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Two Better Than One? Potential Effects of Intraguild Predation on the Biological Control of *Ceratitis capitata* (Diptera: Tephritidae) by the Parasitoid *Aganaspis daci* (Hymenoptera: Figitidae) and the Predator *Pseudoophonus rufipes* (Coleoptera: Carabidae)

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Abstract: The use of more than one species to manage a single insect pest is a common practice among biological control programs. However, the beneficial effects of natural enemies are not always additive, which in many cases may be attributed to interspecific interactions such as intraguild predation (IGP). Herein, we investigated the potential IGP between two relevant natural enemies of the Medfly (*Ceratitis capitata*), the predator *Pseudoophonus rufipes* and the parasitoid *Aganaspis daci*, as well as the possible implications of this phenomenon in their efficiency as biocontrol agents. To this end, we assessed their functional responses and different demographic parameters when acting alone and together against *C. capitata* under laboratory conditions. Coexistence led to a switch in the functional response of both species, from type III to type II in *A. daci* and the opposite in *P. rufipes*. Regarding demographic parameters, coexistence resulted in higher parasitoidism and population reduction by *A. daci* only at low host densities, probably due to competition pressure. In the same circumstances, *P. rufipes* reduced its predatory activity, rejecting those larvae that were presumably parasitized and causing negligible IGP. At high Medfly densities, *A. daci* efficiency decreased, and the reduced encounter probability enhanced the predatory activity by *P. rufipes*. As a result of these trends, Medfly population reduction reached almost 100% at all densities, which suggests an additive effect of both natural enemies and recommends combined releases of these agents as a strategy for the control of the Medfly.

Keywords: Medfly; pest control; intraguild interaction; natural enemies; functional response; combined release

1. Introduction

In biological control programs against agricultural pests, more than one species of natural enemy can be used to manage a single insect pest [1,2]. However, these natural enemies can also interact among themselves, and this interaction may impede the expected additive regulatory effects [3–5]. One of the most studied types of interspecific interactions due to its presumably negative effects on biological control is intraguild predation (IGP), which describes the interaction in which two species that compete for a common resource also engage in a trophic interaction with each other [6–8]. IGP can be uni- or bidirectional, with one or both species attacking the other [9], and may even occur between different types of natural enemies, e.g., a predator and a parasitoid, as long as they share the same prey/host. In predator/parasitoid interactions, unidirectional IGP is more common, with

the predator ('IGP predator') feeding inadvertently on immature parasitoids ('IGP prey') when consuming the prey species [10,11]. Thus, the parasitic performance can be seriously jeopardized, and this should be taken into account in the design and implementation of biocontrol programs involving both types of agents [9,12].

The Mediterranean fruit fly or Medfly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), currently represents one of the main threats to fruit crops and is considered one of the most important agriculture pests worldwide [13,14]. The urgent need to develop efficient and environmentally safe control programs has led to the identification of a substantial number of natural enemies, some of which have demonstrated significant potential [15,16]. During the first phase of the Medfly life cycle, eggs and early larvae develop within the host fruit pulp, after which late-instar larvae jump out the fruit to pupate at a shallow depth in the ground until adult emergence [17]. Potential arthropod natural enemies therefore include both parasitoids that can bore the fruit skin and oviposit into the fly larvae and ground-dwelling predators that can feed on the pupae, late-instar larvae and even freshly emerged adults [15]. Therefore, accidental IGP of Medfly predators on parasitoids is expected to occur in agricultural systems, even when the current scarcity of studies dealing with this phenomenon and its implications suggests the opposite.

Among Medfly natural enemies, the predator *Pseudoophonus rufipes* De Geer (Coleoptera: Carabidae) has emerged as a potential biocontrol agent in Spain after the research of Monzó et al. [18,19]. These authors cited this ground-dwelling beetle as the most abundant carabid in Spanish citrus orchards and reported that it was able to efficiently prey on third instar larvae and pupae of *C. capitata*. This led to the idea that *P. rufipes* could play a relevant role in regulating the Medfly in citrus, wherein the incidence of this pest is significant [20,21]. The dominance of this predator in other southern European crops [22,23] suggests that it could also play an interesting role as a biocontrol agent in other cropping systems.

A current parasitoid being considered for biocontrol is the figitid *Aganaspis daci* (Weld) (Hymenoptera: Figitidae). This larval–pupal solitary endoparasitoid was first detected in Malaysia and Taiwan parasitizing flies from the genus *Bactrocera* [24], and its biocontrol potential against this genus has been well-known ever since [25–27]. However, the first report of *A. daci* as a Medfly parasitoid was not until 2003, when it was found emerging naturally from Medfly larvae on the Greek island of Chios [28]. From then on, Medfly parasitoidism by *A. daci* has been reported to be common in the Mediterranean Basin (Spain, Syria and Tunisia) [29–32]. The high natural parasitism rates observed in the field led to multiple studies assessing the efficacy of this parasitoid in controlling *C. capitata* with promising results [33–35].

