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Bagging of grubbed flowering nassella tussock

Envirolink small advice grant 817-MLDC42

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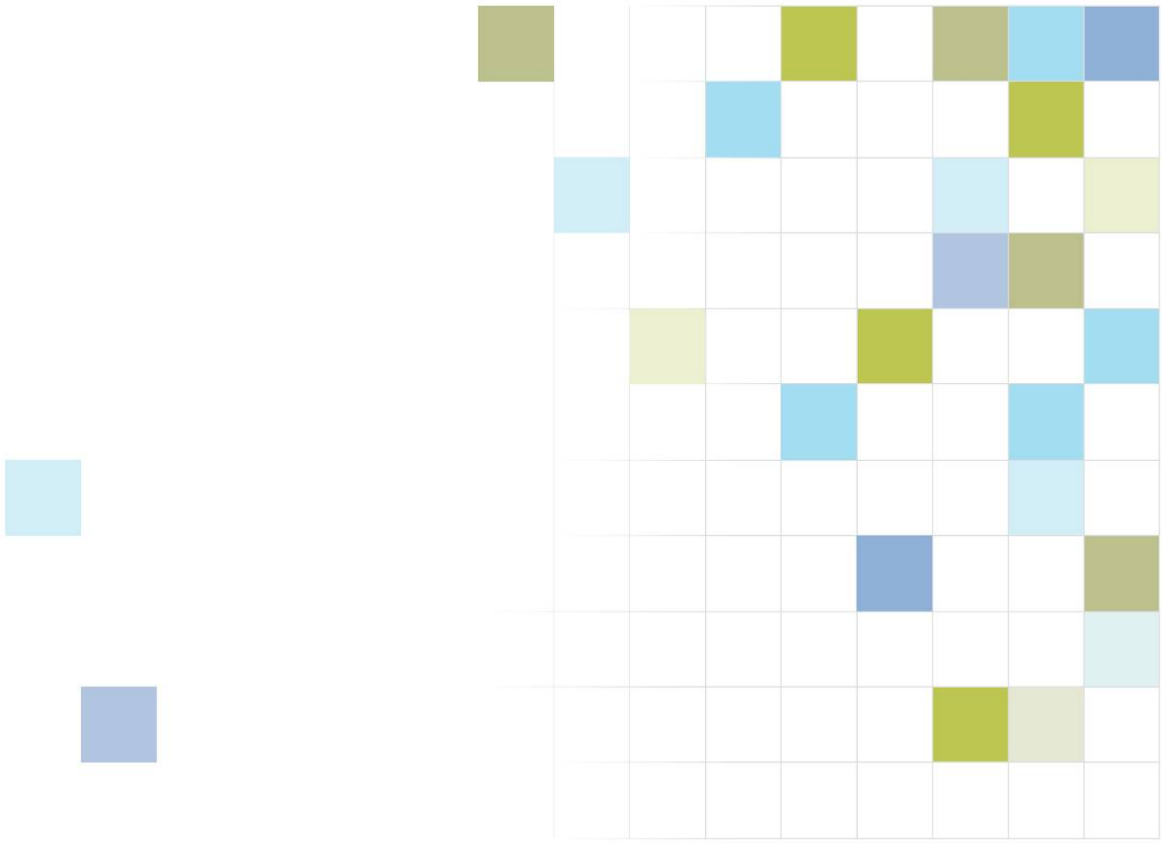

CONTENTS

1. EXECUTIVE SUMMARY

- The Marlborough District Council requested AgResearch to design an experiment to test the idea that the seeds on grubbed nassella tussock plants would be rendered non-viable if stored in plastic bags in the field until the autumn.

- The experiment consisted of grubbed flowering plants of nassella tussock placed into thick and thin black plastic bags tied to a fence, and placed into brown paper bags or knotted and fixed to the ground (the four treatments) on four farms in Marlborough. The experiment ran for three months over the summer of 2009-10.

- The average initial viability of seeds collected from the four farms at the start of the experiment was 58%. After storage for the duration of the experiment at $4^{o}C$, viability averaged 61%.

- For the samples stored in the field, only the heavy black plastic bag treatment significantly lowered the viability of the seed (by 41%) from that of the stored initial samples, but 35% of the seeds under this treatment remained viable.

- The results of this experiment do not support the idea that nassella tussock seeds can be sufficiently reduced in viability by placing grubbed seeding plants in plastic bags (or by leaving them knotted on the ground) to make this a useful management practice for this weed.

