Flupropanate – *in vitro* pasture species susceptibilities

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Abstract Flupropanate, a soil-active herbicide that inhibits lipid bio-synthesis, has become available in New Zealand for the selective control of the stipoid grasses *Nassella neesiana* and *N. trichotoma* in pasture. In commercial spray operations and field trials, the selectivity of the herbicide has been extremely variable. As a first step towards identifying pasture species that could be safely sown following application of the herbicide, the *in vitro* dose-response of 33 species and cultivars (grasses and dicotyledons) was determined. Ranking the cultivars based on the slopes of linear regressions fitted to their radicle lengths (relative to untreated control) against the logarithm of flupropanate dose, suggested that ryegrass and *Nassella* spp. are among the most sensitive whilst plantain, chicory, and some legumes including lucerne are relatively tolerant. The rankings from this study will be subjected to validation in a field trial using a subset of the species.

Keywords pasture herbicide selectivity, herbicide dose-response.

INTRODUCTION

Nassella neesiana Trin. et Rupr. (Chilean needle grass) and *N. trichotoma* (Nees) Hack. ex Arechav. (nassella tussock) are serious weeds in Marlborough, Hawke's Bay and Canterbury (Bourdôt et al. 2010) and are threats to other climatically suitable regions of New Zealand (Kriticos et al. 2004). Their control has relied, historically, on manually digging out tussocks (grubbing or chipping) or the use of the non-selective herbicide glyphosate. In 2011 the Marlborough District Council began working with the Australian producer of Taskforce® (flupropanate-sodium 745 g ai/litre) to get the herbicide registered for use on these two species in New Zealand. The herbicide is widely used for the control of these two species in Australia and is seen to be the most effective control option

for Chilean needle grass (Grech et al. 2012). The purported selectivity and 5-year soil residual activity of the chemical against the two weeds made it potentially an attractive option in hill country pastures. However, the efficacy and selectivity of the chemical in Australia and in council spraying operations and AgResearch field trials in New Zealand has been extremely variable with severe and lasting damage to desirable pasture plant cultivars in some cases (Grech et al. 2014; Saville & Bourdôt 2014).

Staff from Hawke's Bay Regional Council, Marlborough District Council and Environment Canterbury need to make informed recommendations to land managers regarding which species and cultivars can be over-sown into dry hill country sites affected by Chilean needle

grass and nassella tussock that are likely to be treated with flupropanate. The research described in this paper is a first step in understanding how pasture species and cultivars available in New Zealand vary in their inherent tolerance to flupropanate. To do this, a Petri dish dose-response assay commonly used as a “quick test” to identify herbicide-tolerance in weed populations was carried out. The results of this study will be used to select species to include in a field trial to assess their establishment and survival in the presence of flupropanate.

MATERIALS AND METHODS

The tolerances to flupropanate of 33 modern cultivars of pasture species most commonly sown in dry hill country pastures (Table 1), as well as Chilean needle grass and nassella tussock, were compared using an *in vitro* “quick test” method commonly used for confirming herbicide resistance in weed populations (Beckie et al. 2000; Burgos et al. 2013). Prior to treatment, percentage germination was determined for each seed line by placing four replicates of 50 seeds from each line onto moistened paper towels and sealing in individual sealed zip-lock bags. These were placed in a growth chamber for 2 weeks, after which the number of germinated seeds was counted and average percent germination calculated (Table 1). At time of treatment, ten seeds from each cultivar or species were placed in each of three replicate 9 cm diameter plastic Petri dishes lined with two layers of 7 cm diameter filter paper (Whatman®, Grade 1). The three dishes for each treatment were then saturated with 2 ml of one of nine different doses of flupropanate using Taskforce® (PGG Wrightson, 745 g/litre flupropanate as the sodium salt in the form of a water soluble concentrate) plus three water-only controls. The doses made up a log series from the equivalent of 140 g ai/ha to 2.2 g ai/ha using a 0.5 multiplier ($n = 7$ doses) plus 2 further doses of 2.2 kg ai/ha (the label rate of 3 litre/ha Taskforce® for Chilean needle grass) and 0.3 g ai/ha. Except for the highest dose, serial solutions were made using a starting solution of 280 g ai/ha, a solution with the smallest amount of flupropanate that

could be measured by pipette. For the highest dose (2.2 kg ai/ha) the solution was made by extracting the same amount of water as herbicide required and replacing this with the herbicide.

The doses chosen were based on a pilot study testing the susceptibility of cocksfoot and perennial ryegrass to the herbicide; two pasture species thought to be the least and most susceptible grasses to the chemical respectively (G.W. Bourdôt, personal observations). This study employed 19 doses from 17.9 kg to 0.02 g ai/ha plus an untreated water-only control, with four replicates for each dose.

