



## SHORT COMMUNICATION

Attraction of ants by an invasive *Acacia*

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**Abstract.** 1. Invasive plants are often released from the herbivores of their native range, but may also be deprived of their co-evolved mutualists. In southern Portugal *Acacia dealbata* has become naturalised in secondary woodland habitats and is apparently not damaged by local herbivores. It possesses inactive extra-floral nectaries (EFNs).

2. Artificial damage to leaves, mimicking herbivore attack, induced extra-floral nectar production on both adult trees and seedlings. This response was restricted to individual leaves rather than systemic.

3. Following EFN activation, trees were tended by the invasive Argentine ant *Linepithema humile*. Seedlings received a tenfold greater visitation rate from either *L. humile* or the native ant *Plagiolepis pygmaea*, which appears to displace the former. Eight days after the damage treatment the ants and nectar had largely gone.

4. There was no indication that either species of ant would defend the plant against potential herbivores.

5. This is the first recorded ant-plant interaction among two invasive species. Given the lack of natural herbivore damage and the absence of suitable ants in this novel community, nectar induction by *A. dealbata* is likely to be a dysfunctional response to damage in its invasive range, with little possibility of developing into a mutualism.

**Key words.** Ant-plant, defence induction, extra-floral nectaries, invasive species, *Linepithema humile*, Portugal.

## Introduction

*Acacia* trees possess extra-floral nectaries (EFNs), attracting ants which defend them against herbivores. Herbivory commonly triggers increased exudation from EFNs (Agrawal & Rutter, 1998; Heil *et al.*, 2001b, 2004), while ant numbers can be modulated by variable nectar production, allowing defences to be directed toward vulnerable or threatened parts of the plant (Heil *et al.*, 2001a). Pre-emptive EFN activity is unnecessary when the risk of herbivore attack is low (Lach *et al.*, 2009) and damaged plants may reduce nectar production if ants are not attracted (Heil *et al.*, 2000).

Many species of *Acacia* have become invasive, and as such are often released from their usual herbivores (Keane & Crawley, 2002), though it is also likely that they will be deprived of their co-evolved mutualists (Lockwood *et al.*, 2007). *Acacia dealbata* Link is native to Australia but has become naturalised

in Portugal. Its EFNs are located along the midrib of all leaves (see Appendix 1). Little information is available on interactions with ants in its native range, though phylogenetic evidence suggests that extra-floral nectar is inducible rather than constitutive in this species (Heil *et al.*, 2004). Our hypothesis was that EFN induction in its invasive range would attract locally-present ants, but that these would not be co-evolved species capable of forming a mutualistic relationship.

## Materials and methods

The location was an abandoned industrial site south of Lisbon, Portugal (38°38'N, 9°11'E) with secondary woodland dominated by exotic invasive tree species, especially *Acacia* spp. and *Eucalyptus* spp., alongside native species such as *Arbutus unedo* L. There was evidence of herbivore damage on native woody plants (e.g. *A. unedo*, *Cistus* spp.) while invasive species were untouched. Ants were not observed tending any plant species.

Thirty-one trees with foliage between 1–2 m above ground were randomly assigned to either manipulated (16) or control (15) groups. For manipulated trees, the first three branches more

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than 1 m above ground and with a minimum of eight leaves were randomly allocated to fully-damaged (all leaves), partially-damaged (alternate leaves) or control treatments. On control trees three branches were selected as above but were surveyed without any manipulations. Damage was applied using shears by cutting parallel to the midrib on each side and removing half of the leaflets. In addition, eighteen seedlings less than 1 m in height were assigned to either full, partial or control treatments, each applied to the lowest branch with 5–10 leaves. All plants were a minimum of 5 m apart and were initially searched for herbivores or evidence of feeding damage.

Experiments took place from 1st–9th April 2008. Tree observations began at 9 AM on the first day, seedlings at 5 PM on the second day, and both were monitored thereafter at 9 AM and 2 PM each day. EFNs were classed as being open or closed (Appendix 1). Nectar production was measured using 1 µl microcapillary tubes with an average taken from the basal EFN on each of the first three leaves on each branch, using only damaged leaves from the partially-damaged treatment. The ant visitation rate was determined in late morning, the period of peak activity, by observing each branch for 10 min and counting the number of ants moving onto the branch. This was divided by the number of leaves to provide a rate in ants leaf<sup>-1</sup> min<sup>-1</sup>. Sampling was suspended on one day as heavy rain prevented use of microcapillaries and no foraging ants were present.

