Enhanced biocontrol services in artificially selected strains of Orius laevigatus

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Abstract
Augmentative biological control in protected crops relies mainly on omnivorous predators. Their performance as biological control agents (BCA) depends on several characteristics of the species, which in turn may differ among strains within a species. We have recently reported the achievement of two Orius laevigatus (Hemiptera: Anthocoridae) strains showing a significant larger body size or better fitness when feeding on pollen, two characteristics having a key impact on field performance. However, selection towards a specific trait might result in trade-offs, such as reduced predation capacity, which may impair control efficiency. Therefore, the predation capacity of these selected populations was tested in laboratory as a first step prior to its field use. Functional response to different densities of Frankliniella occidentalis (Thysanoptera: Thripidae) (adults and larvae) and Myzus persicae (Hemiptera: Aphididae) (nymphs) were studied in the large-sized and pollen-tolerant O. laevigatus strains in comparison with commercial and wild populations. A type-II functional response was observed regardless of the population. Body size was significantly related to thrips but not to aphid predation. The large-sized strain showed a superior predation capacity, both on thrips larvae and especially on adult thrips, although not on aphids. Therefore, the larger body size of the selected strain may increase its effectiveness as BCA of thrips. Regarding the pollen-tolerant strain, no trade-offs were observed in predation rates on adults or larvae of thrips, but it showed higher predation capacity on aphid nymphs, suggesting an expanded prey range. Implications of such enhanced biocontrol services on crop protection are also discussed.

Keywords Predation capacity · Body size · Omnivory · Zoophytophagy · Selective breeding · Genetic improvement

Key Message
• Biocontrol in protected crops mainly relies on omnivorous predators, such as Orius laevigatus
• We selected two O. laevigatus strains showing larger body size or better fitness feeding on pollen
• Continued selection towards a specific character might result in reduced predation capacity
• The large-sized strain showed superior predation on thrips larvae and adults, but not on aphids
• The pollen-tolerant strain showed no trade-offs in predation on thrips, and higher on aphid nymphs

Introduction
Augmentative biological control is successfully implemented in many crops, particularly in protected crops (Sanchez et al. 2000; van Lenteren et al. 2018, 2020). Different natural enemies are released to control major pests, but generalist predators play a significant role. Among these predators, the genus Orius Wolff (Hemiptera: Anthocoridae) is used worldwide, and the species O. laevigatus (Fieber) in Europe and other African and Asian countries, mainly to
control thrips populations (Sanchez et al. 2000; van Lenteren et al. 2018, 2020).

Performance as biological control agents (BCAs) depends on several characteristics of the species, which in turn may differ among strains within a species. Among those characteristics, body size has a key impact on most biological and ecological traits of any species, including fecundity and longevity (Kingsolver and Huey 2008; Chown and Gaston 2010). Particularly prominent for a predator, body size plays a major role in predator–prey relationships (Kalinkat et al. 2015). The range of prey a predator is capable of attacking is strongly related to body size (Kalinkat et al. 2015).

In addition, the reliability of biological control in protected crops is dependent on the continuous presence of omnivorous predators, such as Amblysieus swirskii Athias-Henriot (Acari: Phytoseidae) and O. laevigatus (Leman and Messelink 2015; Bielza et al. 2020). Their presence throughout the growing season allows an early response to pest density variations. Their omnivory will enable them to survive when prey is absent or scarce feeding on plant material, mainly pollen. However, their reproductive potential is reduced when feeding alternative food (Mendoza et al. 2021).

Therefore, body size and better fitness feeding on pollen (hereafter pollen tolerance) are good candidates for traits to be targeted in genetic improvement of BCAs (Bielza et al. 2020). We have developed selective breeding programmes for several key traits for the field performance of O. laevigatus, such as insecticide resistance (Balanza et al. 2019, 2021a,b). In addition, we have recently reported the successful achievement of a strain showing a significant larger body size (Mendoza et al. 2020) and better fitness feeding on pollen (Mendoza et al. 2021). Both strains exhibited superior reproductive fitness (longevity and fecundity) over wild and commercial populations, both on nutritionally superior (Ephestia kuehniella) Zeller (Lepidoptera: Pyralidae) eggs and inferior (honey bee pollen) food.

