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2 **Ant preference for seeds without awns increases removal of exotic relative to native grass**
3 **seeds**

4 **Running title:** Granivorous ants prefer exotic seeds

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16 **Figures:** 2

17 **Abstract**

- 18 1. The removal of seeds by granivorous ants can affect plant recruitment through either
19 seed loss from predation or the dispersal and recruitment of seeds that are removed but
20 not consumed. Consequently, variation in ant selection preferences can influence
21 patterns of seed removal and affect plant community composition, including the spread
22 of exotic plant species.
- 23 2. We conducted a seed removal experiment to determine whether: 1) rates of removal by
24 ants differed between three native and three exotic grass species in an Australian

25 temperate grassland; and 2) differences in removal rates were associated with the
26 presence or absence of awns.

27 3. We found that seeds of the three exotic species, none of which had awns, were removed
28 by ants at a higher rate than those of the three native species, all of which had awns.
29 Removal rates of native species increased when awns were manually removed,
30 suggesting the awns of native species acted as a removal barrier.

31 4. While we do not know the fate of seeds removed from our experiment, differences
32 among species in removal rates mirrored differences in their spatial spread in a separate
33 seed addition experiment. Exotic species removed by ants at a higher rate in the removal
34 experiment had more widely dispersed seedlings than native species in the seed addition
35 experiment, potentially indicating a role for granivorous ants in dispersing exotic seeds.
36 Identifying ant selection preferences and directly linking removal to seed fate could
37 help explain how exotic grass species move around the landscape.

38 **Keywords:** ant-plant interactions; Australian grasslands; dyszoochory; plant invasion; seed
39 dispersal.

40 **Introduction**

41 Seed removal by granivorous ants can influence plant community composition (MacMahon *et*
42 *al.*, 2000). Two processes are central to this role. First, ants preferentially remove seeds of
43 different species depending on morphological and physiological characteristics (Pulliam &
44 Brand, 1975; Kelrick *et al.*, 1986). Second, the fate of seeds removed by ants can vary
45 depending on whether they are consumed or dispersed (MacMahon *et al.*, 2000). While seed
46 removal by granivorous ants usually results in seed loss through predation, ants can also drop
47 seeds in transit or fail to eat all seeds they store in nests (Retana *et al.*, 2004; Anjos *et al.*, 2020),
48 leading to seed dispersal (dyszoochory).

49 Because ant predation and dispersal can alter spatial patterns of plant recruitment, identifying
50 what drives preferential seed removal by ants could help understand the role of ants in
51 structuring plant communities. In particular, ants could influence the spread of exotic plant
52 species if they preferentially remove either native or exotic seeds (Pearson *et al.* 2018). Humans
53 often introduce exotic species with desired characteristics (e.g. rapid growth or germination)
54 that differ from resident native species (Driscoll *et al.*, 2014). If there are differences between
55 native and exotic seed traits, interactions with granivorous ants may also differ. While several
56 studies report preferential removal of exotic relative to native seeds by granivorous ants
57 (Pearson *et al.*, 2014; Briggs & Redak, 2016), few identify trait differences that might underpin
58 these preferences.

59 We aimed to identify whether ant selection preferences differed between common, co-
60 occurring native and exotic grass species in the Australian Capital Territory (ACT). In the
61 ACT, exotic grasses have been widely introduced as pasture supplements. Many of these
62 grasses have spread into native grasslands, particularly where human land-use has increased
63 moisture or nutrient availability. In the grasslands we studied, an obvious morphological
64 difference was that the seeds of many native grass species had conspicuous awns, while most
65 exotic species lacked awns. We speculated that removal rates would differ between native and
66 exotic species depending on the presence of awns.

