Dispersal of buffel grass (*Cenchrus ciliaris* L.) by ants in Shark Bay, Western Australia

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Summary Despite its continued usefulness to the pastoral industry, buffel grass (*Cenchrus ciliaris* L.) is now recognised as one of the greatest environmental threats to the rangelands of Australia. The aim of this project was to understand the mechanisms of buffel grass seed dispersal in the field. Exclusion cages were placed in buffel grass infested areas with 150 seeds in each. After five days seeds were collected and counted to determine the amount removed. Ants were actively involved in buffel grass seed removal and many seeds were found on and in ant middens and in nests. At least seven seed collecting genera of ants were captured within buffel grass infestations. Buffel grass fascicles collected from ant nests and middens were empty or contained a high proportion of rotten or non-germinating seed.

Keywords Buffel grass, *Cenchrus ciliaris*, seed predation, ants.

INTRODUCTION

Perennial grasses are one of the largest threats to rangeland sustainability however, many of these are still considered useful pasture plants (Grice and Martin 2005). One such controversial species is buffel grass (*Cenchrus ciliaris* L.). Following its accidental introduction to Australia in the 1870s, in 1910 buffel grass was adopted for use in pastoral production for pasture improvement and soil conservation (Marriott 1955). However, buffel grass now occupies a substantial area outside of that used for grazing, impacts native plant biodiversity and is increasingly becoming recognised as one of the greatest threats to rangeland biodiversity (Grice and Martin 2005, Jackson 2005, Friedel *et al.* 2006).

Rapid growth and reproduction allow buffel grass to spread and establish quickly and enables this grass to out compete many native species (Jackson 2005). Buffel grass is a prolific seed producer, yielding between 490–2300 seeds m^{-2} (Hacker 1989). However, only a relatively small proportion, 60–860 viable seeds m^{-2}, have been recovered from the seed bank (Franks 2002) suggesting that a large proportion of seed is removed from the seed bank. Discovering factors that may decrease the soil seed bank of buffel grass could be very useful in developing management plans for this plant in conservation areas.

Seed removal by granivores is one means by which the seed bank can decline. Seed predation is known to occur in natural and agricultural systems across Australia (Predavec 1997, Spafford Jacob *et al*. 2006). There are several granivores in Australia including invertebrates such as ants, beetles, grasshoppers and vertebrates such as mice, rabbits and birds (Predavec 1997, Spafford Jacob *et al*. 2006). The degree of seed predation and the animals likely to consume buffel grass seeds in Australia are unknown.

Parts of the Shark Bay World Heritage Area were previously pastoral lands with large buffel grass pastures; buffel grass persists in these areas today. In this study, buffel grass seed removal was assessed using exclusion cages placed within buffel grass infestations in the Shark Bay World Heritage Area and ants were collected. In the same area, buffel grass seeds were collected from ant nests and middens, counted, and tested for their germination and viability.

MATERIALS AND METHODS

Exclusion cage study In March to June 2007, seed dispersal and predation were measured across six different sites in buffel grass invaded areas in the Shark Bay World Heritage Area. At each of the six sites a 100 m long transect was set up. Points along each transect at 0, 25, 50, 75 and 100 m were marked with a stake. At each point along the six transects, one of each of three types of exclusion cage were placed. The three types of exclusion cages were a small invertebrate access cage (excluding vertebrates), a vertebrate access cage (excluded small invertebrates) and an open cage (did not restrict any granivores) (refer to Spafford Jacob *et al*. 2006 for a description of the cages). Exclusion cages were placed randomly at least one metre away from each other. All cages were placed in a slight soil depression or near vegetation to reduce fascicle removal by wind. Cages were left to settle for two days prior to the commencement of the experiment.

After settling, 150 buffel grass fascicles were placed in each cage and left for five days. A fascicle consists of one or more seeds surrounded by paleas, lemmas and glumes in a cluster of bristles; fascicles used contained an average of two seeds (Hacker 1989). Buffel grass seeds were sourced from Ballard Seeds, Narrogin, Western Australia. At the end of the five day period all remaining fascicles were collected from the cages and placed into zip-lock bags. This procedure was repeated twice, such that there were three collection periods, one in March, May and June 2007. Each collection period was separated by at least 30 days. Exclusion cages were left in place between collections until they were removed after the final collection in June. Remaining fascicles were put through 2 mm and 1 mm sieves to remove any sand and other debris. Samples were then counted and recorded for the number of fascicles remaining. A repeated measures one-way ANOVA was used to determine if the seed removal was different between each cage type over time (Genstat 9, Lawes Agricultural Trust 2003).

Collection of ants Over the five days of the study period the ant community of each transect was sampled by walking approximately parallel to the transect line (5 m away) and collecting ants with aspirators. Collections were made at multiple times during the day and night to ensure capture of a wide cross section of the ant community. Ants were identified to genus (Shattuck 1999). The presence/absence of ants in each genus was calculated for each transect in each sample period (March, May and June). Therefore, there were 18 samples of the ant community. The similarities between ant communities between the transect samples over time were compared using multivariate analyses (Primer 6, Primer-E Ltd 2007).