Nevertheless, the continued selection towards a specific trait might in turn result in detrimental changes in other characters, known as trade-offs, such as reduced capacity to interact with its natural prey, which may impair its control efficiency (Thompson and Hagen 1999; Grenier and de Clercq 2003). Therefore, the predation capacity of the O. laevigatus enhanced strains should be tested prior to its use in biological control programmes. The larger body size and the trophic specialization of the artificially selected strains might extend the range of prey, including larger species or prey life stages.

On the one hand, a larger body size may increase the killing capacity of predatory mites and insects, improving their performance as biocontrol agents (Vangansbeke et al. 2019). Accordingly, our first hypothesis was that the larger O. laevigatus strain had a superior predation rate and range, since increases in average predator body size are correlated with an increase in prey size range (Sabelis and van Rijn 1997; Woodward et al. 2005; Brose et al. 2006) and higher per-capita predation rates (Emmerson and Raffaelli 2004).

On the other hand, selection to non-prey food might lead to a tradeoff hindering the predation capacity (Bielza et al. 2020) altering preferences between animal and vegetal food resources in a zoophytophagous predator (Dumont et al. 2017). In the omnivorous predator mullein bug, Campylomma verbasci (Meyer) (Hemiptera: Miridae), a strain showing preference for pollen was less effective controlling pest populations than the more zoophagous strain (Dumont et al. 2019). Besides, an improved ability to profit from different resources has a key role in prey switching, which is generally associated with population dynamical stability (Murdoch 1969). Indeed, a predator preying on multiple prey can destabilize the predator–prey interaction and give rise to predator–prey cycles (Krivans 1996), leading to efficient pest control.

In the present study, we compared the genetically improved O. laevigatus strains with two commercial and one wild populations under the same conditions in laboratory trials as a first step to assessing their predation capacity. To this end, we carried out bioassays to estimate the functional response of O. laevigatus on its main target prey, the western flower thrips (hereafter WFT) Frankliniella occidentalis (Pergande) (Thysanoptera: Thripidae). We discriminated between larvae and adults since the latter stage represents a frequent challenge for the predator due to its high mobility and larger size (Funderburk et al. 2000; Baez et al. 2004). In addition, predation ability on an alternative non-preferred prey, the peach tree aphid, Myzus persicae Sulzer (Hemiptera: Aphididae) (Alvarado et al. 1997; Messelink et al. 2011; Bouagga et al. 2018) was evaluated to elucidate whether our breeding processes have made any impact on the range of prey typically attacked by the insect.

Materials and methods

Insect rearing

Orius laevigatus enhanced populations with a larger body size and higher fitness on pollen, hereafter BIG30 and 2POL-11, respectively, were obtained through a highly structured selection process as explained in Mendoza et al. (2020, 2021, respectively). Two commercial populations from Agrobio SL (Almeria, Spain, ORIcontrol®) and Koppert Biological Systems (The Netherlands, Thripor-L®) were used as reference, as well as another strain collected in Malaga (Spain) on wild plants of Lantana spp. in 2017. The commercial populations were periodically refreshed with new individuals from the same supplier. All predator
populations were maintained on *E. kuehniella* frozen eggs ad libitum under controlled conditions at 26 ± 1 °C, 70 ± 10% RH and 16:8 L:D photoperiod.

*Frankliniella occidentalis* specimens were obtained from a rearing established at Universidad Politecnica de Cartagena (UPCT) from field populations, originally collected in south-eastern Spain organic greenhouses between 2009 and 2010 and then mixed and reared on green bean pods (*Phaseolus vulgaris* L) as explained in Guilian et al. (2014). Then, synchronized 2nd-instar larvae and young adult samples were provided to conduct these bioassays.

*Myzus persicae* specimens were also obtained from a rearing system conducted at UPCT based on different field populations collected between 2014 and 2015 from peach (*Prunus persica* L.) and nectarine (*Prunus persica* var. *nucipersica* L.) production orchards (Mezei et al. 2020). A mixed (after some generations) and synchronized 2nd-instar (3–4 days old) sample reared in the laboratory on sweet pepper plants, *Capsicum annuum* L. (cv. Herminio, Syngenta), was used to conduct these experiments.

