Oecologia
https://doi.org/10.1007/s00442-021-04874-2

HIGHLIGHTED STUDENT RESEARCH

Evidence of indirect biotic resistance: native ants decrease invasive plant fitness by enhancing aphid infestation

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Received: 20 May 2020 / Accepted: 3 February 2021

Abstract
The biotic resistance hypothesis asserts that native species may hinder the invasion of exotic species, which can occur either directly or indirectly by influencing interactions between exotic and local species. Aphid-tending ants may play a key role in the indirect biotic resistance to plant invasion. Ants may protect aphids, thus increasing their negative effect on exotic plants, but may also deter chewing herbivores, thus benefiting exotic plants. We studied native aphid-tending ants (*Dorymyrmex tener*, *Camponotus distinguendus*, and *Dorymyrmex richteri*) on exotic nodding thistles (*Carduus thoermeri*), which are attacked by thistle aphids (*Brachycaudus cardui*) and thistle-head weevils (*Rhinocyllus conicus*). We evaluated the impact of ants, aphids, and weevils on thistle seed set. We compared ant species aggressiveness towards aphid predators and weevils and performed ant-exclusion experiments to determine the effects of ants on aphid predators and weevils. We analysed whether ant species affected thistle seed set through their effects on aphids and/or weevils. The ant *D. tener* showed the most aggressive behaviour towards aphid predators and weevils. Further, *D. tener* successfully removed aphid predators from thistles but did not affect weevils. Excluding *D. tener* from thistles increased seed set. Analyses supported a negative indirect pathway between the aggressive *D. tener* and thistle seed set through aphid populations, while the other ant species showed no indirect effects on thistle reproduction. Therefore, aggressive aphid-tending ants may enhance biotic resistance by increasing aphid infestation on exotic invasive plants. This study highlights the importance of indirect biotic resistance in modulating the success of invasive species.

Keywords Exotic plants · Aphid · Aphid-tending ants · Biotic resistance · Indirect interactions

Introduction

When exotic species invade a new habitat, the establishment of novel interactions with the local biota can be crucial for their invasion success (Richardson et al. 2000; Mitchell et al. 2006; Prider et al. 2011). These biotic interactions may strongly influence whether exotic species become established, naturalized, and capable of further spread (e.g., Nuñez et al. 2009; Prior et al. 2015). For example, mutualism (Richardson et al. 2000), enemy release (Keane and Crawley 2002), facilitation (Farji-Brener and Ghermandi 2008), allelopathy (Callaway and Ridenour 2004), and invasional meltdown (Simberloff and Von Holle 1999) can enhance species invasion, while competition (Eskelinen and Harrison 2014), predation (DeRivera et al. 2005), and parasitism (Prider et al. 2011) can constrain the invasion. Therefore, to better understand the impact of species invasion, it is important to examine newly established biotic interactions between native and exotic species.

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Combining field observations and experiments, we show that aggressive aphid-tending ants can enhance biotic resistance by increasing aphid infestation on an exotic plant.

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Third-party species may mediate the strength of biotic interactions and this mediation can be critical for species invasion (Strauss and Irwin 2004; Mitchell et al. 2006; Brody et al. 2008; Green et al. 2011). In this regard, the predictions of general hypotheses in invasion ecology may be modified by third-party species (White et al. 2006). The biotic resistance hypothesis posits that exotic species may fail to invade native communities when biotic interactions with local species (e.g., competition, predation, and parasitism) are strong enough to deter their establishment and spread (Elton 1958; Levine et al. 2004; Parker and Hay 2005). If third-party species enhance the negative effects of local species on the exotics, the invasion process should then be hindered (hereafter 'indirect biotic resistance'). Alternatively, the mutualism-facilitation hypothesis asserts that local species can have positive effects on exotics by establishing mutualistic associations and facilitating their establishment and spread (Richardson et al. 2000; Mitchell et al. 2006). Therefore, if third-party species enhance facilitative-mutualistic associations between local and exotic species, the invasion process should be then enhanced (hereafter 'indirect biotic facilitation'). In summary, third-party species may affect species invasion by influencing pairwise interactions between introduced and local species. By studying how these indirect interactions affect the fitness of exotic species we can gain a better understanding of the ecological scenarios driving species invasion. Furthermore, knowledge of the newly established biotic interactions within invaded communities is critical for the development of accurate and sustainable strategies for the management of invasive species.

Ant-aphid mutualisms are suitable models to study whether indirect effects modulate plant invasion. First, aphids are sap-feeders that reduce plant fitness through resource limitation, tissue damage, pathogen infection, and/or pollination interference (e.g., Larson and Whitham 1997; Chalcoff et al. 2019; Devegili and Chalcoff 2020). Second, aphid effects on exotic plant fitness may be mediated by aphid-tending ants (Dixon 1998). On the one hand, ants promote aphid feeding rate and fecundity, protect aphids against natural enemies, and/or reduce the incidence of pathogens on aphid colonies (Banks and Nixon 1958; Stadler and Dixon 2008; Nielsen et al. 2010; Devegili et al. 2020). Therefore, ant attendance can enhance aphid outbreaks (Devegili et al. 2020) leading to negative indirect effects on host plant fitness (e.g., Canedo-Júnior et al. 2017; Ortega-Ramos et al. 2019). On the other hand, ants may deter other (non-aphid) herbivores and thus benefit plants, particularly if those herbivores have a higher impact on plant fitness than aphids (Crutsinger and Sanders 2005; Styrsky and Eubanks 2007; Zhang et al. 2015). Considering that ant species often vary in the defense quality and quantity they provide (Ness et al. 2006; Mooney and Mandal 2010; Yoo et al. 2013; Devegili et al. 2020), the net effect of aphid-tending ants on exotic plant fitness may be influenced by ant identity. Therefore, to predict the impact of aphid-tending ants on exotic plant invasiveness it is important to document the role of the entire native ant assemblage.