2. BACKGROUND

Nassella tussock *(Nassella trichotoma* Nees.*)* is a perennial grass native to South America which is drought tolerant and unpalatable to stock. It is considered a serious weed to pastoral agriculture in New Zealand, Australia and South Africa (Kriticos et al. 2004). Although current densities do not impact on pasture production, ongoing management by grubbing before flowering/seeding each year is considered necessary by farmers in Marlborough and North Canterbury to ensure the weed does not return to the levels that existed in the past (Smith & Lamoureaux 2006).

Nassella plants however, can be difficult to identify before flowering and can be confused with native tussocks. This study aimed to test the hypothesis that by placing grubbed flowering plants into black polythene bags and leaving these out in the sun for three months over summer, the seeds would be rendered non-viable. If proven effective, this bagging method would be a practical way for farmers to leave grubbing until later in the season, and overcome the problem of extra seed entering the seed bank.

3. METHODS

The field experiment was carried out (by MDC staff) according to the protocol "Design Details" in the appendix of this document. The light plastic bags broke down in the sun and were re-bagged into brown paper rubbish bags part-way through the experiment. In other respects the design was unchanged.

Initial samples were received, subsampled and processed in December 2009, then stored at 4^{o}C. At the end of the 3-month-long experiment, bags of tussocks were received at Lincoln on 18th March and stored at 4^{o}C for about 10 days before the processing was done. One sample, No. 37 a brown paper bag, was badly composted and was omitted from the analysis.

The standardized method for testing the viability of seeds was according to instructions in the Tetrazolium Testing Handbook for PoaceaeII (small grasses), *Nassella* (Peters 2000). This test involves imbibing the seeds on moist blotters overnight (12 hours) at 25^{o}C then bisecting them laterally through the testa above the embryo. These are then immersed in a 1% 2,3-5 tetrazolium chloride (TTC) solution overnight at 25^{o}C. Evaluation is by the notes described in the handbook; embryo completely stained (pink or red colour) = 'viable', incompletely stained embryo = 'non viable'.

Upon arrival at Lincoln in December 2009, fifty seeds were sampled randomly from the initial samples from each of the four farms, and tested for viability. These initial samples were re-tested in the same manner on 30/31st March 2010 (after storage at 4^{o}C). For each of the 32 samples received at Lincoln on the 18th March, thirty seeds were sampled randomly and tested. The latter samples were processed in plot order, according to the protocol, and testing was completed over 2 working days (30/31st March 2010).

The percent viability data, and the % reductions in viability (compared to samples stored at 4^{o}C), were analyzed by an analysis of variance for a randomised block design assuming four blocks (=farms) and four treatments (that is, data were pooled over the two blocks within each treatment and farm prior to analysis). The statistical package Genstat, Version 12, was used for this analysis.

4. RESULTS

The viability of the initial seed samples, averaged across the four farms, did not change between the two times of testing (in Dec 2009 and Mar 2010). Here we take the value of 61% measured at the end of March 2010 (for initial samples stored at 4^{o}C) as the control against which to compare the effects of the treatments (Table 1).

The seed in the heavy plastic bag treatment was significantly lower in viability (41% lower) than the seed in the "control samples" (i.e. those stored in the fridge from December 2009 until end of March 2010 (viability 61%)). The viability of the seeds in the other bag treatments and the knotted tussock treatment were not significantly different from the control (since the corresponding % reductions were less than the LSE(5%) of 24%).

The % reduction in viability of the seed in the heavy plastic bag treatment was significantly higher than in both the light plastic bag treatment and the brown paper bag treatment, but was not significantly higher than in the knotted tussock treatment. Also, the % reduction in viability of the seed in the knotted tussock treatment was significantly higher than in the brown paper bag treatment.

The seed in the brown paper bag treatment appeared to have increased in viability when compared to the control, although not significantly (since the 20% increase was less than the LSE(5%) of 24%). This apparent increase may be just experimental variation or it may have been caused by the seeds ripening in the bags, because it was a warm and dry environment.

Table 1. Percent viability of the nassella tussock seeds sampled initially (at the beginning of the experiment), following storage at 4°C, and as affected by the experimental treatments (measured end of March 2010). The % reductions in viability are relative to seed stored at 4°C.