Germination of seed ant nests and middens In areas near transects, several ant nests were carefully excavated using a shovel. Other samples were taken from ant middens. Samples were sieved to remove any sand and other debris. The number of fascicles were then counted and recorded and then were tested for their germination and viability. An additional 100 fascicles were selected from stock seed purchased from a commercial supplier (Ballard Seed, Narrogin, Western Australia) and used as a control. Fascicles were placed on moist paper towel and rolled, then placed in sealable plastic bags. These were stored upright in the incubator which was set at 20°C for 16 hours and 30°C for eight hours on a 24 hour cycle, as per international seed testing guidelines (ISTA 1999). Samples were checked every seven days and germination was recorded for a period of 28 days. At the end of the 28 days non-germinated fascicles were examined for seed, any intact seed was extracted for tetrazolium testing (ISTA 1999). Viability was determined by longitudinally bisecting the seed and examining the embryo for stain uptake (ISTA 1999). Rotten or empty fascicles were also noted. Seed was deemed rotten if obvious fungal infection was observed, or if seed was soft and mushy. Fascicles were noted as empty if no evidence of a seed could be found within the fascicle. Thus an individual fascicle was classified as germinated, non-germinated but viable, non-germinated and non-viable, rotten, or empty.

RESULTS

Exclusion cages Across all study periods an average of 61.9% of seed was removed from the open cages, 29.7% from the small invertebrate access cages and 5.5% from the vertebrate access cages. Across the entire study area an average of 44.6% of buffel grass fascicles were removed by ants and small invertebrates over the three study periods There was a significant interaction between cage type and the sample period on the number of seed removed ($F_{4,174}$ = 10.8, P-value <0.001) (Figure 1). Seed removal in the vertebrate access cages was highest in March and there was no real difference in seed removal between months (Figure 1). For the other two cage types the rate of removal was highest in June, then March, with the lowest rate of removal in May (Figure 1). Of the total amount of fascicles removed from all exclusion cages across all study periods 63.7% were from open access cages, 30.6% from invertebrate access cages and 5.6% from vertebrate access cages.

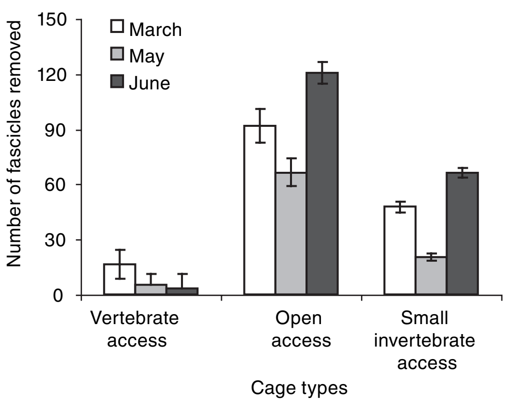

Figure 1. Seed removal from each of the different cage types for each sample period. (Time*Cage type LSD = 17.27).

Collection of ants A total of 1912 ants were collected in the transect sites. Ants in fourteen genera and four sub families were found at these sites. Of those, ants from the genera *Pheidole*, *Rhytidoponera*, *Monomorium*, *Tetramorium*, *Meranoplus*, *Melophorus* and *Iridomyrmex* are known seed consumers. The ant communities were different between months (ANOSIM: Rho = 0.5, P-value = 0.03). The ant communities in June and March were different from one another (R = 0.236, P-value = 0.035) but were not different between March and May (R = 0.06, P-value = 0.27) or May and June (R = −0.11, P-value = 0.78). The ant communities were similar across sites (ANOSIM: Rho = 0.15, P-value = 0.16).

Germination of seed from ant nests and middens Seed was recovered from the nests of ants in three genera: *Pheidole, Iridomyrmex* and *Polyrachis*. Compared to the control of stock seed (46.25% ± 2.75), seed taken from ant nest and middens had a low germinability (Figure 2). The highest germination percentage of buffel grass seed, other than the stock seed, was in that taken from the *Iridomyrmex* ant middens (8.3% ± 2.06). Germination of seed taken from the *Pheidole* midden samples was quite low (2% ± 1). However, the lowest germination percentage was from the *Polyrachis* ant midden, where no seed germinated at all.

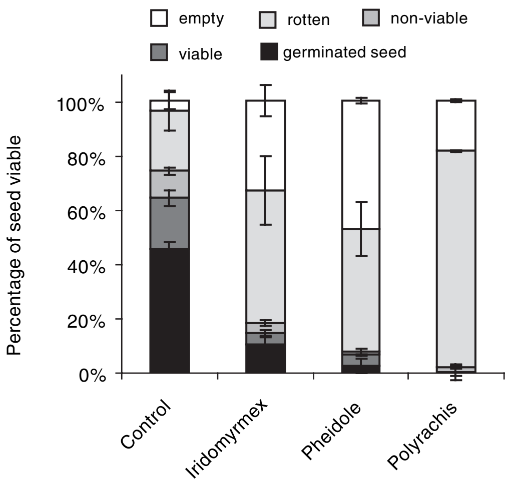

Figure 2. Percentage of seed germinated, rotten, empty, non-germinated but viable and non-germinated and non-viable from each sample. *Iridomyrmex*, *Polyrachis* and *Pheidole* samples were all taken from ant middens or nests of these genera. Rotten, non-viable and viable make up the total non-germinated seed for each sample.