Carduus thoermeri (nodding thistle) is a widespread invasive plant that causes serious impacts on the native community and large economic losses in agriculture (Desrochers et al. 1988; Popay and Medd 1990; Wardle et al. 1991). In north-western Patagonia (Argentina), *C. thoermeri* is usually infested by the exotic aphid *Brachycaudus cardui* and visited by a guild of native aphid-tending ants (Lescano and Farji-Brener 2011). Ant species aggressiveness towards aphid predators (ladybugs and hoverfly larvae) is associated with *B. cardui* population growth on *C. thoermeri* (Devegili et al. 2020). Apart from aphids, thistles are attacked by the weevil *Rhinocyllus conicus*, a biocontrol agent introduced into Argentina to control *Carduus* species (Enrique de Briano et al. 2013). We took advantage of this ecological scenario and performed a comparative analysis along with field and behavioural experiments that allowed us to test the indirect effects of native aphid-tending ants on the reproductive success of *C. thoermeri*.

We examined two hypotheses: i) aphid-tending ants indirectly hinder plant invasion by enhancing aphid infestation, which in turn decreases thistle fitness ('indirect biotic resistance hypothesis') (Fig. 1, upper branch), and ii) aphid-tending ants indirectly facilitate plant invasion by deterring non-aphid herbivores, thus decreasing damage on thistles by these herbivores ('indirect biotic facilitation hypothesis') (Fig. 1, lower branch). Although the ecological relevance of these indirect pathways is well known in ant-plant ecology (Styrsky and Eubanks 2007; Clark et al. 2016, 2019),

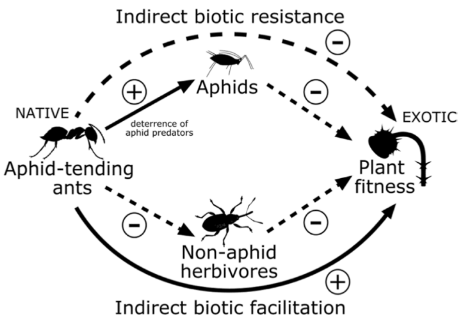

Fig. 1 Potential direct and indirect effects linking aphid-tending ants and aphid-infested exotic plants. We propose two hypotheses. 'Indirect biotic resistance' would occur when aphid-tending ants increase aphid abundance and damage on the exotic plant, thus decreasing plant fitness, while having no positive effect on plant fitness through the deterrence of non-aphid herbivores. 'Indirect biotic facilitation' would occur when aphid-tending ants positively affect the exotic plant fitness by deterring non-aphid herbivores, while having no negative effect on the exotic plant fitness through the aphid pathway

it is unknown whether these indirect effects are relevant for exotic plant invasion. If the consequences on thistle reproduction of ant protection of aphids are stronger than those of weevil suppression, we would find a negative indirect effect of ants on thistle reproduction (i.e., support for the indirect biotic resistance hypothesis). Conversely, if the deterrence of weevils by ants is more significant for the plant than aphid herbivory, we would find a positive effect of ants on thistle reproduction (i.e., support for the indirect biotic facilitation hypothesis). Further, concerning the native aphid-tending ant assemblage, we expected that the most aggressive ant species would have the greatest indirect effect on thistle reproduction, either by better protection of aphids against their natural predators (Devegili et al. 2020) or by deterring weevils more efficiently.

Materials and methods

Study system

The study site, located in NW Patagonia (Argentina, 41.12° S; 71.22° W), is an area with herbaceous and shrub-steppe vegetation. The climate is cold and dry, with a mean annual temperature of 8 ºC and mean annual precipitation of 600 mm falling mostly in winter (Dimitri 1962).

Carduus thoermeri (nodding thistle; Asteraceae) is a widespread biennial weed of Eurasian origin (Shea et al. 2005). It was introduced in the 1920s into Argentina, where it is classified as an agricultural plague (Gobbi et al. 1995). In the Patagonian steppe, it grows in disturbed sites, such as roadsides (Farji-Brener and Ghermandi 2008). *Carduus thoermeri* spends its first year as a rosette and flowers during its second year (Shea et al. 2005). It only reproduces sexually, either by self- or cross-pollination, possessing nectar and pollen as rewards for pollinators (Morales and Aizen 2002). Self-pollination is as effective as cross-pollination, and aphid-tending ants do not interfere with floral visitors (Chalcoff et al. 2019).

Dorymyrmex tener, *D. richteri* (Hymenoptera: Dolichoderinae) and *Camponotus distinguendus* (Hymenoptera: Formicinae) are native ants that visit *C. thoermeri* seeking aphid honeydew. These ant species nest on the ground and/or under rocks near thistle patches. *Dorymyrmex tener,* the most abundant and aggressive species, effectively protects thistle aphids from predators such as ladybugs and hoverfly larvae (Devegili et al. 2020). Moreover, thistle aphids grow faster when *D. tener* is present compared to when the other ant species are present (Devegili et al. 2020).

Brachycaudus cardui (thistle aphid; Hemiptera: Aphididae) is an aphid species native to the Northern Hemisphere (Blackman and Eastop 2006). In NW Patagonia, *B. cardui* feeds on *C. thoermeri*, reducing its seed output (Chalcoff et al. 2019), and it usually forms groups on thistle stems (hereafter 'aphid group'; supplementary material, Fig. S1), where it is preyed on by ladybugs (*Hippodamia variegata*, *Cycloneda* sp., *Eriopis* sp., and *Harmonia axyridis*; Coleoptera: Coccinellidae) and hoverflies (*Allograpta* sp. and *Syrphus* sp.; Diptera: Syrphidae).

Rhynocillus conicus (thistle-head weevil; Coleoptera: Curculionidae) is a biocontrol weevil native to Eurasia (Boldt and Kok 1982) that was introduced into Argentina in the 1980s to mitigate the negative impacts of *Carduus* species (Enrique de Briano et al. 2013). *Rhynocillus conicus* lays eggs on the bracts of thistle flower heads (Zwölfer and Harris 1984). The larval phase has the greatest negative impact on thistle reproduction (Sheppard et al. 1994). Aphid-tending ants patrol thistles during the time in which *R. conicus* feeds, mates, and oviposits (AM Devegili, personal observations). Aphid-tending ants do not attack or damage *R. conicus* eggs, which are protected inside hard cocoons; likewise, they do not attack weevil larvae, which complete pupation inside flower heads (AM Devegili, personal observations).

