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Non-additive effects of multiple natural enemies on aphid populations

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Abstract The question of whether multiple natural enemies often interact to produce lower host mortality than single enemies acting alone has not yet been resolved. We compared the effects of four different combinations of natural enemies—parasitoids, predators, parasitoids plus predators, and no enemies—on caged aphid populations on marsh elder, *Iva frutescens*, in west-central Florida. Using starting densities of natural enemies commonly found in the field, we showed that parasitoid wasps reduced aphid population densities more than predatory ladybird beetles. The addition of predators to cages containing parasites reduced the ability of parasitoids to decrease aphid population densities. Because the experiments ran only over the course of one generation, such a reduction in the effectiveness of parasites is likely caused by interference of predators with parasitoid behavior. Parasitism in the cages containing both parasitoids and predators was reduced when compared to percent parasitism in parasitoid-only cages, but this could also be due to predation. Our experiments showed that ladybird beetles prey on parasitized aphids. Thus over the long-term, the effectiveness of parasites is impaired by the interference of predators on ovipositing parasitoids and by the predation of parasitized aphids. The effects of natural enemies in this system are clearly non-additive.

Key words Aphids · Parasitoids · Predators · Non-additive effects · Biological control

Introduction

Considerable debate exists over whether the effects of multiple natural enemies are additive or non-additive (Gutierrez et al. 1984; Prakasan and Bhat 1985; Hagvar and Hofsvang 1988; Evans 1991; Godfray and Waage 1991; Kindlmann and Ruzicka 1992; Rosenheim et al.

1995). There are at least five recognizable outcomes when more than one natural enemy is released in the field (Fig. 1). First, enemies could act synergistically, resulting in a higher than expected rate of mortality to the prey population. Second, enemies might not interact so that the total level of mortality is equivalent to the individual mortalities combined (additive mortality). Third, the enemies could interact with one another to produce a level of total mortality that was less than additive mortality (non-additive mortality). Fourth, the total mortality might be less than that caused by one natural enemy alone but not the other. Finally, the total mortality could be less than when either enemy acts alone. The last three cases can all be thought of as non-additive effects. There have been, as far as we know, no attempts to assign different terms to describe the three different degrees of non-additive effects.

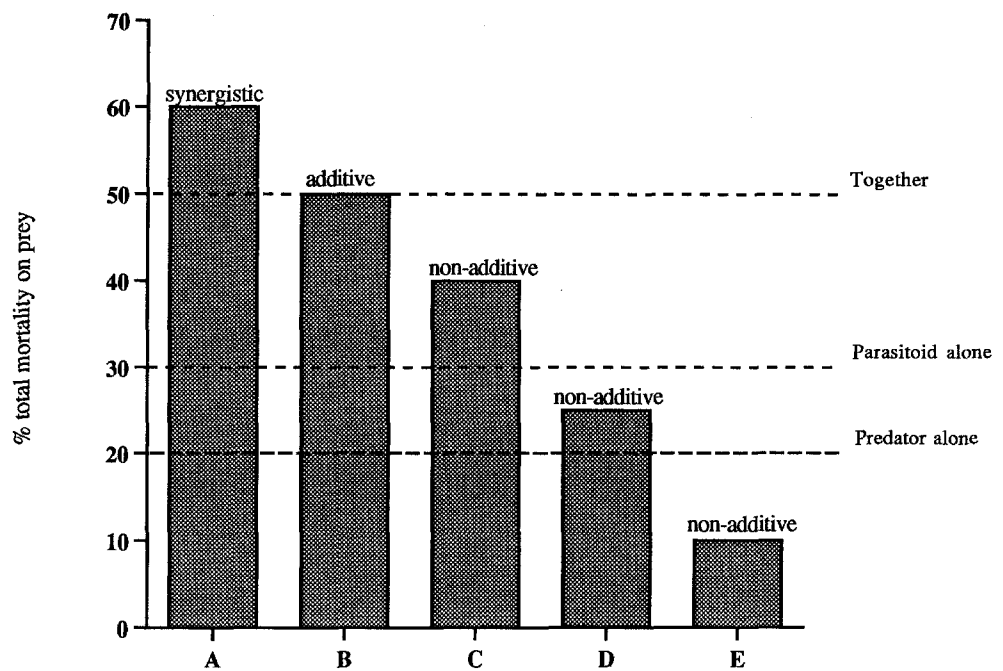
The question of whether combinations of natural enemies act in an additive or non-additive fashion is of interest in many systems, but is particularly important for practitioners of biological control. Such information will help in determining which combination of enemies should be released to maximize mortality of pest populations. Godfray and Waage (1991, p. 448) stated that “a policy of the unlicensed release of all natural enemies” should not be advocated because of the possibility of competitive interactions among the regulatory agents. Interaction of natural enemies has the potential to disturb density-dependent attack of pests by control agents and density-dependence is often regarded as a necessary prerequisite to successful biological control (Stiling 1987). Here we use experiments to examine whether aphids suffer lower rates of mortality when attacked by parasitoids alone, predators alone, or a combination of the two. We also ask whether patterns relating density of aphids and extent of parasitism exist in the field.

