INVADING PARASITOIDS SUFFER NO ALLEE EFFECT: A MANIPULATIVE FIELD EXPERIMENT

Xavier Fauvergue,[1,3] Jean-Claude Malausa,[1] Ludovic Giuge,[1] and Franck Courchamp[2]

[1]*Institut National de la Recherche Agronomique (INRA), 400 Route des Chappes, 06903 Sophia Antipolis Cedex, France*
[2]*Unité Mixte de Recherche, Centre National de la Recherche Scientifique (UNR CNRS 8079), Ecologie, Systématique et Evolution, Université Paris Sud 91405 Orsay Cedex, France*

Abstract. One frequent explanation for the failure of biological invasions is the Allee effect: due to positive density dependence, initially small invading populations may fail to establish and spread. Populations released for biological control are similar to fortuitous invading populations and may therefore suffer from Allee effects. However, unlike fortuitous invasions, biological control allows the experimental manipulation of initial population size and, thus, offers a unique opportunity to test for the occurrence of Allee effects. We manipulated the initial size of 45 populations of a parasitoid wasp introduced for the biological control of a phytophagous insect and followed the population dynamics of both parasitoids and hosts during three years. Our results suggest an absence of Allee effects but clear negative density dependence instead: (1) the probability of establishment after three years was not affected by initial population size; (2) net reproductive rate was highest at low parasitoid density and high host density; (3) the sex ratio, reflecting the proportion of virgin females, did not increase at low density, suggesting that low densities did not impede mate-finding; (4) the depression of host populations did not depend upon the number of parasitoids introduced. This is, to our knowledge, the first experimental test of the Allee effect in an invading parasitoid. It leads us to propose that a number of behavioral and life-history features of many parasitoids could protect them from Allee effects.

Key words: biological control and invasions; density dependence; mate-finding; Metcalfa pruinosa; Neodryinus typhlocybae; *parasitoid introduction; propagule pressure; rescue effect.*

INTRODUCTION

The urgent need to better understand and manage declining populations for conservation biology has recently led to an increased effort toward deciphering fundamental ecological mechanisms at work on small populations. In particular, there has been a dramatic increase in the number of studies focused on the Allee effect, both theoretical and empirical (for reviews see Courchamp et al. 1999, Stephens and Sutherland 1999, Stephens et al. 1999, Boukal and Berec 2002). The Allee effect occurs when a decrease in the fitness of individuals is generated by a decrease of either their density or their numbers (Courchamp et al. 1999, Stephens et al. 1999). The decreased individual fitness lowers the population growth rate, even to negative values in cases of a strong Allee effect, further decreasing the population size or density. As this will further lower individual fitness, it may drive the population into a vortex of extinction. One generally distinguishes two types of Allee effect. The reduction of any component of individual fitness is called a "component Allee effect." The most cited component Allee effect results from the difficulty of

mates finding one another at low density (e.g., Dennis 1989, McCarthy 1997, Wells et al. 1998). The subsequent decrease of the population growth rate is the "demographic Allee effect." Demographic Allee effects are always generated by one or several component Allee effects, but the opposite is not true; component Allee effects do not always result in a demographic Allee effect, because negative density-dependent mechanisms may compensate at the population level (Stephens et al. 1999).

Allee effects can influence, and be studied, at different moments of a population's dynamics, but a major focus of studies is population establishment. In general, populations start with a low number of individuals, and consequently, Allee effects have the most dramatic impact at this stage. A number of theoretical models show that Allee effects influence the relationship between initial population size and the probability of establishment (review in Drake and Lodge 2006). In the simplest case of deterministic population growth with a strong Allee effect, population establishment should follow a step function, with establishment occurring only if the initial population size exceeds a threshold above which the per capita population growth rate is positive (e.g., Hopper and Roush 1993). When the Allee effect is combined with demographic and/or environmental stochasticity, the relationship between the probability of establishment and initial population size

may follow a variety of monotonously increasing functions, with the Allee effect always resulting in lower probabilities of establishment (Grevstad 1999b, Drake 2004). In general, a positive relationship between the number of founders and the probability of establishment is well supported by data from both retrospective meta-analyses (Beirne 1975, Hopper and Roush 1993, Green 1997, Forsyth and Duncan 2001, Duggan et al. 2006) and manipulative experiments (Ebenhard 1989, Memmott et al. 1998, Grevstad 1999a, Hee et al. 2000, Berggren 2001, Memmott et al. 2005). However, these studies do not demonstrate that an Allee effect is responsible for the observed relationship, unless the effect of initial population size on individual fitness and/or per capita growth rate is also assessed. To our knowledge, this demonstration has been made only once (Grevstad 1999a).

Because the Allee effect is expected to influence the establishment stage so dramatically, it should be a crucial process in biological invasions. Indeed, an effect of initial density on individual fitness potentially explains the pervasive relationship between propagule pressure and invasion success (Lockwood et al. 2005). Allee effects may also cause longer lag times and slower spread (review in Taylor and Hastings 2005), and influence the fate of attempts to control invading species (Fagan et al. 2002, Bascompte 2003, Liebhold and Bascompte 2003). Nonetheless, despite the accumulation of indirect evidence for the potential importance of Allee effects in biological invasions, experimental demonstrations are scarce, due to logistic and ethical reasons (but see Grevstad 1999a, Cappuccino 2004, Davis et al. 2004a, b). But as biological invasions are a major cause of biodiversity loss, there is an urgent need to better understand the mechanisms taking place during this process.

A particular type of ecosystem invasion where population establishment is a key stage is biological control: the use of natural enemies to reduce the damage caused by an unwanted population. Rather than enhancing the density of already present natural enemies, classical biological control mostly resorts to introducing new natural enemies into targeted host populations. As biological control is nothing other than a well-controlled biological invasion, the idea that it can be a unique and powerful way of investigating the processes that take place in the course of such invasions is steadily gaining acceptance (Ehler 1998, Fagan et al. 2002). Notably, biological control allows the creation of populations with varying initial conditions, which enables the testing of a number of predictions relating to biological invasion. In particular, two seminal studies used this methodology (based on the introduction of phytophagous insects for the biological control of weeds) to test for the effect of propagule size on population establishment and growth (Grevstad 1999a, Memmott et al. 2005). Nevertheless, given these experimental opportunities, it seems surprising that the

occurrence of Allee effects during biological invasions has not been more thoroughly investigated in the context of biological control.