Field pattern

To examine whether aphids, weevils, and ants influence thistle reproduction, in a thistle patch of 3200 m^2, we randomly selected and marked newly-born rosettes ($N=215$) and followed them during the entire plant cycle (12 surveys during 2 years). We surveyed thistles less often during fall and winter (every 3 months) than spring and summer (every 15 days) because insects are more abundant and active during the warmer seasons. In each survey, we determined the abundance of each target insect. To estimate aphid abundance, we multiplied the number of aphid groups by the average number of aphids in a group. To estimate the average number of aphids in a group, we randomly selected 1–3 aphid groups per plant, took pictures, and counted individuals with ImageJ Software® (Schneider et al. 2012; supplementary material, Fig. S1). Since weevil larva is the most detrimental for thistle reproduction, we counted weevils that successfully developed in the flower heads. On thistles occupied by ants, we identified and counted the number of active workers, calculating the mean ant abundance from 12 surveys. When thistles lacked ants in all the surveys, we categorized them as "plants without ants" ($N=61$). In this group, we found: (1) thistles without aphids or weevils ($N=12$), (2) thistles with aphids only (in $\geq 50\%$ of the surveys; $N=15$), (3) thistles with weevils only ($N=16$), and (4) thistles with aphids and weevils ($N=18$). When thistles hosted a single ant species in $\geq 50\%$ of the surveys and otherwise were ant-free, we categorized them as occupied by this particular ant species (*D. tener*: $N=73$; *C. distinguendus*: $N=31$; *D. richteri*: $N=24$). As our goal was to measure the individual effect of ant species on thistles, we did not include in the analyses plants hosting more than one ant species in the same survey ($N=5$) and plants with ant species replacement during surveys ($N=21$). To estimate seed output, we bagged 1–2 flower heads per thistle and collected them shortly after seed release. At the laboratory, we counted weevils, categorized seeds (viable/unviable) (supplementary material, Fig. S2), and counted seeds using photographs and ImageJ Software®. We defined the seed set as the proportion of viable seeds in the flower head (N viable seeds/[N viable seeds + N unviable seeds]). To compare the seed set of plants from different groups, we used a GLM with plant status as a four-level factor: (1) without insects, (2) with aphids, (3) with weevils, and (4) with aphids + weevils. To account for over-dispersion, we used a quasibinomial error distribution (Zuur et al. 2009), and as post-hoc test, we used Tukey HSD. To examine the relationships between ant species abundance and (1) thistle seed set, (2) aphid abundance, and (3) weevil abundance, we used correlation tests (Pearson's product-moment correlation; Puth et al. 2014). Analyses were done in R (R Core Team 2015).

Behavioural experiments: ant species aggressiveness

To measure ant species aggressiveness, we set pairwise encounters between ants *vs.* aphid predators (ladybugs and hoverfly larvae) and ants *vs.* weevils. Data of experiments involving aphid predators were taken from Devegili et al. (2020), where they were reported separately (ladybugs and hoverfly larvae), while here we pooled them under the category "aphid predators". Results of confrontations between ant species and weevils are first reported in this study.

As described in Devegili et al. (2020), we collected ants (~200 workers per species), aphid predators (ladybugs: $N=76$, hoverfly larvae: $N=91$) and—included in this study—weevils ($N=85$). We collected insects directly from thistles. To account for colony variation in ant aggressiveness, we collected ants from thistles that were at least 30 m apart (5–8 thistles per ant species). This distance was sufficient to assume that ants occupying thistles came from different colonies (Devegili et al. 2020). At the laboratory, each species was housed separately in plastic containers (10 × 8 × 5 cm). All insects had free access to water (moistened cotton). Ants were fed with sugary water and a *Tenebrio molitor* larva; aphid natural enemies were fed with *B. cardui*; weevils were fed with thistle leaves. The confrontation arena was a 3 cm-diameter plastic container with a mobile septum that divided the area into two compartments, thus isolating ants and target insects before the encounter. To elicit ant defensive behaviour, we fitted at the base of the arena a filter paper marked with the odour of the tested ant species. Further, given that aphid presence and honeydew fuel ant activity and trigger ant attacks against aphid predators (Phillips and Willis 2005; Shik and Silverman 2013; McGlynn and Parra 2016), 1 h before the trial we placed a piece of stem infested with *B. cardui* inside the ant colony container. In this way, ants could interact with aphids and collect honeydew before the trials. The confrontation trials lasted 4 min: following a habituation time of 1 min, we removed the septum and video-recorded (20× magnification) the confrontation for the remaining 3 min. For the ant *D. tener* we recorded $N=32$, $N=31$, and $N=31$ confrontations with ladybugs, hoverfly larvae, and weevils, respectively; likewise, for *C. distinguendus* we recorded $N=27$, $N=30$, and $N=26$; and for *D. richteri* $N=28$, $N=30$, and $N=27$. As described in Devegili et al. (2020), we analysed the videos to measure the time to first contact with the target insects (hereafter, encounter time), and we identified six ant behaviours: (1) 'escape', the ant flees after contacting the target insect; (2) 'antennation', antennae contacting over 2 s the target insect; (3) 'threat', ant mandibles open and antennae pointing towards the insect; (4) 'short bites', sudden raid with brief bites (less than 2 s) (5) 'long bites', sustained biting (over 2 s) of any part of the enemy body; and (6) 'gaster flexing', ant bends its gaster towards the insect, releasing a repellent substance (supplementary material, Video S1). We scored ant aggressiveness with an index:

$$\text{Aggressiveness index} = \frac{\sum_i^6 \text{frequency}_i \times \text{behaviour}_i}{\sum_i^6 \text{frequency}_i},$$

where 'frequency' is the occurrence of each interaction and 'behaviour' is a constant that weights the aggression intensity ('escape' = − 1, 'antennation' = 0, 'threat' = 1, 'short bites' = 2, 'long bites' = 3, and 'gaster flexing' = 3). A negative, zero, or positive aggression index means that ants exhibit elusive, neutral or aggressive behaviours toward target insects, respectively. To compare the encounter time of ant species *vs.* aphid predators (or *vs.* weevils), we used a GLM (error distribution: negative binomial) (function *glm.nb* from R package 'Mass', Ripley et al. 2013). To compare the aggressiveness indices between ant species, we used a two-way ANOVA (function *Anova* from R package 'car', Fox et al. 2012) with ant species (levels: *D. tener*, *C. distinguendus*, *D. richteri*) and enemy type (levels: aphid predators, weevils) as explanatory variables. Aggressiveness indices were log-transformed to meet normality assumptions. As post-hoc test, we used Tukey HSD. Analyses were done in R.

