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Experimental protocol to test pasture species susceptibility to the herbicide flupropanate

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CONTENTS

1. EXECUTIVE SUMMARY

- The herbicide flupropanate, recently registered in New Zealand under the product name Taskforce for the selective control of Chilean needle grass and nassella tussock in pastures, has proven to be extremely variable in the damage it causes to desirable hill country pasture species.

- As a result, Hawke's Bay RC, Marlborough DC, and Environment Canterbury have requested protocols for statistically valid field experiments to answer the questions:
    - Which pasture species can be safely over-sown in hill country pastures following an application of the herbicide?
    - Which pasture species, once established in hill country pastures, can be over-sprayed with the herbicide without damage?

- A third, and overarching question posed by AgResearch scientists is "how do pasture species and their cultivars available in NZ that are suitable for dry hill country vary in their inherent tolerance to the herbicide flupropanate"?

- Experimental protocols were developed for all three questions.

- Recommendations:
    - A lab-based dose-response experiment to answer the overarching question should be carried out prior to setting up of the two field-based experiments. This experiment would rank pasture species and cultivars according to their susceptibilities to flupropanate and provide guidance as to which species to include in the field experiments.
    - When carrying out the field experiments, the effect of climatic variability within regions can be reduced by applying the treatments and sowing the seeds at all sites in each region within a 1-week period. A more thorough approach would be to include several different sowing times.
    - If the Councils choose to establish and assess the field experiments themselves, they should:
        - Ensure assessments within each region are made by the same person for the duration of the experiment.
        - Engage a statistician to analyse the data. In that case, the experimental designs that we have suggested here would need to be approved (and perhaps modified) by this statistician.

2. BACKGROUND

The control of Chilean needle grass and nassella tussock historically has relied on manually digging out tussocks (grubbing or chipping) or using the non-selective herbicide glyphosate. In 2011 the Marlborough District Council began working with the Australian producer of a flupropanate-based herbicide (marketed as Taskforce) to get the chemical registered for use on these two species in New Zealand. The purported selectivity of the chemical made it potentially an attractive option for controlling these two weeds in hill country pastures. However, the efficacy and selectivity of the chemical in Council spraying operations and in AgResearch field trials has been extremely variable with extensive damage to pasture species in some cases.

To enable Council staff to make informed recommendations to land managers regarding which species could be over-sown into dry hill country sites affected by Chilean needle grass and nassella tussock, Hawke's Bay RC, Marlborough DC and Environment Canterbury approached AgResearch scientists to design statistically valid experiments to answer the questions:

Question 1 - How do pasture cultivars available in NZ that are suitable for sowing in dry hill country vary in their inherent tolerance to the herbicide flupropanate?

Question 2 - Which pasture species can be safely over-sown in hill country pastures following an application of the herbicide?

Question 3 - Which pasture species, once established in hill country pastures, can be over-sprayed with the herbicide without damage?

The experimental protocols detailed below address these three questions.

3. EXPERIMENTAL PROTOCOL

3.1 Experiment 1 (Question 1)

Assumptions

1. Herbicide susceptibility is primarily a result of biochemical mechanisms within plants that may affect the processes of uptake, translocation, metabolism (to inactive form) or binding (at site of action).
2. A controlled environment dose-response experiment will be the most efficient way of ranking species and cultivars for susceptibility to flupropanate and hence should be conducted first to enable selection of flupropanate-resistant pasture species for field evaluation.

Experimental design

The tolerance to flupropanate of modern cultivars of pasture species most commonly sown in dry hill country pastures may be tested by infusing agar or wetting blotting paper in Petri dishes with an approved nutrient solution and a variety of doses of flupropanate using the commercially available Taskforce. A log series of doses spanning the range from a very small amount of herbicide to 2x the label rate plus an untreated nutrient solution-only control should be used. Fifty seeds of each cultivar should be placed in each of the Petri dishes with four replicates of each of each dose. For example if 50 pasture plant cultivars are tested under 10 different doses of flupropanate with four replicates each then the number of Petri dishes = 50 x 10 x 4 = 2000.

Each lot of 50 seeds would be assessed for seed germination, seedling growth and signs of damage on a weekly basis for 4 – 6 weeks. Dose-response curves fitted to the mortality/damage data and derived LD_{50} would enable the cultivars to be ranked regarding tolerance to the herbicide. Those cultivars with the highest LD_{50} values could then be trialled in the field using the protocol described for Experiments 2 and 3 below.