We have thus used the setting of biological control with parasitoids to address a number of questions with direct relevance to Allee effects in biological invasion. Is there an effect of initial population size or density on (1) population establishment probability, (2) population growth rate, (3) mating probabilities, or (4) control of the host population? To address these questions, we used an experimental design manipulating propagule size of the parasitoid *Neodryinus typhlocybae* (Ashmead) (Hymenoptera: Dryinidae), introduced in the South of France for the biological control of a phytophagous insect, *Metcalfa pruinosa* (Say) (Homoptera: Flatidae). In our setting, all four questions can be answered negatively, suggesting the absence of an Allee effect. We then attempt to generalize these results, arguing that a number of behavioral and life-history features of many parasitoid species should prevent their suffering from Allee effects.

MATERIALS AND METHODS

Characteristics of the introduced organism

Allee effects were investigated by introducing various numbers of *Neodryinus typhlocybae*, a specialist parasitoid of the phytophagous flatid planthopper *Metcalfa pruinosa*. In Mediterranean conditions, the life cycle of *N. typhlocybae* is as follow: eggs are laid inside third- to fifth-instar larvae of *M. pruinosa*, generally during late spring to early summer. Larvae undergo solitary development up to a prepupal stage outside their hosts. They then leave the host cadaver and spin a cocoon on the supporting leaf (Dean and Bailey 1961). Most individuals enter into quiescence at that stage and overwinter until the following spring, but some individuals emerge and produce a second parasitoid generation during the summer. In the laboratory, adults can mate soon after emergence (Mazzon and Girolami 2002). Unmated females produce offspring through arrhenotokous parthenogenesis, albeit only males (Olmi 2000, Santi and Maini 2000). Males are short lived: 2–3 days in outdoor conditions, 7–10 days in the laboratory (Girolami and Conte 1999). Females live much longer (3–6 weeks), and lay 3–4 eggs per day for a total of 40–60 eggs laid over the lifetime; females also kill and feed on an average of two host larvae per day (Girolami and Conte 1999, Mazzon and Girolami 2002). Dispersal distances of adult *N. typhlocybae* were estimated to be 10–100 m at the beginning of the study (Girolami and Conte 1999), but we later realized that such a small distance likely represented only neighborhood moves that were complemented by dispersal events at a much larger spatial scale (at least several kilometers [Malausa et al. 2003]).

All the 4141 individuals introduced in May 2001 originated from a population at Cap d'Antibes (Fig. 1) that had established from the first introduction of

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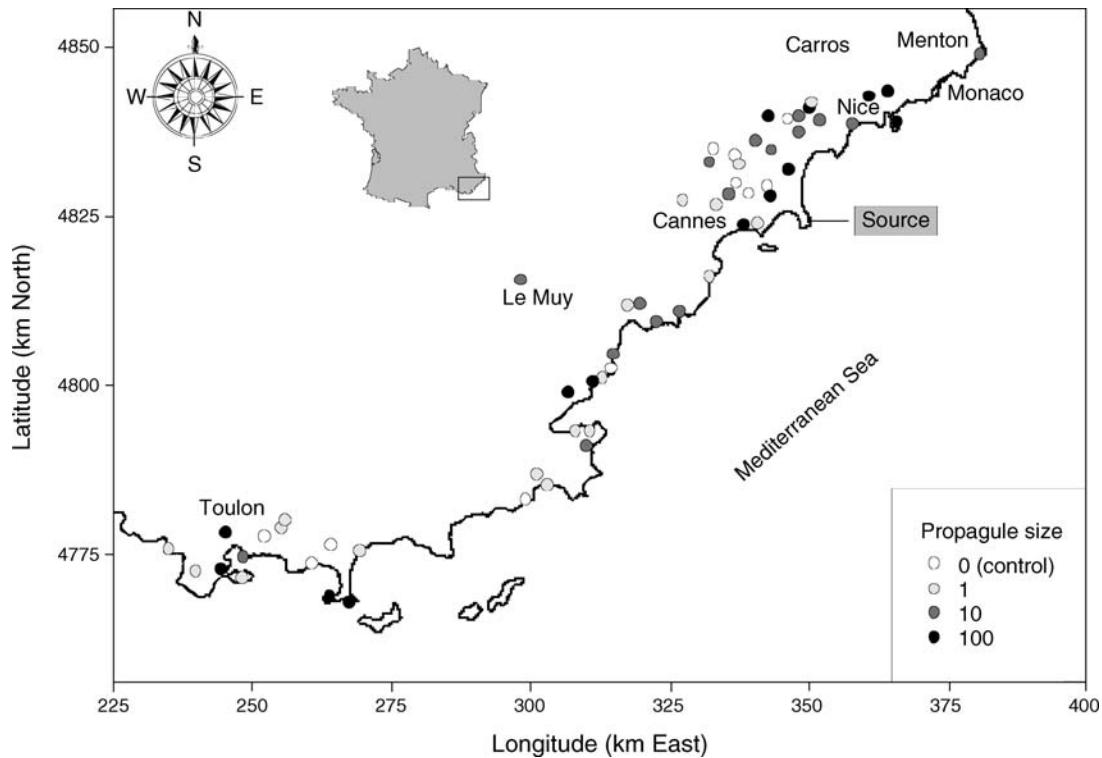
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Fig. 1. Location of the 60 experimental sites along the Mediterranean Sea in southeastern France. Propagule size corresponds to the planned number of females released per site in May 2001. All males and females released originated from the source population established since June 1996 on the Cap d'Antibes.

parasitoids in 1996. This population was derived from an Italian one, itself resulting from the introduction of individuals in 1990–1992 originating from the North American home range of *M. pruinosa*. It is therefore possible that the population at Cap d'Antibes, which will henceforward be referred to as the "source" population, was more adapted to local conditions than the ancestral American population. Integration within the recipient community had indeed started, as some individuals were attacked by local hyperparasitoids such as *Pachyneuron muscarum* and *Cheiloneurus boldyrevi*. In addition, for our experiment, all introduced cocoons had been collected eight months before the introductions (in September 2000) and handled in outdoor conditions during fall and winter to synchronize the quiescence of individuals with the local climate, and hence, the phenology of the hosts.

