Integrated Control of Plant-Feeding Mites
IOBC/wprs Bulletin Vol. 50, 2009
pp. 83-94

Multiple predators, intraguild interactions and biological control of a single spider mite species

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Abstract: To test whether biological control of spider mites is promoted by the use of multiple instead of a single predator species, experiments have been published that employed either an additive design (initial density of each predator is kept constant) or a replacement design (initial density of all predators together is kept constant). The variable under test is usually some measure of pest abundance. We argue that such tests are not always adequate to infer positive or negative effects among multiple predator species and their impact on the pest. Instead, there is a need for experiments elucidating predator-predator interactions and their impact on the pest at a mechanistic level, using a series of experiments ranging in focus from the individual level to the population level, from the laboratory to the field, from short-term to long-term and from small scale to large scale. To illustrate this, two experiments will be discussed, the first based on a replacement design and the second on an additive design: (1) Combined releases of *Phytoseiulus persimilis* and *Neoseiulus californicus* for control of spider mites on strawberries in Turkey, and (2) Predator removal with respect to established populations of *Typhlodromalus aripo* and *Amblydromalus* (*Typhlodromalus*) *manihoti*, which are supposed to control green mites on cassava in Africa. These examples show that microhabitat specialization of the predators in the field reduces negative effects of intraguild predation among predator species and promotes their synergistic effect on pest suppression.

Key words: Multiple enemies, biological control, Phytoseiidae, Tetranychidae, strawberry, cassava, experimental design

Introduction

A major question in biocontrol theory is how the interactions among multiple predator species affect herbivore populations and how herbivores respond to each of the predators (Losey & Denno, 1998; Sih *et al.*, 1998; Relyea, 2003; Cardinale *et al.*, 2006; Prasad & Snyder, 2006; Snyder *et al.*, 2006). One interaction among predators is intraguild predation (Polis *et al.*, 1989). Theory on the effects of intraguild predation has been developed over the last decades. It predicts that the densities of a herbivore shared as prey by two predators will increase if intraguild predation affects the predator that is superior in competing for the herbivore that is attacked by both predator species (Rosenheim *et al.*, 1995; Prasad & Snyder, 2006) or if the herbivore partially avoids its predators. However, if escape from one predator species increases the vulnerability of the herbivore to another predator (Soluk & Collins, 1988; Losey & Denno, 1998; Magalhães *et al.*, 2002), the total rate of predation incurred by the herbivore may increase. Provided intraguild predation is weak enough, herbivore densities may then become lower when exposed to multiple instead of single predator species (Snyder *et al.*, 2006). Such a synergistic impact on the herbivore population is not only of interest to ecologists, but it is also to biological control workers when deciding whether to use a single most efficient predator species to control a pest in agriculture or to use multiple predator species (Briggs, 1993). Whereas many studies have shown effects of multiple enemies on the density of a shared prey, only few have been appropriately designed for statistical analysis (Symondson *et al.*, 2002). These few studies employed a replicated design and included experimental manipulations of predator populations in order to separate effects of each enemy alone and all enemies together (Sih *et al.*, 1985; Denoth *et al.*, 2002; Stiling & Cornelissen, 2005; Cardinale *et al.*, 2006). To assess the conditions for 'two predators to be better than one' it is necessary to carry out manipulative field experiments and to interpret the results against a background of insight into the underlying behavioural and physiological responses of predators and prey, as well as in possibilities for predators to switch to other than the focal prey (Prasad & Snyder, 2006). Moreover, design and analysis should be such that effects of predator identity composition and those of overall predator abundance are not confounded (Straub & Snyder, 2006).