Field experiments

We used the most aggressive ant species (*D. tener*, see results) to test in natural conditions (1) whether ants are capable of deterring aphid predators and weevils from thistles and (2) whether the exclusion of ants from thistles impacts seed set. We selected thistles ($N = 30$) naturally infested with *B. cardui* and patrolled by *D. tener* and in each plant we selected two similar stems. We then excluded ants in one stem (ant-exclusion treatment) and left the ants undisturbed in the other stem (control treatment). To exclude ants, we wrapped the stems with duct tape (Ductac®) gently coated with lithium grease (Lubrigras®). We further removed the remaining ants with an insect aspirator. To test ant capacity to deter aphid predators and weevils, we selected one aphid group per treatment and drew with a permanent marker a reference spot at 2 cm. We then placed on the spot the target insects, which were transferred singly using soft tweezers (supplementary material, Video S2). Each trial lasted 5 min. The target insects were considered 'deterred' when they were ejected from the plant and 'not deterred' when they stayed on the plant during the whole trial (supplementary material, Video S2).

To test whether *D. tener* ants impact seed set, we selected thistles with *B. cardui* and *D. tener* ($N = 20$) and marked two stems in each plant, excluding ants in one stem as described above and leaving the other as a control. Two months later we collected 1–2 inflorescences per treatment and determined seed set. We also estimated aphid density (individuals/cm^2) in one randomly selected aphid group in each treatment. We counted aphids with photos plus ImageJ Software® and determined the area occupied by the aphid group using the same photos. We estimated aphid density in three plant surveys (every 15 days from ant exclusion) and then averaged those values.

To analyse the fate of weevils and aphid predators on thistle stems, we used logistic regressions (GLM, error distribution: binomial). To test the effect of ant exclusion on the seed set, we performed a GLM; to account for over-dispersion, we used a quasibinomial error distribution. To test the effect of ant exclusion treatment on aphid density we performed a GLM (error distribution: negative binomial). Analyses were done in R.

Direct and indirect pathways between ant species and thistle seed set

To integrate the results and examine the 'indirect biotic resistance' and the 'indirect biotic facilitation' hypotheses, we conducted path analyses based on structural equation models (Grace 2006). Following ecological knowledge, we proposed an a priori model showing expected relationships between the biotic variables: ants (root variable), aphid predators, aphids, weevils, and thistle seed set (Fig. 4a; supplementary material, Fig. S3). The a priori model was built including all the aphid-tending ant species. To examine whether the model is consistent with our data, we used the model chi-square statistic (where a P value < 0.05 indicates a mismatch between the model and the data; Grace 2006) and comparative fix index CFI (where CFI ~ 1 indicates a good model fit and CFI ~ 0 indicates no model fit; Grace 2006). Path coefficients estimates were obtained using the maximum likelihood estimation technique; they are equivalent to standardized partial regression coefficients and are interpreted as relative effects of one variable upon another (Grace 2006). The variable 'aphid predators' corresponds to pooled data of the abundance of adult ladybugs (four species, see study system) and hoverfly larvae (two species, see study system) in six thistle surveys covering three months. The variables 'ants' and 'aphids' correspond to the average abundance of ants and aphids in twelve thistle surveys. The variable 'aphids' corresponds to the aphid *B. cardui* and 'weevils' to the abundance of adult *R. conicus* in thistle inflorescences. We used the model to examine direct and indirect pathways between each aphid-tending ant species (*D. tener*, *C. distinguendus*, or *D. richteri*) and thistle seed set. We considered the pathway linking ants with seed set through aphid predators and aphids as support for the 'indirect biotic resistance' hypothesis (Fig. 4a, upper branch), and the pathway linking ants with thistle seed set through weevils as support for the 'indirect biotic facilitation' hypothesis (Fig. 4a, lower branch). We built the structural equation models and examined their goodness of fit with *lavaan* and *AICcmodavg* packages in R (Mazerolle 2013; Rosseel 2012).

Results

Field pattern

The aphid *Brachycaudus cardui* was found in 69% of thistles, with an abundance of 70.7 ± 6.6 aphids per plant (mean ± SE). The weevil *Rhynocillus conicus* was found in 36% of the flower heads, with an abundance of 1.4 ± 0.24 weevils per capitulum (mean ± SE). The seed set (mean ± SE) of thistles attacked by aphids (0.64 ± 0.02) or weevils (0.55 ± 0.05) was 0.22% or 34% lower than the seed set of thistles without herbivores (0.83 ± 0.04) (GLM, aphid presence: $LR\chi^2 = 18.48$, $df = 1$, $P < 0.001$, weevil presence: $LR\chi^2 = 15.03$, $df = 1$, $P < 0.001$; Fig. 2a). The seed set of thistles with both aphids and weevils was even lower (0.43 ± 0.03) than the seed set of thistles with only aphids or weevils; however, there was no interaction between the effects of these herbivores on thistle seed set (GLM, aphid presence*weevil presence: $LR\chi^2 = 2.44$, $df = 1$, $P = 0.12$; Fig. 2a). Thistles with ants represented 62% of the surveyed plants, with the ant *D. tener* being present in 33% of the cases, *C. distinguendus* in 16%, and *D. richteri* in 13%; the average abundance (mean ± SE) of ants in twelve thistle surveys was 16.2 ± 2.1 for *D. tener*, 5.2 ± 2.1 for *C. distinguendus*, and 3.3 ± 1.1 for *D. richteri*. Thistle seed set was negatively correlated with the abundance of *D. tener* (correlation