There is a distinct scarcity of research on the IGP involving *C. capitata* natural enemies. The joint action of different species of natural enemies on the Medfly and other fruit flies has been widely assessed [36–38]. However, to our knowledge, IGP and its implications on Medfly control had only been specifically studied for *P. rufipes* and *Spalangia cameroni* Perkins (Hymenoptera: Pteromalidae), another important Medfly parasitoid [39]. Herein, we assessed the consequences of IGP on the parasitic and predatory efficiency of *A. daci* and *P. rufipes* on the Medfly, in view of possible combined releases of these natural enemies for pest control purposes. Based on their nature and considering previous works such as [39], a certain mutual effect between these species could be expected. However, the direction and intensity of this interaction is completely unpredictable. Specifically, we measured (i) the functional responses and (ii) different demographic parameters (parasitoidism, fertility, induced mortality, population reduction and offspring sex ratio for *A. daci* and predation for *P. rufipes*) of these agents when acting both alone and together against *C. capitata* under laboratory conditions.

2. Materials and Methods

2.1. Study Center and Insect Rearing

All experiments in this study were performed in compliance with current Spanish law and carried out in 2018 at the facilities of the Museu Valencià d'Història Natural

(MVHN) and the Instituto Valenciano de Investigaciones Agrarias (IVIA). Adult specimens of *P. rufipes* were captured from citrus fields in the Valencian community (Spain) using pitfall traps, then transported to a climate cabinet in the laboratory. Specimens were reared in a $50 \times 50 \times 50$ cm methacrylate box with an upper mesh covering and fed with water and sucrose solution for 2 days prior to the assays. Medfly larvae and *A. daci* adults were obtained from their regular semimassive rearings, established at IVIA [40,41], and then maintained in the same type of terraria mentioned above before being used in the experiments. The climatic conditions were slightly different for natural enemies ($22 \pm 2\,^{\circ}$C, $65 \pm 10\%$RH, 16:8 (L:D)) and the Medfly ($27 \pm 2\,^{\circ}$C, $65 \pm 10\%$RH, 16:8 (L:D)).

2.2. Experimental Design

Four experiments were conducted throughout the present research. Experiments 1, 2 and 3 aimed to assess the functional response (defined by Solomon [42] as 'the relationship between the number of prey or hosts attacked by a predator or parasitoid as a function of prey density') of *A. daci* and *P. rufipes* on *C. capitata*, both when acting alone (Experiments 1–2) and together (Experiment 3). The last experiment consisted of a comparison of several demographic parameters of these natural enemies when acting alone and also together on *C. capitata*.

2.2.1. Experiment 1: Functional Response of *A. daci*

In this experiment, newly emerged (<20 h) adults of *A. daci* were collected from the terraria, then sexed and paired in 10 mL plastic tubes. The tubes were sealed with a piece of cotton and contained honey for the parasitoids to feed on. Breeding pairs were left undisturbed for 2 days, after which they were assumed to have mated. Then, the parasitoids were introduced into 'experimental units'. Each cage consisted of a lidded translucent plastic box ($20 \times 15 \times 10$ cm). The lids were adapted with a 4 cm diameter gauze-covered hole for ventilation. Three breeding pairs were introduced in each experimental unit, which was also provided with water and honey as feeding sources. For 24 h over 6 consecutive days, Medfly late larvae (=L3) were supplied to the experimental units, by placing them into a Petri dish (60 mm ø) with 15, 60 or 120 per unit (i.e., 5, 20 or 40 per breeding pair). Eight repetitions (=experimental units) were employed per density in each replicate (=block), and two replicates were performed. To assess natural mortality in the host, a 'control' treatment was also included in each block, consisting of 8 experimental units each containing 15 larvae but no parasitoids at all. After exposure, larvae were recovered and put in ventilated Petri dishes (one per experimental unit) until parasitoid emergence. Petri dishes were kept in a Sanyo MLR 350 climate cabinet (Sartorius, Barcelona, Spain) at $24.5 \pm 0.5\,^{\circ}$C, $60 \pm 10\%$ RH and 16:8 (L:D). Emergences of the parasitoid were counted to assess both fertility (=number of total emergences) and percentage parasitoidism (=emergence rate, i.e., the number of emergences per exposed larvae).

2.2.2. Experiment 2: Functional Response of *P. rufipes*

Three *P. rufipes* adults (males or females, but never mixed) were introduced in the same type of experimental units as in Experiment 1, with water and honey and a Petri dish with Medfly larvae. Additionally, units contained a thin layer of moistened perlite (Floreal, Agroperlita F-13; Piquer Morte S.L., Rafelbunyol, Valencia, Spain) to facilitate predator locomotion. The density of larvae, number of repetitions and replicates, exposure time and control treatment were all the same as in Experiment 1. Predation, taken as the number and percentage of prey killed by *P. rufipes*, was recorded.