Materials and methods

In Florida, the saltmarsh aphid *Dactynotus* sp. (hereafter referred to by genus) is commonly found on the coastal plant *Iva frutescens* L. (marsh elder). *I. frutescens*, a member of the family Astera-

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Fig. 1 Possible outcomes when two natural enemies, for example a predator and a parasitoid, which alone inflict 20% and 30% mortality respectively, act together. The mortality from either natural enemy can increase or decrease resulting in five separate scenarios: *A* synergistic effects: total mortality higher than 50%; *B* additive mortality: both enemies are relatively unaffected by one another and total mortality is about 50%; *C* non-additive mortality which results in a total mortality higher than that caused by either enemy alone but is less 50%; *D* non-additive mortality that results in total mortality less than that caused by the parasitoid alone, but more than that caused by the predator alone; *E* non-additive mortality that results in total mortality less than that caused by either enemy acting alone



ceae, reaches a maximum height of approximately 3.5 m. It is a perennial and is found primarily in the upper reaches of coastal salt marsh communities ranging from Texas through Florida and north to Nova Scotia (Bertness et al. 1992). *Dactynotus* is preyed on by the coccinellid *Cycloneda sanguinea* L. and, at our sites, is the primary host of the aphidiid parasitoid *Aphidius floridaensis* Smith. Parasitized aphids (mummies) are cemented by the pupating parasitoid larvae to either side of leaves or stems of the host plant and have a conspicuous golden appearance, providing a relatively simple means of comparing parasitism levels in the field.

Experiments to examine interference between natural enemies

Field experiments were conducted at Upper Tampa Bay Park, Hillsborough County, Florida. *Iva frutescens* is abundant at this site, but, unlike at most other sites, natural aphid colonies were small and uncommon, coccinellids were very rare, and mummies were never found. These conditions minimize interference from naturally occurring aphid enemies on experimental patches.

In preliminary studies, experimental populations of aphids not enclosed within cages all disappeared within 4 days. Even when populations were built up in cages to hundreds of aphids on each *Iva* stem, as soon as cages were removed, numbers of aphids diminished to zero in 4 days. Hacker and Bertness (1995) also noted rapid disappearance of aphids on uncaged *Iva* stems in New England. As a result, their experiments on aphid growth, and ours on the interaction of natural enemies, were conducted in cages. While aphid disappearance is an interesting phenomena, and may be related to crowding-induced dispersal, this was not the focus of this paper.

In our experiments, ten aphids from spoil islands (see next section) were placed inside a 60 cm long by 38 cm diameter cylindrical cage (large enough to enclose an entire *Iva* branch) and their numbers allowed to increase prior to exposure to natural enemies (~10–15 days). Cages were constructed of AGRYL fabric, an insect-resistant polyester fiber cloth commonly used in agriculture. AGRYL fiber is fine enough to completely prevent the escape of all insects inside and to prevent any "outside" insects from entering the cage. Cages also captured any mummified aphids that became dislodged from the plant – but these were extremely rare.