Cocoons of *N. typhlocybae* were crucial at three steps of this study (see Plate 1). First, cocoons are ideal to release; because they protect the individuals inside, they can be handled at low risk. This reduces initial demographic stochasticity and the chance of observing an effect of the number released on establishment rate, which does not result from an Allee effect. Second, cocoons are also ideal for nondestructive sampling. Cocoons observed on plant leaves at the end of the summer indicate the reproduction of adults during the season, and therefore the number of adults that will

reproduce in the next season (i.e., cocoons that have survived over the winter). Third, the sex of individuals can be determined from the size of the larva's compartment inside the cocoon. A threshold of 4 mm separates males and females, with males being smaller than females (X. Fauvergue, L. Giuge, and J. C. Malausa, *unpublished data*).

Field sites

The size of parasitoid propagules was manipulated in 60 experimental sites scattered along the French east end of the Mediterranean coast (Fig. 1). In order to decrease the variability among replicates, field sites were selected among a number of private gardens that fulfilled four conditions. (1) Each site had a hedge of *Pittosporum tobira* (Rosales: Pittosporaceae; see Plate 1) measuring at least 10 m in length. The hedge served as a standardized environment to release the parasitoids and to measure the demography of hosts and parasitoids. *P. tobira* was chosen because it is the most widespread ornamental plant of the French Riviera that *Metcalfa pruinosa* uses as a host plant. (2) During a one-man-hour survey carried out on every hedge a few months before the introductions, we had observed symptoms of the host (white filaments of waxy exudates), but no cocoon of the parasitoid. This insured that the host was present before the introductions, and that the parasitoid was either absent or present but at

TABLE 1. Summary statistics for the number of parasitoids introduced per site and the number of experimental sites for each level of treatment.

Mean number of individuals released			Number of sites			
Planned	Females	Males	Planned	2001	2002	2003
0	0.0 [0–0]	0.0 [0–0]	12	12 (6)	12 (6)	12 (6)
1	0.9 [0–1]	8.2 [6–11]	18	17 (12)	16 (11)	16 (10)
10	8.8 [6–10]	15.1 [6–21]	15	15 (8)	15 (8)	14 (7)
100	94.7 [90–106]	116.7 [75–134]	15	15 (9)	15 (9)	14 (8)

Notes: Numbers within brackets indicate the range in number of individuals released. Numbers within parentheses indicate the number of sites judged too far from the source population for the sites to be colonized by parasitoids (>20 km).

undetectable densities. (3) The hedge was neither trimmed nor treated with pesticides during the whole experiment, two processes which could have substantially decreased the size of both host and parasitoid populations. (4) At least 3 km separated neighboring sites, a distance which was thought to guarantee independence between sites given our initial estimate of dispersal distance. Sites fulfilling these requirements were found by selecting among 344 volunteers who responded to an announcement published in a local newspaper eight months before the introductions (Rosso 2000). A posteriori, it appeared that a few hedges were <10 m long, but this was easily handled by incorporating hedge size in statistical models. More importantly, four hedges were sprayed with insecticides or trimmed in the three-year course of the experiment. Data from such sites were kept in analyses before trimming or spraying, but removed thereafter, leading to a slight decrease in the number of replicates over time (Table 1). For each site, latitude and longitude were measured with a global positioning system. The leaf density of each hedge was estimated by counting the number of leaves in a 1-m^2 quadrat representative of the entire hedge.

Manipulation

Propagule size was manipulated by introducing either 1, 10, or 100 female *N. typhlocybae* per site, together with an overabundance of conspecific males (Table 1), both sexes being in the form of quiescent pupae overwintering inside cocoons. Such a release protocol insured that the released females were mated at emergence and could potentially found a new population. In control sites, no parasitoids were introduced. These four levels of treatment were completely randomized across the 60 sites (Fig. 1), and to prevent any anthropogenic biases, site owners were not informed about the treatment (all were told that the introduction consisted of 100 wasps). The number of cocoons introduced was manipulated by packing leaves with and without cocoons in wire baskets in proportions such that each basket (one per site) contained the planned number of cocoons but the same number of leaves. Such baskets allowed the emergence and natal dispersal of adult parasitoids through the wire. The 60 baskets were hung at the center of the 60 hedges between the 14 and 18 May 2001, a couple of weeks before expected emergence (at the beginning of June in the south of

France [Malausa et al. 2000]). Baskets were recovered after emergence, in July 2001, in order to count cocoons with and without emergence holes, and hence, to estimate the actual number of adults introduced (Table 1). Of all the cocoons introduced, 84% of the parasitoids emerged, slightly decreasing the actual number of individuals introduced. Subsequently, one site out of the 18 sites planned with one female was further assimilated as a control, because the female had not emerged.