2.2.3. Experiment 3: Functional Response of *A. daci* and *P. rufipes* When Acting Jointly on the Medfly

Parasitoids and predators were introduced together in the experimental units, set up as in Experiment 2. Three *P. rufipes* adults and three *A. daci* mated females were introduced in each unit. The density of larvae, number of repetitions and replicates, exposure time

and control treatment were all the same as the previous experiments. After exposure, the number of pupae predated by *P. rufipes* was recorded, and the remainder were reared as in Experiment 1 to determine parasitoid emergence.

2.2.4. Experiment 4: Demographic Parameters of *A. daci* and *P. rufipes*

The last experiment aimed to determine the effect of sharing host/prey on several life history parameters of both natural enemies. Experiments 1, 2 and 3 were repeated, but new variables were measured for *A. daci*, such as induced mortality, population reduction and offspring sex ratio. Induced mortality was determined as the proportion of pupae that did not eclose after 2 months and had evidence of parasitoid activity, namely oviposition holes and/or parasitoid immatures. Thus, every pupae that had not eclosed after this period was dissected and examined under the microscope. The other measured variables were the same as in previous experiments, i.e., fertility and percentage parasitoidism for *A. daci* and predation for *P. rufipes*.

2.3. Statistical Analyses

Functional responses (Experiments 1–3) were analyzed by a generalized linear model (GLM), aiming to discriminate between the two most common types of responses, type II and type III (see Discussion). The data were fit to a binomial distribution with Logit link function, then fit to their corresponding functional response equation [43–45]. Attack rate coefficient is known to be constant and independent of prey/host density in type II responses, while in type III responses, it varies with prey/host density and can be obtained as follows [46]: $a' = b \times x/(1 + c \times x)$, where x is the prey/host density and b and c are constants of itself. Data were fitted through a non-linear least-squares regression by means of the Levenberg–Marquardt iterative estimation procedure [47]. The parameters of the functional response, attack rate (a') and handling time (Tm) were extracted from this regression.

The fertility, percentage parasitoidism, induced mortality and population reduction attributed to *A. daci* and the predation displayed by *P. rufipes* were tested when acting alone and when sharing host/prey (Experiment 4) by a two-way factorial analysis of variance (ANOVA), considering the replicates (block effect) as a random factor. Offspring sex ratio of the parasitoid was also compared between both situations (alone vs. sharing host/prey) by a Pearson's chi squared (χ^2) test. When the assumptions of normality and homogeneity of variance were not fulfilled, data were transformed to meet those assumptions. Values are given as means $\pm$ standard error (SE) or percentage (%). Data were analyzed using IBM SPSS Version 25.0 (Chicago, IL, USA), with a significance set at $p = 0.05$.

3. Results

3.1. Experiment 1: Functional Response of A. daci

A generalized linear model for the proportion of emerged parasitoids revealed that the estimated values for the linear and quadratic coefficients were both positive (Table 1), which reveals a functional response from type III [43]. The estimated handling time and the b and c parameters are shown in Table 2. Based on these parameters, the estimated attack coefficients were 0.37, 0.68 and 0.78 days^{-1} for a density of 15, 60 and 120 larvae, respectively. The estimated maximum number of successfully emerging offspring was 85 per female in 24 h (Figure 1).

Table 1. Maximum likelihood estimate parameters from the generalized linear model of the proportion of host/prey parasitized/hunted as a function of initial host/prey densities by *Aganaspis daci* mated females and *Pseudoophonus rufipes* adults acting alone (Experiments 1 and 2, respectively) and together (Experiment 3) (in all cases, assessed under laboratory conditions).

	Parameter	Estimate	SE	χ^2	df	p
A. daci	Linear	0.035	0.0025	194.828	1	<0.0001
	Quadratic	0.00001	1.5552×10^{-5}	159.518	1	<0.0001
P. rufipes	Linear	−0.070	0.0037	361.345	1	<0.0001
	Quadratic	0.00001	2.1748×10^{-5}	180.264	1	<0.0001
A. daci (with *P. rufipes*)	Linear	−0.012	0.0025	24.203	1	<0.0001
	Quadratic	6.109×10^{-5}	1.5428×10^{-5}	15.582	1	<0.0001
P. rufipes (with *A. daci*)	Linear	−0.016	0.0026	36.714	1	<0.0001
	Quadratic	-8.926×10^{-5}	1.618×10^{-5}	30.429	1	<0.0001

Table 2. Functional response type (FR), attack rate (a) (days^{-1}) and estimated handling time (T_h) (days) estimated from non-linear regression of the number of host/prey by *Aganaspis daci* mated females and *Pseudoophonus rufipes* adults acting alone (Experiments 1 and 2, respectively); and by *A. daci* mated females sharing the experimental unit with *P. rufipes* adults and vice versa (Experiment 3) (in all cases, assessed under laboratory conditions).