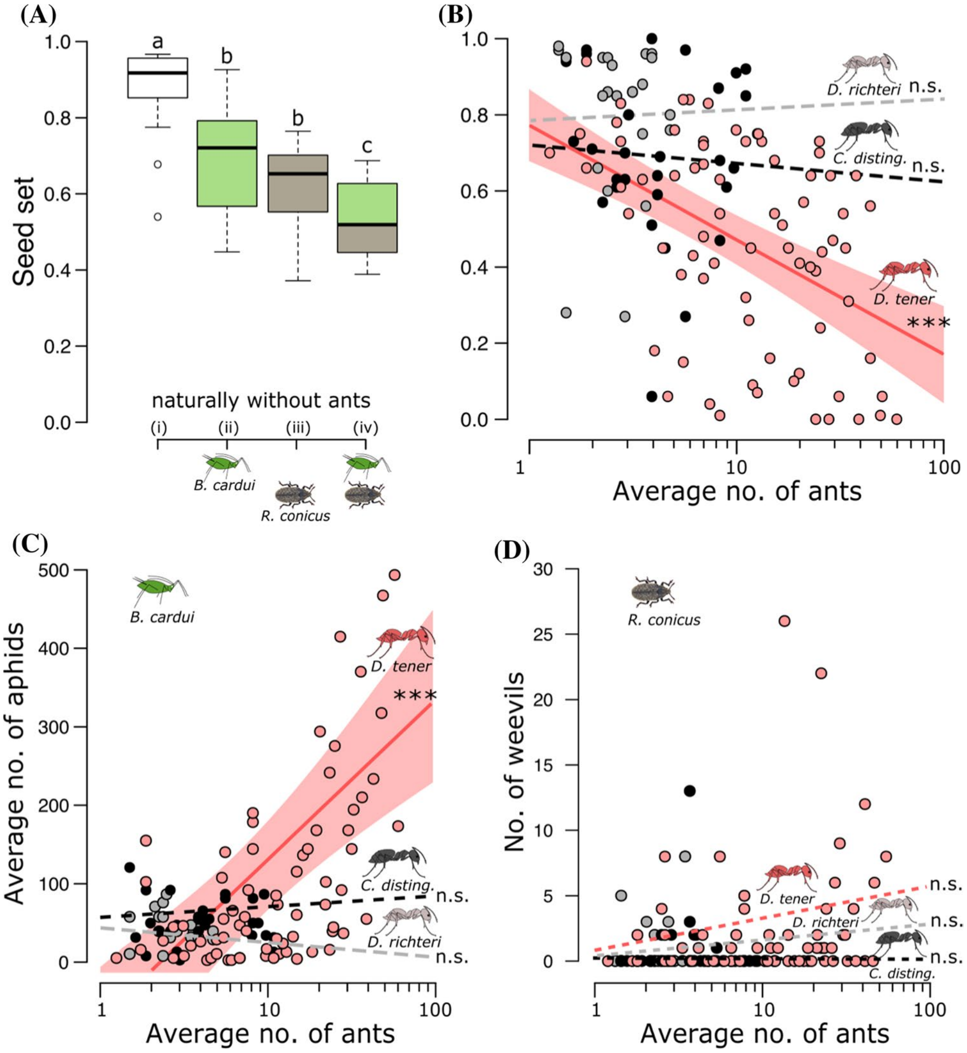

Fig. 2 Field patterns. **a** Boxplots of seed set of *Carduus thoermeri* thistles under different natural conditions of herbivore load: (i) herbivore-free ($N = 12$), (ii) with *Brachycaudus cardui* aphids ($N = 15$), (iii) with *Rhinocyllus conicus* weevils ($N = 16$), and (iv) with both *B. cardui* and *R. conicus* ($N = 18$). All thistles were naturally ant-free to isolate the effect of herbivores on seed set. Seed set is the proportion of viable seeds in the flower head. Groups sharing lowercase letters are not significantly different (Tukey HSD test following GLM). **b** Relationships between the abundances of aphid-tending ant species and thistle seed set: red, *Dorymyrmex tener*; black, *Camponotus distinguendus* and grey, *D. richteri*. Dots represent individual thistles. Error shadows (95% CI) are shown only if the relationship is significant (Pearson's product-moment correlation; $^{***}P < 0.001$, *n.s.* not significant). The average number of ants = mean ant abundance across twelve surveys over the entire thistle life cycle. **c** Relationships between the abundances of aphid-tending ant species and the average number of aphids on thistles (mean aphid abundance across twelve surveys over the entire thistle life cycle). Analyses as in **b**. **d** Relationships between the abundances of aphid-tending ant species and the number of weevils in the thistle flower head. Analyses as in **b**

test, $r = -0.45$, $t = -4.21$, $df = 73$, $P < 0.001$), but not with the abundance of the other ant species (Fig. 2b). The seed set (mean ± SE) of thistles with *D. tener* (0.48 ± 0.03) was %40 lower than the seed set of thistles without ants (0.81 ± 0.03). The abundance of thistle aphids *B. cardui* was positively correlated with the abundance of *D. tener* (correlation test, $r = 0.72$, $t = 8.66$, $df = 73$, $P < 0.001$), but not with the abundance of the other ant species (Fig. 2c). None of the ant species showed an association between their abundances and the abundance of thistle-head weevils *R. conicus* (correlation test, *D. tener*: $P = 0.06$, *C. distinguendus*: $P = 0.47$, *D. richteri*: $P = 0.49$; Fig. 2d). Apart from thistle aphids and thistle-head weevils, no other herbivores were found on thistles.