Two types of experimental design

To test the impact of multiple predator releases against that of single predator releases, two types of experimental design have been commonly used: (1) the additive design, in which the initial density of each predator species is kept constant, (2) the replacement design, where the total density of predators is kept constant. The additive treatment design results in a more diverse enemy assemblage with the number of enemies equal to the sum of all enemies used in the less diverse (or single species) assemblages. The strength of the additive design is that enemy density and diversity are intentionally confounded so that one can statistically establish departures from the expectation of independent, additive species effects (Sih *et al.*, 1998). The additive design mimics the natural correlation between total enemy abundance and diversity as is often found in the field and it allows assessing whether enemy diversity has effects exceeding those anticipated from the effects of higher enemy abundances only. A further benefit of the additive design is that intra- and interspecific interactions are less likely to be confounded across levels of diversity – a problem that can hinder interpretation of treatment effects in the replacement-series design where the density of each species is inversely proportional to species richness (Connolly; 1988, Snaydon, 1991; Jolliffe, 2000). Whereas additive designs are more appropriate to answer ecological questions (Cardinale *et al.*, 2006), biocontrol workers are more interested in the impact of multiple predators given a constant effort in mass rearing and predator-release effort, which is more properly addressed with a replacement design. A further benefit of the replacement design is that intra- and interspecific interactions are less likely to be confounded if their strength depends on the ratio of predators to prey – a problem that can hinder interpretation of treatment effects in the additive design.

In this paper, we discuss the merits and drawbacks of both designs, exemplified with the results of two experiments: (1) Combined and separate releases of the predatory mites *Phytoseiulus persimilis* and *Neoseiulus californicus* for control of spider mites on strawberries (based on a replacement design) (Cakmak *et al.*, 2009), (2) Predator removal vs non-removal experiments in established populations of the predatory mites *Typhlodromalus aripo* and *Amblydromalus* (*Typhlodromalus*) *manihoti*, which are supposed to control green mites on cassava (implying an additive design) (Onzo *et al.*, 2009). Whereas each of these two studies provides interesting conclusions, their interpretation is fraught with problems due to the release design employed. We will conclude by providing a perspective for the use of mathematical modelling as an aid in the interpretation of experiments to test the impact of single vs multiple predators.

Combined releases to control carmine spider mites on strawberry

To compare the effects of multiple and single predator releases on prey density, we (Cakmak *et al.*, 2009) used a replacement design: the same total number of predators was released in all treatments. This design allows for straightforward interpretation of the results only in two cases (ignoring the trivial case of all treatments yielding equal prey suppression): (1) if the prey density after multiple predator releases is suppressed more than after any of the single predator releases, then the predator species should have acted synergistically, (2) if the prey density with multiple predator releases is suppressed less than with any of the single predator releases, then the predator species should have acted antagonistically. If, however, multiple enemy releases have an effect intermediate between those of the single predator releases, then this may arise from synergism, antagonism or absence of any interference between the predator species. This is because each predator species in a multiple enemy release is represented by a lower number of individuals than in the single enemy release. Moreover, predators and prey reproduce at the time scale of the experiments, so that it is not possible to predict the effect of multiple predators on prey density in the absence of predator interference. Thus, if multiple predator releases yield results intermediate between the two single predator releases, no pertinent conclusions can be drawn. As we will see below, this case applies to the results of Cakmak *et al.* (2009).

Cakmak *et al.* (2009) found that the release of *N. californicus* alone, at a 1:20 predator-prey ratio, did not result in the successful control of *T. cinnabarinus* (in agreement with Sato *et al.*, 2007; Greco *et al.*, 2005; Fraulo & Liburd, 2007), whereas the release of *Phytoseiulus persimilis* alone, at the same predator-prey ratio, gave effective pest control, as did the the combination of *P. persimilis* and *N. californicus* with each species released at a 1:40 predator-prey ratio. However, the combination of the two species did not result in significantly better control than *P. persimilis* alone. Similarly, Barber *et al.* (2003) reported that *P. persimilis* and *N. californicus* eliminated spider mites on dwarf hops in the UK faster than *N. californicus* alone, but slower than *P. persimilis* alone. On strawberries in Florida, Rhodes *et al.* (2006) found qualitatively similar results in short term (2 weeks) studies in greenhouses, but after 4 weeks in the greenhouse and in two consecutive full season experiments they found that the combined release of *P. persimilis* and *N. californicus* and the release of *N. californicus* alone gave equally effective spider-mite control and even better control than *P. persimilis* alone. Thus, the experiments of Cakmak *et al.* (2009) and the two referred above lead to the conclusion that multiple predator releases result in prey suppression intermediate between that achieved under single predator releases. Hence, given that all three studies employed a replacement design, no definitive conclusions can be drawn as to synergy or antagonism among the released predator species in their effect on the spider mites.