	FR	a	±SE	95% CI	Th	±SE	95% CI	R^2
A. daci	III	$b = 0.041$	0.031	−0.020–0.101	0.004	0.003	−0.002–0.010	0.744
		$c = 0.044$	0.053	−0.061–0.149				
P. rufipes	II	1.549	0.233	1.090–2.008	0.024	0.002	0.021–0.027	0.584
A. daci / *P. rufipes*	II	0.572	0.055	0.464–0.681	0.002	0.002	−0.002–0.005	0.659
P. rufipes / *A. daci*	III	$b = 0.050$	0.042	−0.032–0.132	0.003	0.003	−0.003–0.010	0.791
		$c = 0.104$	0.111	−0.115–0.322				

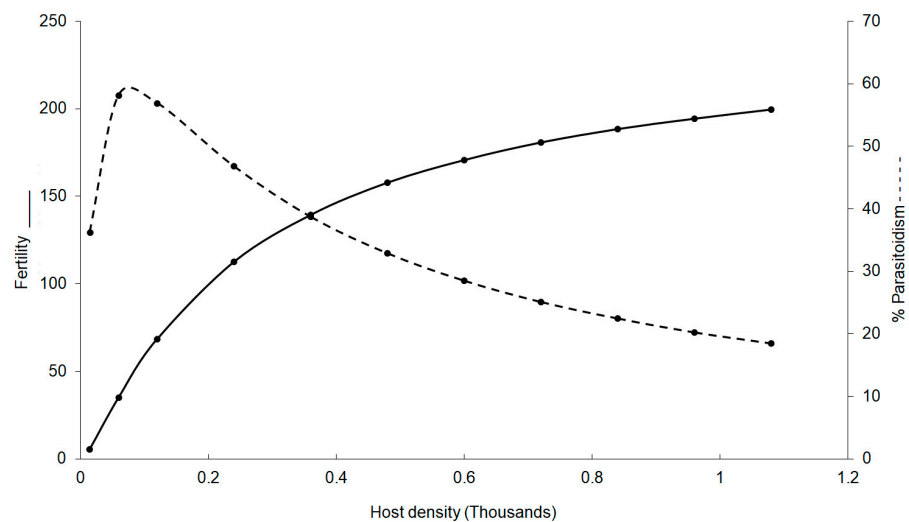R^2 = are the coefficients of determination from R^2 of each regression.

Figure 1. Functional response curve fit by non-linear least squares regression of *Aganaspis daci* mated females parasitizing *Ceratitis capitata* larvae exposed in the laboratory, acting alone (type III). Host density: 5, 20 or 40 larvae per mated female (three) and day.

3.2. Experiment 2: Functional Response of P. rufipes

A generalized linear model for the proportion of killed pupae showed a negative estimated value of and a positive quadratic (Table 1), revealing a type II response [43]. The estimated attack rate coefficient and handling time are shown in Table 2. The estimated maximum number of predated pupae per adult was 14 specimens in 24 h (Figure 2).
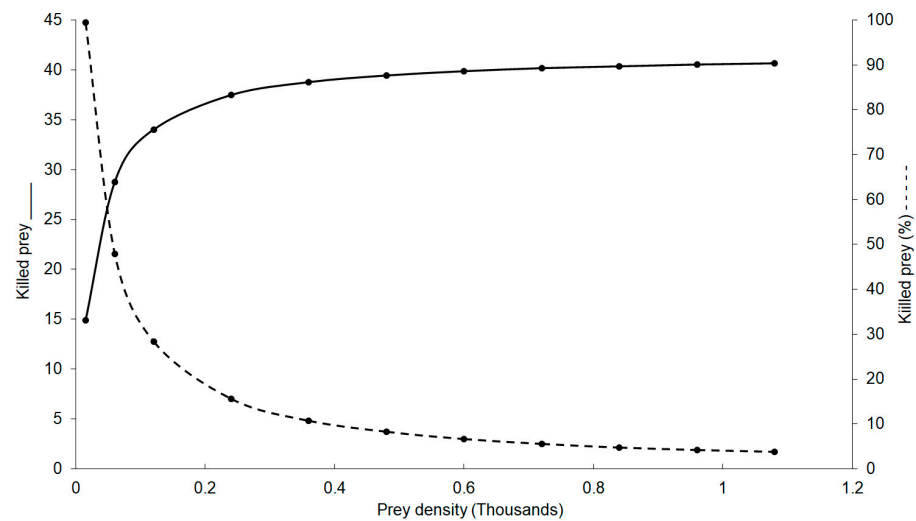
Figure 2. Functional response curve fit by non-linear least-squares regression of *Pseudoophonus rufipes* adults attacking *Ceratitis capitata* pupae exposed in the laboratory, acting alone (type II). Host density: 5, 20 or 40 larvae per adult (three) and day.