Treatment solutions were applied to each replicate of 35 Petri dishes (33 cultivars + 2 weed species) randomly, with new solutions being made for each replicate and applied within 2 h. The Petri dishes were positioned randomly on a growth room bench, kept at 25°C with 12:12 h light:dark and rotated and checked daily and topped up with additional water as required until assessments were carried out 12–15 days after treatment. At assessment, the radicle length of each seedling was measured using digital callipers (Kincrome 150 mm Vernier Caliper) and, by default, the number of germinated seeds counted (i.e. a radicle length of 0 implies the seed did not germinate). Data from all three replicates were combined for statistical analysis.

Traditionally, dose-response data are analysed using a nonlinear log-logistic regression model (Seefeldt et al. 1995). However, because the radicle length data in the present experiment did not seem to follow a logistic curve in any of the cultivars, it was not an appropriate model to compare dose responses between the cultivars. Instead, a mixed effects linear regression model was fitted to the means of the radicle lengths (relative to the average of the controls), with the $\log_{10}$ of the dose as a fixed effect and cultivar as a random effect. By including cultivar as a random effect, the model fitted an average slope to which each cultivar slope was compared thereby allowing the cultivars to be ranked in order of their susceptibility to flupropanate. Radicle lengths were normalised relative to the untreated controls to overcome the very large differences

Table 1 Pasture and weed species tested for their susceptibility to the herbicide flupropanate. Endophytes, where known, are indicated in parentheses after the cultivar name.

Scientific name	Common name	Cultivar	% germination
<i>Bromus sitchensis</i>	Upland brome	Hakar ¹	100
<i>B. stamineus</i>	Grazing brome	Gala ¹	79
<i>B. stamineus</i>	Grazing brome	Bareno ⁶	98
<i>B. willdenowii</i>	Prairie grass	Atom ³	96
<i>B. willdenowii</i>	Prairie grass	Matua ¹	89
<i>Cichorium intybus</i>	Chicory	Chico ²	79
<i>C. intybus</i>	Chicory	Puna II ³	74
<i>Dactylis glomerata</i>	Cocksfoot	Tekapo ¹	79
<i>Lolium multiflorum</i>	Italian ryegrass	Sonik ²	100
<i>L. perenne</i>	Perennial ryegrass	Alto (AR37) ⁶	96
<i>L. perenne</i>	Perennial ryegrass	Matrix ²	100
<i>L. perenne</i>	Perennial ryegrass	Samson (AR37) ¹	93
<i>L. perenne</i>	Perennial ryegrass	Nui ¹	97
<i>L. perenne</i>	Perennial ryegrass	Ultra ²	98
<i>L. perenne</i> x <i>Schedonorus pratensis</i>	Festulolium hybrid	N/A ²	82
<i>Lotus pedunculatus</i>	Lotus	Trojan ¹	100
<i>Medicago sativa</i>	Lucerne	Torlesse ¹	86
<i>Nassella neesiana</i>	Chilean needle grass	N/A ⁴	83
<i>N. trichotoma</i>	Nassella tussock	N/A ⁵	86
<i>Phalaris aquatica</i>	Phalaris	Maru ¹	78
<i>Plantago lanceolata</i>	Narrow-leaved plantain	Tonic ²	100
<i>S. arundinaceus</i>	Tall fescue	Easton ¹	96
<i>S. arundinaceus</i>	Tall fescue	Quantum II ³	96
<i>S. arundinaceus</i>	Tall fescue	Flecha ¹	84
<i>Trifolium fragiferum</i>	Strawberry clover	Onwards ¹	78
<i>T. pratense</i>	Red clover	Relish ¹	90
<i>T. pratense</i>	Red clover	Tuscan ⁶	91
<i>T. repens</i>	White clover	Mainstay ³	93
<i>T. repens</i>	White clover	Huia ¹	94
<i>T. repens</i>	White clover	Nomad ³	72
<i>T. repens</i>	White clover	Demand ²	100
<i>T. repens</i>	White clover	Tahora II ¹	95
<i>T. subterraneum</i>	Subterranean clover	Whatawhata selection ¹	15

Seed provided by: ¹Grasslanz, ²Cropmark, ³PGG Wrightson, ⁴Marlborough District Council, ⁵AgResearch, Lincoln, ⁶Agriseeds

between cultivars in the absolute lengths of their radicles. Because there was no significant difference in the number of non-germinated seeds between the treated and control Petri dishes, it was assumed that the non-germinated seeds were dead or dormant and zeros (non-germinated seeds) were excluded when estimating the means.

