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

3

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13

14 **Abstract**

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

31

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

33

34 **Introduction**

35 Seeddispersal is one of the most ecologically significant plant-animal mutualisms. This
36 mutualism influences seedling recruitment, population dynamics, species distributions, plant-
37 community composition and gene flow (Nathan and Muller-Landau 2000). Due to their
38 ubiquitous occurrence in terrestrial ecosystems, ants are one of the most important animal
39 groups involved in seed dynamics (Lengyel et al. 2010). They contribute significantly to
40 maintaining the structure and diversity of different natural communities (Lengyel et al. 2009).

41 Elimination of native ants following invasion and particularly by the Argentine ant
42 (*Linepithema humile*) is widely reported to lead indirectly to the near-complete collapse of seed
43 dispersal services (Christian 2001; Carney et al. 2003). The Argentine ant is seen as an
44 imperfect substitute for the full range of native ants that disperse elaiosome-bearing seeds
45 (Rodriguez-Cabal et al. 2009b). Homogenization of native ant fauna following its invasion
46 further diminishes seed dispersal services for native plant species (Lach 2003), and in some
47 cases can favour the spread of highly invasive plants (Rowles and O'Dowd 2009).

48 As in other parts of its introduced range, replacement of native ants by *L. humile* in Southern
49 Europe has led to changes in the relationships between ants and plants, including
50 myrmecochory (Gómez et al. 2003; Gómez and Oliveras 2003). Argentine ants have been
51 present and affected the local ecosystem of Corsica, a French Mediterranean island, for more
52 than 60 years (Blight et al. 2014). They spread mainly on the coasts, invading modified but also
53 natural habitats including sites that serve as a habitat for an endemic myrmecochorous plant,
54 *Anchusa crispa* Viv. (Blight et al. 2009). *Anchusa crispa* is a Mediterranean endemic species
55 occurring on the islands of Corsica (France) and Sardinia (Italy). It occurs in isolated
56 populations in coastal habitats. *Anchusa crispa* has been listed under the Berne Convention
57 since 1979 and has been on the European Community list of protected species since 1992. This
58 species is classified as endangered by International Union for Conservation of Nature.

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

66

67 **Materials and Methods**

68 *Experimental sites*

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

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

82

83 *Ant assemblages*

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

91

92 Offering tests

93 We determined the behavioural reaction of ants to mature seeds of *A. crispera* in the field by
94 directly offering seeds to workers at both sites in June 2009. By using tweezers, we placed seeds
95 on the ground next to randomly selected solitary workers that prospected for food. We offered
96 80 seeds at Portigliolo to the three most abundant species (28 seeds to both *Messor minor* and
97 *Tapinoma sp* and 24 seeds to *Aphaenogaster spinosa*) and 80 seeds to the Argentine ant, the
98 only species present at Cannella. Each ant's response was categorized following the Culver and
99 Beattie (1978) classification : (i) antennate and examine: the ant touched the seed with antennae
100 and then continued to forage (ii) pick-up attempt: the ant touched the seed with antennae,
101 inspected it and then released it without removing it and (iii) remove: the ant held the seed
102 between its mandibles and removed it. When possible, we also recorded the number of seeds
103 transported over less than 10 cm, between 10 cm and 50 cm, between 50 cm and 1 m and beyond
104 1 m. Seeds were collected directly from the plant and offered to ants at the sites where they
105 were collected 24 hours after their collection. Differences in proportion of ant-seed interactions
106 and in proportion of seeds dispersed over less than 50 cm were compared using Fisher's exact
107 test.