The species tested will include modern and readily available cultivars of rye grass (*Lolium perenne*) sub-clover (*Trifolium subterraneum*), red clover (*T. pratense*), white clover (*T. repens*), cocksfoot (*Dactylis glomerata*), phalaris (*Phalaris aquatica*), chicory (*Cichorium intybus*), plantain (*Plantago lanceolata*), lotus (*Lotus pedunculatus*) and Birdsfoot trefoil (*L. corniculatus*) reccommended for use in dry hill country pastures.

Analysis

The relationship between germination success or growth measurements and dose of flupropanate should be statistically analysed by fitting dose-response curves using a probit or logit regression model. From these, the species susceptibility to the chemical can be ranked (Fig 1 as an example).

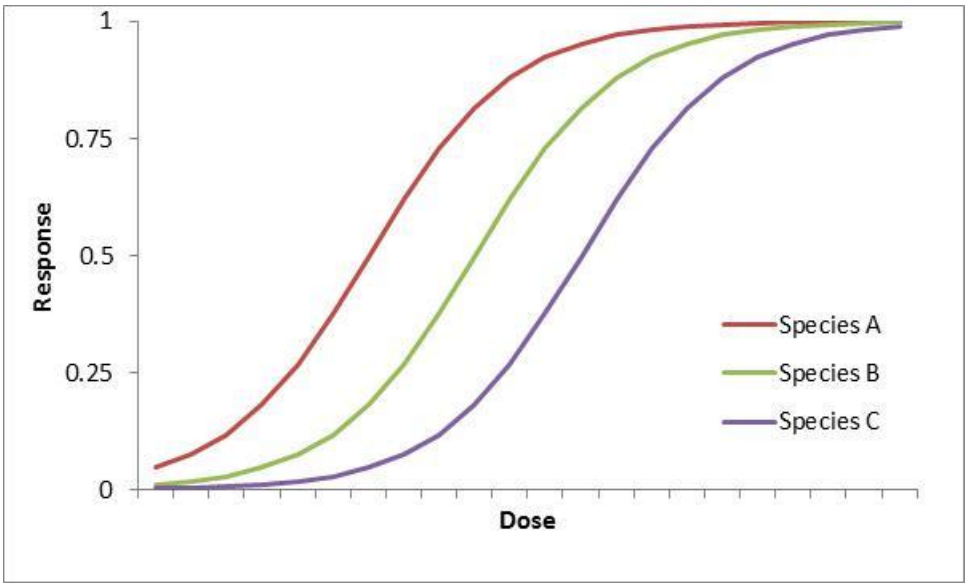

Figure 1. An example of fitted dose-response curves. In this curve Species C is least susceptible and therefor one to consider before Species B or A for field experiments.

Cost

The cost for a CRI to carry out the above experiment would be dependent on the number of species and cultivars tested. For the example given above (50 cultivars, under 10 different doses with four replicates) the cost would be approximately $50k. This would include laboratory supplies, reviewing the relevant literature to ensure the dose-response experiment protocol is robust, sourcing appropriate seed cultivars, setting up and assessing the experiment, preliminary runs of the experiment to ensure suitable spacing and range of doses of the herbicide, statistical analysis and writing up results in a report.

3.2 Experiments 2 and 3 (Questions 2 and 3)

Assumptions

1. Herbicide will be applied by air over a relatively large area of a property. This creates logistical issues regarding obtaining statistically valid control (untreated) plots but a "work-around" is proposed.
2. Flupropanate will move downhill in the soil. This will result in the herbicide moving out of treated plots reducing the effective dose application and also into untreated

plots, rendering them ineffective as controls. Appropriate buffer zones and plot arrangement across slopes will be necessary to avoid these potentially serious issues.

3. Environmental variability will greatly affect pasture species establishment and herbicide activity.

Experimental design

For Experiment 2, plots should be established on at least two properties in each region (Hawke's Bay, Marlborough, Canterbury). Since Experiment 2 will run for 2 years and Experiment 3 for 3 years (1 year for pasture establishment + 2 years post chemical application) the land managers need to be willing to allow access to their property for up to 3 years.

