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Provost

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Interaction between a threatened endemic plant (*Anchusa crispa*) and the invasive Argentine
ant (*Linepithema humile*)

Blight^{1*} Olivier, Orgeas¹ Jérôme, Le Menn¹ Aline, Quilichini² Angélique and Provost¹ Erick

¹ Institut Méditerranéen de Biodiversité et d'Ecologie, Avignon Université, UMR CNRS IRD
Aix Marseille Université, Avignon, France.

² CNRS, UMR Ecologie des Forêts de Guyane, Campus Agronomique, F-97379 Kourou cedex,
France.

* Corresponding author:

Email: olivier.blight@imbe.fr

Abstract

Seed dispersal mutualisms are essential to ensure the survival of diverse plant species and communities worldwide. Here we investigated whether the invasive Argentine ant can replace native ants by fulfilling their functional role in the seed dispersal of the rare and threatened endemic myrmecochorous plant, *Anchusa crispa*, in Corsica (France). Our study addressed the potential of *Linepithema humile* to disperse elaiosome-bearing seeds of *A. crispa*, examining *L. humile*'s effects on (1) the composition of communities of ants removing seeds, (2) the number of seed removals, (3) seed preference, (4) the distance of seed dispersion, and (5) seed germination. We caught seven native species at the control site, but only the Argentine ant at invaded sites. *L. humile* removed *A. crispa* seeds in greater numbers than did native ants, respectively 66% and 23%, probably due to their higher worker density. The invader was similar to native ants with respect to distance of seed transport. Finally, rates of seed germination were not significantly different between seeds previously in contact with either Argentine ants or not. Taken all together, these results suggest that the Argentine ant is unlikely to pose a threat to *A. crispa* population. These results have important implications for the management of this rare and threatened endemic plant and provide an example of non-negative interactions between invasive and native species.

Keywords: invasive ant; endemic plant; seed dispersal; conservation.

Introduction

Seed dispersal is one of the most ecologically significant plant-animal mutualisms. This mutualism influences seedling recruitment, population dynamics, species distributions, plant-community composition and gene flow (Nathan and Muller-Landau 2000). Due to their ubiquitous occurrence in terrestrial ecosystems, ants are one of the most important animal groups involved in seed dynamics (Lengyel et al. 2010). They contribute significantly to maintaining the structure and diversity of different natural communities (Lengyel et al. 2009). Elimination of native ants following invasion and particularly by the Argentine ant (*Linepithema humile*) is widely reported to lead indirectly to the near-complete collapse of seed dispersal services (Christian 2001; Carney et al. 2003). The Argentine ant is seen as an imperfect substitute for the full range of native ants that disperse elaiosome-bearing seeds (Rodríguez-Cabal et al. 2009b). Homogenization of native ant fauna following its invasion further diminishes seed dispersal services for native plant species (Lach 2003), and in some cases can favour the spread of highly invasive plants (Rowles and O'Dowd 2009). As in other parts of its introduced range, replacement of native ants by *L. humile* in Southern Europe has led to changes in the relationships between ants and plants, including myrmecochory (Gómez et al. 2003; Gómez and Oliveras 2003). Argentine ants have been present and affected the local ecosystem of Corsica, a French Mediterranean island, for more than 60 years (Blight et al. 2014). They spread mainly on the coasts, invading modified but also natural habitats including sites that serve as a habitat for an endemic myrmecochorous plant, *Anchusa crispa* Viv. (Blight et al. 2009). *Anchusa crispa* is a Mediterranean endemic species occurring on the islands of Corsica (France) and Sardinia (Italy). It occurs in isolated populations in coastal habitats. *Anchusa crispa* has been listed under the Berne Convention since 1979 and has been on the European Community list of protected species since 1992. This species is classified as endangered by International Union for Conservation of Nature.

In the past 20 years, the Argentine ant has significantly increased its population density in Corsica (Blight et al. 2009), this study therefore aimed to evaluate its effects on the population of the rare and threatened endemic myrmecochorous plant, *A. crispa*, investigating the direct and indirect effects of the Argentine ant invasion on patterns of seed dispersal. Specifically, our study addressed the potential of *L. humile* to disperse elaiosome-bearing seeds of *A. crispa* and its effects on (1) the composition of ant communities removing seeds, (2) the number of seed removals, (3) seed preference, (4) the distance of seed dispersion and (5) seed germination.