Natural enemies were placed in the appropriate cages when the aphid population achieved densities found in the field, ~100–150

individuals per branch (field frequency of numbers of aphids per branch, 0–200 = 88%, 201–400 = 6%, 401–600 = 6%, $n = 50$). Each of five bushes received the following treatments: (1) aphids with no parasitoids or predators; (2) aphids with predators only; (3) aphids with parasitoids only, and (4) aphids with both predators and parasitoids. *Cycloneda sanguinea* were also collected from *Iva* on spoil islands, by gently knocking them off a branch into a plastic vial with a small paintbrush. They were then placed in a predator cage within 24 h of capture. One adult ladybird was placed in each predator cage; again, as this is the number most often found per branch in the field (field frequency of numbers of ladybirds per branch, 1 = 79.4%, 2 = 11.8%, 3 = 7.3%, 4 = 1.5%, $n = 68$). *Aphidius floridaensis* were collected as mummies of *Dactynotus* from the spoil islands. One to three mummies are the most common number found per branch in nature, though up to 90 have been recorded, so three adult female parasitoids were placed in each of the nine parasitoid cages (field frequency of *A. floridaensis* mummies per branch, 1–3 = 83.1%, 4–6 = 11.3%, 7–9 = 2.8%, >10 = 2.8%, $n = 71$). Three adult wasps were also introduced into each of the predator plus parasitoid cages and an adult ladybird was also placed in each cage.

The numbers of aphids and mummies were recorded every two days in each of the cages for 3 weeks, until parasitoids first began to emerge from mummies. After 3 weeks the volume of honeydew produced by the aphids was so great that the cages and *Iva* leaves became fouled with sooty mold. Thus, it was only possible to examine the effects of natural enemies over the course of one generation of parasitoids. All cages were opened carefully to facilitate counting of aphids and marking of mummies. All insects were relatively sedentary and we did not disturb aphids or parasites enough to cause them to drop off branches. We never recorded any escapees during counts. In the parasitoid cages, mummies were marked with a black permanent marker. This provided a means of estimating the loss of parasitized aphids to ladybird predation. All experiments and counts were not on the same exact calendar schedule but counts began 10 February 1993 and ended 24 March 1993.

A two-factor repeated measures ANOVA (with or without parasitoid and with or without predator) was employed to test for non-additive effects. Data were $\log(x+1)$ transformed prior to analysis. Another repeated measures ANOVA was used to compare rates of parasitism between the parasitoid and the parasitoid plus predator cages, using arcsine square-root transformed data.

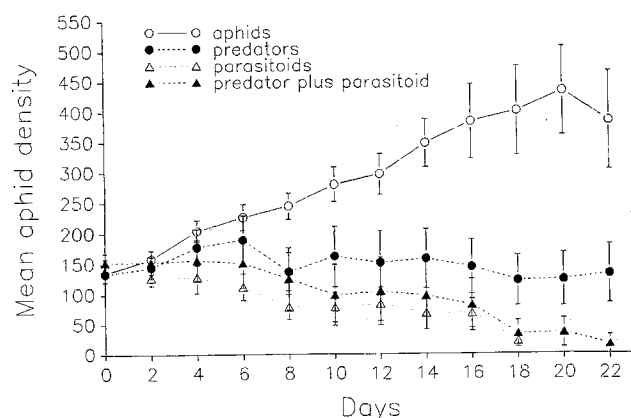


Fig. 2 Average (\pm SE) aphid densities within cages of each of the four treatments: aphids only (○), aphids and predators (●), aphids and parasitoids (△), and aphids, predators, and parasitoids combined (▲)

Table 1 Repeated-measures ANOVA on aphid densities for parasitoid (+/-) and predator (+/-) treatments on caged *Iva frutescens* stems at Upper Tampa Bay Park, 1993. One of the aphids-only treatments ended prematurely; hence, error $df=15$

Source	Sum of squares	df	Mean square	F-ratio	P
Between subjects					
Parasitoids	47.530	1	47.530	11.209	0.004
Predators	1.964	1	1.964	0.463	0.506
Parasitoids	11.391	1	11.391	2.686	0.122
xPredators					
Error	63.602	15	4.240		
Within subjects					
Time	31.244	11	2.840	12.032	<0.001
Parasitoidsxtime	21.570	11	1.961	8.307	0.001
Predatorsxtime	2.391	11	0.217	0.921	0.522
Parasitoids	5.843	11	0.531	2.250	0.014
xpredators					
xtime					
Error	38.950	165	0.236		