Behavioural experiments: ant species aggressiveness

Ant aggressiveness varied with ant species and enemy type (aphid predators or weevils) (GLM, ant species: $LR\chi^2 = 466.69$, $df = 2$, $P < 0.001$; enemy type: $LR\chi^2 = 6.92$,

$df = 1$, $P = 0.008$). The ant *D. tener* was the most aggressive species against aphid predators and weevils, *C. distinguendus* showed intermediate levels of aggressiveness, and *D. richteri* was the least aggressive (Fig. 3a). *Dorymyrmex tener* showed higher aggressiveness against aphid predators than against weevils (Fig. 3a). The most common behaviours displayed by *D. tener* towards aphid predators were aggressive behaviours such as 'threat', 'short bites', and 'long bites', while the most common behaviours against weevils were passive behaviours such as 'antennation' (supplementary material, Fig. S4). Conversely, the ants *C. distinguendus* and *D. richteri* exhibited primarily passive ('antennation') and elusive ('escape') behaviours towards aphid predators and weevils (supplementary material, Fig. S4). Ant species differed in their encounter time in the arena with aphid predators (GLM, $LR\chi^2 = 70.3$, $df = 2$, $P < 0.001$) and with weevils (GLM, $LR\chi^2 = 81.99$, $df = 2$, $P < 0.001$), being *D. tener* the fastest species to contact weevils and aphid predators (supplementary material, Fig. S4).

Field experiments

Supporting the 'indirect biotic resistance' hypothesis, the probability for aphid predators of being expelled from the plant increased with *D. tener* abundance (GLM, $z = 7.16$, $P < 0.001$) (Fig. 4a). Particularly, ≈20 ants were able to expel aphid predators with an 80% probability. Moreover, when *D. tener* was present on thistle stems, the density (mean ± SE) of thistle aphids (34.9 ± 3.5 aphids/cm^2) increased by %44 with respect to stems with ant-exclusion (24.3 ± 2.8 aphids/cm^2) (GLM, $LR\chi^2 = 5.43$, $df = 1$, $P = 0.02$; Fig. 4b). Supporting the 'indirect biotic resistance' hypothesis, the seed set (mean ± SE) of stems with *D. tener* (0.38 ± 0.06) was 31% lower than the seed set of stems with ant-exclusion (0.55 ± 0.04) (GLM, $LR\chi^2 = 5.72$, $df = 1$, $P = 0.02$; Fig. 4b). Contrary to the 'indirect biotic facilitation' hypothesis, *D. tener* abundance was not associated with the probability for thistle-head weevils of being expelled from the plant (GLM, $z = 1.29$, $P = 0.20$) (Fig. 4a). Neither aphid predators nor weevils deliberately dropped off or flew away when placed on stems without the ant *D. tener*.

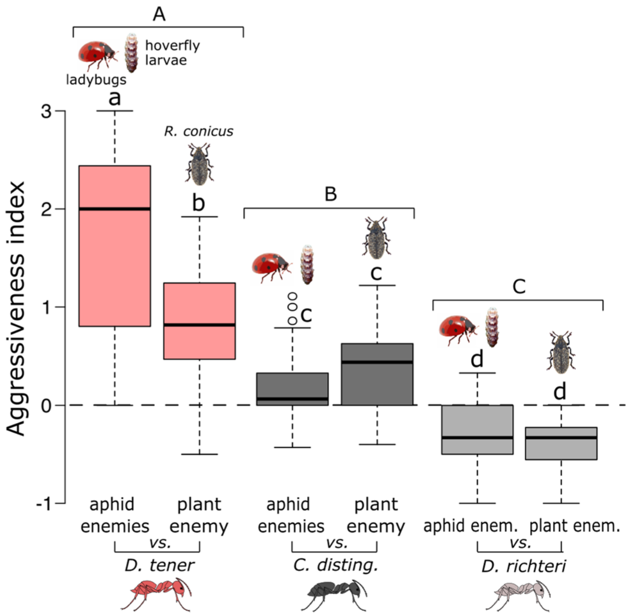

Fig. 3 Behavioural experiments: ant species aggressiveness towards aphid predators and weevils. Boxplots of aggressiveness indices of aphid-tending ant species (*Dorymyrmex tener*, *Camponotus distinguendus*, and *D. richteri*) in pairwise encounters with aphid predators (data pooled from four ladybug species and two hoverfly species) and weevils (*Rhinocyllus conicus*). Sample size range: 26–31 for each ant species. Lowercase letters depict significant differences between all groups and uppercase letters between aphid-tending ant species (Tukey HSD test following two-way ANOVA)

Direct and indirect pathways between ant species and thistle seed set

Our a priori model (Fig. 4a; supplementary material, Fig. S3) was consistent with the data, as indicated by the goodness-of-fit tests employed ($\chi^2 = 24.66$, $P = 0.16$, $df = 4$; CFI = 0.87). Supporting the 'indirect biotic resistance' hypothesis, (1) the abundance of the ant *D. tener* was negatively associated with the abundance of aphid predators (SEM path analysis, $P < 0.001$, $R^2 = 0.12$), (2) the abundance of aphid predators was negatively associated with thistle aphid abundance (SEM path analysis, $P = 0.001$, $R^2 = 0.08$), (3) the abundance of aphids was negatively associated with thistle seed set (SEM path analysis, $P < 0.001$, $R^2 = 0.35$), and (4) the abundance of the ant *D. tener* showed a negative indirect effect on thistle seed set (SEM path analysis, $P = 0.02$; Fig. 5b). Contrary to the 'indirect biotic facilitation' hypothesis, *D. tener* abundance showed no association with the abundance of thistle-head weevils (SEM path analysis, $P = 0.20$) and, accordingly, *D. tener* abundance showed no indirect effect on thistle seed set via thistle-head weevils (SEM path analysis, $P = 0.23$; Fig. 5b). The pathways involving the ants *C. distinguendus* or *D. richteri* showed no support for either the 'indirect biotic resistance' or 'indirect biotic facilitation' hypotheses (supplementary material, Fig. S5). For more results and details, see Table S1 in the supplementary material.