Prior to applying flupropanate, a minimum of 10 replicate 20m-wide x 40m-long main-plots should be located per farm with the plots arranged along the contour to avoid any cross contamination via down-hill leaching of the herbicide. Also, these main plots should be located so as to cover as many different areas of the face as possible to ensure a wide representation of any local variability. Each of these main-plots should be divided into two 20m x 20m paired sub-plots (sub-plot 1 & 2), each member of the pair being randomly assign one of the two treatments, +flupropanate or –flupropanate (e.g., by tossing a coin for each main-plot, heads = sprayed sub-plot 1, tails = not sprayed sub-plot 1). Within the centre of each sub-plot, peg one 0.5m x 0.5m quadrant sub-sub-plot for each species being sown with 0.5m between each of them. For example if 9 species are being sown, peg out 9 sub-sub-plots (Fig 2).

Note that the buffer around the central seed-sowing sub-sub-plots is designed to minimise any "edge effects" that may otherwise arise through the movement of chemical though the soil. If the herbicide is not going to be applied aerially, position plots so that there are no other plots directly up slope to minimise any down hill leaching of the herbicide.

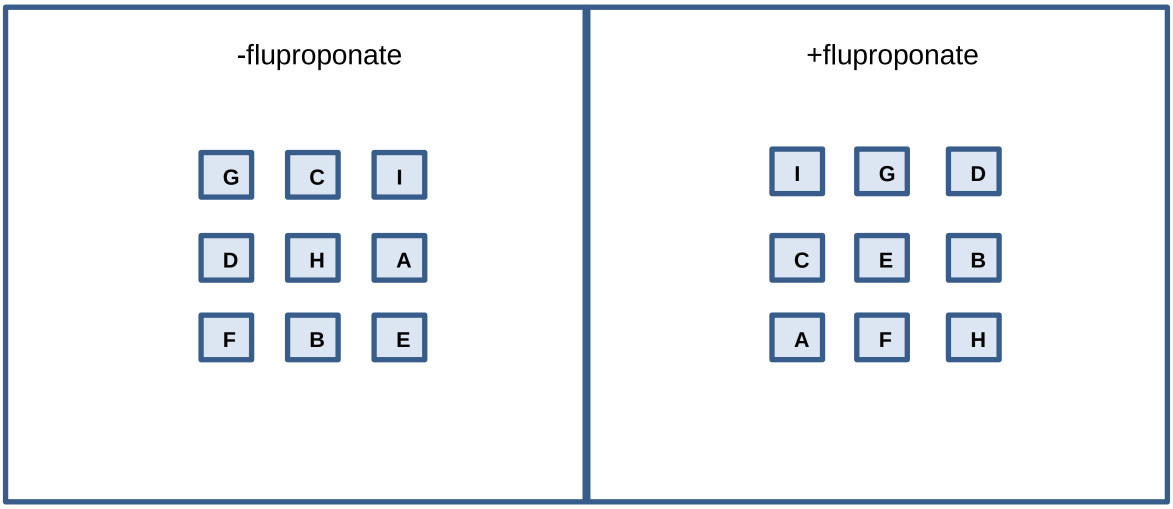

Figure 2. General layout of paired plots with nine subplots. The letters within the subplots represent a single pasture species randomly allocated to the subplots.

On the day of spraying, secure one 20mx20m tarpaulin over each of the untreated (no flupropanate) plots to prevent herbicide application. Once the herbicide has dried, the tarpaulins can be removed. Ideally, the herbicide should be applied within a 1-week time frame over all farms within each region.

At over-sowing time, (after a minimum of 100mm rain and at a time suitable for sowing in the region) one pasture species should be randomly allocated to each sub-plot. Hand sow the species within their allocated subplot at the recommended rate ensuring even coverage of the subplot. (NB resident vegetation type and density will have a huge effect on establishment of over sown species. Therefore, you may want to consider adding in a 3rd treatment +flupropanate+Roundup).

So that results can be compared between farms and regions, the seed used and the sowing rate should be the same for all farms and regions. In additon, as with herbicide application it would be ideal to have all the seed sown on all farms within a region within a one week period.

For Experiment 2 in particular, the interval between spraying and over-sowing will have critical and interacting effects on sown species' response and this will be affected by rainfall and the decay rate/binding of the herbicide in the soil (the latter being affected by soil type and possible microbial metabolism of the herbicide). To help explain the expected variation in responses between sites (within and across regions), "sown time after spraying" and soil type need to be noted and rainfall measured at all field sites.

For Experiment 3, the methodology is the same as for Exp 2 but with pasture species sown at least one year in advance of the flupropanate application.

In addtion to the above:

- Desirable - Due to annual climatic variablilty it would be desirable to replicate the experiment in a second year.
- Desirable but optional - Additional subplot for "no seed added" treatment could be included in each subplot group.