**Predation capacity and functional response**

*Frankliniella occidentalis* 2nd-instar larvae and adults and *M. persicae* 2nd-instar nymphs were used as prey for the *O. laevigatus* populations tested. The strains of *O. laevigatus* were tested in two phases, firstly BIG30 and Agrobio, and secondly the other strains, 2POL-11, Koppert and Malaga. Within each phase, the three prey types were tested simultaneously at the different prey densities. The prey populations tested in each phase were the same and their body size did not change.

To study the predation capacity on *M. persicae*, replicates of 5, 10, 20 and 30 2nd-instar nymphs were placed into polypropylene cages (38 mm diameter, 20 mm height) with a circular Sect. (38 mm diameter) of a sweet pepper leaf centered upside down on a 1% agar layer. A ventilated lid with a fine metal mesh was used to cover each cage. Prior to experiments freshly emerged *O. laevigatus* females (in the last 24 h) were offered *M. persicae* nymphs in excess for 24 h and then the prey was removed for 24 h more. Finally, one single *O. laevigatus* female was transferred to each cage. A control was also evaluated to test aphid nymphs’ survival in the absence of predator.

In the case of *F. occidentalis*, either 2nd-instar larva or adult individuals were transferred to 5 mL-plastic vials with a rectangular Sect. (30×5 mm) of sweet pepper leaf inside and covered by a carefully punctured lid. In the case of 2nd-instar thrips, densities of 20, 30, 50 and 100 thrips (the last one only for BIG30, according to preliminary bioassays) per vial were assessed, as well as a negative control. For adults, densities of 5, 10 and 30 individuals were provided and a control was also evaluated. After 24-h contact between adult *O. laevigatus* and the prey and 24-h fasting, one single *O. laevigatus* female was transferred into each plastic vial.

Only three to four prey densities were used since additional densities would not add substantial benefits to assess predation. In the preliminary tests with both prey species lower densities were fully predated so the predation capacity would not be adequately compared among *O. laevigatus* strains due to saturation. For the highest densities tested, *O. laevigatus* females killed less prey than half of those offered.

There were ten replicates per *O. laevigatus* strain, prey density and prey species. After 24 h, the predators were removed from experimental arenas and the dead preys with symptoms of having been killed and emptied (totally or partially) were recorded as predated. In the controls without predators the dead individuals were counted. Dead *O. laevigatus* females (only 2 cases out of 510) were excluded from the analysis. Finally, *O. laevigatus* females were frozen and the width of the pronotum was measured with an optical micrometer at 50x (Leica S9 E).

**Statistical analysis**

Data were checked for normality by the Shapiro–Wilk’s test and homogeneity of variance (homoscedasticity) by the Levene test. For the analysis of variance (ANOVA), we transformed counts to their square root values and proportions to their arcsine values if assumptions of normality and homogeneity were not met. Variation of body size (pronotum width) among populations were analyzed by using one-way ANOVA. For each prey type, differences in predation consumption among populations within prey density or among prey density within population were assessed with one-way ANOVA.

Data were checked for normality by the Shapiro–Wilk’s test and homogeneity of variance (homoscedasticity) by the Levene test. For the analysis of variance (ANOVA), we transformed counts to their square root values and proportions to their arcsine values if assumptions of normality and homogeneity were not met. Variation of body size (pronotum width) among populations were analyzed by using one-way ANOVA. For each prey type, differences in predation consumption among populations within prey density or among prey density within population were assessed with one-way ANOVA tests. When differences among treatments were significant, comparison among means were conducted using Fisher’s LSD post-hoc test at \( P \leq 0.05 \). Finally, one-way ANOVA was used to compare the numbers of dead prey recovered from controls.

Correlation tests between the averaged proportion of killed prey and pronotum width of each *O. laevigatus* population were conducted to determine the influence of females’ size on their predation capacity. Only data of the highest prey density was used for each prey type in order to avoid predation limitation due to low prey availability.