	Farm 1	Farm 2	Farm 3	Farm 4	Mean % viability	% reduction in viability
Initial samples (Dec, 09)	72	58	38	65	58.3	
Initial samples after storage at 4°C at AgResearch Lincoln (end Mar, 10)	70	62	52	60	61.0	
Treatments						
Light black polythene bag on fence	68.0	50.0	48.0	73.5	59.9	2%
Heavy black polythene bag on fence	28.5	43.5	48.5	20.0	35.1	41%
Brown paper bag on ground	79.5	63.0	83.0	63.5	72.2	-20%
Knotted tussock on ground	64.5	36.5	60.0	35.0	49.0	19%
Least Significant Difference(LSD)(5%)					20.6	34%
Least Significant Effect(LSE)(5%)					-	24%

5. DISCUSSION

Although leaving grubbed tussocks in heavy plastic bags outside in the sun for three months over summer reduced the seed viability significantly (by 41%), many of the seeds (35%) remained viable. Based on these results, this practice could not be recommended as a method of dealing with nassella tussock plants grubbed during the seeding period. It is possible that leaving them out for longer may reduce the viability further, but there is no way of predicting from the current results, how long seeds would need to remain in the bags for them to all become nonviable. It may take several summers for this to occur. During extended time in the sun the plastic bags may break down, as the light bags had done, so this may not be a safe solution. Removing them from the field and burning them may be a safer, though less practical option.

It is possible that if the samples were wetted before being placed into bags the composting process would have been more effective and more seed would have been destroyed. This was evident in one of the samples which was badly composted and had much lower viability than the other samples within that treatment. This was the only sample that obviously had moisture in the bag, whereas all of the other 31 samples were quite dry.

6. ACKNOWLEDGEMENTS

We wish to thank the farmers for the use of their land throughout the study. This study was funded through Envirolink small advice grant 817-MLDC42 .

7. REFERENCES

Peters J ed. 2000. Tetrazolium Testing Handbook. Lincoln, Association of Official Seed Analysts.

Kriticos D, Lamoureaux S, Bourdôt G, Pettit W 2004. Nassella tussock: current and potential distributions in New Zealand. New Zealand Plant Protection 57: 81-88.

Smith LP, Lamoureaux SL 2006. When science meets policy - nassella tussock in New Zealand as a case study. In: Preston C, Watts JH, Crossman ND ed. 15th Australian Weeds Conference. Pp. 450 - 453.

8. APPENDICES

Design details, "Bagging of grubbed flowering nassella tussock" study, Envirolink small advice grant 817-MLDC42

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and
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Sept 4, 2009

Introduction

"Nassella tussock is more obvious when in flower, but grubbing this late promotes population growth. If grubbed flowering tussock is placed in black polythene bags, the heat in the bag may render the seed non-viable, preventing population growth."

This small study will test whether this occurs. Two types of black polythene bags will be tested, one a heavy plastic bag and one a light plastic bag. One or other may be bio-degradable. These will be tied to a fence or shrub. To serve as "comparative controls", a brown paper bag will be tested along with a "knotted tussock", both secured to the ground. The viability of the seed that is placed in the bags (or knotted) will also be tested at the outset, and after storage in optimum conditions at Lincoln.

Number of farms

The study is to be spread over four farms.

Number of blocks per farm

The four experimental treatments will be randomly allocated within each of four blocks on each farm, as shown in the diagram on the next page (note the word blocks is simply a word for groups, not to be read as different blocks within each farm). However, only two of these blocks will be sampled at the end of the experiment on March 31, 2010. The other two blocks are present simply as "backup" in case stock interfere with the bags or knotted tussocks. Note also that the "random allocation" can only be done to the positions on the fence or on the ground.

Number of nassella tussock plants per farm

On each farm, there will need to be enough nassella tussock plants to fill 8 polythene bags (packed in as tightly as in the intended usage), plus enough to fill 4 paper bags, plus 4 plants for knotting.

Procedure at time of setting up in December 2009

As they are grubbed, *soil and roots need to be removed* from all tussocks to make the AgResearch laboratory work feasible**.**

As each bag is filled, a *few panicles should be placed in a brown paper bag for an initial bulk sample (one per farm)* to be sent immediately down to AgResearch at Lincoln (avoid overheating in transit!). For each farm, this sample will be split upon arrival at Lincoln into two samples. From one such sample, 50 seeds will be immediately tested (in December) for initial viability. The other sample will be stored under optimum conditions

until the end of the experiment, when 50 seeds will be tested for viability, simultaneously with the experimental samples from that farm.