Materials and Methods

Experimental sites

Anchusa crispa, a short-lived (up to 4 years with 2–3 flowering years) hemicryptophyte, grows on coastal dunes and back-dunes, on sandy soils in open herbaceous vegetation. The fruits comprise four fused carpels that develop into nutlets which bear an elaiosome.

In Corsica, three populations occur on the west coast and two on the east coast. The total number of plants in each population in Corsica varies from less than 10 individuals to several hundreds. Given the low number of *A. crispa* populations and their small size, we could perform our experiments on only the two biggest sites: one uninvaded, on the West coast (Portigliolo) and one on the East coast (Cannella) that is invaded by the Argentine ant (Blight et al. 2009). Both populations of *A. crispa* covered approximately 80m². All the populations are located near a river mouth. The station on the West coast is located near a river in the Gulf of Propriano. This site is fenced off from car access (Portigliolo). The second population, on the East coast (Cannella), is approximately 45km away and located on a dune sloping down from a restaurant and a camp site.

Ant assemblages

To determine local ant richness and species occurrence, we placed at both sites 20 plastic pitfall traps (35 mm in diameter and 70 mm deep) partly filled with a non-attractive mixture (ethylene-glycol) as a capture and preservation fluid, at one-metre interval along two transects of 10 meters set in parallel. The two transects were separated by 6m. After 48 hours, all pitfall samples were collected and the ant species were sorted and identified to the species level. Ant assemblages were evaluated in June (optimum of fructification) 2008 and 2009, two weeks before the experimental tests were conducted.

Offering tests

We determined the behavioural reaction of ants to mature seeds of *A. crispa* in the field by directly offering seeds to workers at both sites in June 2009. By using tweezers, we placed seeds on the ground next to randomly selected solitary workers that prospected for food. We offered 80 seeds at Portigliolo to the three most abundant species (28 seeds to both *Messor minor* and *Tapinoma sp* and 24 seeds to *Aphaenogaster spinosa*) and 80 seeds to the Argentine ant, the only species present at Cannella. Each ant's response was categorized following the Culver and Beattie (1978) classification : (i) antennate and examine: the ant touched the seed with antennae and then continued to forage (ii) pick-up attempt: the ant touched the seed with antennae, inspected it and then released it without removing it and (iii) remove: the ant held the seed between its mandibles and removed it. When possible, we also recorded the number of seeds transported over less than 10 cm, between 10 cm and 50 cm, between 50 cm and 1 m and beyond 1 m. Seeds were collected directly from the plant and offered to ants at the sites where they were collected 24 hours after their collection. Differences in proportion of ant-seed interactions and in proportion of seeds dispersed over less than 50 cm were compared using Fisher's exact test.

Seed preference and removal events

To test seed attractiveness and seed transport rates at the community level, we placed in a 5.5 cm (diameter) plastic petri dish one seed of each of three plants, *A. crisper*, *Silene sericea* and *Lotus cytoides* at the uninvaded site (Portigliolo) and *A. crisper*, *S. sericea* and *Malcolmia ramosissima* at the invaded site (Cannella), where there was no *L. cytoides*. This test was conducted in June 2008 and June 2009. *Anchusa crisper* seeds are the only seeds bearing an elaiosome and are larger than the seeds of the three other species (19 mg for *A. crisper*, < 10 mg for *S. sericea*, *L. cytoides* and *M. ramosissima*) that were dominant at their sites. We included non-elaisome bearing seed species to compare attractiveness of *A. crisper* seeds to the two dominant plant species in each site. We placed two petri dishes separated by one meter at one-metre intervals along the same two transects used for the ant sampling, one petri dish on each side of the transects. We recorded the seeds remaining on each petri dish every 30 min for two hours and then every hour until the end of the test. Observations started at 6:00 a.m. and finished at 11:00 a.m. Ants were not active after 11:00 a.m. when temperatures reach more than 40°C on the soil surface. We conducted this experiment over three consecutive days on the same two transects. A total of 120 seeds for each of the three plants on both sites were deposited. Seeds were collected directly on plants. Differences in proportions of seeds dispersed between plant species were tested using Fisher's exact test.