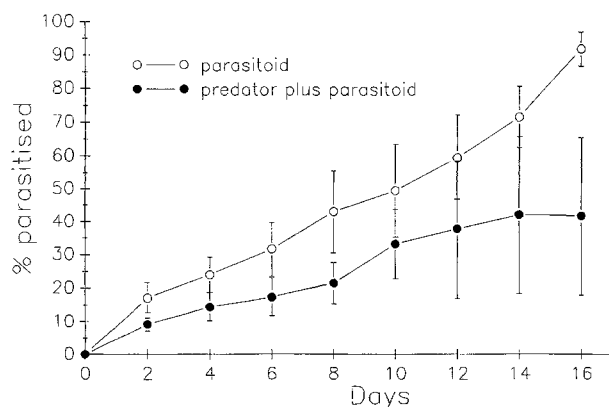


Fig. 3 Average (\pm SE) percent parasitism of aphids in cages with aphids and parasitoids only (○), and in cages with aphids, parasitoids, and predators (●)

Field censuses to detect density-dependent parasitism

Natural population densities of aphids, parasitoids, and ladybirds were censused on ten small (1000–2500 m²) spoil islands along the intercoastal waterway of Pinellas County, Florida. These islands run parallel to the mainland, and are regularly spaced (0.5–1.5 km) from one another (Stiling and Rossi 1994). Weekly counts were made on each of three marked bushes of *I. frutescens* per island and the numbers of aphids, coccinellids, and mummies found on 30 haphazardly chosen stems were recorded. Counts were done in late winter and spring (February and April of 1992) when aphid populations are at their seasonal peak (P. Stiling, unpublished work). We then determined whether percent parasitism was correlated with aphid density at three separate scales: between stems on the same bush (between stems); between summed 30-stem counts on different bushes on the same island (between bushes); and between summed three-bush counts on different islands (between islands).

Results

Experiments to detect interference between natural enemies

Aphid densities increased dramatically when protected from natural enemies (Fig. 2). Other studies have demonstrated a similar increase of aphid densities in enemy exclusion cages (Frazer et al. 1981; Chambers et al. 1983; Carroll and Hoyt 1984; Chiverton 1986; Hacker and Bertness 1995). Parasites had a significant effect on aphid densities but the effect of predators and the interaction of parasitoids by predators were not significant (Table 1). However, there was a significant and consistent parasitoids by predators by time interaction, indicating that there was a non-additive effect of the natural enemies. This indicates that predators interfered with the behavior of the parasites, causing them to reduce aphid population density less than in parasitoid only cages.

The percentage of aphids parasitized was marginally, but consistently lower in the parasitoid plus predator cages than in the parasitoid only cages (Fig. 3), ($P = 0.075$; Table 2). This could be because the predators affect the behavior of ovipositing wasps, because predators eat mummies, or both. Counts of marked mummies showed that in the predator plus parasitoid cages an average of 15–30% of mummies were lost to predation by *C.*

Table 2 Repeated measures ANOVA on the rate of parasitism of *Dactynotus* spp. by *Aphidius floridaensis* in cages with and without *Cycloneda sanguinea*

Source	Sum of squares	df	Mean square	F-ratio	P
Between subjects					
Treatment	12271.826	1	12271.826	4.176	0.075
Error	23506.528	8	2938.316		
Within subjects					
Time	14162.443	7	2023.206	7.935	<0.001
Treatment	1236.519	7	176.646	0.693	0.678
xtime					
Error	14278.106	56	254.966		