Scrutiny of the densities of the two predator species in the experiments of Cakmak *et al.* (2009) did not reveal definitive evidence for facilitation or interference. On the one hand, there was evidence for interspecific interference because the densities of *P. persimilis* in the presence of *N. californicus* were similar to those in absence of *N. californicus* (despite the fact that their numbers at release were twice as low as a consequence of the replacement design) and the densities of *N. californicus* seemed lower in the presence of *P. persimilis*, albeit not significantly so. On the other hand, the same trends in the densities of the two predator species might have arisen from a lower predation impact of *N. californicus*, thereby first allowing the spider mite population to build up and then the *P. persimilis* population to reach a larger size. In addition, it might have been that the densities of *N. californicus* in the presence of *P. persimilis* were lower than in its absence, simply as a result of the lower numbers released under the replacement design. Thus, it is not possible to infer interference from the densities of the two predator species and, for much the same reasons, this also applies to other studies employing a replacement design (Rhodes *et al.*, 2006; Barber *et al.*, 2003; Fitzgerald *et al.*, 2007). Absence of evidence for interference was also concluded from another study on *N. californicus* and *Stethorus punctillum* (Rott & Ponsonby, 2000).

All these arguments, however, should not be taken as 'evidence for absence' of interference. Bidirectional intraguild predation has been found in laboratory experiments, with both species feeding on each other's juvenile stages (Walzer & Schausberger, 1999; Walzer *et al.*, 2001; Cakmak *et al.*, 2006). Moreover, neither of the two predators avoided prey patches with the heterospecific competitor, both when inexperienced with the other predator and when experienced with prey patches occupied by the heterospecific predator (Cakmak *et al.*, 2006). Nevertheless, *P. persimilis* hardly suffered from intraguild predation by *N. californicus* as long as the shared prey was present (Cakmak *et al.*, 2006). Perhaps this is one of the reasons why it is hard to detect intraguild interference between these two predators in the field, even if it would occur occasionally.

Because the initial numbers of each predator were not equal across treatments in Cakmak *et al.* (2009), evidence for interference or facilitation can only be detected by analyzing the population growth rates of the predators. The weekly growth rates of both predators became negative earlier in presence of the other species than when alone. As the initial densities of each species were lower in the combined releases of the predators than in the single releases, this suggests that there was interference between the two species.

Scrutiny of the growth rates of the predators revealed that, when densities of *T. cinnabarinus* declined, *N. californicus* was able to maintain positive growth rates for a longer period than *P. persimilis*. This indicated that *N. californicus* is better capable of persisting at low prey densities. Because these low densities arose at the end of the experiment, no clear advantage of *N. californicus* might have become manifest with respect to spider mite control. In case lower densities would arise earlier in the crop-growing season and/or locally in the space occupied by a crop, *N. californicus* might add to overall spider-mite control by its ability to persist longer at lower densities. Perhaps this is the reason why Rhodes *et al.* (2006) found better performance of *N. californicus* with and without *P. persimilis* during two cropping seasons. Thus, an advantage of releasing *N. californicus* in addition to *P. persimilis* may still become evident when biocontrol experiments last long enough for prey densities to become low.