Seed germination

To test for an effect of Argentine ant workers on seed germination, we collected 112 seeds and placed them in petri dishes containing cotton moistened with distilled water. Before the beginning of the experiment, 55 seeds were deposited for 72h in a foraging arena connected to an artificial nest containing an Argentine ant colony. Workers were then allowed to remove seeds to the nest. The remaining 57 seeds were placed in the same container but without any

ants. The dishes were placed in a single randomised block. Seeds were germinated at $18\pm 2^{\circ}\text{C}$, under day-night conditions, and distilled water was added as needed. Seed germination was recorded every two days during two months, after which no further germination occurred.

Results

Ant assemblages

At the uninvaded site (Portigliolo), in 2008, we caught seven native species (Table 1). *Messor minor*, *Tapinoma sp* and *Aphaenogaster spinosa* were the three most dominant species occurring in 60%, 80% and 50% of the traps respectively. In 2009, we caught five species. *Tapinoma sp* and *A. spinosa* were the most abundant species, occurring in 75% and 55% of the traps respectively. Although we did not catch *M. minor* workers in 2009 during the 48h trapping session, they were present a few metres away from the *A. crista* population. *Tapinoma sp* was identified as *Tapinoma nigerrimum* but according to a recent review of this genus, it is probably *Tapinoma magnum* (Seifert et al. 2017).

At the invaded site, in both years we collected only one species, the Argentine ant. In 2008 Argentine ants occurred in 100% of the pitfall traps whereas in 2009 they were caught in 70% of the pitfall traps (Table 1). The difference in Argentine ant occurrence between the two years may be explained by the flooding of this study site one week before experimentation started.

Offering tests

The rates of seed removal at Portigliolo (*Messor minor* (n=28), *Tapinoma sp* (n=28) and *Aphaenogaster spinosa* (n=24)) and Cannella (*L. humile*) were similar, respectively 46/80 (57.5 %) and 41/80 (51 %) ($df = 1$, $P = 0.21$, Fisher's exact test). All *A. crista* seeds offered to the seed harvester *M. minor* were systematically removed, taken to the ant's nest more than 1 metre away (range 1 m – 3 m) and deposited in the nest (n = 28). *Tapinoma sp* and the Argentine ant

behaved similarly with seeds. Removal of seeds represented 50% and 51.3% of seed interactions for *Tapinoma sp* and the Argentine ant respectively ($df = 1$, $P = 0.55$, Fisher's exact test), but only 29% of *A. spinosa* seed interactions (Fig. 1). All seeds transported by *A. spinosa* workers were carried directly to the nest more than one metre away.

Moreover, both *Tapinoma sp* and *L. humile* transported seeds over short distances, dropping them along foraging trails. *Tapinoma sp* workers transported 38% (5/13) of seeds over less than 10 cm, 38% (5/13) between 10 cm and 50 cm and 23% (3/13) between 50 cm and 1 m (Table 2). *Linepithema humile* transported 41% (14/34) of seeds over less than 10 cm, 32% (11/34) between 10 cm and 50 cm and the remaining 26% (9/34) were transported over distances ranging between 50 cm and 1 m (Table 2). Proportions of seeds dispersed by *Tapinoma sp* and *L. humile* over less than 50 cm were similar: 77% (10/13) and 74% (25/34) respectively ($df = 1$, $P = 0.56$, Fisher's exact test).

Seed preference and removal events

At the uninvaded site, the proportion of *A. crista* seeds dispersed did not differ significantly between years, respectively 28/120 (23%) in 2008 and 19/120 (16%) in 2009 (Fig. 2) ($df = 1$, $P = 0.10$, Fisher's exact test). *Anchusa crista* seed dispersal was continuous throughout the five-hour tests. Twenty-six seeds (22 %) of *Lotus cytisoides* were dispersed in 2008 and 3/120 (2.5%) in 2009 ($df = 1$, $P < 0.0001$, Fisher's exact test). Fourteen (12%) seeds of *Silene sericea* were dispersed in 2008 and 4/120 (3.3%) in 2009 ($df = 1$, $P = 0.01$, Fisher's exact test). Both in 2008 and 2009, we recorded a significant relationship between the proportion of dispersed and remaining seeds and plant species ($df = 2$, $P < 0.05$, Chi-square test). In 2008, the numbers of *A. crista* and *L. cytisoides* seeds dispersed did not differ significantly ($df = 1$, $P = 0.43$, Fisher's exact test), whereas in 2009 it was mainly *A. crista* seeds that were dispersed.