3.3. Experiment 3: Functional Response of A. daci and P. rufipes When Acting Jointly on the Medfly

The joint action of both natural enemies resulted in *A. daci* showing a type II functional response, with a negative estimated value of the linear coefficient and a positive quadratic coefficient [43] (Table 1). The estimated attack rate coefficient and handling time are shown in Table 2. In this case, the estimated maximum number of emergences per female was 167 specimens in 24 h (Figure 3).
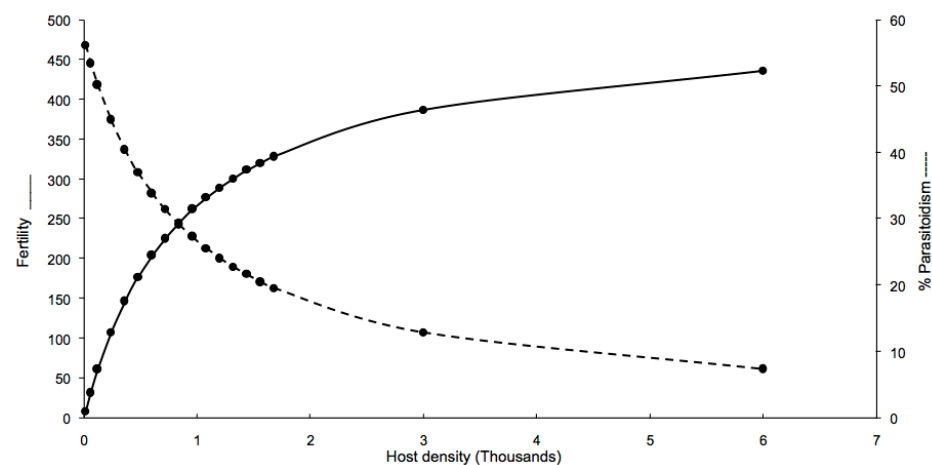

Figure 3. Functional response curve fit by non-linear least-squares regression of *Aganaspis daci* mated females parasitizing *Ceratitis capitata* larvae and sharing the experimental unit with *Pseudoophonus rufipes* adults attacking *C. capitata* larvae (type II). Host density: 5, 20 or 40 larvae per *A. daci* mated female and *P. rufipes* adult (both species sharing the same experimental unit).

For its part, *P. rufipes* functional response switched to type III when sharing prey, with both linear and quadratic coefficients showing negative estimated values [43] (Table 1). The estimated *b* and *c* parameters (see Table 2) led to the following attack rate coefficients: 0.29 days^{-1} (for a prey density of 15 pupae), 0.41 days^{-1} (60 pupae) and 0.44 days^{-1} (120 pupae). The estimated maximum number of predated pupae per adult was 111 specimens in 24 h (Figure 4).
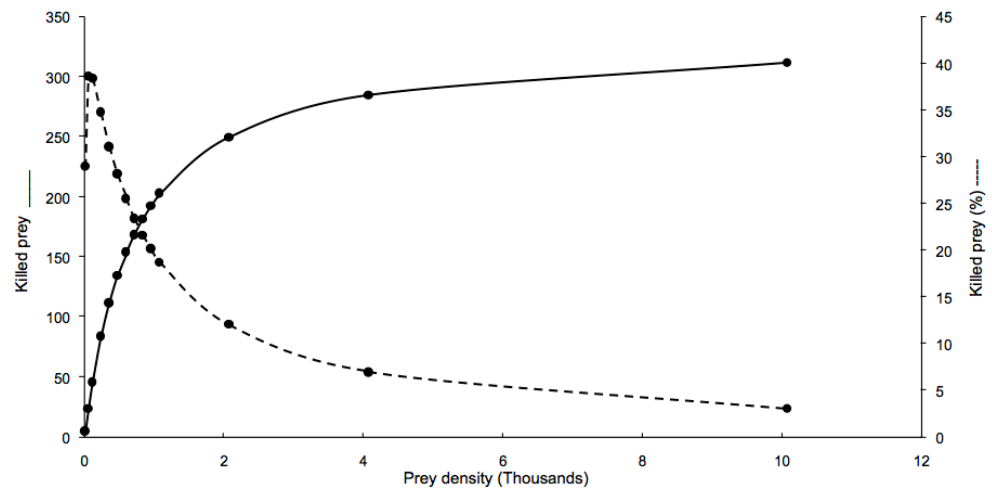
Figure 4. Functional response curve fit by non-linear least-squares regression of *Pseudoophonus rufipes* adults attacking *Ceratitis capitata* larvae and sharing the experimental unit with *Aganaspis daci* mated females parasitizing *C. capitata* larvae (type III). Host density: 5, 20 or 40 larvae per *P. rufipes* adult and *A. daci* mated female (both species sharing the same experimental unit).