Predator removal to study impact on control of cassava green mites

Onzo *et al.* (2009) studied the impact of predator removal on the interaction between an apex-inhabiting predatory mite, *Typhlodromalus aripo* (an exotic species introduced in 1993), leaf-inhabiting predatory mites, either *Amblydromalus* (=*Typhlodromalus*) *manihoti* (another exotic species introduced in 1989) or *Euseius fustis* (an endemic species), and a herbivorous spider mite, the cassava green mite *Mononychellus tanajoa*, (a pest inadvertently introduced in Africa around 1971), on cassava plants in the field. The apex-inhabiting predatory mite resides in the plant tips during the day, but comes out at night to forage for cassava green mites on the upper leaves (Onzo *et al.*, 2003a). The leaf-inhabiting predatory mites are never found in the apex and occur on the leaves day and night. The study was conducted in South-Western Benin in cassava fields where either *T. aripo* and *A. manihoti* or *T. aripo* and *E. fustis* co-occurred. Within groups of four plants in a field, each plant received a different treatment: (1) no predatory mites, (2) *T. aripo* alone, (3) *T. aripo* + either *A. manihoti* or *E. fustis*, and (4) *A. manihoti* alone or *E. fustis* alone. Whether *A. manihoti* or *E. fustis* were involved, depended on which of the two was the dominant leaf-inhabiting phytoseiid in a given field. The treatment without predatory mites involved manual removal of all the leaf-inhabiting phytoseiids (i.e. *A. manihoti* or *E. fustis*) using a camel hair brush, whereas the apex-inhabiting phytoseiids (i.e. *T. aripo*) were excluded chemically by dabbing the apex of cassava plants with a paint brush (size 0), impregnated with a solution of a pyrethroid insectide, known to have a very brief action period and to have no toxic effect on cassava plants (Permethrin 20 EC at 6.25 g AI/100 litres; Hanna *et al.*, 1998). The '*T. aripo* alone' treatment involved manual removal of all leaf-inhabiting phytoseiids, whereas the '*A. manihoti* alone' or the '*E. fustis* alone' treatments involved chemical elimination of *T. aripo*, as described above. To avoid removing *T. aripo* from the plants together with leaf-inhabiting phytoseiids, and to succeed in killing all *T. aripo* in the '*A. manihoti* alone', '*E. fustis* alone' or 'Predator-free' treatments, predator exclusion, either from apices or from leaves, was carried out always between 10:00 hours and 16:00 hours when all *T. aripo* individuals reside in the apices of cassava plants (Onzo *et al.*, 2003a). Predator exclusion was repeated every week, immediately after taking the leaf samples.

These predator removal experiments are much akin to an additive design. Such experiments have a clear interpretation if the prey density under multiple predator releases is suppressed more than expected from the combination of the single predator releases. In that case, the predator species should have acted synergistically. The problem, however, is that the prey suppression expected under multiple predator presence is not simply obtained by adding or multiplying the percentages of prey density reduction obtained for each of the predator species (as done by e.g. Ferguson & Stiling, 1996). The reasons for this will be given in the final section of this paper. In the absence of such an expectation, it is not possible to infer synergistic interactions from additive design experiments. The same applies vice versa for antagonistic interactions. Below we first discuss whether the treatments applied by Onzo *et al.* (2009) showed statistical differences and then return to the question whether these results allow conclusions on synergistic or antagonistic interactions.

In the predator removal experiments of Onzo *et al.* (2009), densities of *M. tanajoa* were reduced mainly by the apex-inhabiting predator *T. aripo*. In addition, there was an effect of the leaf-inhabiting predator *A. manihoti* on *M. tanajoa* densities when together with *T. aripo*. The presence of the leaf-inhabiting predator *E. fustis* in addition to *T. aripo* had no significant effect on *M. tanajoa* densities. While confirming the key role of *T. aripo* in the biological control of *M. tanajoa* on cassava in Africa (Yaninek and Hanna, 2003; Hanna *et al.*, 2005), these results also point at a joint effect of *T. aripo* in the apex and *A. manihoti* (but not *E. fustis*) on the leaves.