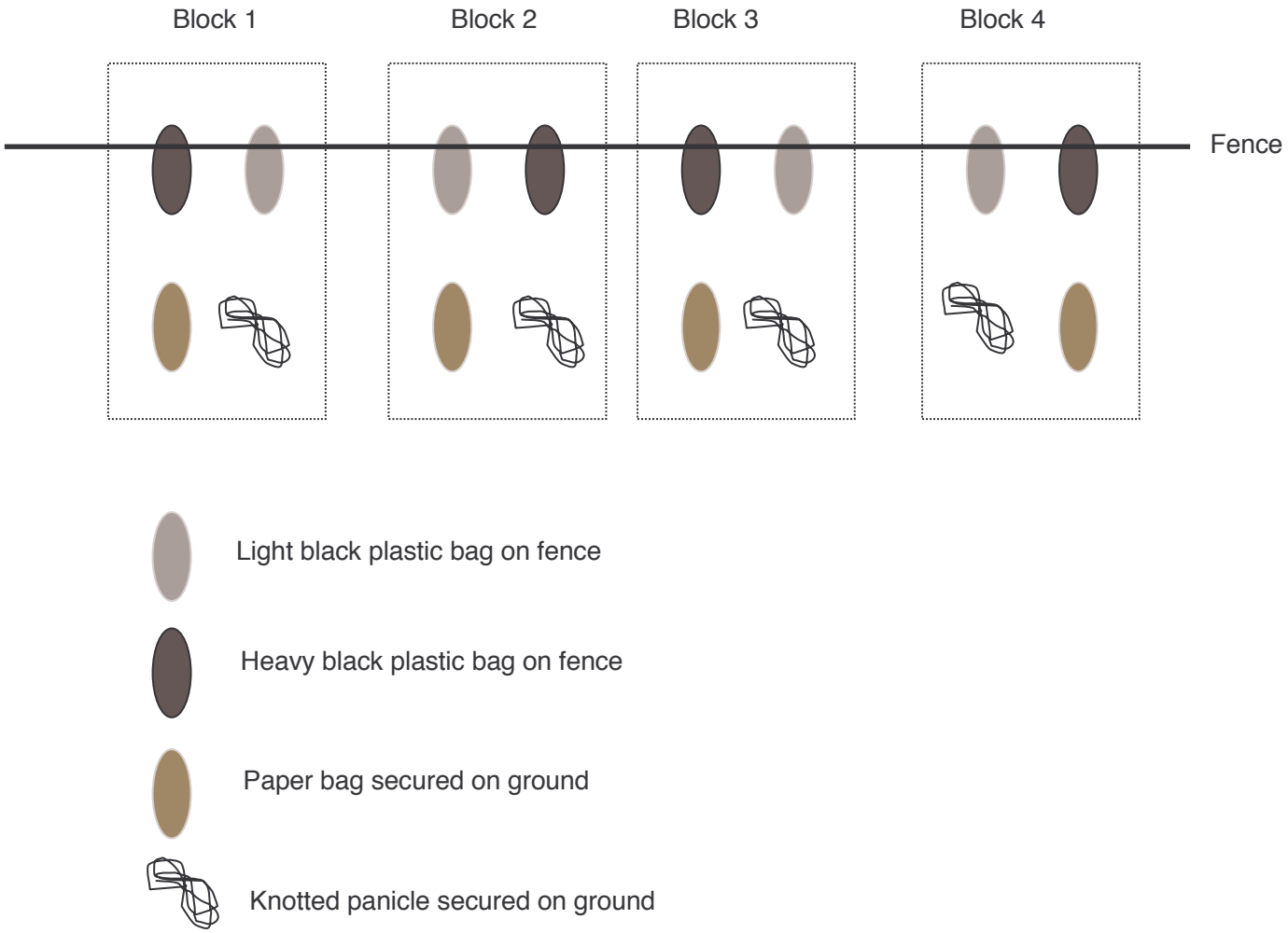
The order of setting up of the experimental treatments has been randomised, and is given on the following pages as the unique plot number (1 to 64). **All bags or knotted tussocks should be identified by their plot number.** *This order should be followed at set up and at time of sample collection (about March 31, 2010).*

An example of the set up on one farm is diagrammatically depicted below.

Procedure at time of sample collection (about March 31, 2010)

All samples should be collected in plot order and brought back to the MDC depot (after placing the knotted tussock plants into a brown paper bag labelled with the appropriate plot number). The "spare" blocks should then be stored at the depot under optimum storage conditions. The bags from the other blocks should be couriered down to AgResearch Lincoln (with care taken to avoid overheating in transit). Processing at Lincoln will then be in plot order.