Discussion

Indirect effects can be critical in shaping the structure and dynamics of ecological communities (Strauss 1991; Strauss and Irwin 2004; Ohgushi 2005, 2008; Schmitz 2008) but we still know little about their contribution to

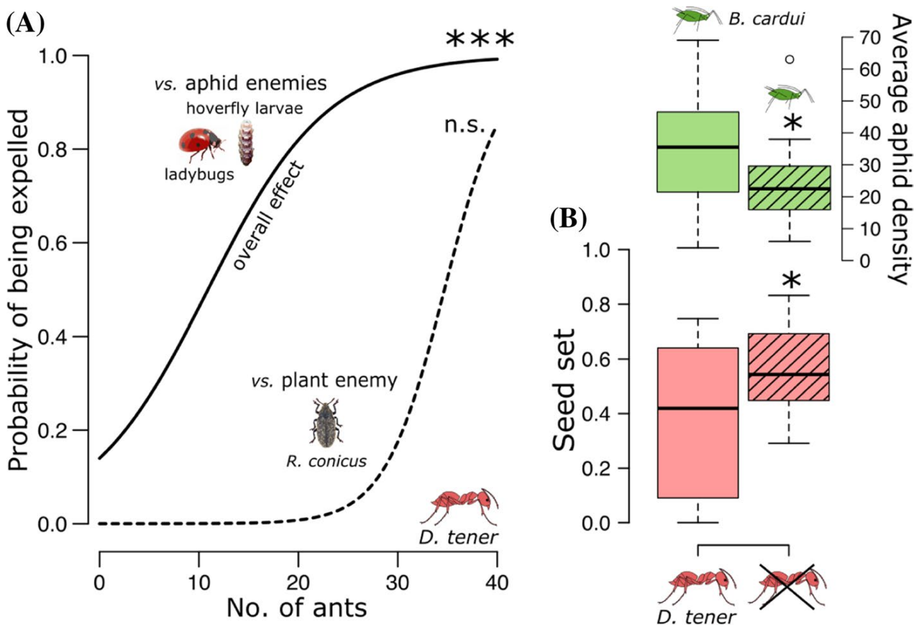

Fig. 4 Field experiments: effects of the most aggressive ant species on aphid predators, weevils, and thistle seed set. **a** Relationships between the number of the most aggressive ant species (*Dorymyrmex tener*) and the probabilities for aphid predators (data pooled from four ladybug species and two hoverfly species; solid line) and for weevils (*Rhinocillus conicus*; dashed line) of being expelled from thistle stems (*Carduus thoermeri*) (ants *vs.* aphid predators: $N = 191$, ants vs. weevils: $N = 30$). ***$P < 0.001$, *n.s.* not significant (logistic regressions). Neither aphid predators nor weevils deliberately dropped (or flew away) when placed on stems with ant-exclusion ($N = 10$–19 trials per target species). **b** Effects of experimentally excluding *D. tener* from thistles on aphid density and thistle seed set. Ant-exclusion (striped boxes) and control (solid boxes) treatments were randomly assigned to different stems of the same thistle plant ($N = 20$). Average aphid density (number of individuals/cm^2) is the mean value of three plant surveys. The seed set is the proportion of viable seeds in the flower head. *$P < 0.05$, ***$P < 0.001$ (GLM)

biological invasions (White et al. 2006; Ricciardi et al. 2013). We found that native aphid-tending ants indirectly reduce invasive plant reproduction by protecting aphids from predators and hence allowing greater aphid numbers –and damage– on plants. Particularly, the thistle seed set was reduced by 40% when patrolled by the ant *D. tener*, the most aggressive species in the aphid-tending assemblage, while the seed set was not affected when patrolled by the rather passive ant species *C. distinguendus* or *D. richteri*. *Dorymyrmex tener* successfully removed aphid predators from thistles and when it was excluded from thistle stems, aphid density decreased by 44% and thistle seed set increased by 31%. However, *D. tener* did not expel weevils from thistles. Accordingly, path analysis supported an indirect pathway between *D. tener* and thistle seed set through aphid predators and aphids, while there was no such pathway through weevils. Collectively, results suggest that aggressive aphid-tending ants may bring about ‘indirect biotic resistance’ by increasing aphid infestation on invasive plants without affecting weevil performance. This outcome supports earlier contentions that knowledge of the pairwise, first-order interactions among plants, sap-feeders, ants, and chewing insects could not be enough to predict the fitness consequences of ant presence on plants (Messina 1981). Therefore, to fully understand the role that the local species plays in biological invasions, it is important to consider both direct and indirect effects.

Although it is well recognized that ants can directly affect exotic species fitness (Jensen and Six 2006; Farji-Brener and Ghermandi 2008; Alba-Lynn and Henk 2010; Mascioc-chi et al. 2010), ants may also trigger indirect effects with important impacts on species invasion (e.g., invasional meltdown: O’Dowd et al. 2003; Green et al. 2011). We found that native aphid-tending ants can negatively affect thistle reproduction through the protection and maintenance of exotic aphid colonies. Ants engaged in mutualistic interactions with aphids become more aggressive towards any competing arthropod, deterring aphid natural enemies such as coccinellids, hoverfly larvae, neuropteran larvae, and aphid parasitoids (Kaneko 2003; Devegili et al. 2020). As a consequence, ant attendance may enhance aphid outbreaks on aphid-infested plants (Devegili et al. 2020) leading to a decrease in exotic plant fitness and a potential impact on plant invasion. However, aphid-tending ants can also have a positive effect on the host plants by reducing the abundance of non-aphid herbivores and so decreasing herbivore damage (Rosumek et al. 2009) and this can affect plant invasion. This positive indirect pathway, that we named as ‘indirect