Measurements

During the third and fourth week of September 2001, 2002, and 2003, we sampled the 60 hedges to estimate the density and sex ratio of the parasitoid *N. typhlocybae* and the density of the host *M. pruinosa*. For this, we used a nested sampling design. Each hedge was subdivided in 10 1-m blocks, with five blocks on each side of the release point (or fewer blocks for smaller hedges). Each block was further subdivided into five horizontal strata, from the ground to human height (i.e., 2 m). In each stratum, we sampled 20 *Pittosporum* leaves selected at random. We thus sampled 1000 leaves per hedge in standard 10 m long hedges. Given average leaf density (761 leaves/m^2), this corresponds to ~7% of the leaves that can be observed in a 20 m^2 surface. On each sampled leaf, we counted the cocoons of *N. typhlocybae* and the exuviates from fifth-instar larvae of *M. pruinosa*. When cocoons were found, we measured the larval compartment to determine the parasitoid's sex. An estimation of the efficiency of this sampling method was carried out independently on 10 additional hedges. For this sampling, 25 cocoons, half of the maximum fecundity of *N. typhlocybae* (Mazzon and Girolami 2002), were placed at random on one side of a hedge by one person, and the sampling was carried out by another person. On average, one-fourth of the cocoons were found (6.1 cocoons; 95% confidence interval 4.7–7.8 cocoons). Hence, there is a nonnegligible probability of finding no cocoon in a hedge where some cocoons are present. This probability will be further estimated to analyze establishment and extinction rates correctly.

Data analysis

Our data had either a binomial distribution (presence/ absence; male/female) or a Poisson distribution (number of insects), and were repeated over time. We therefore

used generalized linear models (GLM), implemented with these respective distributions, and fitted to raw data by maximum likelihood estimation of the parameters associated with the explanatory variables. When analyzing repeated measures, we based the models on generalized estimated equations (GEE; Liang and Zeger 1986), making it possible to account for the correlation structure among measurements made on the same hedge but at different times. For model selection, we used the backward procedure recommended by Crawley (1993). The significance of parameters was assessed by means of likelihood ratio (G) tests that we compared to a χ^2 distribution, or an F distribution when data were overdispersed. When parameters for class variables were found significant, means were compared via pairwise contrasts, and the significance of P values was asserted by the method of false discovery rate (Benjamini and Hochberg 1995, Garcia 2004). One peculiarity of our statistical models was the use of an offset, i.e., a variable included with the explanatory variables but for which the parameter is not estimated (it is set to one). Except when mentioned, all analyses were performed with Proc Genmod, using SAS software (SAS Institute 1999).

Results

Establishment/extinction

Because our sampling allows the observation of only one-fourth of the cocoons present in a hedge, there may be densities below which cocoons will be present in the hedge but not in the sample. We thus need a statistical criterion to infer establishment/extinction from presence/absence data. A simple way to approach this criterion is to compute the probability of finding no cocoon in the smallest possible population, i.e., one in which a single female has reproduced. We had six such populations in the first year of our experiment, based on the following criteria: (1) we had released a single female; (2) no parasitoids were found in control sites at the same distance from the source population (>20 km, see further), suggesting that the cocoons found were descending from the released female only, and not from immigrants; (3) we found at least one cocoon in the sample at the end of the reproductive season, so that the released female had indeed reproduced. In the smallest possible populations, such as these, we found an average of two cocoons in 1000 leaves (4, 3, 2, 1, 1, and 1 cocoon). The probability of finding zero cocoons in 1000 sampled leaves is thus $P = 0.14$ (from a Poisson distribution with a mean of 0.002), and the probability that this occurs over two consecutive years is $P = 0.018$. We will thus consider that observing no parasitoid over two consecutive years reveals a true absence of parasitoids. Therefore, over three consecutive years, a population may (1) fail to establish if parasitoids are never observed; (2) go extinct if parasitoids are observed the first year only; (3) be rescued by immigration if parasitoids are observed the third year only; (4) persist, in other cases. Cases 1 and 2 are considered extinctions, whereas cases 3 and 4 are considered establishment.

According to these criteria, the proportion of established populations was not affected by propagule size: 14/16 for 1 and 15/15 for both 10 and 100 (Fisher's exact test: $P = 0.3188$). The establishment of populations initiated with a single female was the result of either persistence (11/16) or rescue (3/16). Unexpectedly, half of the control sites had established populations (7/12). The probability of establishment for these sites decreased with the distance from the source (GLM with a binomial distribution and a logit link function, $G = 17.25$, $P < 0.0001$), but was not affected by host density ($G = 1.06$, $P = 0.3040$). All control sites with established populations were <20 km from the source. Establishment in control sites could be a consequence of higher rates of migration close to the source.

Parasitoid net reproductive rate

Our three-year time series allowed the estimation of parasitoid net reproductive rate (N_t/N_{t-1}) over three consecutive periods: from the release in May 2001 to the first sampling in September 2001, from September 2001 to September 2002, and from September 2002 to September 2003. The first period reflects the reproduction of the introduced individuals just after their release. In this case, N_{t-1} is the number of females introduced in May 2001, and N_t is the number of their offspring after one season of reproduction. We do not anticipate a mate-finding Allee effect during this period because females were released together with abundant males. For the two subsequent periods, the number of cocoons measured at the end of the reproductive season of year $t - 1$ (N_{t-1}) is used as an index of the number of adults reproducing during year t, and N_t is the number of their offspring. During these two periods, released individuals had scattered in their new habitat, so that mate-finding Allee effects, as well as other types of component Allee effects, are possible. A demographic Allee effect should manifest itself as a positive effect of N_{t-1} on N_t/N_{t-1}. A genetic Allee effect (e.g., resulting from inbreeding depression) may result in a positive effect of propagule size on N_t/N_{t-1}, whatever the value of t.

For each of the three periods, we fitted a GLM to N_t with N_{t-1} included as an offset. Such a procedure allows statistical predictions on N_t/N_{t-1}. Models were based on a Poisson distribution and a log link function. As explanatory variables, maximal models were based on one discrete variable, the propagule size (N_0, with three levels: 1, 10, and 100), and three continuous variables, including the density of parasitoids (N_{t-1}), the density of hosts (H_t), and the distance from the source (D). This latter variable was included to handle the problem of migration between sites. If the establishment of new populations in empty sites close to the source is a result of higher migration rates in this area, net reproductive rates should decrease with increasing distance from the

TABLE 2. Results from generalized linear models fitted to parasitoid net reproductive rate (N_t/N_{t-1}) for the three consecutive reproductive periods (each from $t-1$ to t).