At Cannella, the invaded site, the proportion of *A. crispera* seeds dispersed differed between years, respectively 79/120 (66%) in 2008 and 26/120 (22%) in 2009 (Fig. 2) ($df = 1$, $P < 0.0001$, Fisher's exact test). In 2008, approximately 50% of *A. crispera* seeds were already dispersed 30 min after the test started. Eleven seeds (9%) of *M. ramosissima* were dispersed in 2008 and 4/120 (3%) in 2009 ($df = 1$, $P = 0.05$, Fisher's exact test). Eight (7%) seeds of *S. sericea* were dispersed in 2008 and 6/120 (5%) in 2009 ($df = 1$, $P = 0.39$, Fisher's exact test). At Cannella, both in 2008 and 2009, we recorded a significant relationship between the proportion of dispersed and remaining seeds and plant species ($df = 2$, $P < 0.05$, Chi-square test). Both in 2008 and 2009, it was mainly *A. crispera* seeds that were dispersed. Proportions of *A. crispera* seeds dispersed differed between sites in 2008 ($df = 1$, $P < 0.0001$, Fisher's exact test) but were similar in 2009 ($df = 1$, $P = 0.16$, Fisher's exact test).

Seed germination

We recorded no significant difference in the number of germinated seeds between the group of seeds that were in contact with Argentine ants for 72h and the control ($df = 1$, $P = 0.19$, Chi-square test). At the end of the test, 51% of the seeds that were in contact with Argentine ant workers and 37% of the seeds in the control germinated.

Discussion

The results of our study indicate that the Argentine ant is attracted to, and able to remove, the elaisome-bearing seeds of *A. crispera*, without affecting seed germination. Argentine ants behaved similarly to the presumed main native seed disperser *Tapinoma sp* with respect to their inherent seed-removing ability and to the distances of transport. However, at the community level, they collected and dispersed three times more seeds than did all the native ants together.

Comparisons of seed dispersion rates between *A. crispera*, an elaiosome-bearing seed species, and three non-elaiosome-bearing seed species confirmed Argentine ants' preference for elaiosome-bearing seeds. Both in 2008 and 2009, *A. crispera* seeds were by far the most dispersed seeds by Argentine ants. At the uninvaded site, in 2008, the numbers of *A. crispera* and *L. cytoides* seeds dispersed did not differ significantly, whereas in 2009 it was mainly *A. crispera* seeds that were dispersed. This drastic decrease of seed dispersion rates of non-elaiosome-bearing seed species (22% in 2008 and 2.5% in 2009 of *L. cytoides* seeds removed, and 12% and 3.3% seeds removed for *S. sericea*) between years at the uninvaded site may be explained by the low activity of the harvester ant *M. minor* during the three days of experimentation in 2009. At the opposite, *A. crispera* seed removal rates were similar between years (23% in 2008 and 16% in 2009), probably because *Tapinoma sp* continued to disperse them.

Although their inherent ability to remove seeds does not seem to exceed that of native ants, the proportion of dispersed seeds was higher when Argentine ants were present in 2008. The high densities reached by Argentine ant colonies may explain this result, increasing the likelihood of seed/worker contacts by a factor of nearly five. In 2009, when the Argentine ant occurrence decreased, we recorded similar rates of seed removal between the two sites. Similar results were reported for *Solenopsis invicta*, which was found to be superior to native species in seed removal at the community level, but not in their inherent ability to exploit resources (Stuble et al. 2010). The presence of a "handle" on *A. crispera* seeds is also probably an important factor, facilitating their transport by Argentine ant workers despite their weight (20mg mass) (see Fig. 3). By attaching an artificial handle to seeds, Gómez et al. (2005) demonstrated that this structure allowed small ant species to displace seeds that they had initially been unable to transport.

The ratio between seed mass and Argentine ant body size usually explains the lack of dispersal of large seeds and the dispersal distances observed in invaded sites (Christian 2001). Dispersal distance generally depends on ant body size, large ants being likely to displace larger seeds and over longer distances than smaller ants (Ness et al. 2004). According to the classification of ants and seeds used by Ness et al. (2004), ants with body length under 5 mm being classified as “small”, both *Tapinoma sp* and the Argentine ant can be considered small ants and *M. minor* a “large” ant. and *A. crisper* seeds can be considered “large”.