3.4. Experiment 4: Demographic Parameters of A. daci and P. rufipes

Two-way ANOVA revealed that the percentage parasitoidism of *A. daci* only differed between the two situations (acting alone vs. acting together with *P. rufipes*) at a density of 15 larvae (Tables 3 and 4). The same applied to fertility and population reduction, while induced mortality did not differ between both situations in any case (Tables 3 and 4). When differences were detected, values were always higher when in the presence of the predator (Table 4). Regarding offspring sex ratio, Pearson's chi squared test showed significant differences at all densities (15 larvae: $\chi^2 = 18.282$, df = 1, $p < 0.001$; 60 larvae: $\chi^2 = 108.789$, df = 1, $p < 0.001$; 120 larvae: $\chi^2 = 16.444$, df = 1, $p < 0.001$; 15–120 larvae (global): $\chi^2 = 104.454$, df = 1, $p < 0.001$). Offspring were female-biased in all cases, and the bias was especially marked when in the presence of *P. rufipes* (Table 4).

Table 3. Two-way ANOVAs comparing percentage parasitoidism, fertility, induced mortality and population reduction of *A. daci* and predation of *P. rufipe*, between the two situations considered (acting alone vs. acting jointly) at the three Medfly densities established (15, 60 and 120 larvae/pupae) and globally (15–120 larvae/pupae). df = degrees of freedom; RV = residual variance; BV = block variance. Asterisks (*) indicate significant differences in each comparison.

	Parameter		15 Larvae	60 Larvae	120 Larvae	Global (15–120 Larvae)
A. daci	Percentage parasitoidism	F	36.481	0.843	53.183	0.220
		df	*1188*	*1188*	*1188*	*1572*
		p	0.003 *	0.527	0.087	0.721
		σ^2 (RV)	7.445	27.555	12.333	27.120
		σ^2 (BV)	1×10^{-6}	1×10^{-7}	1×10^{-8}	1×10^{-7}
	Fertility	F	146.689	0.875	53.127	0.007
		df	*1188*	*1188*	*1188*	*1572*
		p	0.002 *	0.548	0.089	0.948
		σ^2 (RV)	5.678	27.322	12.480	20.120
		σ^2 (BV)	1×10^{-6}	1×10^{-7}	1×10^{-8}	1×10^{-7}
	Induced mortality	F	0.212	0.016	1.591	0.156
		df	*1188*	*1188*	*1188*	*1572*
		p	0.725	0.921	0.427	0.761
		σ^2 (RV)	12.717	32.444	28.111	14.101
		σ^2 (BV)	1×10^{-6}	1×10^{-7}	1×10^{-7}	1×10^{-7}
	Population reduction	F	220.061	2.250	2.317	0.414
		df	*1188*	*1188*	*1188*	*1572*
		p	0.043 *	0.374	0.370	0.636
		σ^2 (RV)	17.040	25.111	27.123	29.037
		σ^2 (BV)	1×10^{-6}	1×10^{-7}	1×10^{-6}	1×10^{-7}

Table 3. *Cont.*

	Parameter		15 Larvae	60 Larvae	120 Larvae	Global (15–120 Larvae)
P. rufipes	Predation	**F**	1019.434	177.929	224,240.743	56.006
		df	*1188*	*1188*	*1188*	*1572*
		p	0.020 *	0.048 *	0.001 *	0.085
		σ^2(RV)	11.321	17.888	10.555	16.112
		σ^2(BV)	1×10^{-7}	1×10^{-8}	1×10^{-7}	1×10^{-8}

Table 4. Demographic parameters of *A. daci* (percentage parasitoidism, fertility, induced mortality, population reduction and offspring sex ratio) and *P. rufipes* (predation) acting alone and together under laboratory conditions on different host densities of *C. capitata* larvae. In each column, asterisk indicates significant differences for a same parameter.

	Parameter	Host Density		
		15 Larvae	60 Larvae	120 Larvae
A. daci (alone)	Parasitoidism (%) (Range; Mean ± SE)	0–80; 35.90 ± 2.41 *	0–83.3; 58.00 ± 1.92	0–88.3; 57.17 ± 2.00
	Fertility (Range; Mean ± SE)	0–12; 5.38 ± 0.36 *	0–50; 34.80 ± 1.15	0–106; 68.60 ± 2.40
	Induced mortality (%) (Range; Mean ± SE)	0–20; 9.44 ± 0.70	0–18.3; 8.07 ± 0.62	0–16.67; 6.63 ± 0.59
	Population reduction (%) (Mean ± SE)	45.34 ± 1.86 *	66.07 ± 1.84	66.61 ± 2.01
	Sex ratio (♂♂, ♀♀; ♀♀/♀♀+ ♂♂)	196, 321; 0.62 *	1352, 1654; 0.55 *	2053, 4083; 0.62 *
A. daci (with *P. rufipes*)	Parasitoidism (%) (Range; Mean ± SE)	26.67–100; 62.43 ± 2.38 *	23.33–93.33; 53.73 ± 2.08	20.83–90.83; 51.97 ± 2.03
	Fertility (Range; Mean ± SE)	4–15; 9.37 ± 0.36 *	14–56; 32.23 ± 1.25	25–109; 62.37 ± 2.44
	Induced mortality (%) (Range; Mean ± SE)	0–33.3; 8.61 ± 1.27	0–31.67; 7.86 ± 1.52	0–40; 9.98 ± 1.44
	Population reduction (%) (Mean ± SE)	71.11 ± 1.45 *	61.59 ± 1.26	61.96 ± 1.10
	Sex ratio (♂♂, ♀♀; ♀♀/♀♀+ ♂♂)	243, 657; 0.73 *	990, 2105; 0.68 *	1715, 4004; 0.70 *
P. rufipes (alone)	Predation (Range; Mean ± SE) [Percentage: Mean ± SE]	9–15; 13.56 ± 0.23 * [90.41 ± 1.56]	9–48; 31.18 ± 1.56 * [51.97 ± 2.61]	9–51; 32.62 ± 1.59 * [27.18 ± 1.32]
P. rufipes (with *A. daci*)	Predation (Range; Mean ± SE) [Percentage: Mean ± SE]	0–11; 4.31 ± 0.22 * [28.85 ± 1.44]	4–46; 23.04 ± 0.76 * [38.40 ± 1.26]	11–93; 45.64 ± 1.32 * [38.04 ± 1.11]