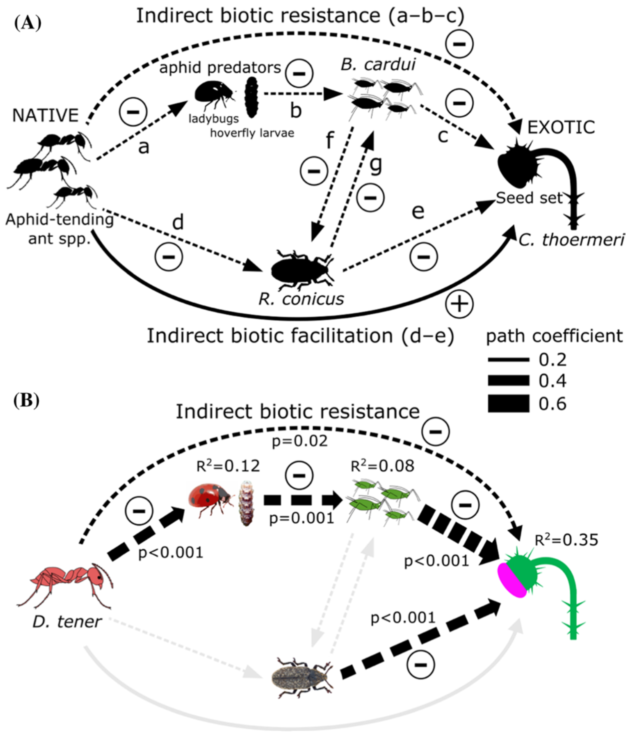

Fig. 5 Direct and indirect pathways between ant species and thistle seed set. **a** Hypothesized model: a, negative effect of aphid-tending ants on aphid predators; b, negative effect of aphid predators on aphid population; c, negative effect of aphids on plant fitness; d, negative effect of aphid-tending ants on weevils; e, negative effect of weevils on plant fitness; f and g, negative effects between aphids and weevils via competition for thistle resources. **b** Resulting pathways linking the abundance of the native aphid-tending ant *Dorymyrmex tener* and the seed set of the exotic *Carduus thoermeri*. Aphid predators correspond to pooled data from four ladybug species and two hoverfly species (larvae). Black arrows depict significant pathways ($P < 0.05$) while grey arrows represent non-significant pathways. Path coefficients are standardized coefficients. Path analyses based on structural equation models (see text)

biotic facilitation’, was not supported in our study system. We found no association between the abundances of any of the ant species and the abundance of the weevil *R. conicus*, and we evidenced that even the most aggressive ant species failed to expel adult weevils from plants. The fact that the most aggressive aphid-tending ant species failed to expel weevils might be explained by size differences (*R. conicus* is ~ 3 times larger than the aggressive ant *D. tener*) and/or by the strong sclerotization of weevil bodies, which makes them resistant to ant bites (Byk and Del-Claro 2010; Alves-Silva et al. 2015). In summary, aggressive native ants promoted aphid infestation and damage on invasive thistles but were unable to mitigate the strong negative effects of weevils on thistle fitness. These results support the notion that aggressive aphid-tending ants may bring about indirect biotic resistance to plant invasion by enhancing aphid infestation, while not affecting chewing herbivory.

Understanding the biotic interactions within invaded communities is crucial for making accurate management decisions. The management of invasive species is often based on the introduction of new elements to the invaded community, such as biocontrol agents which can have detrimental non-target effects on the native species (Louda et al. 2003; Rand and Louda 2004). However, biotic communities often comprise exotic and native species in a network of interactions through direct and indirect effects. Thus, an alternative management strategy is the manipulation of elements and/or interactions already present in the invaded community. Here we found that aggressive native ants negatively affected exotic thistle fitness via enhanced herbivory by specialist aphids (‘indirect biotic resistance’). Consequently, fostering the association between aggressive native ants and aphids could be an accurate strategy for the management of invasive thistles. In NW Patagonia, this strategy seems very promising since (1) the aggressive ant *D. tener* is the most abundant species in the native assemblage (Farji-Brener et al. 2002), (2) *D. tener* is particularly prone to visit aphid-infested thistles (Lescano and Farji-Brener 2011; Devegili et al. 2020), and (3) *D. tener* protects thistle aphids against their predators without affecting the performance of the thistle biocontrol agent *R. conicus*. In summary, knowledge of the indirect biotic effects within invaded communities may provide novel strategies for the management of invasive species, such as the strengthening of the indirect biotic resistance towards the exotic species.

When exotic species arrive at a new community, they establish novel interactions with local species, and these new interactions may enhance or hinder their establishment and spread (Richardson et al. 2000; Mitchell et al. 2006). Ants and aphids are present in almost all plant communities and their mutualistic association represents ‘keystone interactions’ (Styrsky and Eubanks 2007; Clark et al. 2019). Therefore, interactions between aphid-infested exotic plants and native aphid-tending ants are expected to be frequent and ecologically relevant. Despite two meta-analyses failed to find a significant effect of aphid-tending ants on plant reproductive output (Rosumek et al. 2009; Zhang et al. 2012), we showed that aggressive native ants indirectly affected thistle fitness through an effect on aphid abundance, and hence on sap-feeding damage. This is one of a few studies showing that a well-studied invasion biology mechanism, the biotic resistance hypothesis (Elton 1958), can be indirectly modulated by third-party species. Knowledge of the extent of indirect effects within invaded communities will enhance predictions of the potential impacts of invaders and will refine management strategies for exotic species invasion.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00442-021-04874-2.

Acknowledgements Thanks to M Rodriguez-Cabal who gave valuable suggestions to an earlier version of the manuscript. Thanks to A Lovrich, T Gonzalez, and D Gonzalez who assisted in the field, and L Ammassari who processed samples in the laboratory. Thanks to an anonymous reviewer who provided numerous recommendations that improved the clarity and quality of the manuscript. AM Devegili is supported by a doctoral fellowship from the Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina.

Author contribution statement All authors conceived and designed the experiments; AMD collected the data and performed the experiments; AMD analysed the data; AMD led the writing of the manuscript, to which all authors contributed.

Funding This study was conducted without funding.

Data availability The datasets generated during and/or analysed during the current study will be available in the Figshare Data Repository (https://figshare.com/).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Statement of human and animal rights All applicable institutional and/or national guidelines for the care and use of animals were followed.

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