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Version: Accepted Version

Article:
Wandrag, Lizzie, Copeland, Hannah and Duncan, Richard (2020) Ant preference for seeds without awns increases removal of exotic relative to native grass seeds. Ecological entomology. ISSN: 0307-6946

https://doi.org/10.1111/een.12980

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Accepted for publication at Ecological Entomology, November 2020.

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

Running title: Granivorous ants prefer exotic seeds

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Words: 1490

References: 20

Figures: 2

Abstract

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

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

3. We found that seeds of the three exotic species, none of which had awns, were removed by ants at a higher rate than those of the three native species, all of which had awns. Removal rates of native species increased when awns were manually removed, suggesting the awns of native species acted as a removal barrier.
4. While we do not know the fate of seeds removed from our experiment, differences among species in removal rates mirrored differences in their spatial spread in a separate seed addition experiment. Exotic species removed by ants at a higher rate in the removal experiment had more widely dispersed seedlings than native species in the seed addition experiment, potentially indicating a role for granivorous ants in dispersing exotic seeds. Identifying ant selection preferences and directly linking removal to seed fate could help explain how exotic grass species move around the landscape.

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

Introduction

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

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

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

Materials and Methods

Seed removal experiment

We conducted a seed removal experiment in Austral summer 2018 in grassland on the University of Canberra campus, ACT. We selected three native (*Bothriochloa macra*, *Chloris truncata*, *Rytidosperma caespitosum*) and three exotic (*Dactylis glomerata*, *Eragrostis curvula, Phalaris aquatica*) grass species that co-occur locally (Wandrag *et al.*, 2019). Seeds of the 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 m^2) 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*

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

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

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

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.

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

Acknowledgements: This work was funded by Australian Research Council Grant DP150101839 to RPD, funding from the University of Canberra to RPD, and a University of New England Postdoctoral Fellowship to EMW. We thank Jon Lewis and Philip Barton for ant identification. We have no conflict of interest to declare.

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

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

Supporting Information:

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

Appendix S2. Histogram of the raw data.

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

Appendix S4. Diaspore losses from control dishes.

Appendix S5. Seed addition experiment.

Data S1: Raw data.

References

Andersen, A.N. & Ashton, D.H. (1985) Rates of seed removal by ants at heath and woodland sites in southeastern Australia. *Australian Journal of Ecology*, **10**, 381–390.

Anjos, D.V., Andersen, A.N., Carvalho, R.L., Sousa, R.M.F. & Del-Claro, K. (2020) Switching roles from antagonist to mutualist: a harvester ant as a key seed disperser of a myrmecochorous plant. *Ecological Entomology*, **45**, 1063–1070.

Briese, D.T. & Macauley, B.J. (1981) Food collection within an ant community in semi-arid Australia, with special reference to seed harvesters. *Australian Journal of Ecology*, **6**, 1–19.

Briggs, C.M. & Redak, R.A. (2016) Seed selection by the harvester ant Pogonomyrmex rugosus (Hymenoptera: Formicidae) in coastal sage scrub: interactions with invasive plant species. *Environmental Entomology*, **45**, 983–990.

Cooke, J., Ash, J.E. & Groves, R.H. (2012) Population dynamics of the invasive, annual species, Carrichtera annua, in Australia. *Rangeland Journal*, **34**, 375–387.

Driscoll, D.A., Catford, J.A., Barney, J.N., Hulme, P.E., Inderjit, Martin, T.G., *et al.* (2014) New pasture plants intensify invasive species risk. *Proceedings of the National Academy of Sciences*, **111**, 16622–16627.

Eminniyaz, A., Qiu, J., Tan, D., Baskin, C., Baskin, J. & Nowak, R. (2013) Dispersal mechanisms of the invasive alien plant species Buffalobur (Solanum rostratum) in cold desert sites of Northwest China. *Weed Science*, **61**, 557–563.

