

## An improved method for measuring the germinable soil seed banks of tropical pastures

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### Summary

The accuracy of 2 methods for determining germinable soil seed banks was compared in soil cores containing known seed loads of 5 tropical grasses placed on sand columns in pots. A sprayed method watered soil cores for 30 minutes per day, and a capillary method watered cores by capillary action from shallow trays.

The sprayed method produced significantly ( $P < 0.05$ ) higher seedling emergence than the capillary method and gave more reliable estimates of seed load. In a second pot experiment, using soil cores from a grazing experiment, the sprayed method again resulted in significantly ( $P < 0.05$ ) higher seedling emergence than the capillary method.

We consider that the sprayed method more closely simulates the natural process in a field situation, where seeds are subjected to daily fluctuations in moisture potential rather than being continuously wet, as occurs with the capillary method.

### Introduction

The determination of both size and composition of soil seed banks is important in understanding changes in composition in grazed pastures (Harper 1977). Size and composition of the soil seed bank can be determined either by a germination method or by separation of seeds from the soil. For germination, soil cores are usually watered so that seeds germinate and seedlings are identified and counted. For separation, seeds are usually washed or floated from the soil, identified

and counted. In Australian tropical pastures, the germination method is commonly used for grass species, which have no lasting dormancy mechanisms (Orr 1991), and the separation method for legumes, which can have lasting dormancy mechanisms (Jones and Bunch 1988). Further reasons for using a germination method include: (1) ease of dealing with small seeds which are difficult to recover and count; and (2) greater confidence when dealing with a wide range of species whose seeds are difficult to tell apart.

Using a germination method, preliminary estimates of seed banks of branched wiregrass (*Aristida ramosa* var. *speciosa*) in black speargrass (*Heteropogon contortus*) grassland (Orr *et al.* 1991) and of feathertop wiregrass (*A. latifolia*) and white speargrass (*A. leptopoda*) in Mitchell grass (*Astrebla* spp.) grassland (J.R. Day and D.M. Orr, unpublished data) were low as few seedlings emerged. Similarly, no seedlings of the annual Flinders grass (*Iseilema* spp.) emerged from soil cores from north-west Queensland, even though plants of this species were recorded in the pasture (Orr 1992). A common feature of these studies was the relatively high (35%) clay content of the soils.

The germination method is unable to distinguish between soil cores which contain no seed of a particular species and those which contain seeds that fail to germinate because of seed dormancy or the technique used. Since seeds of the wiregrasses and Flinders grass should have been present in the cores described above and little dormancy would still exist in the seeds (Silcock *et al.* 1990), the failure of seedlings to emerge suggests that seeds of these species were not responding to the watering regime employed.

Both studies described above used a germination method in which soil cores remained continuously wet (Jones and Bunch 1988). Throughout our paper, this method is referred to as the capillary method. In this paper, we report the results of 2 experiments designed to compare this capillary method with an alternative method,

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referred to as sprayed; the comparison is made in terms of the germination and emergence of seed contained in soil cores.

## Materials and methods

### Germination methods

Drained plastic pots of 15 cm diameter were lined with shade cloth (to prevent loss of soil) and filled with coarse sand. This was compacted by dropping the pot on the bench until the top of the sand layer was 3 cm from the top of the pot. A 2 cm thick layer of the test soil was placed on top of the sand.

For the capillary method, pots were placed in metal trays (160 × 75 × 8 cm) in which water was maintained at a depth of 2–6 cm. Under this system, the surface remains moist because of capillary movement of water through the coarse sand. For the sprayed method, pots were placed on a steel mesh bench and watered with a fine spray by overhead sprinkler for 30 minutes each day.

Using these techniques, 2 experiments were conducted in a plant house at Brian Pastures Research Station, Gayndah (25°39'S, 151°45'E), starting in November 1991 and March 1993. No overt temperature control was used.

### Experiment 1

The 2 germination methods – capillary and sprayed – were compared using known soil seed loads of a range of tropical grasses. Estimates made by the 2 methods were compared with the known load of viable seed.