67 **Materials and Methods**

68 *Seed removal experiment*

69 We conducted a seed removal experiment in Austral summer 2018 in grassland on the
70 University of Canberra campus, ACT. We selected three native (*Bothriochloa macra*, *Chloris*
71 *truncata*, *Rytidosperma caespitosum*) and three exotic (*Dactylis glomerata*, *Eragrostis curvula*,
72 *Phalaris aquatica*) grass species that co-occur locally (Wandrag *et al.*, 2019). Seeds of the
73 three native species have conspicuous awns (Figure 1) such that the unit of dispersal (diaspore;

74 Peart, 1979) comprises the seed plus awn. The three exotic species lack awns. Diaspore weights
75 varied among species (Appendix S1). No species were adapted for ant dispersal
76 (myrmecochory), so we assumed seed removal was by granivorous or omnivorous ants. We
77 caught *Pheidole* spp. and *Iridomyrmex rufoniger* removing seeds. Both species remove seeds
78 in Australia (Cooke *et al.*, 2012), though the omnivore *I. rufoniger* is often associated with
79 scavenging and nectar harvesting (Briese & Macauley, 1981).

80 We compared removal rates of diaspores that were: 1) unmodified (native diaspores comprised
81 seeds plus awns); and 2) modified such that we manually removed awns and other appendages
82 from native seeds. This gave a total of nine treatments (unmodified diaspores for all six species
83 plus modified diaspores for the three native species). For each treatment, we placed 100
84 diaspores in a closed Petri-dish with two holes approximately 0.5 cm high and 2 cm wide cut
85 into the side, allowing ant access but excluding larger animals (Andersen & Ashton, 1985;
86 Wandrag *et al.*, 2013). To estimate diaspore loss due to factors other than ant removal (e.g.
87 wind), each dish was paired with a no ant-access control: a dish containing 100 diaspores placed
88 on a sheet of plastic coated with insecticide (Go Natural Tree Guard). A nail pushed through a
89 central hole secured dishes in place, entry holes were flush with the ground.

90 We carried out the experiment over nine days (between 6-19 April 2018), each day laying out
91 18 Petri-dishes (one for each treatment plus its associated no-access control). Paired dishes
92 were located by dividing the study area (approximately 50 m²) into 1 m grid-squares and
93 randomly assigning each treatment to the corner of a square. Dishes were put out at 1 pm each
94 day and collected at 11 am the following morning. After recording the number of diaspores
95 remaining in each dish, we randomly assigned treatments to a new location.

96 *Data analysis*

97 Each treatment was paired with a no-access control. For each pair of dishes on each day, we
98 estimated diaspore removal by ants ('removal rate') as the number of diaspores lost from
99 treatment dishes minus the number lost from their corresponding no-access control (Appendix
100 S2). Using this estimate as our response variable, we fitted two models within a Bayesian
101 framework (Appendix S3) using the software JAGS v4.2.0 (Plummer, 2003) implemented in
102 R (R Development Core Team, 2020) using jagsUI v1.5.1 (Kellner, 2015). This allowed us to
103 account for non-independence in the data due to observations being clustered by day and to
104 propagate all uncertainties associated with estimating the parameters.

105 The first model estimated mean removal rates and associated credible intervals for unmodified
106 diaspores of each species. We included 'species' as a categorical predictor variable and
107 modelled variation in removal rate as normally distributed. To account for non-independence
108 in the data due to measurements taken on the same day we included 'day' as a random effect,
109 specifying a different mean for each day with those means drawn from a normal distribution
110 with mean zero and standard deviation estimated from the data. We assigned the mean for each
111 species a normal prior distribution having mean zero and variance 100 and assigned the
112 standard deviation for the 'day' term a uniform prior (0 – 100).

113 The second model estimated mean removal rates for exotic diaspores, and for modified and
114 unmodified native diaspores ('treatments'). We again modelled variation in removal rates as
115 normally distributed but set 'treatment' as the predictor variable and included both 'species'
116 and 'day' as random effects. We assigned the mean for each treatment a normal prior
117 distribution with mean zero and variance 100 and assigned random effects a non-informative
118 uniform prior (0 – 100) on the standard deviation.

119 **Results and Discussion**

120 Seed removal rates differed for native and exotic seeds. While on average between 20 (*Dactylis*
121 *glomerata*) to 35 (*Phalaris aquatica*) more exotic diaspores were lost from dishes with ant
122 access relative to no-access controls (Figure 2A, the 95% credible intervals did not include
123 zero), losses of unmodified native diaspores differed little between ant access and no-access
124 controls (Figure 2B).