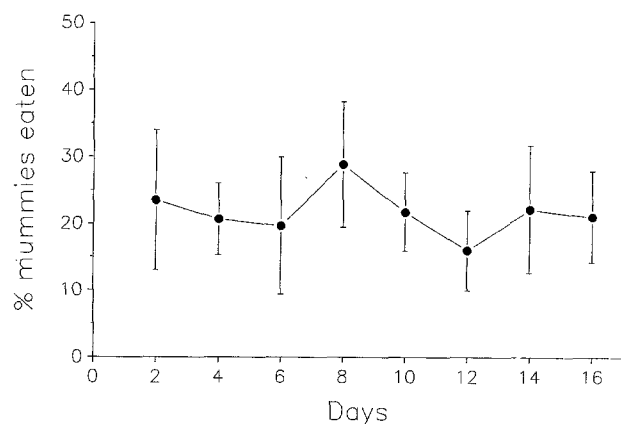


Fig. 4 Mean (\pm SE) percentage of mummies eaten by *Cycloneda sanguinea* in the predator plus parasitoid cages

sanguinea between each sampling period (Fig. 4). Thus predation of mummies and interference with parasitoid behavior are both possible.

Field studies to detect density-dependent parasitism

Little evidence was found of density-dependent parasitism of *Dactynotus* at any scale (Pearson correlation coefficients, between islands, February = 0.527, April = 0.798; between bushes, February = -0.174, April = 0.428; between stems, February = -0.085, April = 0.234). Only at the largest scale, between islands, on the April date, was there a significant relationship between aphid density and parasitism, but after correcting for experiment-wise error, the significance was lost.

Discussion

Considerable debate concerning the relative efficiencies of predators and parasitoids as biocontrol agents remains within the scientific community. Mills (1982) argued that adult aphidophagous coccinellids are unlikely to be reliable agents for controlling aphid populations because they are easily satiated at moderate to high aphid densities, exhibit little prey specificity, and their development rate is slow relative to that of aphids. Hofsvang (1990) concluded that parasitoids provide more effective and reliable control of aphids than do predators. Our experiments show that natural levels of parasitoids inflict higher levels of mortality on aphid populations than natural levels of predators.

What happens when both types of natural enemy are used together? In some systems the release of multiple enemies results in more effective control than the release of single species (e.g., Gutierrez et al. 1988; Kindlmann and Ruzicka 1992). In others, intraguild predation results in an increase in prey numbers (Rosenheim et al. 1994). Our research shows that, over the short term, the effects of the enemies were non-additive and resulted in less

mortality than when the parasitoids alone were present (scenario D in Fig. 1).

Our results add to the growing list of non-additive effects between natural enemies that have now been shown to occur among a number of different taxa. For example, Tscharntke (1992) demonstrated that birds often consume the contents of parasitized galls over unparasitized ones. Gall predation by birds was on average 70% but actually accounted for only a 30% increase in midge mortality because many of the gall midges eaten by birds had already been killed by parasites. Jones (1987) demonstrated that the effects of ant predation and wasp parasitism of the juvenile cabbage butterfly were not cumulative because the ants preyed on parasitized individuals at a greater rate than on unparasitized individuals. Non-additive effects have also been shown to occur between coccinellid predators and hymenopteran parasitoids of mealybugs (Prakasan and Bhat 1985; Gutierrez et al. 1988). Our experiments are unusual in that they show different ways in which non-additive effects can occur. In our case predators interfered with adult parasitoids, causing them to parasitize fewer aphids. This is a novel result. They also had the potential to reduce the effect of the parasitoids by feeding on mummies, though, because our experiments were terminated before second generation wasps emerged, our experiments were not able to demonstrate this.

Lastly, the lack of detection of a density-dependent pattern of oviposition by *A. floridaensis* in the field is consistent with the idea that interactions between natural enemies could obscure patterns relating prey density and attack rate by natural enemies. While this is by no means proved, it is interesting that Mackauer and Volkl (1993) also suggested that density-dependence by aphidiid wasps may not be detected because of the effects of hyperparasitoids. Instead of laying all their eggs in the most dense patch of hosts, female aphidiid wasps oviposit within several host patches as a form of spatial refuge for their eggs from hyperparasitoids. A similar strategy could reduce losses due to predation by ladybirds. For biological control practitioners, this means that the search for parasites that oviposit in a density-dependent fashion should not be the holy grail that it was once thought to be.

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