108

109 *Seed preference and removal events*

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

127

128 *Seed germination*

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

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

137

138 **Results**

139 *Ant assemblages*

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

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

152

153 Offering tests

154 The rates of seed removal at Portigliolo (*Messor minor* (n=28), *Tapinoma sp* (n=28) and
155 *Aphaenogaster spinosa* (n=24)) and Cannella (*L. humile*) were similar, respectively 46/80 (57.5
156 %) and 41/80 (51 %) ($df = 1$, $P = 0.21$, Fisher's exact test). All *A. crispera* seeds offered to the
157 seed harvester *M. minor* were systematically removed, taken to the ant's nest more than 1 metre
158 away (range 1 m – 3 m) and deposited in the nest (n = 28). *Tapinoma sp* and the Argentine ant

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

163 Moreover, both *Tapinoma sp* and *L. humile* transported seeds over short distances, dropping
164 them along foraging trails. *Tapinoma sp* workers transported 38% (5/13) of seeds over less than
165 10 cm, 38% (5/13) between 10 cm and 50 cm and 23% (3/13) between 50 cm and 1 m (Table
166 2). *Linepithema humile* transported 41% (14/34) of seeds over less than 10 cm, 32% (11/34)
167 between 10 cm and 50 cm and the remaining 26% (9/34) were transported over distances
168 ranging between 50 cm and 1 m (Table 2). Proportions of seeds dispersed by *Tapinoma sp* and
169 *L. humile* over less than 50 cm were similar: 77% (10/13) and 74% (25/34) respectively ($df =$
170 1, $P = 0.56$, Fisher's exact test).

171

172 *Seed preference and removal events*

173 At the uninvaded site, the proportion of *A. crispera* seeds dispersed did not differ significantly
174 between years, respectively 28/120 (23%) in 2008 and 19/120 (16%) in 2009 (Fig. 2) ($df = 1,$
175 $P = 0.10$, Fisher's exact test). *Anchusa crispera* seed dispersal was continuous throughout the
176 five-hour tests. Twenty-six seeds (22 %) of *Lotus cytisoides* were dispersed in 2008 and 3/120
177 (2.5%) in 2009 ($df = 1, P < 0.0001$, Fisher's exact test). Fourteen (12%) seeds of *Silene sericea*
178 were dispersed in 2008 and 4/120 (3.3%) in 2009 ($df = 1, P = 0.01$, Fisher's exact test). Both in
179 2008 and 2009, we recorded a significant relationship between the proportion of dispersed and
180 remaining seeds and plant species ($df = 2, P < 0.05$, Chi-square test). In 2008, the numbers of
181 *A. crispera* and *L. cytisoides* seeds dispersed did not differ significantly ($df = 1, P = 0.43$, Fisher's
182 exact test), whereas in 2009 it was mainly *A. crispera* seeds that were dispersed.

183 At Cannella, the invaded site, the proportion of *A. crispata* seeds dispersed differed between
184 years, respectively 79/120 (66%) in 2008 and 26/120 (22%) in 2009 (Fig. 2) ($df = 1, P < 0.0001$,
185 Fisher's exact test). In 2008, approximately 50% of *A. crispata* seeds were already dispersed
186 30 min after the test started. Eleven seeds (9%) of *M. ramosissima* were dispersed in 2008 and
187 4/120 (3%) in 2009 ($df = 1, P = 0.05$, Fisher's exact test). Eight (7%) seeds of *S. sericea* were
188 dispersed in 2008 and 6/120 (5%) in 2009 ($df = 1, P = 0.39$, Fisher's exact test). At Cannella,
189 both in 2008 and 2009, we recorded a significant relationship between the proportion of
190 dispersed and remaining seeds and plant species ($df = 2, P < 0.05$, Chi-square test). Both in
191 2008 and 2009, it was mainly *A. crispata* seeds that were dispersed. Proportions of *A. crispata* seeds
192 dispersed differed between sites in 2008 ($df = 1, P < 0.0001$, Fisher's exact test) but were similar
193 in 2009 ($df = 1, P = 0.16$, Fisher's exact test).

194

195 *Seed germination*

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

200

201 **Discussion**

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

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

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

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

238 The fact that *L. humile* and *Tapinoma sp*, disperses seeds over short distances may not imperil
239 *A. crisper* populations. If it is of no value to the majority of myrmecochores (e.g. Kjellsson,
240 1991), seed dispersal over short distances may provide benefits to *A. crisper*. Indeed, the progeny
241 produced by related individuals shows greater fitness than the progeny produced by unrelated
242 individuals, through outbreeding depression (Quilichini et al. 2001). Moreover, the fact that
243 both *Tapinoma sp* and the Argentine ant loses seeds on route to nests may not be a limiting
244 factor for seed germination. It removes direct competition from the mother plant and under the
245 action of wind or rain, abandoned seeds are rapidly covered by a fine layer of sand that may
246 facilitate their germination. The highest germination rates were observed when seeds remained
247 within the top two centimetres of sand (Quilichini and Debussche 2000). This is confirmed by
248 our observation of several plants growing along main Argentine ant trails.