The type of functional response was determined by logistic regression analysis of the proportion of prey consumed \( (N_c/N_0) \) as a function of the initial prey density \( (N_0) \) using the categorical modelling procedure (PROC CATMOD) of the Statistical Analysis System (SAS Institute 2001) as follows (Trexler and Travis 1993):

\[
N_c/N_0 = \frac{\exp(P_0 + P_1N_0)}{1 + \exp(P_0 + P_1N_0)}
\]
where $N_C$ is the number of prey consumed, $N_0$ is the initial prey density, $P_0$ and $P_1$ are the constant and linear coefficients, respectively. To determine the significance of the linear term from Eq. 1 and the type of the functional response, the log likelihood-ratio test was used to determine whether equation coefficients differed significantly from 0 (Trexler and Travis 1993). In this test, the difference in log-likelihoods is a chi-square value ($\chi^2$) with 1 degree of freedom. For a type I, the curve of $N_C/N_0$ versus $N_0$ has a linear shape if the linear term from Eq. 1 was not significantly different from 0, whereas a significant negative value indicated a type II response (the proportion of prey consumed decreases gradually as the initial number of offered prey increases) and a significant positive value indicated a type III functional response (the proportion of prey consumed is positively density-dependent) (Juliano 2001).

Then, the Holling disc equation (Holling 1959) was fitted to the results using least-square nonlinear regression procedure (PROC NLIN, SAS Institute 2001) to estimate the parameters values of the predator as follows:

$$N_C = \frac{a N_0 T}{1 + a N_0 T_h}$$

(2)

where $a$ is the instantaneous attack rate of a predator (searching efficiency), $T$ is the total amount of time available for searching (24 h in this experiment) and $T_h$ is the handling time per prey caught (proportion of the exposure time that a predator spends in identifying, pursuing, killing, consuming and digesting prey). Afterwards, $T_h$ values were used to calculate maximum attack rate as $T/T_h$ (Hassell 1978), which represent the maximal number of prey individuals that could be consumed by *O. laevigatus* during 24 h. The curves of the number of prey consumed by *O. laevigatus* to different prey densities were depicted by Excel software.

### Results

Body size (pronotum width) of *O. laevigatus* females used as predators differed consistently between the populations studied, with the largest size (mean ± SE: 0.825 ± 0.004 mm) for the selected BIG30 strain; intermediate values for the commercial strains Agrobio and Koppert (0.768–0.779 ± 0.004 mm); a lower size (0.751 ± 0.003 mm) for the pollen tolerant population 2POL-11; and the lowest value for the wild population Malaga (0.717 ± 0.003 mm) (Table 1).

Prey consumption differed among *O. laevigatus* populations both on larval and adult WFT, but also for aphid nymphs (Table 1). Thus, BIG30 showed the highest values for thrips, killing 66% more WFT larvae than commercial ones when offered prey in excess. Even more significant was the difference when the prey was the adult WFT, with 145%...
more predation capacity in BIG30 females than in Agrobio’s at the maximum prey density. However, when aphid nymph was offered as prey, only 2POL-11 showed a significantly higher predation rate despite being smaller in size than larger and commercial strains. No significant differences in thrips predation were observed between the pollen tolerant strain 2POL-11 and the commercial populations.

Female size (pronotum width) had a consistent effect on predation rate per population when thrips were offered in excess as prey (when predation consumption is not limited by number of prey available), but not for aphids (Table 1). Indeed, the correlation between size and predation rate was very strong for larval (n = 5, \( r = 0.95, P = 0.015 \)) and adult (n = 5, \( r = 0.96, P = 0.009 \)) thrips, and but not significant for aphid nymphs (n = 5, \( r = 0.43, P = 0.461 \)).

A strong and relatively constant positive relationship was found between prey density and prey consumption in \( O. \) laevigatus females for both thrips stages, 2nd-instar larva (one-way ANOVA: \( F_{3, 156} = 103.94; P < 0.001 \)) and adult WFT (\( F_{2, 149} = 49.43; P < 0.001 \)), regardless the population tested (Table 1, Fig. 1), as well as for aphids (\( F_{3, 198} = 63.53; P < 0.001 \)). For WFT larvae, the predation rate of BIG30 at 50 larvae density was 88.8%, significantly higher than that obtained for the other populations (43.6–61.0%), making it necessary to test a higher dose (100) for the large strain with the aim of reaching an asymptote.