	May 2001–Sep 2001			Sep 2001–Sep 2002			Sep 2002–Sep 2003		
Variable	Estimate	G	P	Estimate	G	P	Estimate	G	P
Intercept	−1.5691			1.2482			0.7638		
N_0		26.82	0.0001		*0.32*	*0.7315*		*1.44*	*0.2488*
1	2.2805			*0.2225*			*0.4238*		
10	0.9265			*0.0118*			*0.2768*		
100	0.0000			*0.0000*			*0.0000*		
D	−0.0080	9.69	0.0033	−0.0176	28.60	0.0001	*0.0037*	*0.78*	*0.3827*
N_{t-1}	*−0.0312*	*1.59*	*0.2151*	−0.0337	39.98	0.0001	−0.0099	16.86	0.0002
H_t	0.0051	31.81	0.0001	0.0066	44.21	0.0001	0.0056	21.57	0.0001

Notes: Parameter estimates and likelihood ratio statistics for the most parsimonious models are non-italic. Those for variables that were removed from maximal models are set italic and correspond to the step immediately preceding their removal. N_0 is the planned number of parasitoids released. (Note that results of likelihood ratio tests [G and P] are for all three levels of N_0.) D is the distance from the source population; N_{t-1} is the number of parasitoids in the preceding reproductive season; H_t is the number of hosts during the reproductive season.

source. Additionally, all analyses were performed with and without sites close (<20 km) to the source.

We found no Allee effect in any of the three reproductive seasons analyzed. Instead, our data demonstrate clear negative density dependence. Parasitoid net reproductive rates were positively affected by host density and negatively affected by parasitoid density (Table 2, Fig. 2). The effect of propagule size (N_0) was significant in the first season only, where it represented the negative effect of parasitoid density. (The actual number of females released was also included as a continuous form of N_{t-1}, but this variable was not in the most parsimonious model, probably because it covaried with the planned propagule size N_0.) Net reproductive rates were also likely affected by immigration. In the first two years, higher rates were observed close to the source, where new populations had established in control sites (Table 2).

Immigration did not invalidate our demonstration of negative density dependence and the absence of Allee effects. When the analyses were restricted to sites far away from the source (>20 km), where no immigration had been observed in control sites, the most parsimonious models were similar to the ones obtained from analyses with all the data. For each of the three reproductive seasons, we found net reproductive rates to be affected negatively by parasitoid density in the previous season (2001–2001, $\beta = -0.0602$, $G = 4.47$, $P = 0.0456$; 2001–2002, $\beta = -0.0465$, $G = 30.08$, $P < 0.0001$; 2002–2003, $\beta = -0.0064$, $G = 7.42$, $P = 0.0124$), and positively by host density in that season (2001–2001, $\beta = 0.0039$, $G = 18.77$, $P = 0.0002$; 2001–2002, $\beta = 0.0075$, $G = 29.55$, $P < 0.0001$; 2002–2003, $\beta = 0.0035$, $G = 9.54$, $P < 0.0054$). Data for distant sites only are plotted in the right panels of Fig. 2.

Parasitoid density

Propagule size (N_0, 1, 10, 100) affected parasitoid population density (N_t) only in 2001 ($G = 34.21$, $P < 0.0001$). Multiple comparisons of means showed that populations initiated with 100 females were significantly larger than populations initiated with either 10 females or a single female (20.6, 4.0, and 2.0 individuals per 1000 leaves, respectively). Hence, despite a process of negative density dependence occurring as soon as populations were introduced, we did manipulate initial density by manipulating propagule size.

The number of parasitoids per 1000 leaves, which we refer to as population density, could nonetheless have reflected population size but not population density if females had aggregated their progeny by laying their eggs in nearby hosts. This could have prevented some types of Allee effects, including the difficulty of finding mates (Stephens et al. 1999), and could explain our results. We thus analyzed the relationship between the number of cocoons found on 1000 leaves of hedge and the average distance separating the nearest cocoons. This relationship appeared significantly negative, whether we considered the nearest distance between any kind of cocoon ($G = 16.14$, $P < 0.0001$), or the nearest distance between male and female cocoons only ($G = 17.37$, $P < 0.0001$). Hence, by manipulating the number of parasitoid females released, we also manipulated the actual density of parasitoids in the *Pittosporum* hedge.