Seeds of black speargrass, branched wiregrass and dark wiregrass (*A. calycina*) were collected from a black speargrass pasture at Brian Pastures Research Station. Seeds of curly Mitchell grass (*Astrebla lappacea*) were collected near Charleville and feathertop wiregrass seed was collected near Longreach. All collections were made in autumn 1991.

The germinability of seeds of these 5 species was determined by germinating 25 seeds in each of 3 replicates for 21 days in October 1991 using a cabinet set at 30°C with alternating periods of 12 hours light/dark.

In November 1991, soil, which had been collected from the surface 10 cm of a prairie soil of 35% clay content (Uf 6, Northcote 1979) and

sterilised, was spread as a 2 cm layer on top of each of 60 pots prepared as described earlier. Using the seed viability results, sufficient seed of each of the 5 grass species was buried just below the surface of the sterilised soil layer in order that 10 seedlings should emerge per pot. To achieve this, we sowed 22 seeds of branched wiregrass, 24 seeds of Mitchell grass and black speargrass and 50 seeds of feathertop and dark wiregrass. Ten pots were not sown with seed and remained as control pots to check for contamination.

The design for Experiment 1 was, for each of the 2 germination methods, a randomised block of 5 species plus nil seed control × 5 replicates. The 30 pots for each germination method were re-randomised, within germination methods and replicates/blocks, each week for the 6 weeks of the experiment. The numbers of seedlings per pot were counted after 6 weeks of treatment.

### Experiment 2

Emergence of a range of species was compared using soil cores taken from a grazing experiment in Mitchell grass grassland at Toorak Research Station, Julia Creek (Orr 1992). The grazing experiment together with an ungrazed control was established in 1984. This vegetation type was chosen for this study because of its diverse species composition (Orr 1981) and high (60%) clay content (Turner *et al.* 1993). The unrepliated grazing experiment examined 5 different grazing pressures but the soil cores taken were replicated to allow us to test for differences between germinable soil seed loads of the treatment paddocks for each of the major pasture species.

In October 1992, before summer rainfall occurred, 24 soil samples were collected at random from each of the 6 paddocks. Each sample was a block of soil from an area of 12 × 12 cm and 2.5 cm deep. Samples were stored individually at Brian Pastures Research Station until March 1993, when all seed was at least 12 months of age and dormancy should have been overcome (Silcock *et al.* 1990).

For each of the 6 forage utilisation treatments, the 24 soil cores were randomly allocated (12 each) to the 2 germination methods. After breaking any large clods and thorough mixing, each of the 144 cores was subdivided and spread on top of coarse sand in 2 pots which were

randomly assigned to either Block 1 or Block 2 of a randomised block layout.

The complete design was therefore: 2 germination methods  $\times$  2 blocks  $\times$  12 internal replicates  $\times$  6 forage utilisation treatments, comprising a total of 288 pots; the 72 pots in each block of each watering system were fully randomised. The capillary and sprayed treatments were applied for 8 weeks.

Seedlings of each species were identified and counted after 8 weeks. Because of the relatively high density of Flinders grass, an initial plant count of this species was made after 6 weeks and these plants were removed.

#### Statistical analysis

For Experiment 1, a square root transformation (count + 0.5) of the data was applied. The data from both germination methods were analysed together, using a combined-over-methods split-plot type ANOVA model: the between-replicates (within germination methods) mean squares were pooled to provide an error term for an approximate F-test of the germination methods main effect; the main effect of species and the germination method  $\times$  species interaction were tested against the pooled experimental error.

For Experiment 2, for each species, block, germination method and paddock treatment, plant counts from the 12 individual pots were averaged and then square-root transformed (count + 0.5) prior to analysis using a split-plot

ANOVA model: 2 blocks  $\times$  2 germination methods split for 6 paddock treatments. Species data were analysed separately.