The linear coefficient \( (P_1) \) of the logistic regression of \( O. \) laevigatus females on \( M. \) persicae, larval and adult WFT were negative for all populations tested, thus indicating a type-II functional response (Table 2). They are characterized by a hyperbolic curve: starting at low prey densities on the abscissa, \( O. \) laevigatus predation rate first increases almost linearly as prey density increases until it gradually slows down to reach an upper limit, a plateau (Fig. 1). Now then, both the slope and the asymptote values were higher for BIG30 than for unselected populations when fed either on larvae or adults WFT. Again, no differences were observable when aphids were offered as prey, except for 2POL-11, which showed a slightly higher predation pattern than the rest of the populations assayed (Fig. 1).

The coefficients of attack rate and handling time were the parameters used to determine the extent of the functional response of \( O. \) laevigatus. In our bioassays, the different populations also responded differently here to the increase in prey density, with the two parameters reasonably fitted to a Type-II functional response (Table 3). Except for BIG30, the lowest attack rate and the highest handling times were registered for adult thrips, ranging 0.01–0.03 h\(^{-1}\) and 1.1–2.8 h,

![Fig. 1](image-url)
respectively; tripling the time spent feeding on thrips larvae (0.4–0.6 h). BIG30 presented higher values for attack rate (adult WFT: 0.04 h⁻¹; larva WFT: 0.06 h⁻¹) and lower values for handling time (adult WFT: 0.60 h; larva WFT: 0.26 h) than reference populations regardless of the thrips instar. However, significant differences were observed only for handling time. We found the widest difference between populations for this parameter on adult WFT, with a 72% lower value for BIG30. Regarding 2POL-11, this enhanced strain was the only one to show statistically favorable values for attack rates on aphids compared to Koppert and Malaga strains, together with short handling times not only for aphids but also on thrips larvae. These results are further supported by the higher theoretical maximum predation rate (T/Th) observed for the enhanced O. laevigatus strains, with values for BIG30 almost twice those of the commercial populations Agrobio and Koppert for WFT larvae and twice or more for adult WFT. In the case of 2POL-11, a higher value for this parameter on M. persicae compared to the rest of the populations assayed was registered (26.03 against 14.20–22.94).

Prey mortalities were very low in the control vials. The numbers of dead insects observed in controls into the vials were on the range of 1.4–4.0% of those from similar prey species and density but in the presence of O. laevigatus, without significant differences among prey species and stages (F₂, 149 = 2.32; P = 0.102).

### Discussion

The functional response to prey density is one of the most important methods for estimating the biocontrol efficiency of a predator (Wiedenmann and Smith 1997). It refers to

| Population | Prey Parameters | Parameters* Estimate ± SE | χ²** | P value |
|------------|----------------|---------------------------|-------|---------|
| BIG30 | Myzus persicae | P₀ | 1.685 ± 0.243 | 48.25 | < 0.001 |
|          |                 | P₁ | −0.066 ± 0.010 | 43.91 | < 0.001 |
|          | 2nd instar F. occidentalis | P₀ | 4.894 ± 0.265 | 342.04 | < 0.001 |
|          |                 | P₁ | −0.047 ± 0.003 | 282.42 | < 0.001 |
|          | Adult F. occidentalis | P₀ | 2.369 ± 0.316 | 56.32 | < 0.001 |
|          |                 | P₁ | −0.069 ± 0.012 | 34.31 | < 0.001 |
| 2POL-11 | Myzus persicae | P₀ | 3.074 ± 0.320 | 92.29 | < 0.001 |
|          | 2nd instar F. occidentalis | P₀ | 4.002 ± 0.284 | 198.35 | < 0.001 |
|          | Adult F. occidentalis | P₀ | 0.381 ± 0.217 | 3.07 | 0.080 |
|          |                 | P₁ | −0.042 ± 0.009 | 21.09 | < 0.001 |
| Agrobio | Myzus persicae | P₀ | 1.936 ± 0.244 | 63.05 | < 0.001 |
|          | 2nd instar F. occidentalis | P₀ | 3.263 ± 0.223 | 205.39 | < 0.001 |
|          | Adult F. occidentalis | P₀ | 0.198 ± 0.218 | 0.83 | 0.036 |
|          |                 | P₁ | −0.047 ± 0.009 | 24.80 | < 0.001 |
| Koppert | Myzus persicae | P₀ | 0.896 ± 0.220 | 16.31 | < 0.001 |
|          | 2nd instar F. occidentalis | P₀ | 3.127 ± 0.243 | 166.02 | < 0.001 |
|          | Adult F. occidentalis | P₀ | 0.487 ± 0.218 | 1.17 | 0.083 |
|          |                 | P₁ | −0.043 ± 0.009 | 26.04 | < 0.001 |
| Malaga | Myzus persicae | P₀ | −0.177 ± 0.223 | 0.63 | 0.429 |
|          | 2nd instar F. occidentalis | P₀ | 1.149 ± 0.169 | 46.44 | < 0.001 |
|          | Adult F. occidentalis | P₀ | −0.986 ± 0.259 | 14.49 | < 0.001 |
|          |                 | P₁ | −0.025 ± 0.012 | 4.89 | 0.027 |