Of the seed that did not germinate 29% ± 3 of the stock seed (control) remained viable; the remaining fascicles contained non-viable or rotten seed (Figure 2). Few fascicles were empty (Figure 2). In comparison, the samples collected from ant nest and middens had a greater proportion of empty fascicles and more rotten seeds (Figure 2). Proportionally, the number of viable and non-viable seeds that did not germinate also appeared to be lower if the seeds were collected from ant nest or middens. For example, only 6.67% ± 0.92 of the non-germinated seed collected from *Iridomyrmex* ant middens remained viable, whereas, 2.9% ± 0.95 was non-viable. Only 4% ± 2.5 of intact non-germinated seeds from *Pheidole* middens were viable but 0.75% ± 0.25 was non-viable. There were very few intact non-germinating seeds collected from *Polyrachis* middens (Figure 2).

DISCUSSION

The amount of buffel grass seed removed was higher from small invertebrate access cages than vertebrate access cages suggesting that small invertebrates such as ants are the main collectors of buffel grass seed. It is clear, based on the results from the exclusion cage field experiment, observations of ants carrying buffel grass seeds, and the recovery of large numbers of seeds from middens and nests, that ants are actively removing and may be consuming buffel grass seeds in the Shark Bay area. However, despite the obvious involvement of ants in seed removal, it was not clear which genera were most responsible for seed removal.

Although, the ant community changed over time, there did not appear to be a relationship between particular ant genera and rates of removal. Further work may indicate a dominant seed collector for buffel grass. But for now it appears that a multitude of genera may be involved with buffel grass removal.

Of the genera found in close association with buffel grass infestations, the greatest percentages of empty fascicles were in the ant midden samples from *Pheidole* and *Iridomyrmex* middens, suggesting that these ants are actually consuming buffel grass seed. *Pheidole* are known seed consumers and are abundant across most environments in Australia (Shattuck 1999). *Iridomyrmex* are generalist scavengers that also have a wide distribution across Australia (Shattuck 1999). Fascicles were also found in middens of *Polyrachis*. However most of these fascicles contained rotten seed rather than being empty. This suggests that although *Polyrachis* ants are likely to gather seed they may not consume it. Crist and Friese (1993) found that ants preferred uninfected seed to infected seed. This may have been the case for *Polyrachis*, thus there were more rotten seed rather than empty fascicles. Further investigation is required to determine the proportion of buffel grass seed in the diets of these ants.

Seed found in ant middens had a relatively low percentage of germination and viability compared with the stock seed (control). The low germination and viability may be due to exposure to high temperatures and variable conditions, characteristic of Shark Bay area. It may be that the reduction in germination and viability is due to burial by the ants in the case of the seeds collected from within nests or exposure as in the case of the seeds collected from middens. A glasshouse experiment has shown that burial of buffel grass seed below 2 cm or leaving it on the surface does reduce germination and viability (P. Goldsmith 2007 pers. comm.).

A large number of rotten seeds were also observed in samples collected from the nests and middens. Factors that affect seed survival in soil are soil moisture, aeration fertility and activity of soil microorganisms. Humidity in ant nests is usually higher than the outside environment, making conditions more favourable for pathogens to infect stored seed. Furthermore, small seed, like buffel grass seed, may be more likely to be decomposed by pathogens (Crist and Friese 1993). Dostál (2005) found higher seed mortality in ant mounds compared with inter-mound spaces in a temperate mountain grassland. Identification of pathogens that decrease buffel grass seed viability would be useful towards management of the seed bank. However, it may be that the dramatic effects of the pathogen were only evidenced once the seeds that had been in the ant middens (and in contact with the pathogen) were exposed to laboratory conditions. Consequently, field studies of pathogen infection and its impact on buffel grass seed germination and viability are warranted.

The greatest amount of seed was removed from open access cages, probably due to several vectors working together. Spafford Jacob *et al*. (2006) similarly found the greatest seed removal from open access cages placed in an agricultural field. Of the 60% of fascicles removed from open cages in our study we can assume that 30% were removed from open cages by small invertebrates and 5% by small vertebrates. This leaves 25% removed by unknown vectors. No day during any of the study periods (March, May and June) was calm (average wind speeds ranged from 12–37 km h^{-1}) and during the study periods seed was occasionally observed being blown out of exclusion cages. Further investigation may be required to adequately assess the removal of seed by wind or to stop wind removal from exclusion cages, allowing a more accurate measure of seed removal by granivores.

The results of this study indicate that ants are dispersers of buffel grass seed and ants also reduce the viability of buffel grass seed probably through burial or placement in middens. In addition fungal or bacterial pathogens also reduce the viability of buffel grass seed even of seed on the soil surface. The combined activity of ants and pathogens has the potential to reduce the buffel grass seed bank substantially.

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