As a result of negative density dependence, parasitoid populations deriving from different inocula in May 2001 converged toward a common density in the course of the experiment. Average densities of populations initiated with 1, 10, and 100 females were statistically similar, with 14.8, 27.7, and 34.5 individuals per 1000 leaves, respectively, in 2002, and 58.7, 47.4, and 54.3 individuals per 1000 leaves, respectively, in 2003. For all three reproductive seasons, the density of cocoons in one year increased with increasing density in the previous year, and increased also with increasing host density. Not surprisingly, if the immigration rate is higher close to the source, parasitoid density decreased with increasing distance from the source in 2001 and 2002 (but not 2003).
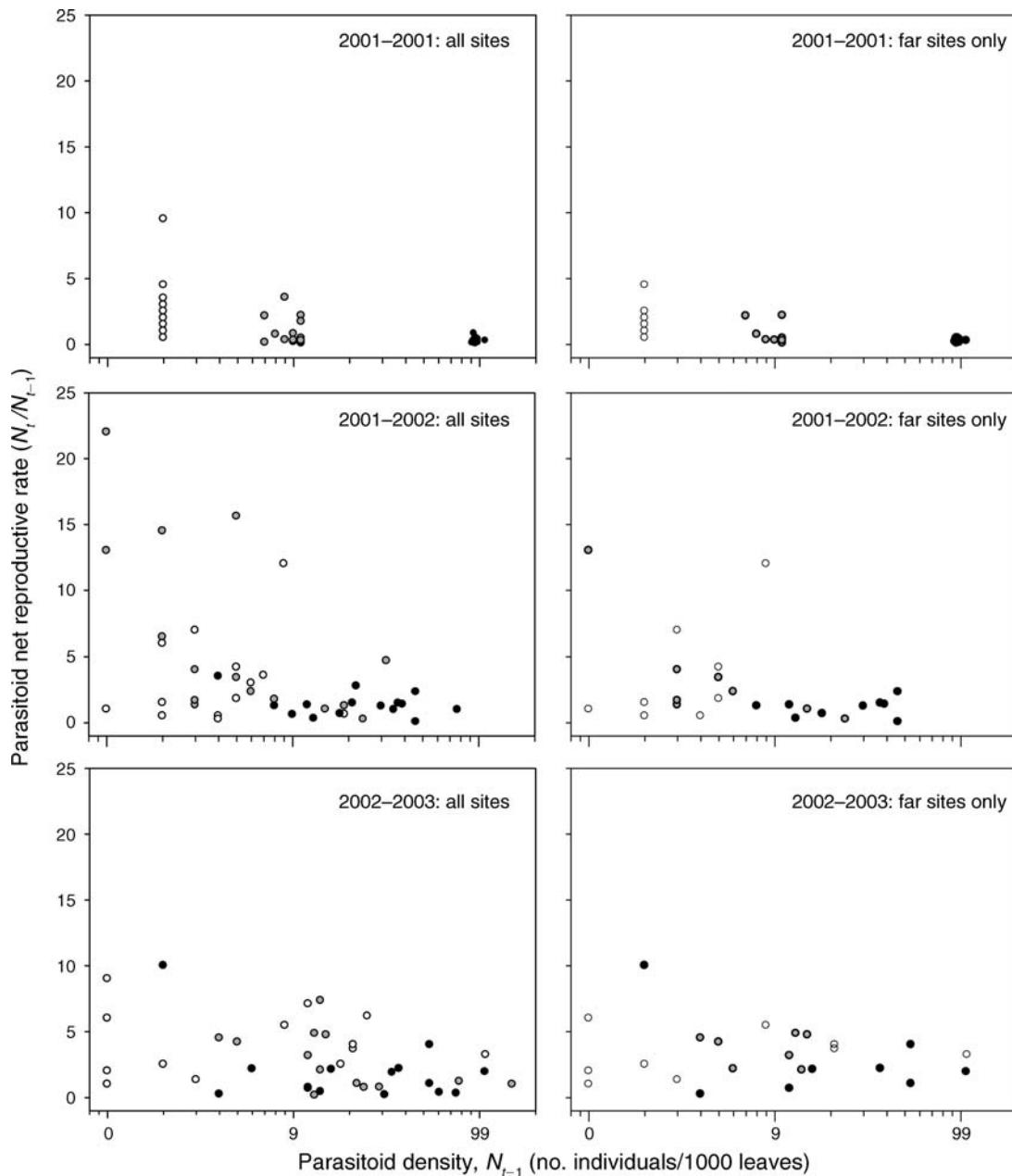
FIG. 2. Parasitoid net reproductive rate (N_t/N_{t-1}) vs. parasitoid density (N_{t-1}) for populations inoculated with one (open circle), 10 (gray circles), or 100 females (solid circles) and for the three reproductive seasons. Here, N_{t-1} and N_t are the number of parasitoids per 1000 sampled leaves in September of two consecutive years, except for the first season (2001–2001), where N_{t-1} is the number of females actually released. Data are plotted for all sites (left panels) or for sites far (>20 km) from the source population (right panels), the distance at which control sites were not colonized by parasitoids.

Sex ratio

If male density affects the probability that females are mated, then because virgin females of *N. typhlocybae* produce only sons, male density during a given reproductive season should affect the offspring sex ratio. To test this prediction, we fitted a GLM based on a binomial distribution and a logit link function to the proportion of males (measured at the end of a reproductive season), with male density (assessed from the number of male cocoons at the end of the previous season) as an explanatory variable. In 2002, the proportion of males was not affected by male density ($G = 1.84$, $P = 0.1749$). In 2003, the proportion of males was affected by male density ($G = 6.78$, $P = 0.0092$), but the relationship was opposite to that expected from a difficulty of finding mates at low density: fewer males in 2002 resulted in fewer males in 2003 (Fig. 3).

Parasitoid impact on host dynamics

To assess the consequences of introducing different numbers of parasitoids on the population dynamics of the host *M. pruinosa*, we fitted host densities across the three years with a GLM for repeated measures based on a Poisson distribution and a log link function. As explanatory variables, we included the parasitoid propagule size (N_0, 0, 1, 10, and 100) as a discrete variable, and year and distance from the source as continuous variables. We also included the interaction between propagule size and year as a specific way to test the effect of the number of parasitoids released on host dynamics. The number of sampled leaves was introduced as an offset. Fitting this model to the data resulted in an insignificant effect of propagule size as a main effect as well as when interacting with time. The most parsimonious model revealed the effect of year only ($G = 16.15$, $P < 0.0001$). Whatever the number of parasitoids introduced, the density of hosts decreased in the three-year course of the experiment. The same conclusion was derived after selecting only sites far from the source ($G = 9.83$, $P = 0.0017$).

DISCUSSION

This article is the first, to our knowledge, to experimentally study the Allee effect in an introduced parasitoid. Through an experimental release of individuals in a standardized, natural environment, we were able to assess the importance of the number of individuals introduced on the probability of population establishment and on the rate of population growth. By manipulating initial population size under natural conditions, with sizes as small as one single female, our objective was to gather the conditions to detect a demographic Allee effect, if one were to exist in this species. This would enable us either to characterize the requirements for the presence of an Allee effect or to identify the reasons for its absence. For this, we also studied the role of population density on mating success. Finally, as this work was conducted in the context of biological control, we assessed the role of initial population size of the species introduced, a parasitoid, on the population density of its host. Unexpectedly, our results are unambiguous and consistent in showing that *Neodryinus typhlocybae* was not subject to Allee effects in our setting; population establishment probability was not dependent on the number of individuals released and the subsequent growth rate was negatively correlated to the propagule size. Similarly, we found no correlation between mating probability and density, which may explain the lack of Allee effect. Consequently, the control efficiency of the parasitoid did not depend on the initial parasitoid population size.