These conclusions prompted Onzo *et al.* (2009) to pose two questions: (1) which properties make *T. aripo* a key predator (compared with *A. manihoti* and *E. fustis*), and (2) which properties make *A. manihoti* a more suitable predator in combination with *T. aripo*, compared with *E. fustis*? Clues to answer these questions are to be sought first in the large differences in abundance of the predator species. In the experiments of Onzo *et al.* (2009), *T. aripo* densities per plant were 10 times higher than *A. manihoti* densities and 6 times more abundant than *E. fustis* densities. These relative differences in abundance may be caused by species-specific traits, such as (a) predation, reproduction and survival in response to *M. tanajoa* density, (b) use of alternative food (pollen, plant exudates) or alternative prey (the spider mite *Oligonychus gossypii*), and (c) interference among predator species. Below these possible causes are discussed.

In terms of laboratory-assessed rates of population increase and rates of predation on *M. tanajoa* the predator species rank as *A. manihoti* > *T. aripo* > *E. fustis* (Gnanvossou *et al.*, 2003a; 2005). Clearly, these traits do not explain the differences in abundance observed in the field experiments of Onzo *et al.* (2009) (which ranked as *T. aripo* > *E. fustis* >*A. manihoti*).

Onzo *et al.* (2004) found that *A. manihoti* thrives better than *T. aripo* at high *M. tanajoa* densities, but *T. aripo* did better at low prey densities. Since densities of *M. tanajoa* in the experiments of Onzo *et al.* (2009) were relatively low, the relatively higher abundance of *T. aripo* compared with *A. manihoti* is in agreement with the results of Onzo *et al.* (2004). It is, however, not entirely clear why *T. aripo* performs relatively better at low prey density. Possibly, it is more efficient in utilizing prey for egg production (fewer prey consumed per egg produced) (Magalhães *et al.*, 2003).

Another explanation may be that the three predator species differ in their ability to use alternative food and alternative prey. All three species are known to feed on plant foods, such as phloem exudates of cassava and maize pollen (Bakker & Klein, 1992; Bruce-Oliver *et al.*, 1996; Magalhães & Bakker, 2002; Gnanvossou *et al.*, 2005; Hanna *et al.*, 2005), and they all feed on spider mites such as *M. tanajoa* and *O. gossypii* (Bruce-Oliver *et al.*, 1996; Gnanvossou *et al.*, 2003a). However, *E. fustis* prefers maize pollen to spider mites (Bruce-Oliver *et al.*, 1996). By the time the experiments of Onzo *et al.* (2009) were initiated (end of maize harvest), maize pollen was rare and could therefore not promote the abundance of *E. fustis* to the extent observed by Onzo *et al.* (2003b) in earlier experiments carried out across seasons and included periods with abundant maize pollen availability. This may partly explain why *E. fustis* had no significant impact on *M. tanajoa* in the experiments of Onzo *et al.* (2009) (whether alone or together with *T. aripo*) compared with the results of the experiments of Onzo *et al.* (2003b), where pollen was more readily available. Instead of pollen, the field experiments of Onzo *et al.* (2009) included an alternative prey, *O. gossypii*. This prey resource may have promoted *E. fustis* populations more than those of the other two predator species, because the experiments of Onzo *et al.* (2009) revealed a significant impact of *E. fustis* on *O. gossypii*, but not of *A. manihoti* and not of *T. aripo* (in '*A. manihoti*' fields). Moreover, *A. manihoti* and *T. aripo* prefer *M. tanajoa* over *O. gossypii* when given a choice, probably because these predators achieve a much higher population growth rate when offered *M. tanajoa* (Gnanvossou *et al.*, 2002). These differences in prey preference are also reflected in the way the three predator species distribute themselves within a plant relative to the two prey species. Whereas *M. tanajoa* inhabits the upper and mid stratum of the plant, *O. gossypii* is more frequently found in the lower and mid strata (Bonato *et al.*, 1995). Indeed, *T. aripo*, inhabiting the apices, forages at night mainly in the upper stratum (Onzo *et al.*, 2003a; 2009) and *A. manihoti* forages in the upper and mid strata (Bonato *et al.*, 1999), but *E. fustis* forages mainly in the lower and mid strata (Zannou *et al.*, 2007). The three predator species thus differ in their feeding rates on the two herbivorous mites found on cassava and occupy different microhabitats.