Sub-plot assessment
For Experiment 3, the percent cover of each sown pasture species in each sub-sub-plot must be assessed prior to spraying with flupropanate; this pre-spray measurement wll be the baseline against which the post-spraying measurments are compared. After spraying, for both experiments, the percent cover of each sown pasture species should be assessed at 6-monthly intervals over 2 years using the foliage cover chart for guidance (Fig 3). If possible, assessments should be carried out by one person per region to reduce any observer bias. An example field recording sheet is included in Appendix 1.

Analysis
We recommend the data collected from the two field experiments be analysed by a statistician. The analysis should reflect the design of these experiments; a split-plot design consisting of two treatmens as main-plot factor and sown species as sub-plot factor. A simple graphical representation of the data would include time along the x-axis and percent cover along the y-axis for both experiments.

Cost
The cost of Experiments 2 and 3 will depend largely on the number of sites and their geographic locations, number of replicates and frequency of sampling.

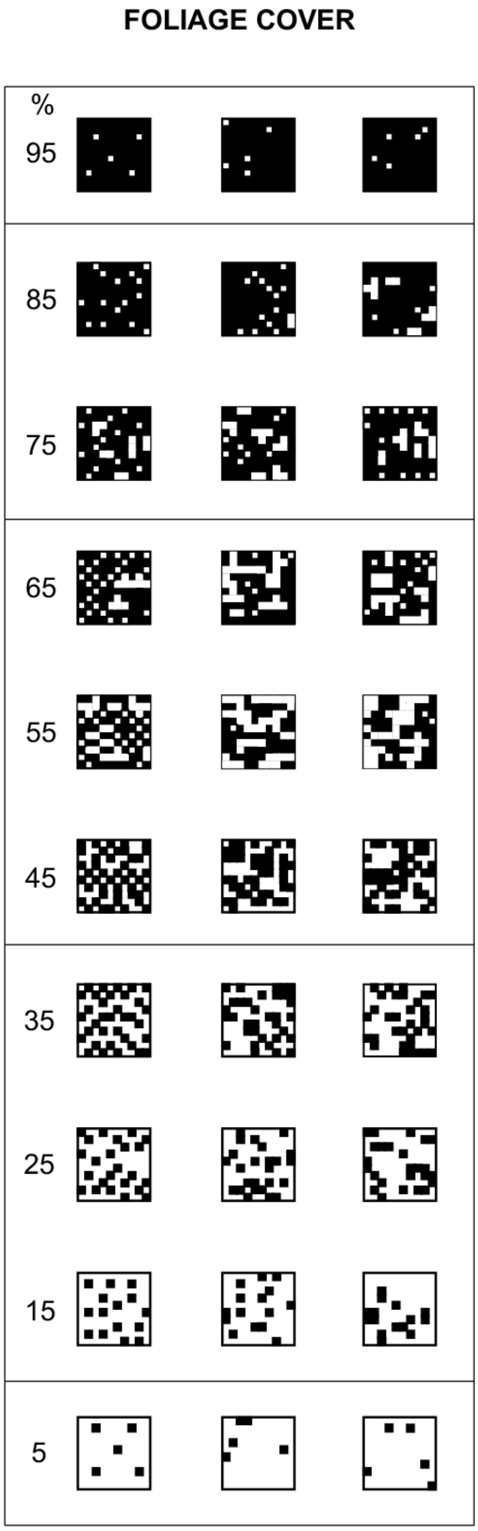

Figure 3. Comparison charts for use in the field to visually estimate the foliage cover of each pasture species.

4. RECOMMENDATIONS

- A lab-based dose-response experiment to answer the overarching question should be carried out prior to setting up of the two field-based experiments. This experiment would rank pasture species and cultivars according to their susceptibilities to flupropanate and provide guidance as to which species to include in the field experiments.

- When carrying out the field experiments, the effect of climatic variability can be reduced by applying the treatments and sowing the seeds at all sites in a region within a 1-week period. A more thorough approach would be to include several different sowing times.

- If the Councils choose to establish and assess the field experiments themselves, they should:
    - Ensure assessments within each region are made by the same person for the duration of the experiment.
    - Engage a statistician to analyse the data. In that case, the experimental designs that we have suggested here would need to be approved (and perhaps modified) by this statistician.

5. ACKNOWLEDGEMENTS

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6. APPENDIX 1

Sample data sheet for recording the cover of species in the field

Date:			Observer:	
Property:				
Main-plot (1-10)	Sub-plot (1-9)	Treatment (+,- flupropanate)	Species	% Cover