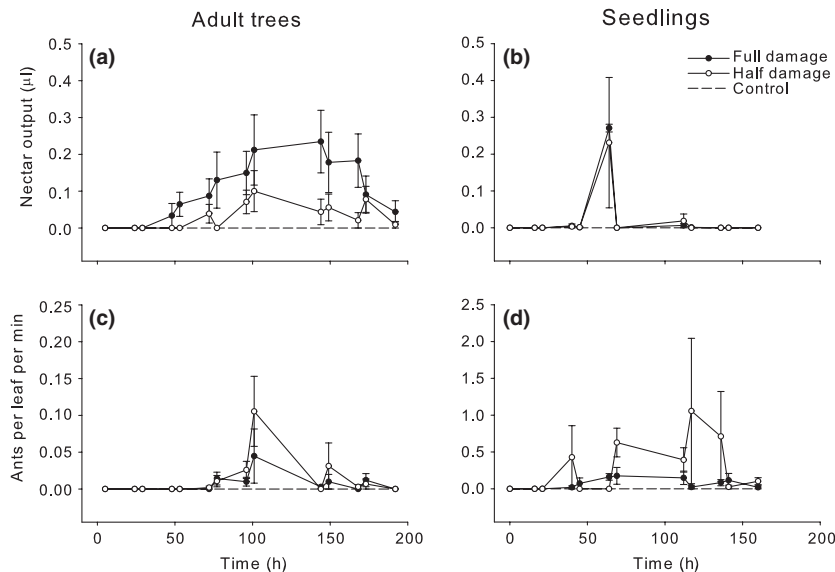
Sweep-netting of *A. dealbata* foliage failed to reveal any potential insect herbivores. We therefore collected insects from neighbouring grasslands. Potential folivores were placed in Petri dishes with two intact *A. dealbata* leaflets for 48 h in a no-choice feeding trial. Those which fed on the leaflets were killed by

freezing then used to test ant responses. Eight treated trees with foraging ants were selected and potential herbivores placed on all three treatment branches and observed for 10 min, a length of time adequate for removal of most introduced herbivores in a similar study (Agrawal, 1998). This was performed intermittently over the course of the study, in late morning, following the visitation rate assessments.

Statistical analysis examined the maximum recorded standing nectar levels and ant visitation rates over the study period. Paired *t*-tests were used for comparisons within treatment trees (or Wilcoxon's *T* test for matched pairs for non-normal data), and independent samples *t*-tests for comparisons between trees and seedlings and between seedling treatment groups (or Kolmogorov-Smirnov *Z*-tests).

## Results and discussion

There was no initial nectar production by any *Acacia*, nor evidence of herbivores or feeding damage, and their EFNs were closed. Of the 16 trees subjected to damage, 10 produced measurable nectar on either damaged branch (Fig. 1a). Elsewhere it may have been removed by foraging ants. Maximum recorded nectar levels were greater on fully-damaged branches ( $0.47 \pm 0.07$  µl per leaf, mean  $\pm$  S.E.) than partially-damaged ( $0.18 \pm 0.04$  µl per leaf;  $t = 3.27$ , d.f. = 9,  $P = 0.010$ ). All EFN pores were open, even on trees where no nectar was visible. Control branches and trees and intact leaves from partially-damaged branches showed no signs of nectar production and EFN pores remained closed, suggesting that induction was localised to individual leaves.



**Fig. 1.** Nectar production and ant visitation rates for *Acacia dealbata* trees and seedlings, means  $\pm$  SE. (a) EFN nectar production on fully- ( $n = 9$ ) and half-damaged ( $n = 8$ ) tree branches, and (b) fully- ( $n = 3$ ) and half-damaged ( $n = 5$ ) seedlings which produced at least one measureable nectar volume. (c) Ant visitation of all fully- ( $n = 11$ ) and half-damaged ( $n = 8$ ) tree branches and (d) fully- ( $n = 6$ ) and half-damaged ( $n = 5$ ) seedlings with at least one record. Note the order of magnitude difference in scale on the y-axis of plots (c) and (d). No nectar or ants were recorded on control seedlings; these records are marked with a single line.

Three of the six fully-damaged seedlings produced visible nectar, compared to five partially-damaged seedlings (Fig. 1b). Maximum recorded nectar levels were  $0.17 \mu\text{l}$  per leaf [0.05–0.28] (median [inter-quartile range]) and did not differ between fully- and partially-damaged seedlings ( $Z = 1.01$ ,  $P = 0.18$ ) nor from trees ( $Z = 0.843$ ,  $P = 0.48$ ). All EFN pores were open on damaged leaves, but no nectar was observed on control leaves and EFNs remained closed.

Though no ants were initially present on plants, the majority (13) of damaged trees were visited (Fig. 1c), exceeding the number (10) with recorded nectar production, indicating that ants were removing nectar. Notably all three trees without recorded ants had nectar present. Ant arrival occurred more slowly than in a comparable study by Agrawal (1998) in which ant densities peaked after around 8–12 min and remained high for at least 24 h. In the latter case this aggressive recruitment occurred more rapidly than EFN responses, and was therefore an active defence, whereas in our study the longer time span was likely a result of the delay in activating inert EFNs. Maximum recorded visitation rates of  $0.08 \text{ ants leaf}^{-1} \text{ min}^{-1}$  [0.03–0.10] did not differ between fully- and partially-damaged branches ( $T = 5$ ,  $P = 0.89$ ). No ants were observed on control branches or trees.