## Results

### Experiment 1

The sprayed method produced significantly ( $P < 0.05$ ) more plants than the capillary method and the species main effect was also significant ( $P < 0.05$ ) (Table 1). With the sprayed method, all 5 species approximated the target of 10 plants/pot within the 95% confidence interval. The capillary method failed to reach this target for any of the 5 species.

### Experiment 2

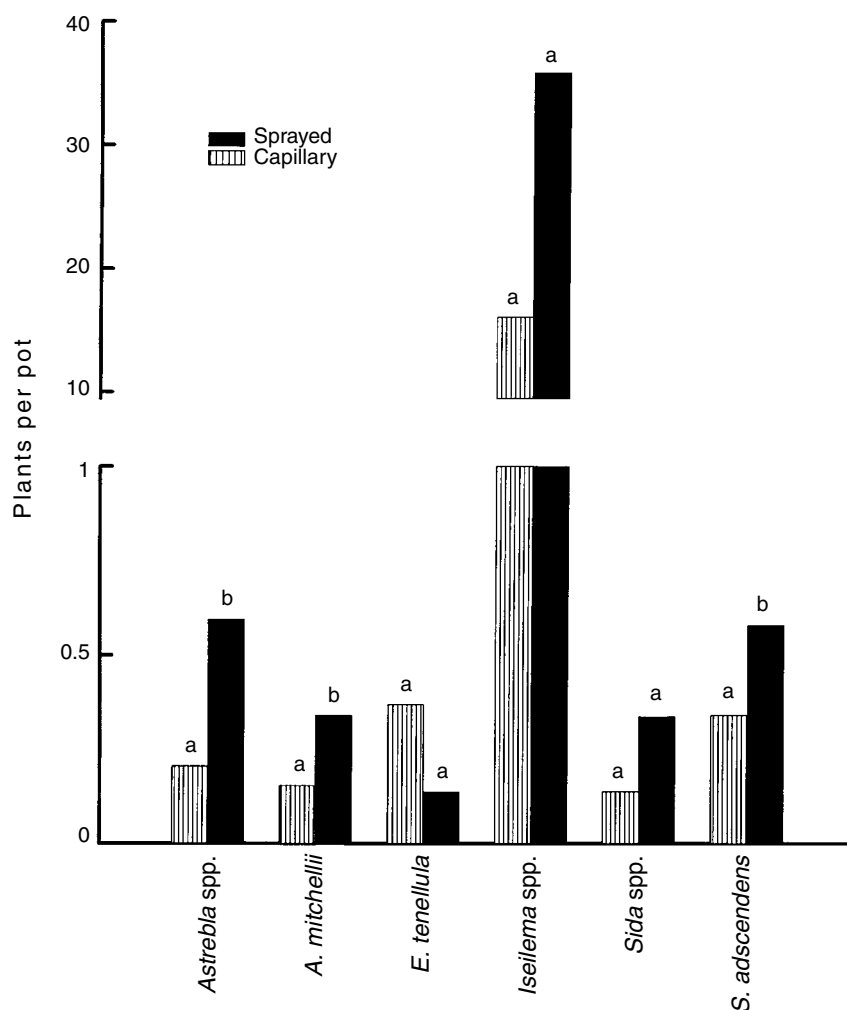
Three grasses, Mitchell grass, Flinders grass and delicate lovegrass (*Eragrostis tenellula*), and 3 forbs, mint bush (*Streptoglossa adscendens*), boggabri (*Amaranthus mitchellii*) and sida (*Sida* spp.), had an overall plant density greater than 0.25 plants per pot (equivalent to approximately 20 seeds/m<sup>2</sup>). Of these, Mitchell grass, boggabri and mint bush had a significantly ( $P < 0.05$ ) higher plant density under the sprayed than the capillary method (Figure 1). For both Flinders grass and sida, the mean density under spraying was twice that with capillary watering, but the difference was not statistically significant ( $P > 0.05$ ).

**Table 1.** Mean number of seedlings per pot after 6 weeks with 2 germination methods. [Values are square-root transformed (count + 0.5) data with back-transformed data in brackets].