125 We found strong evidence that awns reduced removal rates. While losses from control dishes
126 were similar for both modified (awns removed) and unmodified native diaspores (Appendix
127 S4), modified native diaspores were over five times more likely to be removed from treatment
128 dishes than unmodified native diaspores (Figure 2C). Awn removal could not completely
129 account for differences in native and exotic removal rates. Removal rates for modified native
130 diaspores were still around half those of exotic diaspores, possibly due to unmeasured seed
131 traits, such as nutritional content (Kelrick *et al.*, 1986).

132 Granivorous ants act as both predators (Pearson *et al.*, 2014) and dispersers (Eminniyaz *et al.*,
133 2013) and removal rates alone do not tell us about seed fate. Interpreting our results in the
134 context of a separate, seed addition experiment (Appendix S5) suggests that in our system,
135 granivorous ants could disperse exotic grass seeds. Specifically, four months after adding seeds
136 of the same six species used here to grassland plots, we recorded three species (*D. glomerata*,
137 *P. aquatica* and *C. truncata*) establishing in >45% of plots to which seeds were not originally
138 added. For the native *C. truncata*, establishment in new plots appeared due to stoloniferous
139 spread from seed addition plots. However, the exotic *D. glomerata* and *P. aquatica* frequently
140 recruited near ant nests in plots to which seeds were not added. Given the results in the
141 experiment we present here, we speculate that this movement was due to dispersal by
142 granivorous ants.

143 Although ant dispersal occurs over relatively short distances, it can enhance recruitment by
144 moving seeds to microsites that promote germination and survival. For example, ant nests
145 typically have higher moisture content than surrounding soils (MacMahon *et al.*, 2000),
146 promoting recruitment in arid locations (Farji-Brener & Werenkraut, 2017). Consequently,
147 understanding the dual role of ants as predators and dispersers (Anjos *et al.*, 2020) may help
148 understand the success and failure of exotic species' spread. This is particularly important in
149 places like Australia, where many non-native species have spread into native plant
150 communities. The potential for granivorous ants to play a dual role in plant invasions,
151 functioning as both predators and dispersers of exotic seeds, remains largely unexplored. Our
152 finding that ants preferentially removed seeds of common exotic species, together with the
153 observation that species with high ant removal rates were also widely dispersed in our seed
154 addition experiment, highlights the need to explore this role. This will require tracking seed
155 fate and directly linking seed removal to recruitment outcomes.

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159 identification. We have no conflict of interest to declare.

160 **Data Accessibility Statement:** Data is available as Supplementary Material (Data S1).

161 **Author contribution:** This study formed HRC's third year research project as part of a larger
162 study looking at exotic plant invasion in Australian grasslands. HRC, RPD and EMW designed
163 the study, HRC collected the data, HRC, RPD and EMW analysed the data. EMW wrote the
164 first draft of the manuscript and all authors edited the manuscript.

165 **Supporting Information:**

166 **Appendix S1.** Relationship between mean diaspore weight and diaspore removal rates.

167 **Appendix S2.** Histogram of the raw data.

168 **Appendix S3.** Details of the analysis used in the main manuscript.

169 **Appendix S4.** Diaspore losses from control dishes.

170 **Appendix S5.** Seed addition experiment.

171 **Data S1:** Raw data.

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226

227 **Figure Legends.**

228 **Figure 1.** Seeds of each of the three exotic (a; left to right: *Dactylis glomerata*, *Eragrostis*
229 *curvula* and *Phalaris aquatica*) and three native (b; left to right: *Bothriochloa macra*, *Chloris*
230 *truncata* and *Rytidosperma caespitosum*) grass species used in this experiment. Scale bar in
231 each photo shows 1 mm intervals. Photo credit: Philip Barton.

232 **Figure 2.** Mean difference in the number of seeds removed (out of 100) from dishes where ants
233 had access relative to no-access controls for three exotic (a) and three native grass species (b),
234 and mean removal rate for each treatment group (c): unmodified exotic (Exotic), modified
235 native (awns removed: Native -) and unmodified native (awns intact: Native +) species. Thin
236 bars represent 95% credible intervals (there is a 95% chance that the interval contains the true
237 value), thick bars represent 50% credible intervals. Credible intervals that do not cross zero
238 indicate that seed losses from treatment dishes did not differ from seed losses from no-access
239 controls.

240 **FIGURE 1**



241

242 **FIGURE 2**