*P₀ and P₁ are the constant and linear coefficients, respectively

**Chi-square value with 1 df
changes in the number of prey consumed by a predator per unit time in relation to initial prey density (Solomon 1949; Holling 1959), and shows whether a predator is capable to regulate the density of its prey or not (Jervis and Kidd 1996). Our results show that the strain selected for larger body size BIG30 improved predation performance on its natural prey, both on thrips larvae but especially on adults. For the pollen tolerant strain, 2POL-11, we found a slightly improvement in the efficacy to predate thrips larvae, but mainly, higher efficiency in hunting and killing aphid nymphs.

The linear coefficient (P1) of the logistic regression of *O. laevigatus* females at 26 °C was negative regardless of the prey and population tested, which indicated a type II functional response. With increases in prey density, the net prey consumption in every *O. laevigatus* population tested increased until a plateau was reached. This functional response has been reported for *O. laevigatus* (F. occidentalis; Montserrat et al. 2000; Aphis glycines Matsumura (Hemiptera: Aphididae): Hassanpour et al. 2020, Pehlivan et al. 2020) but also for other *Orius* species, such as *O. majusculus* (Reuter) [F. occidentalis and Trialeurodes vaporariorum (Westwood) (Hemiptera: Aleyrodidae): Montserrat et al. 2000], *O. insidius* (Say) (A. glycines: Rutledge and O’Neill 2005), *O. albipennis* (Reuter) [Thrips tabaci Lindeman (Thysanoptera: Thripidae): Madadi et al. 2007; Aphis fabae Scopoli (Hemiptera: Aphididae): Rashedi et al. 2020], *O. niger* (Wollf), *O. minutus* (L) (Tetranychus urticae Koch (Acari: Tetranychidae) and *T. tabaci*: Fathi and Nouri-Ganbalani 2010), *O. insidiosus* (A. gossypii Glover: Veiga et al. 2014), *O. tricolor* (White) (*Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae): Queiroz et al. 2015) or *O. strigicolis* (Poppius) [{*Bemisia tabaci* Gennadius) and *T. vaporariorum* (Hemiptera: Aleyrodidae): Rehman et al. 2020].

*Orius laevigatus* is considered a generalist predator attacking a wide array of arthropod prey. However, the results of our study on commercial, wild and selected populations show that the type of prey can have a considerable impact on its performance as predator. Predation capacities of the studied anthocorids showed large variations when they were offered different prey species and life stages. Our results indicate that *F. occidentalis* larvae are the most suitable prey, amongst those tested, for all populations tested. This suitability over other prey is suggested by higher numbers of prey killed, higher attack rates (*α*) and shorter handling times (*Tth*) of the predatory populations when fed WFT larvae, resulting in shorter time for searching prey and higher predation rates. These findings coincide with previous studies not only involving other *O. laevigatus* strains (Arno et al. 2008; Bonte and de Clercq 2010) but also for other *Orius* species, such as *O. insidiosus* (Isenhour and Yeargang 1981; Tommasini and Nicoli 1993), *O. sauteri* (Kohno and Kashio 1998), *O. thripoborus* and *O. naivashae* F. (Bonte et al. 2015).