Farm 1 (of 4)



Plot order (to be followed for set up and final sampling)

Farm no	Plot no	Block no	Fence/ground	Treatment	Status of block
1	1	1	Ground	Brown paper bag	Spare block
1	2	1	Ground	Knotted tussock	
1	3	1	Fence	Heavy black polythene bag	
1	4	1	Fence	Light black polythene bag	
1	5	2	Fence	Light black polythene bag	To be sampled about March 31, 2009
1	6	2	Fence	Heavy black polythene bag	
1	7	2	Ground	Brown paper bag	
1	8	2	Ground	Knotted tussock	
1	9	3	Fence	Heavy black polythene bag	To be sampled about March 31, 2009
1	10	3	Fence	Light black polythene bag	
1	11	3	Ground	Brown paper bag	
1	12	3	Ground	Knotted tussock	
1	13	4	Ground	Knotted tussock	Spare block
1	14	4	Ground	Brown paper bag	
1	15	4	Fence	Light black polythene bag	
1	16	4	Fence	Heavy black polythene bag	
2	17	5	Fence	Heavy black polythene bag	Spare block
2	18	5	Fence	Light black polythene bag	
2	19	5	Ground	Brown paper bag	
2	20	5	Ground	Knotted tussock	
2	21	6	Fence	Light black polythene bag	To be sampled about March 31, 2009
2	22	6	Fence	Heavy black polythene bag	
2	23	6	Ground	Brown paper bag	
2	24	6	Ground	Knotted tussock	
2	25	7	Fence	Heavy black polythene bag	To be sampled about March 31, 2009
2	26	7	Fence	Light black polythene bag	
2	27	7	Ground	Knotted tussock	
2	28	7	Ground	Brown paper bag	
2	29	8	Fence	Light black polythene bag	Spare block
2	30	8	Fence	Heavy black polythene bag	
2	31	8	Ground	Brown paper bag	
2	32	8	Ground	Knotted tussock	
3	33	9	Fence	Heavy black polythene bag	Spare block
3	34	9	Fence	Light black polythene bag	
3	35	9	Ground	Brown paper bag	
3	36	9	Ground	Knotted tussock	
3	37	10	Ground	Brown paper bag	To be sampled about March 31, 2009
3	38	10	Ground	Knotted tussock	
3	39	10	Fence	Heavy black polythene bag	
3	40	10	Fence	Light black polythene bag	
3	41	11	Ground	Brown paper bag	To be sampled about March 31, 2009
3	42	11	Ground	Knotted tussock	
3	43	11	Fence	Heavy black polythene bag	
3	44	11	Fence	Light black polythene bag	
3	45	12	Ground	Brown paper bag	Spare block
3	46	12	Ground	Knotted tussock	
3	47	12	Fence	Heavy black polythene bag	
3	48	12	Fence	Light black polythene bag	
4	49	13	Ground	Brown paper bag	Spare block
4	50	13	Ground	Knotted tussock	
4	51	13	Fence	Light black polythene bag	
4	52	13	Fence	Heavy black polythene bag	
4	53	14	Fence	Light black polythene bag	To be sampled about March 31, 2009
4	54	14	Fence	Heavy black polythene bag	
4	55	14	Ground	Brown paper bag	
4	56	14	Ground	Knotted tussock	
4	57	15	Ground	Brown paper bag	To be sampled about March 31, 2009
4	58	15	Ground	Knotted tussock	
4	59	15	Fence	Light black polythene bag	
4	60	15	Fence	Heavy black polythene bag	
4	61	16	Ground	Brown paper bag	Spare block
4	62	16	Ground	Knotted tussock	
4	63	16	Fence	Heavy black polythene bag	
4	64	16	Fence	Light black polythene bag	

Note that the trial design given above is also available in an excel file (from which it has been copied).

Processing at Lincoln

For each of the 32 sample bags that arrive at Lincoln in early April 2010, 30 seeds will be randomly selected and subjected to tetrazolium viability testing. The bags will be processed in plot order, to statistically remove any bias due to order of processing. The 50 seeds per farm from the initial sampling that have been stored at Lincoln will also be processed simultaneously with the seeds from newly-arrived bags for that same farm (being inserted into the sequence of plot numbers using a random number to determine its order of processing).

Total number of seeds examined at Lincoln

50 seeds x 4 farms = 200 seeds (December 2009)
32 bags x 30 seeds = 960 seeds (April 2010)
50 seeds x 4 farms = 200 seeds (April 2010)

TOTAL seeds = 1360