This form of partial microhabitat specialization and differential prey preference may reduce encounters between the three predator species. Moreover, the predator species may avoid each other (Gnanvossou *et al.*, 2003b; 2003c). Interspecific interference among the predators is therefore expected to be low. Indeed, the field experiments of Onzo *et al.* (2009) provided no indication for intraguild predation because the densities of each of the predators did not significantly differ between experiments where they occurred alone and experiments where they co-occurred on the same plant with another species. Intraguild predation among the three predator species does occur in small arenas in the laboratory (Zannou *et al.*, 2005) and on small cassava plants in screenhouses (Onzo *et al.*, 2004). On full grown plants, however, with different spider mite species, as in the field experiments of Onzo *et al.* (2009), the predators tend to concentrate in different microhabitats within the plant and to prefer different prey species and plant foods (pollen, exudate). This type of niche segregation is likely to reduce intraguild predation and promote the coexistence of multiple predator species (Polis *et al.*, 1989; Rosenheim *et al.*, 1995; Finke & Snyder, 2008).

Given niche segregation among predator species and lack of predator-predator interference, one may expect enhanced control of prey populations through predator facilitation (Losey & Denno, 1998; 1999; Straub & Snyder, 2006; 2008; Finke & Snyder, 2008), a phenomenon also observed on cassava in southern Africa with a slightly different predator species compositions (Zannou *et al.*, 2007). The field experiments of Onzo *et al.* (2009) lend support to the hypothesis that suppression of *M. tanajoa* on cassava is promoted by the presence of two predator species, the apex-inhabiting predator *T. aripo* and the leaf-inhabiting predator *A. manihoti*. This is probably because *M. tanajoa* cannot escape from predation by moving up or down in the plant. In response to *T. aripo* foraging down from the apex at night, *M. tanajoa* moves down in the plant (Onzo *et al.*, 2003a; Magalhães *et al.*, 2002) and in response to *A. manihoti* on mid stratum leaves *M. tanajoa* tends to move up in the plant (Magalhães *et al.*, 2002). Thus, when there is a predator species near the apex and another on the mid and upper stratum leaves, there is no escape from predation: the herbivorous mite is squeezed between two dangers. If instead of *A. manihoti*, *E. fustis* is present together with *T. aripo*, enhanced suppression of *M. tanajoa* does not seem to occur, perhaps because *E. fustis* consumes relatively less *M. tanajoa*, inhabits the lower strata of the plant and may not have had sufficient alternative food (maize pollen) to become numerically abundant enough to suppress *M. tanajoa* densities significantly. Moreover, *E. fustis* seems to prefer *O. gossypii* and has more effect on the densities of this herbivorous mite than on densities of *M. tanajoa*, especially when together with *T. aripo*. Onzo *et al.* (2009) therefore argued that on cassava in Africa *M. tanajoa* and possibly also *O. gossypii* are better controlled by the complex of all three predator species.

The above interpretations of the predator removal experiments by Onzo *et al.* (2009) were obtained by combining information on statistical differences in prey density between treatments and information from laboratory and greenhouse observations on the behaviour. However, the method by which the information is combined is of a qualitative nature. Given the behavioural data on niche segregation, the lack of evidence for intraguild interactions in the field and the significant prey suppression with multiple (as opposed to single) predators, one may suspect synergistic interactions between two predator species (*T. aripo* and *A. manihoti*). However, this expectation is based on a qualitative argument and it is not quantitatively testable because the predator removal experiments are much akin to an additive design. As argued above, one needs to calculate the prey suppression expected under multiple predator presence in absence of synergism from the prey suppression under single predator presence. Below, we will explain why this cannot be simply done by adding or multiplying the percentages of prey density reduction obtained under single predator presence (as in Ferguson & Stiling, 1996).