All six fully-damaged seedlings were tended by ants, and five of the partially-damaged seedlings; the only damaged seedling without ants had visible nectar. Nectar removal is likely to account for the observation above that only half of the fully-damaged seedlings had standing nectar. Maximum recorded visitation rates of  $1.08 \pm 0.47 \text{ ants leaf}^{-1} \text{ min}^{-1}$  did not differ between fully- and partially-damaged seedlings ( $t = 1.84$ , d.f. = 9,  $P = 0.10$ ; Fig. 1d) though were an order or magnitude greater on damaged seedlings than trees ( $Z = 1.88$ ,  $P = 0.002$ ). This may be due to the lower overall height of seedlings relative to damaged tree branches, reflecting either greater accessibility or willingness of ants to forage at lower heights. This may also account for the observation that nectar was visible for less of the experiment than on trees (Fig. 1b). No ants were observed on control seedlings.

Trees were exclusively visited by the Argentine ant *Linepithema humile* (Mayr), a widespread invasive in Mediterranean climate zones throughout the world (Suarez *et al.*, 2001). Little work has been done on the responses of invasive ants to EFNs (Holway *et al.*, 2002), though *L. humile* is known to visit EFNs in both its native and invasive ranges (Koptur, 1979; Holway *et al.*, 2002), aided by a modified crop which allows it to ingest liquid foods (Davidson, 1998). Seedlings were also visited by *Plagiolepis pygmaea* (Latreille), with no difference in abundance between the two species ( $Z = 1.12$ ,  $P = 0.16$ ). For images of the two ant species see Appendix 2. Only one ant species was found on any given seedling. On five occasions, between days 3–6, seedlings occupied by *L. humile* switched to *P. pygmaea*; the only seedling on which the opposite occurred reverted the following day. This was unexpected given that in California *L. humile* repelled native ants from sugar baits, as well as discovering them more quickly, recruiting in greater numbers (Human & Gordon, 1996; Holway *et al.*, 2002) and showing greater aggression in defending them (Human & Gordon, 1999). Native and invasive ants are known to compete for access to nectar resources (Oliver *et al.*, 2008). It is unknown how many other

species of ants are present within these secondary forests in Portugal.

In no-choice feeding trials, only common field grasshoppers (*Chorthippus brunneus*, Orthoptera: Acrididae) demonstrated any ability to feed on leaves of *A. dealbata*. Dead herbivores placed on experimental trees with *L. humile* ants were ignored, though this is an imperfect test of their response to an active herbivore. *L. humile* has a reputation as an aggressive hunter, even preying upon wasp broods in defended nests (Gambino, 1990). In Portuguese pine plantations it is thought to control populations of the pine processionary moth (*Thaumetopoea pityocampa* Den. & Schiff.) through predation on nests of young larvae (Way *et al.*, 1999). It has been recorded to reduce the abundance of a range of invertebrates following invasion (Holway *et al.*, 2002; Lach, 2008). Some evidence suggests that *L. humile* can be beneficial to EFN plants in deterring herbivores (Koptur, 1979), although there are no studies known which compare their performance to native ants (Ness & Bronstein, 2004). In a comparable study examining whether invasive *Solenopsis invicta* might act in defence of the native tree *Catalpa bignonioides* Walter in Georgia, USA, the invasive ant provided incidental protection from herbivores but also attacked their parasitoids (Ness, 2003). Since they infrequently visited EFNs and did not actively harvest nectar, this could not be considered an ant-plant mutualism.

On the basis of the present study, instigation of the ant-plant interaction by *A. dealbata* can only be viewed as dysfunctional, and though the cost of the maintenance and induction of a system of extra-floral nectaries is unknown, it is unlikely to be negligible. Ant-plant interactions are vulnerable to exploitation by parasitic ants (e.g. Raine *et al.*, 2004), but such effects in an invasive plant species have not been previously documented. In this case it would appear that *A. dealbata* has become a successful invasive while remaining undamaged by local herbivores, suggesting that any interaction with ants is superfluous. Should an herbivore from its native range be introduced, perhaps as a means of biological control, or if a native herbivore acquires the ability to feed on *Acacia*, it will be intriguing to observe whether *L. humile* proves to be an effective defender of the trees.

## Acknowledgements

G. Bradford and A. Birch assisted with data collection, B. Taylor identified the ant species and F.S. Gilbert, T. Reader and two anonymous referees provided helpful comments on the manuscript.

## Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: doi: 10.1111/j.1752-4598.2010.00121.x

**Appendix S1.** Typical extra-floral nectaries from damaged (left) and undamaged (right) leaves of *Acacia dealbata* seedlings. Note the rounded openings of the pores on damaged leaves compared to the closed slit on an undamaged leaf. Credit: R. du Feu.

**Appendix S2.** High-magnification images of (a) *Linepithema humile* and (b) *Plagiolepis pygmaea* specimens collected during experiments.

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Accepted 29 September 2010

First published online 11 November 2010

Editor/associate editor: Simon R. Leather