RESULTS AND DISCUSSION

The subterranean clover had a very low germination rate and the lotus seeds were prone to drying out during the experiment so these two species have been omitted from the analysis. The mixed model regressions and the means to which they were fitted are shown in Figure 1.

The overall mean slope was significantly negative ($P < 0.001$) showing that radicle growth was reduced as the dose of flupropanate increased with those cultivars having a steeper slope being more susceptible. The differences between the individual cultivar slopes and the overall average slope were not significantly different. However, using these differences, the cultivars were ranked in order of their susceptibility to the herbicide (Figure 2). With the exception of perennial ryegrass 'Alto', all of the ryegrass cultivars had slopes lower than the overall average slope, making them some of the most susceptible cultivars. Many of the other grass cultivars also had slopes lower than the average, suggesting that they too were relatively susceptible to flupropanate. In contrast, most of the broad leaved species had slopes greater than the average indicating that they were relatively less susceptible to the herbicide; plantain, strawberry clover, chicory 'Chico' and lucerne were amongst the least susceptible of the cultivars (Figure 2).

The effect of flupropanate on desirable pasture species has been examined in the field in Australia with conflicting results. In two studies comparing the effect of different rates of flupropanate in 18-month-old improved pasture, it was shown that the ground cover of sown legumes (*Trifolium subterraneum* and *T. repens*) was reduced by rates greater than 0.75 kg ai/ha but the ground cover of sown grasses (*Festuca arundinacea*, *Phalaris aquatica* and *Dactylis glomerata*) was not affected by rates up to 1.11 kg ai/ha (Campbell 1997; Campbell et al. 2002). However, in another study comparing the use of flupropanate (1.12 kg ai/ha) in combination with different grazing regimes there was an increase in the percent cover of bare ground and broad leaf weeds and a decrease in desirable grass species (*F. arundinacea*, *P. aquatica*, *D. glomerata* and *L. perenne*) in those plots sprayed with flupropanate in the presence of grazing. However, there was no difference in the absence of grazing (Grech et al. 2014). The results of this study concur with the findings of Grech et al (2014) that the chemical does have a deleterious effect on desirable species and should therefore be used with caution in grazed pasture.

The original intention of this experiment was to use GR_{50} values (the dose that causes 50% inhibition of growth), derived from fitting traditional log-logistic regression models to the data, to rank the cultivars in order of their susceptibility to flupropanate. Based on the pilot test study, the range of doses used in this experiment should have given sufficient data at both ends (asymptotes) of the responses to fit the traditional curve. However, the data unexpectedly did not fit these models due to the high variability of data and absence of data points at the lower end of the logistic tail (i.e. values near zero at higher herbicide doses) for many of the cultivars. A possible explanation for this unexpected result is that the time required for the herbicidal effect of flupropanate to become evident may be longer than the 12–15 days after treatment when the effects on radicle length were measured in this study. Unfortunately, fungal ingress and the restriction of seedling growth by the height of the Petri dish precluded an extension of the assessment period.

Despite the methodological challenges, the ranking of the species according to their relative susceptibilities to flupropanate suggests consistent trends with a recent Australian field trial (Grech et al. 2014), with grasses being more susceptible than broadleaf species. This has provided a guideline as to which species to test further under New Zealand field conditions. The results of this experiment suggest that many of the ryegrass cultivars traditionally used in hill country pasture may not be suitable for over sowing into nassella tussock or Chilean needle grass-infested pastures that have been, or may be, treated with flupropanate. However, three grass cultivars seem to have lower susceptibilities to flupropanate and could possibly be used in place of perennial ryegrass. These cultivars are upland brome, and the two prairie grass cultivars, Atom and Matua. In addition, plantain, strawberry clover, chicory 'Chico' and lucerne could also warrant testing under field conditions. Chicory and lucerne have the added advantage of being deep rooted and therefore better adapted to the dry hill country conditions found in the infested

Figure 1 The response of 29 pasture species and cultivars and two weeds (*Nassella trichotoma* and *N. neesiana*) to increasing dose of flupropanate. Mixed-effect regression lines (95% confidence intervals shown as greyed area) fitted to the means ($n = 3$ Petri dishes, each with 10 seeds) of the radicle lengths relative to the non-treated control are shown. The log of the dose of flupropanate (g/ha) was used as a fixed effect and cultivar as a random effect.

Figure 2 Cultivars ranked from least susceptible (top) to most susceptible (bottom) to the herbicide flupropionate based on the difference between the slope of radicle length relative to untreated and the average slope. Bars indicate 95% confidence intervals.

regions. However, these two species would require special management practices, which would need to be taken into account on steep hill country. The results of this study will be subjected to validation using a subset of cultivars in a field trial carried out in Hawke's Bay, Marlborough and North Canterbury.

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