Therefore, the optimal release strategy for *N. typhlocybae*, defined as the one that would maximize the number of populations established with a given number of individuals to release (Shea and Possingham 2000), would be many introductions of a single female. This
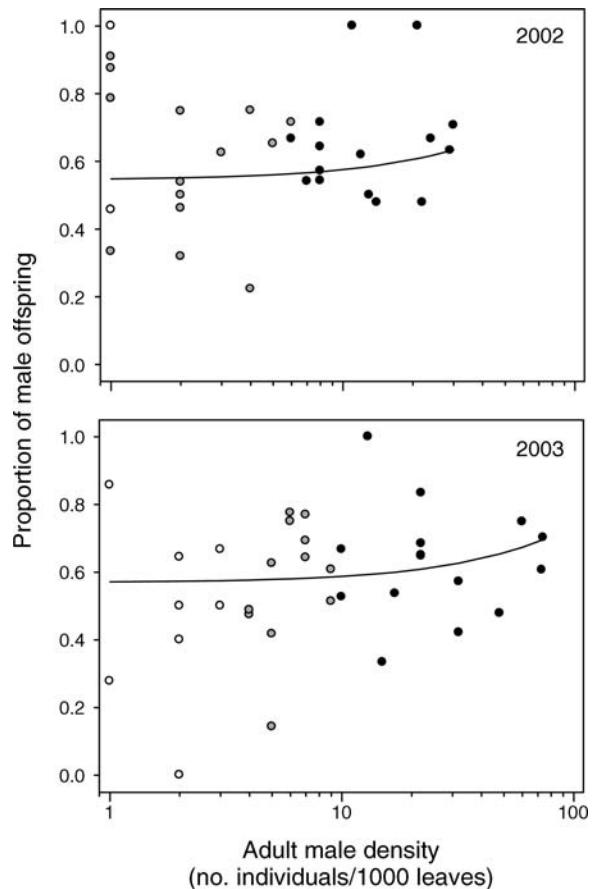

FIG. 3. The proportion of male cocoons produced during a given field season (measured at the end of the season) vs. adult male density during that season (measured as the number of male cocoons at the end of the previous season; note the log scale) for populations inoculated with one (open circle), 10 (gray circles), or 100 females (solid circles). Lines represent predictions from a binomial regression. The absence of negative correlation shows that low male density does not result in more virgin females.

result comes from the high probabilities of establishment of *N. typhlocybae* at low density; even if there were no rescue effect, with 100 females, one could establish 69 populations with 100 releases of one mated female ($100/1 \times 11/16$), 10 populations with 10 release of 10 females ($100/10 \times 15/15$), and only one population with one release of 100 females ($100/100 \times 15/15$).

Obviously, it is important to distinguish between the demonstration of the absence of Allee effects, and the failure to demonstrate its potential presence. We claim that the evidence we present here is unambiguous: with the setting we used, no Allee effect occurred in the establishment, building, and persistence of introduced *Neodryinus typhlocybae* populations, with clear negative density dependence occurring instead.

Because a number of pioneering theoretical and empirical studies had highlighted the possible importance of the Allee effect in biological control (Beirne

PLATE 1. Leaves of *Pittosporum tobira* (Rosales: Pittosporaceae) with cocoons of the introduced parasitoid *Neodryinus typhlocybae* (Hymenoptera: Dryinidae). Such a system facilitated the experiment at different steps: (1) cocoons were ideal to introduce parasitoids with no harm in experimental release sites; (2) parasitoid density was estimated by counting cocoons on the 1000 sampled leaves of each *Pittosporum* edge; (3) parasitoid sex ratio was estimated by measuring the larva's compartment inside each cocoon found, as a threshold of 4 mm separates males and females, with males being smaller than females.

1975, Hopper and Roush 1993, Memmott et al. 1998, 2005, Grevstad 1999a, b, Shea and Possingham 2000, Fagan et al. 2002), we did not anticipate its absence here. The most pronounced claim that an Allee effect occurs during biological control introductions is that of Hopper and Roush (1993). Based on a reaction–diffusion model of invasion and a meta-analysis of past attempts to introduce parasitoids and predators to control lepidopteran pests worldwide, they argued that the difficulty of mate-finding at low density could explain the positive relationship between the number introduced and the proportion of established populations. Their results suggested a threshold of ~1000 insects per release to insure establishment. In their discussion of the implications of their findings for biological control introduction design, they nonetheless acknowledge that "limitations in retrospective analyses and current knowledge indicate the need for an experimental approach to introductions" (Hopper and Roush 1993). These limitations motivated the three experiments on the biological control of weeds with herbivores (Memmott et al. 1998, 2005, Grevstad 1999a) as well as the present study on parasitoids.

It may thus come as a surprise that our main finding is inconsistent with the presence of Allee effects, and the next exciting question, assuming our study is devoid of bias, obviously relates to the generality of this counter-intuitive finding. Although up to now considered the authoritative study in the field, the study by Hopper and Roush (1993) did not in fact prove the presence of any Allee effect. What their analysis clearly shows is that the probability of establishment increases with increasing number released. However, they also observed a tight correlation between the number released and the number collected, which inevitably tangles inferences based solely on the Allee effect. For example, a low number collected could result from rarity in the environment of origin, for such reasons as poor adaptation to the host. When released in a different environment but against that same host (which is a usual practice in classical biological control), such individuals could have very low fitness and drive the population extinct. Demographic stochasticity or other reasons that are not linked to an Allee effect could also lead to extinctions of small populations. Manipulative experiments where individual fitness and/or demography are followed over several generations may unravel the processes underlying the extinction of small, introduced populations. At present, one such experiment does show a demographic Allee effect (Grevstad 1999a), but the other does not (Memmott et al. 2005). So we argue here that despite previous generally admitted claims, control agents might not always suffer from Allee effects.