249 It is commonly argued that invasive ants disrupt seed dispersal, leading to a near-complete
250 collapse of dispersal services and a decrease in seedling recruitment (Lach 2003). Reducing
251 predation and competition from related plants, minimizing the effects of fire, and depositing
252 seeds under suitable conditions are the usually benefits that plants receive from native ants
253 (Giladi 2006), and which Argentine ants have usually failed to provide (Ness & Bronstein,
254 2004; Rodriguez-Cabal, Stuble, Nuñez, & Sanders, 2009). However, invasive ants do not
255 systematically interact to the detriment of native plants. In some cases, they even facilitate

256 native species. For instance, Imported Fire ant has been described as responsible for more than
257 half of all removals facilitating dispersal of elaiosome-bearing plant species (Stuble et al. 2010).
258 Generally, exotic species facilitate native myrmecochores when they replace native ants, fulfil
259 their functional role and provide seeds with a means of escape from enemies or competitors
260 (Rodriguez 2006). Here, in addition to seed removal there is no direct negative effect on seed
261 germination. Furthermore, the Argentine ant may decrease seed predation by displacing
262 granivorous insects like the native ant *M. minor*. A previous study demonstrated that the
263 presence of the Argentine ant could prevent seed predation by local *Messor* species (Oliveras
264 et al. 2007). Similarly, *Solenopsis invicta* creates heterogeneity in seed survival by reducing
265 seed predation by arthropods in the vicinity of their nest (Chandler and Orrock 2016). Among
266 seed predators, harvester ants are considered primary seed consumers (Beattie and Hughes
267 2002). They may decrease seed germination by direct predation (Schöning et al. 2004) although
268 they could play a dispersal role when they drop seeds in suitable conditions for germination in
269 midtransport or in refuse piles (Retana et al. 2004; Bulot et al. 2016). However, this positive
270 effect may be very limited. For example, ant-dispersed diaspores accounted for only 0.1% of
271 seeds harvested in grasslands by *Messor barbarus* (Detrain and Tasse 2000). In the time of the
272 experimentation, we never noted adult plants or seedlings in the immediate vicinity of *M. minor*
273 nests (e.g. refuse piles), suggesting the species likely negative impact on *A. crispa* seeds.

274 Although, the absence of replication due to the very low number and small size of *A. crispa*
275 populations in Corsica may limit our conclusions, the results obtained from the different
276 experimentations suggest a functional replacement of local ants in *A. crispa* seed dispersal by
277 the Argentine ant. In the line of this hypothesis, long-term surveys of *A. crispa* populations
278 showed an increase in the number of individuals over 10 years at the invaded site, Cannella
279 (Bosc 2009). From 2007 onwards, the number of plants is higher at Cannella than at Portigliolo,
280 which was initially the biggest population in Corsica (Bosc 2008).

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

288

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293

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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).

