing beneath them from the spray and those seedlings commenced to replace them as the bigger plant died.

At all sites, killing the existing *Pimelea* plants did not remove the sizeable soil seedbank of *Pimelea* and favourable germination rains the next year saw large numbers of *Pimelea* plants emerge to pose as great a threat as ever to livestock. We do not know how large those seedbanks are but the numbers emerging indicate that it is sizeable. Metsulfuron-methyl is reputed to have some residual herbicidal activity in the soil (Extoxnet, 2010) but we cannot confirm that from our observations of later *Pimelea* populations.

The minimal dose that was effective on *P. trichostachya* was 75% of the commonly recommended dose for many broad-leafed plants, irrespective of whether 2,4-D, 2,4-DB or metsulfuron-methyl was used. So a dose as low as 375 g a.i. of 2,4-D ha^{-1} or 3.5 g of metsulfuron-methyl ha^{-1} may be appropriate or 240 g of 2,4-DB ha^{-1} to avoid damage to associated legumes. Products containing 2,4-D may work slightly better on *P. elongata* and metsulfuron-methyl may be more effective if cold winter conditions are expected after spraying. We are unable to say how effective any of these herbicides might be on *P. simplex* which is the third main troublesome species in Australia's cattle-raising regions. However, the broad sensitivity to Group I chemicals and glyphosate, plus its very close taxonomic and phenological affinity, would indicate that they would be effective on it also.

4.2. Change in Pimelea toxin levels due to herbicides

There seems to be no cause for concern about toxin levels increasing dramatically as a result of spraying *Pimelea* with any of the three herbicides tested in the dose trial. 2,4-DB amine may increase slightly the concentration of simplexin in the sprayed tops. There is no hope of the sprays causing a dramatic fall in toxin concentration of half-dead *Pimelea* plants, although the standing dead remnants would be expected to lose their toxin over several months just like plants that die from other causes show (Fletcher et al., 2009). The rapid stop put to flowering by the effective herbicides would also stop seedfill and thus the formation of the most potent and enduring source of toxin found in dead *Pimelea* plants, the seeds (Fletcher et al., 2009).

4.3. Options for herbicide use in property management

Because the troublesome *Pimelea* plants are native and occur mainly in the extensive pastoral lands of inland Australia, herbicide use to control *Pimelea* will be strongly influenced by the cost of application and the persistence of their effect. Cost of chemical is relatively low at $\$^{AUS}$ 4.25 ha^{-1} for 2,4-D amine but even that is expensive when viewed in terms of over 10,000 ha of infested land on some properties. In reality, spraying such extensive areas would never be considered, with any herbicide use confined to strategic locations where application is easy and management benefits would be great, such as around yards and watering points. Even metsulfuron-methyl ($\$^{AUS}$ 1.90 ha^{-1} for chemical) may be considered too expensive over large areas. Metsulfuron-methyl is also toxic to many woody plants (Dupont, 2009) and so would have to be used carefully from ground equipment where sparse tree and shrub cover has to be retained. Annual native herbage that is so important for good lambing rates in pastoral Australia would also be seriously damaged by 2,4-D and metsulfuron-methyl but 2,4-DB may be a better option if the health of such plants is critical.

Mostly only one *Pimelea* species or subspecies needs attention at any time but it is not uncommon for *P. elongata* to be in a mixture with either of the other two main species. In that case, a decision would have to be made about which species presents the greater risk and to then select the best herbicide to control it. Metsulfuron-methyl can be mixed with most Group I herbicides and glyphosate (Dupont, 2009). Glyphosate is compatible also with some formulations of Group I herbicides such as 2,4-D ester (Nuturf, 2009).

Other research shows that *Pimelea* seed in the soil has variable degrees of dormancy at any time and that all seeds never germinate in the same recruitment event (Fletcher et al., 2009). Also plants can begin to ripen new seed within 6 weeks of emerging from seed. Thus repeated spraying would be needed after each recruitment event if the plant was to be virtually eradicated. Any missed seeding event could see hundreds of seeds per square metre returned to the soil (Dadswell et al., 1994) to restart the long term regeneration of *Pimelea* in that area. Complete removal of such a well-adapted native species is improbable, so minimization of the problem will be an ongoing management challenge. In a few regions where wheat farming occurs in *Pimelea*-infested country, herbicides may be an option in combination with normal cropping practices such as zero-till farming.

Herbicides may also have a realistic role where *Pimelea* populations are confined to discrete areas such as roadsides, powerline easements, run-on flats where extra water collects after small falls of rain, or creek-beds. If *Pimelea* is confined to those specialized habitats, which they often are, the area to be sprayed is much less and, in the case of roadsides, is readily accessible to ground-based spray equipment. Similarly, if seedling recruitment tends to occur in disturbed areas around waterpoints or along stock pads, spraying there alone may deliver a relatively cost-effective outcome. In that instance, removal of the stock from the paddock for a short period (depending on the label instructions) may be necessary if any of these chemicals becomes registered for use on *Pimelea*. Currently there are no herbicides registered in Australia for use in controlling *Pimelea* species. There should be minimal need to spray for *Pimelea* where there is dense timber because the species prefer growing with direct sunlight (Fletcher et al., 2009). Thus application issues that may impact on biodiversity and difficulty in accessing rough, timbered country with ground equipment would mostly only be encountered along watercourses.

Spraying as early as possible after a high-risk population of *Pimelea* has been detected would be desirable because ideal *Pimelea* recruitment conditions are found in very open, autumn pasture. However, spraying early in the cooler months with post-emergence herbicides may find later winter or spring rains germinating a further wave of seedlings that would require a second spraying. Leaving spraying until early flowering or early spring, when flowering usually peaks, runs the risk of some persistent unexpectedly poor weather delaying application until a significant amount of seed has already ripened.

Acknowledgements

We thank very much the owners of the properties who assisted us to conduct our trials on their land and took a great interest in what we were trying to achieve. Technical assistance and herbicide trial expertise was provided by Earl Sparkes while Kerrin Morrissy, Matt Pyke and Clare McNeale assisted with laboratory analyses. David Mayer provided his biometrical skills in the design of the trials and the statistical analysis of the results. Financial impetus for this work came from the Australian Natural Heritage Trust Fund (NHT #60436) through AgForce Qld.

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