The fact that *L. humile* and *Tapinoma sp*, disperses seeds over short distances may not imperil *A. crisper* populations. If it is of no value to the majority of myrmecochores (e.g. Kjellsson, 1991), seed dispersal over short distances may provide benefits to *A. crisper*. Indeed, the progeny produced by related individuals shows greater fitness than the progeny produced by unrelated individuals, through outbreeding depression (Quilichini et al. 2001). Moreover, the fact that both *Tapinoma sp* and the Argentine ant loses seeds on route to nests may not be a limiting factor for seed germination. It removes direct competition from the mother plant and under the action of wind or rain, abandoned seeds are rapidly covered by a fine layer of sand that may facilitate their germination. The highest germination rates were observed when seeds remained within the top two centimetres of sand (Quilichini and Debussche 2000). This is confirmed by our observation of several plants growing along main Argentine ant trails.

It is commonly argued that invasive ants disrupt seed dispersal, leading to a near-complete collapse of dispersal services and a decrease in seedling recruitment (Lach 2003). Reducing predation and competition from related plants, minimizing the effects of fire, and depositing seeds under suitable conditions are the usually benefits that plants receive from native ants (Giladi 2006), and which Argentine ants have usually failed to provide (Ness & Bronstein, 2004; Rodriguez-Cabal, Stuble, Nuñez, & Sanders, 2009). However, invasive ants do not systematically interact to the detriment of native plants. In some cases, they even facilitate

native species. For instance, Imported Fire ant has been described as responsible for more than half of all removals facilitating dispersal of elaiosome-bearing plant species (Stuble et al. 2010). Generally, exotic species facilitate native myrmecochores when they replace native ants, fulfil their functional role and provide seeds with a means of escape from enemies or competitors (Rodriguez 2006). Here, in addition to seed removal there is no direct negative effect on seed germination. Furthermore, the Argentine ant may decrease seed predation by displacing granivorous insects like the native ant *M. minor*. A previous study demonstrated that the presence of the Argentine ant could prevent seed predation by local *Messor* species (Oliveras et al. 2007). Similarly, *Solenopsis invicta* creates heterogeneity in seed survival by reducing seed predation by arthropods in the vicinity of their nest (Chandler and Orrock 2016). Among seed predators, harvester ants are considered primary seed consumers (Beattie and Hughes 2002). They may decrease seed germination by direct predation (Schöning et al. 2004) although they could play a dispersal role when they drop seeds in suitable conditions for germination in midtransport or in refuse piles (Retana et al. 2004; Bulot et al. 2016). However, this positive effect may be very limited. For example, ant-dispersed diaspores accounted for only 0.1% of seeds harvested in grasslands by *Messor barbarus* (Detrain and Tasse 2000). In the time of the experimentation, we never noted adult plants or seedlings in the immediate vicinity of *M. minor* nests (e.g. refuse piles), suggesting the species likely negative impact on *A. crispa* seeds. Although, the absence of replication due to the very low number and small size of *A. crispa* populations in Corsica may limit our conclusions, the results obtained from the different experimentations suggest a functional replacement of local ants in *A. crispa* seed dispersal by the Argentine ant. In the line of this hypothesis, long-term surveys of *A. crispa* populations showed an increase in the number of individuals over 10 years at the invaded site, Cannella (Bosc 2009). From 2007 onwards, the number of plants is higher at Cannella than at Portigliolo, which was initially the biggest population in Corsica (Bosc 2008).

This study has important implications for the management of this rare and threatened endemic plant. Even if the Argentine ant may not be a threat to *A. crista* survival, the invaded population needs to be regularly monitored, for example every 5 years. Indeed, the fact that *A. crista* seed dispersal relies on one species can make the resilience of this service lower than in the uninvaded site (concept known as the “insurance hypothesis”, Yachi and Loreau 1999), especially because invasive ant populations can vary substantially over time and experience dramatic population declines and local extinctions (Lester and Gruber 2016).

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385 Figure 1 Proportion of ant-seed interactions for the three most abundant native species *Messor*
386 *minor*, *Tapinoma sp* and *Aphaenogaster spinosa* and the invasive Argentine ant. In parentheses,
387 the number of seeds offered to ants.