191 Farji-Brener, A.G. & Werenkraut, V. (2017) The effects of ant nests on soil fertility and plant
192 performance: a meta-analysis. *Journal of Animal Ecology*, **86**, 866–877.
193 Kellner. (2015) *A wrapper around rjags to streamline JAGS analyses.* R package version
194 1.3.1. Rerieved from https://github.com/kenkellner/jagsUI.
195 Kelrick, M.I., Macmahon, J.A., Parmenter, R.R. & Sisson, D. V. (1986) Native seed
196 preferences of shrub-steppe rodents, birds and ants: the relationships of seed attribtues and
197 seed use. *Oecologia*, **68**, 327–337.
198 MacMahon, J.A., Mull, J.F. & Crist, T. (2000) Harvester ants (Pogonomyrmex spp.): their
199 community and ecosystem influences. *Annual Review of Ecology and Systematics*, **31**, 265–
200 291.
201 Pearson, D.E., Icasatti, N.S., Hierro, J.L. & Bird, B.J. (2014) Are local filters blind to
202 provenance? Ant seed predation suppresses exotic plants more than natives. *PLoS ONE*, **9**, 1–
203 11.
204 Pearson, D.E., Ortega, Y.K., Eren, Ö. & Hierro, J.L. (2018) Community assembly theory as a
205 framework for biological invasions. *Trends in Ecology and Evolution*, **33**, 313–325.
206 Peart, M.H. (1979) Experiments on the biological significance of the morphology of seed
207 dispersal units in grasses. *Journal of Ecology*, **67**, 843–863.
208 Plummer, M. (2003) JAGS: A program for analysis of Bayesian graphical models using
209 Gibbs sampling. In *Proceedings of the 3rd International Workshop on Distributed Statistical*
210 *Computing (DSC 2003)*. pp. 20–22.
211 Pulliam, H.R. & Brand, M.R. (1975) The production and utilization of seeds in plains
212 grassland of Southeastern Arizona. *Ecology*, **56**, 1158–1166.
213 R Development Core Team. (2020) *R: a language and environment for statistical computing.*
214 Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.r-
215 project.org/.

Retana, J., Picó, F.X., Rodrigo, A., Oikos, S. & May, F. (2004) Dual role of harvesting ants as seed predators and dispersers of a non-myrmechorous Mediterranean perennial herb. *Oikos*, **105**, 377–385.

Wandrag, E.M., Catford, J.A. & Duncan, R.P. (2019) Quantifying niche availability, niche overlap and competition for recruitment sites in plant populations without explicit knowledge of niche axes. *Journal of Ecology*, **107**, 1791–1803.

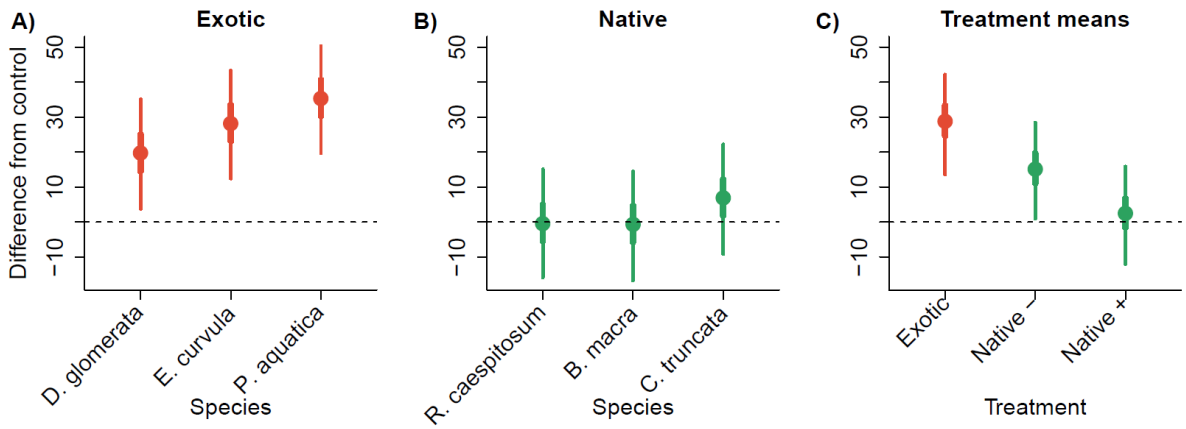
Wandrag, E.M., Sheppard, A., Duncan, R.P.P. & Hulme, P.E.E. (2013) Mutualism vs. antagonism in introduced and native ranges: Can seed dispersal and predation determine Acacia invasion success? *Perspectives in Plant Ecology, Evolution and Systematics*, **15**, 171–179.

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 to 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**