The problem of defining a null-hypothesis under the additive design

Whereas testing the results obtained from multiple predator release against those from the single predator releases under the replacement design helps to detect some (but not all) forms of synergism and antagonism between predator species, the additive design also does not help in detecting all forms of synergism or antagonism unless additional assumptions are made on how to predict the prey suppression expected under multiple release and in absence of synergism and antagonism. Ferguson & Stiling (1996) proposed that the effect of multiple predators is non-additive when the total mortality observed is higher or lower than the *sum* of the prey mortalities observed for either predator alone. It is easy to see why this proposed null hypothesis is incorrect. Suppose that the ultimate mortality from predator A alone, M_A, and that from predator B alone, M_B, arise from constant per capita mortality rates, m_A and m_B

respectively (implying that predator numbers stay constant). If prey do not reproduce and die only from predation, it follows that for a fixed time period t the fraction of prey killed by predator A is equal to: $M_A = 1 - S_A$ with the fraction surviving $S_A = \exp[-m_A t]$ and that killed by predator B is equal to:

$$M_B = 1 - S_B \text{ with the fraction surviving } S_B = \exp[-m_B t].$$

The mortality expected from the two predators together under an additive design and in absence of synergism or antagonism is then equal to:

$$M_{A+B} = 1 - \exp[-(m_A + m_B)t] = 1 - S_A S_B.$$

Hence, Ferguson & Stiling (1996) are not right when stating that the mortality expected from two predators should equal the *sum* of the prey mortalities observed for either predator alone (i.e. $M_A + M_B$). Survival from the two predators is given by the *product* of the survival fractions (i.e. $S_A S_B$) and the mortality from the two predators is equal to the complementary fraction.

However, multiplication of the fractions surviving from either predator alone (i.e. $S_A S_B$) also does not provide a correct null hypothesis if prey do not only die from predation, but also reproduce, as was the case in the experiments of Ferguson & Stiling (1996) with aphids and two of their enemies (a hymenopteran parasitoid and a coccinellid predator). This can be seen from a model calculating changes in prey number (N) by the use of a differential equation with a constant death rate m and a birth term that is given by the product of N and a relative per capita birth rate r:

$$dN/dt = rN - m$$

Integrating this equation and some rearrangement yields the following formula:

$$N_t / N_0 = 1 + (1 - m/(rN_0))\,[\exp(rt) - 1], \text{ where } m = m_A + m_B$$

Thus, the ratio of surviving prey to initial prey number depends on t, r, N_0 and m, the latter being the sum of the relative mortality rates of each predator alone. Clearly, the prediction under the null hypothesis is not simply obtained by multiplying survival fractions assessed at the end of the experiments. Would Ferguson & Stiling (1996) have used the model discussed above, they would have predicted prey extinction by the two predators together within a period of 10 days ($N_0 = 130$ aphids; $r = 0.07$/day; $m_A = 7.8$ aphids/day; A is represented by one coccinellid predator; $m_B = 10.5$ aphids per day; B is represented by three parasitoids). Because their observations showed prey decimation but not yet full extinction within 22 days, they could have made a strong inference that the two predators negatively interact with each other in their impact on the prey. Instead, they infer this from a repeated measures ANOVA with a marginally significant difference between the rate of parasitism with and without the coccinellid predator.

Without further proof we conjecture that – if the interactions are even more complex than those considered above (e.g. predators do not remain constant, but reproduce and die) – the sum of prey mortalities due to each predator alone or the product of survival fractions from each predator alone are incorrect predictors of prey mortality when the different predators are together. We therefore advocate the use of models based on explicit assumptions to calculate the prey mortality expected when there are multiple predators and no synergism nor antagonsim. Such model predictions provide null hypotheses that help in the interpretation of experimentally assessed prey mortalities by real predators that may interact synergistically or antagonistically. An application of this approach to the data sets from Cakmak *et al.* (2009) and Onzo *et al.* (2009) will be published elsewhere.

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