388 Figure 2 Percentage of *Anchusa crispera* seeds dispersed in both invaded and uninvaded sites, in
389 2008 and 2009. A total of 120 seeds were used in both sites. $a \neq b$ (Fisher's exact test).

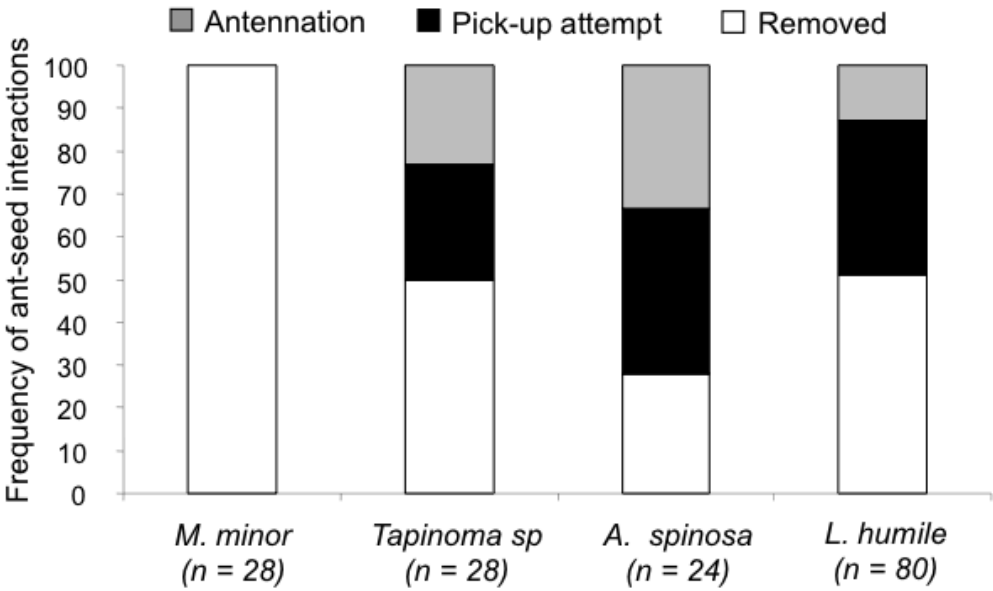
390 Figure 3 An Argentine worker transporting a seed of *Anchusa crispera* by the handle.

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393 Fig. 1

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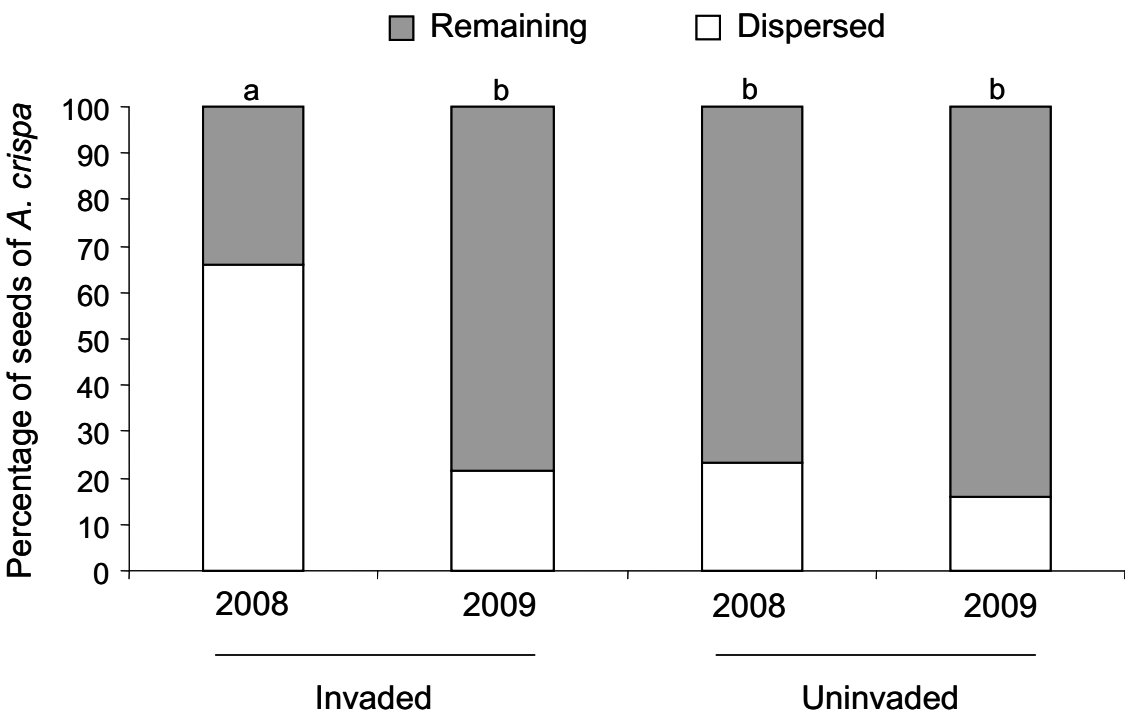
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399 Fig. 2