Species	A.cal <sup>1</sup>	A.ram	A.lat	H.con	A.lap	Mean <sup>2</sup>
Germination method						
Capillary	1.39 (1.4)	1.16 (0.8)	1.14 (0.8)	1.88 (3.1)	1.60 (2.1)	1.43b (1.6)
Sprayed	3.36 (10.8)	3.32 (10.5)	2.67 (6.6)	3.88 (14.6)	3.86 (14.4)	3.42a (11.2)
Mean	2.38abc (5.2)	2.24bc (4.5)	1.91c (3.1)	2.88a (7.9)	2.73ab (7.0)	

<sup>1</sup>A.cal = *Aristida calycina*, A.ram = *Aristida ramosa* var. *speciosa*, A.lat = *Aristida latifolia*, H.con = *Heteropogon contortus*, A.lap = *Astrelba lappacea*.

<sup>2</sup>Marginal means followed by different letters are significantly different ( $P < 0.05$ ). The species  $\times$  germination method interaction was not significant ( $P > 0.05$ ).



**Figure 1.** Seed bank (plants/pot) of 6 species from *Astrebla* grassland using 2 methods of seed germination (Experiment 2). Within a species, germination methods labelled with different letters are significantly different ( $P < 0.05$ ).

## Discussion

This study has shown quite conclusively that more seedlings emerged under the sprayed than the capillary germination method, and that plant densities with the sprayed method were close to the potential predicted from a preliminary germination test.

In Experiment 2, seedling densities of both Flinders grass and sida under the sprayed method were twice those under the capillary method but differences were not significant. This may be a function of the limited precision of the significance test of the germination methods with

only one error degree of freedom. Although the difference in Flinders grass was not statistically significant, we consider it was biologically significant.

Reduced emergence and survival of seedlings under the capillary method probably occurred because seeds were kept continuously wet, whereas seeds under the sprayed method were moistened daily and then experienced progressively greater moisture potential as moisture evaporated.

The daily wetting and drying with our sprayed method may also promote seed germination. In Experiment 1, we aimed to achieve 10 plants per

pot based on the initial germination test. However, Mitchell grass, dark wiregrass and black speargrass achieved higher plant densities in our sprayed treatment suggesting that germination in these grasses may be promoted either by prolonged cycles of wetting and drying or alternating temperatures. An alternative explanation could be that seeds of these species usually overcome innate dormancy at this time of year (October–November) and this may be reflected in the higher seedling emergence we recorded 1 month later.

The dead seedlings of branched wiregrass and feathertop, observed at the time of seedling counts in the capillary pots in Experiment 1, indicate that seedlings were damped off, possibly by microbial or fungal attack and possibly because the soil remained too wet. This may be particularly important for species of wiregrass as we observed no deaths in seedlings of either Mitchell grass or black speargrass. The disadvantages (poor aeration) of the capillary method were probably exacerbated by the use of clay soils, which allow closer contact between the germinating seed and the continuously wet clay particles than would occur in coarse-textured soils.

The sprayed method was clearly superior in clay soils in our experiments, but the capillary method has been used successfully in coarse-textured soils. For example, total seed levels of 40 000 and 45 000 seeds/m<sup>2</sup> have been recorded in light soils from tropical and subtropical pasture systems using the capillary method (McIvor and Gardener 1991; Jones *et al.* 1991). Clearly, more research is necessary to determine the interaction between soil types and germination methods.

The role of residual dormancy in seeds present in soil cores was not addressed in our study. However, we subjected cores from Experiment 2 to a second cycle in March–April 1994 and found that at least 90% of the total grass seed bank had emerged in the first year. Similarly, Andrew (1988) reported that 95% of Flinders grass (*I. fragile*) and 40% of forb seed banks emerged in the first year of a 3-year study.

The results of these experiments have shown that the sprayed watering of soil samples will result in a reliable assessment of the size and

composition of the germinable seed banks in soils supporting tropical pastures.

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