In the case of *P. rufipes*, the two-way ANOVA revealed that predation was significantly affected by the presence of *A. daci* at the three densities (15, 60 and 120 pupae) but not globally (15–120 pupae). Predation was higher when *P. rufipes* acted alone at densities of 15 and 60 pupae, but, with the highest prey availability, predation was greater when in the presence of *A. daci* (Tables 3 and 4).

4. Discussion

Many insect pests are attacked by both predators and parasitoids, which frequently interact with each other while exerting their biocontrol activity [10,11]. Among these interactions, intraguild predation (IGP) has been reported to be especially common [48,49]. IGP between predators and parasitoids is asymmetric: while predators can frequently kill parasitoids when feeding on hosts that are already parasitized, parasitoids cannot cause direct mortality to predators [50–54]. Moreover, the presence of predators with whom they must share the same resource usually leads to alterations in the behaviour of parasitoids that can cause reduced parasitic efficiency [55]. In short, the coexistence with predators often leads to the disruption of the biological control by parasitoids, and this impedes the desired complementary action of both natural enemies [48,56–59].

In the present study, we studied the functional responses shown by the parasitoid *A. daci* and the predator *P. rufipes* when feeding on the Medfly as a way to understand the trophic interactions between these three species. Functional responses are classified into three types (type I to III), with type II being the most common for invertebrate parasitoids and predators [60]. This type of response leads to inverse density-dependent predation or parasitism [44], with values increasing with prey/host density at a constantly decreasing rate towards a maximum value determined by the maximum attack rate. Type III functional response, although more common among vertebrates, could also be characteristic for invertebrate natural enemies, especially when external factors affect their behavior [61,62]. This type of response resembles type II but includes an acceleration at low prey/host densities [63], which is believed to be due to learning behaviors and the subsequent

increased searching efficiency and decreased host-handling time [60]. Therefore, under these circumstances, it is the only functional response that may lead to direct density dependence and can potentially stabilize predator–prey interactions [46].

We observed a type III functional response for *A. daci* in the absence of the predator, as was the case in the two previous works that assessed the functional response of this species under laboratory conditions. In de Pedro et al. [64], conditions were very similar to ours, with the only difference that hosts were offered an artificial Medfly diet. This could explain the lower handling times exhibited by the parasitoid when compared to the present study. In de Pedro et al. [65], the Medfly larvae received the artificial diet, and, moreover, *A. daci* was in the presence of the braconid parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae), which also parasitizes the late larval instars of *C. capitata* [66,67]. Competition, in this case, resulted in higher handling times and lower attack rates but did not affect the functional response of *A. daci*. In contrast, in the present study, the presence of *P. rufipes* caused a shift to a type II response. The shift to a different functional response seems to be a common phenomenon in parasitoids when they compete for hosts with predators, whose presence may have different effects on parasitoid efficiency. For example, Vanaclocha et al. [45] observed that the coccinelid *Chilocorus circumdatus* (Gyllenhall) (Coleoptera: Coccinellidae) modified the functional response of the aphelinid *Aphytis lingnanensis* Compere (Hymenoptera: Aphelinidae) on armored scales exclusively via IGP at low host densities. In most cases, however, the shift is linked to behavioral changes caused by the presence of the predator. In this regard, Martinou et al. [55] described an increase in the handling time of the parasitoid *Aphidius colemani* Viereck (Hymenoptera: Aphidiinae) when coexisting with the mirid *Macrolophus caliginosus* Wagner (Hemiptera: Miridae), while our study, as well as the one by Tormos et al. [39], revealed the opposite effect on *A. daci* and *S. cameroni*, respectively, in the presence of *P. rufipes*. The fact that these Medfly parasitoids displayed opposite responses could be attributed to differences in their searching ability, probably derived from the different ways in that hosts were offered in each case.