The generality of our results now needs to be assessed. One step toward this important goal is to elucidate the reasons for the apparent absence of Allee effect. Understanding the reasons for Allee effect absence could indeed lead to deciphering those for its presence in other cases. This will enable us not only to categorize species into those with and those without Allee effects, but also to learn how to avoid Allee effects in the future, both constituting an enormous advantage for biological control as well as for biological conservation. Six possible, nonexclusive explanations could be invoked to elucidate the apparent absence of Allee effect in this species, as well as in other parasitoid species:

1) Parasitoids generally experience high levels of intraspecific competition for the host resource, through mechanisms ranging from interference to superparasitism (Godfray 1994). Consequently, the effect of any component Allee effect would readily be counterbalanced by intense negative density dependence, which would prevent any demographic Allee effect.

2) Parasitoids are known for their very efficient system of location, either of hosts or of mates, notably with pheromones (Fauvergue et al. 1999), which may allow them to avoid component Allee effects. This could lead to a limitation or even prevention of mate-finding Allee effects, even at very low densities. Although a positive relationship between male density and mating success was suggested from behavioral studies (Fauvergue et al. 1995), we have no evidence that this relationship does occur in field populations of parasitoids. In our study, mating probability did not decrease at low density.

3) Another mechanism that could allow parasitoid populations to avoid Allee effects relates to a shift in the reproductive behavior of individuals after they have traveled to isolated patches. Both theory and data show that parasitoids should reproduce more intensively on a host patch after a long travel time (Charnov 1976, Cronin and Strong 1999, Boivin et al. 2004, Thiel and Hoffmeister 2004, Tentelier et al. 2006). Moreover, based on field data, Cronin and Strong (1999) showed that the resulting aggregation of parasitism would be kept at a level theoretically sufficient to promote locally stable parasitoid–host dynamics in isolated patches. Therefore, increased foraging effort with distance traversed could counter Allee effects in colonization and increase spatial spread of populations of natural enemies.

4) Many parasitoids belong to the order Hymenoptera, and are therefore haplodiploids, a characteristic that may substantially alleviate of the difficulty of mate-finding at low density. In haplodiploids, arrhenotokous parthenogenesis is the most common mode of reproduction: males develop from unfertilized eggs and females from fertilized eggs. Virgin females can reproduce, but they are constrained to produce males only (Godfray 1990). Consequently, a shortage of males in one generation does not lead to a decrease in birth and a subsequent decrease in density in the next generation, but rather, to a sex ratio bias toward males. This effect generates a negative feedback, which lowers potential Allee effects. A difficulty of mate-finding at low density results in more males being produced, which in turn reduces that difficulty. In agreement with such a scenario, models and data are consistent in showing that Allee effects should be less frequent in haplodiploids than in diploids (Hopper and Roush 1993).

5) Another mechanism resulting from haplodiploidy is that genetic load is purged through haploid males. Consequently, inbreeding depression at low population size may be less severe in haplodiploids than in diploids (Henter 2003), making species of hymenopteran parasitoids less likely to suffer from genetic Allee effect. A notable exception, however, concerns species with single-locus complementary sex determination (sl-CSD), where homozygosity at the sex locus results in unviable or sterile males. In such species, decreased population size may drive populations into an extinction vortex, as it leads to decreased allelic richness at the sex locus, increased proportion of diploid males, and in turn, decreased growth rate (Zayed and Packer 2005, see also Hedrick et al. 2006). For such reasons, the genetics of sex determination has been hypothesized as an important factor for the success of biological control (Stouthamer et al. 1992). Whether it is of significant importance nonetheless depends on the pervasiveness of CSD among parasitoids used for biological control, something that remains unknown (van Wilgenburg et al. 2006).

6) In a metapopulation perspective, the dispersal of individuals may contribute to rescue small populations from extinction (Kuussaari et al. 1998). Our study shows that parasitoid establishment, growth rate, and population densities are positively affected by the presence of a nearby source population, a result that could be explained by source–sink migrations. Such a relationship between population connectivity and colonization has seldom been documented in parasitoids (van Nouhuys and Hanski 2002), and a generalization is also difficult given the scarcity of data on long-distance dispersal in these organisms (but see Antolin and Strong 1987).

Hence the absence of major components Allee effects, in conjunction with strong intraspecific competition and rescue effects, would then combine to lower the possibility of demographic Allee effects in *Neodryinus typhlocybae*. With perhaps the exclusion of the last one, all of the above explanations hold true for many parasitoid species that are currently used as biological control agents, especially hymenopterous parasitoids. It is therefore likely that the apparent absence of Allee effect in our study is also a general outcome for those parasitoids. Evidently, the veracity of this assertion remains to be confronted with hard data as needed on a case-by-case basis.

With this study, we support the view that applied ecology in general and biological control in particular

directly benefit from expanding the conceptual and theoretical frameworks of fundamental ecology. Biological control can be a very powerful avenue of knowledge for applied ecology, and in particular for invasion biology. Mechanisms involved in both processes are similar, to such a point that we claimed that biological control introductions could and should be used as controlled experiments about the course of biological invasions. We have seen here, however, that some fundamental mechanisms that are likely present in biological invasions might be lacking in biological control. Namely, the Allee effect has been proposed as an important process hindering establishment of introduced populations (Hopper and Roush 1993, Grevstad 1999b, Drake 2004, Drake and Lodge 2006), but we propose here that such is not necessarily always the case for biological control. One reason is that biological control is normally designed to succeed, notably putting control agents in very favorable conditions (especially in terms of resource, the target pest). Another reason is that a favored organism for biological control, parasitoid wasps, are, and this is not fortuitous, species with life-history parameters allowing them a high success in population establishment. Consequently, the theoretical framework used to describe and understand biological invasions might not always be adequate to biological control. We thus urge for further experimental studies to prove or disprove the generalization of our finding of an apparent absence of Allee effect in a species used for biological control.

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