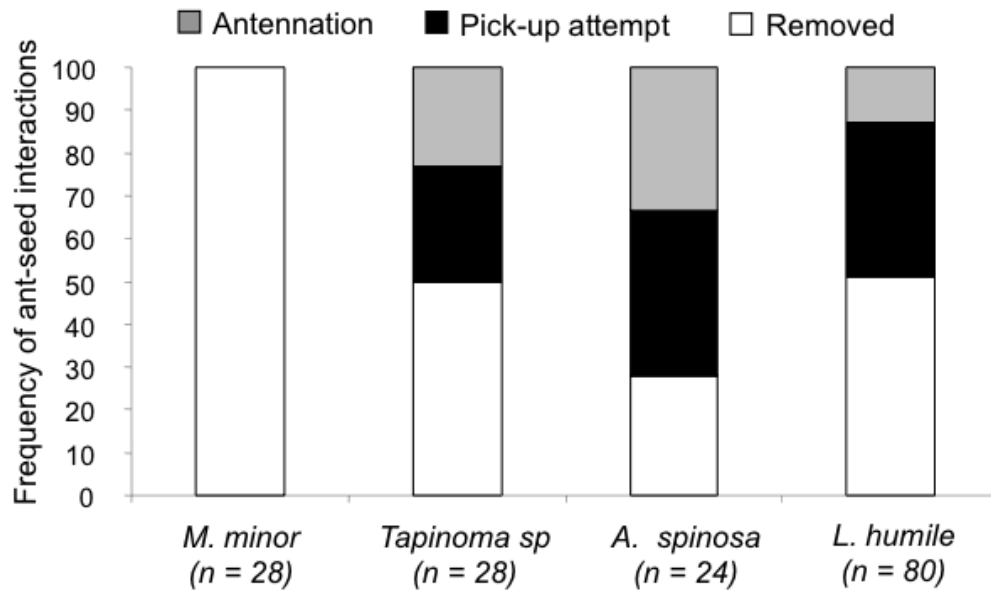
390 Figure 3 An Argentine worker transporting a seed of *Anchusa crispera* by the handle.

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393 Fig. 1

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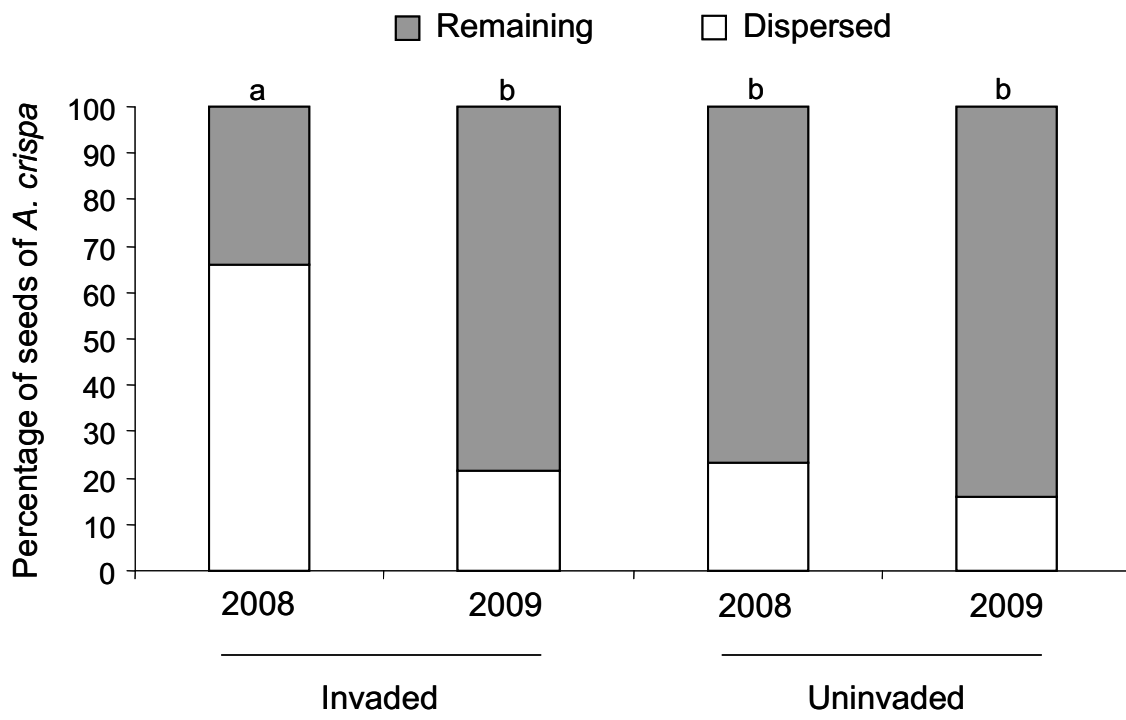
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399 Fig. 2

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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).

417

	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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