For its part, the functional response of *P. rufipes* also changed in the presence of *A. daci*, in this case from a type II to a type III response, suffering a significant decrease in handling time but also in the attack rate. Type II responses seem to be the most typical when *P. rufipes* acts alone on *C. capitata* [18,39], whereas the shift to type III in competition had never been observed prior to the present work. The attack rate and handling time we recorded are similar to those scored by Monzó et al. [18] for third instar Medfly larvae and significantly lower than the values obtained by the same author and Tormos et al. [39] for Medfly pupae, which seems to confirm that the latter is the preferred instar of this beetle species.

None of the *A. daci* parameters were globally affected by the presence of the predator, but some effects were observed at certain host densities. For example, at low densities, percentage parasitoidism and fertility were higher in the presence of *P. rufipes*. Under these circumstances, the parasitoid efficiency and, more specifically, the attack rate seems to be enhanced by the predator, probably via competition pressure. Induced mortality was very low (below 10%) in any case and was neither affected by host density nor by the presence of the carabid. This was unexpected considering that both factors seem to have a negative effect on superparasitism, which in turn seems to be one of the main causes of the induced mortality caused by Medfly parasitoids [68]. The stability of induced mortality led to population reduction by *A. daci* following a similar trend to percentage parasitoidism, with higher values in the presence of with *P. rufipes* at low host densities. Since Medfly population reduction is the final goal of biocontrol programs, the combined use of both natural enemies seems to be a proper strategy from the perspective of *A. daci*. Another argument in favor of this strategy is the strongly female-biased offspring recorded for *A. daci* in the presence of *P. rufipes* at all host densities. Female-biased sex ratio is very common among hymenopteran parasitoids and is always desirable within biocontrol programs because only females exert parasitic activity [69,70]. Until now, only temperature had been highlighted as a decisive factor affecting *A. daci* offspring sex ratio, with the

proportion of females rising with increasing temperatures in the suitable developmental temperature range [33].

The predation of *P. rufipes* was significantly lower when in the presence of *A. daci* at low and medium host densities, and the opposite was the case when prey were abundant. As we mentioned before, it seems that at low densities, when the probability of encounter is higher, the competition pressure exerted by *P. rufipes* stimulated the parasitic activity of *A. daci*, which negatively affected the performance of the predator. At high host densities, the efficiency of the parasitoid stabilized, and the abundance of *C. capitata* was enough to ensure an optimal activity of both natural enemies. These results support the observation of Tormos et al. [39] that *P. rufipes* is able to discriminate between parasitized and unparasitized hosts, expressing a preference for the latter, which is common among predators in coexistence with parasitoids [48,71–73]. The main novelty in the present study is that *P. rufipes* discrimination seems to occur at all prey densities, even when the Medfly was scarce. The reason why Medfly juveniles parasitized by *A. daci* are avoided by *P. rufipes* to such an extent should be addressed in future research, as this phenomenon could have serious implications on predator survival under low prey availability. In any case, we can conclude that IGP between *P. rufipes* and *A. daci* is negligible, and this allows an additive effect of both natural enemies that improves their individual performance. The population reduction of the pest under the joint action of both natural enemies, i.e., the sum of the population reduction caused by *A. daci* and the percentage predation of *P. rufipes*, reached 100% or very close values at all densities. Therefore, combined releases of *A. daci* and *P. rufipes* seems to be a suitable strategy for the control of the Medfly regardless of the density of the pest. This could be greatly advantageous in comparison to other predator–parasitoid systems whose effects are only additive over a certain pest density threshold due to IGP [39,45,74]. Firm conclusions, however, will not be drawn until field studies are conducted to assess the real behavior of these species in nature.

5. Conclusions

The present work represents the first study of intraguild interactions involving *A. daci*, one of the most studied Mediterranean parasitoids of the Medfly in recent years [32,41,75]. Our results suggest that, when acting together against Medfly larvae, *A. daci* and *P. rufipes* affect each other's functional response. At low host densities, *A. daci* improves its parasitic efficiency, while *P. rufipes* reduces its predatory activity due to an extreme host discrimination that causes the rejection of parasitized larvae. As host availability increases, the parasitic activity of *A. daci* reaches a maximum value and the reduced encounter probability enhances predation by *P. rufipes*, which even exceeds the values obtained in the absence of the parasitoid. The result of all these trends is a practically negligible IGP and a combined deleterious effect on Medfly populations that almost completely eliminate *C. capitata* at all densities. This lends support to the recommendation of combined releases of *A. daci* and *P. rufipes* in biocontrol programs against the Medfly. However, further ecological studies, especially under natural conditions, will be necessary to elucidate the long-term effects that competition may have on the population dynamics and the biocontrol performance of these natural enemies.

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