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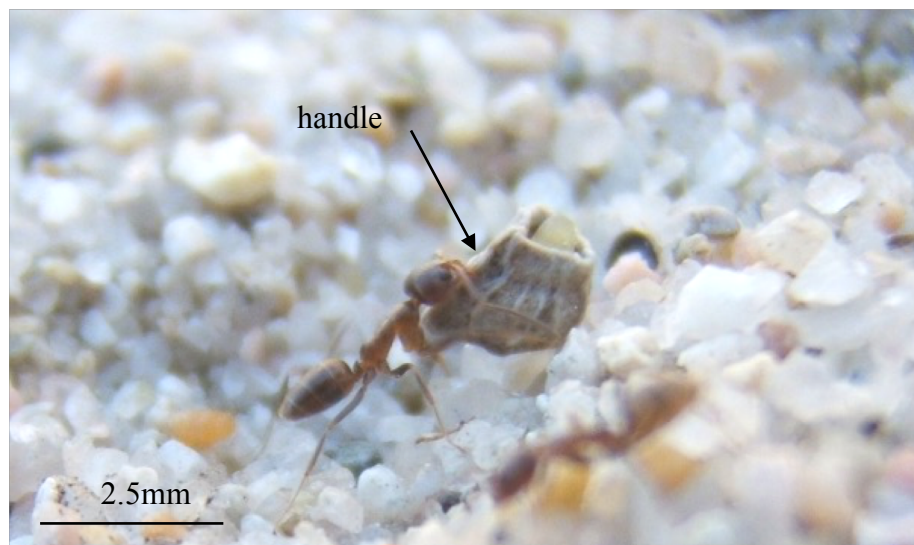
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405 Fig. 3



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409 Table 1 Species occurrences (20 pitfall traps) at each site (uninvaded: Portigliolo and invaded:
 410 Cannella) in 2008 and 2009. Blank boxes correspond to an absence of the species in the traps.

<i>Species</i>	Uninvaded (Portigliolo)		Invaded (Cannella)	
	2008	2009	2008	2009
<i>Aphaenogaster spinosa</i>	65%	55%	-	-
<i>Camponotus vagus</i>	5%	-	-	-
<i>Crematogaster scutellaris</i>	15%	5%	-	-
<i>Formica cunicularia</i>	5%	5%	-	-
<i>Lasius niger</i>	-	5%	-	-
<i>Linepithema humile</i>	-	-	100%	70%
<i>Messor minor</i>	60%	-	-	-
<i>Tapinoma sp</i>	80%	75%	-	-
<i>Tetramorium complex caespitum</i>	5%	-	-	-

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414 Table 2 Number of seeds of all species transported within five distances categories by *Tapinoma*
 415 *sp* (n=13), *Linepithema humile* (n=34), *Messor minor* (n=28), and *Aphaenogaster spinosa*
 416 (n=7).

	Distances of seed removal (cm)			
	< 10	10 - 50	51 - 100	> 100
<i>Aphaenogaster spinosa</i>	0 (0%)	0 (0%)	0 (0%)	7 (100%)
<i>Messor minor</i>	0 (0%)	0 (0%)	0 (0%)	28 (100%)
<i>Tapinoma sp</i>	5 (38%)	5 (38%)	3 (23%)	0 (0%)
<i>Linepithema humile</i>	14 (41%)	11 (32%)	9 (26%)	0 (0%)

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