It is reasonable to assume that adults have a greater ability to escape because they are winged and move faster. Therefore, adults will be more challenging to catch successfully, and the predator may abstain from attacking before it is fully satiated, as the energy required for the attack may exceed the energy gained from feeding (van den Meiracker and Sabelis 1999). WFT larvae are more vulnerable than adults because

### Table 3 Parameters of functional response (FR) of *Orius laevigatus* feeding on different prey

| Population | Prey                  | FR Type | α ± SE (95% CI) | *Tth* ± SE (95% CI) | *T/Tth* | *r*² | df |
|------------|-----------------------|---------|----------------|---------------------|--------|------|----|
| BIG30      | *Myzus persicae*      | II      | 0.045 ± 0.007 (0.031–0.059) | 1.074 ± 0.166 (0.738–1.411) | 22.35  | 0.73 | 39 |
|            | 2nd instar *F. occidentalis* | II      | 0.062 ± 0.007 (0.049–0.076) | 0.269 ± 0.027 (0.215–0.324) | 89.22  | 0.82 | 36 |
|            | Adult *F. occidentalis* | II      | 0.043 ± 0.004 (0.034–0.052) | 0.599 ± 0.098 (0.399–0.798) | 40.07  | 0.91 | 29 |
| 2POL-11    | *Myzus persicae*      | II      | 0.056 ± 0.003 (0.050–0.062) | 0.922 ± 0.044 (0.833–1.011) | 26.03  | 0.96 | 39 |
|            | 2nd instar *F. occidentalis* | II      | 0.055 ± 0.004 (0.047–0.062) | 0.394 ± 0.036 (0.321–0.467) | 60.91  | 0.89 | 29 |
| AgroBio    | *Myzus persicae*      | II      | 0.025 ± 0.004 (0.017–0.033) | 1.330 ± 0.278 (0.768–1.892) | 18.05  | 0.72 | 29 |
|            | 2nd instar *F. occidentalis* | II      | 0.051 ± 0.012 (0.027–0.074) | 1.161 ± 0.230 (0.669–1.623) | 20.67  | 0.75 | 39 |
|            | Adult *F. occidentalis* | II      | 0.026 ± 0.007 (0.011–0.041) | 2.143 ± 0.534 (0.833–1.101) | 11.20  | 0.40 | 29 |
| Koppert    | *Myzus persicae*      | II      | 0.034 ± 0.005 (0.024–0.044) | 1.046 ± 0.195 (0.650–1.441) | 22.94  | 0.77 | 39 |
|            | 2nd instar *F. occidentalis* | II      | 0.054 ± 0.004 (0.045–0.063) | 0.426 ± 0.045 (0.335–0.517) | 56.34  | 0.89 | 39 |
| Malaga     | *Myzus persicae*      | II      | 0.027 ± 0.005 (0.022–0.035) | 1.062 ± 0.226 (0.702–1.426) | 22.60  | 0.57 | 39 |
|            | 2nd instar *F. occidentalis* | II      | 0.020 ± 0.007 (0.007–0.034) | 1.690 ± 0.719 (0.235–3.146) | 14.20  | 0.39 | 39 |
|            | Adult *F. occidentalis* | II      | 0.037 ± 0.004 (0.028–0.046) | 0.564 ± 0.091 (0.379–0.749) | 42.55  | 0.79 | 39 |

CI: Confidence intervals, α: attack rate (h⁻¹), *Tth*: handling time (h), *T/Tth*: theoretical maximum predation rate, *r*² are the coefficients of determination obtained from *r*² = 1−(sum of squares of residuals/ total sum of squares), and df are the degrees of freedom of analysis of variance (ANOVA) for nonlinear regression procedure (PROC NLIN, SAS Institute 2001) to estimate the parameters values.
they move less and more slowly, while adults are more active and so more likely to evade predation (Sabelis and van Rijn 1997; van den Meiracker and Sabelis 1999). The apparent preference and consumption of the larvae by our predator is consistent with Funderburk et al. (2000), Ramachandran et al. (2001) and Reitz et al. (2006), who recorded lower numbers of thrips larvae than adults in their collection trials using O. insidiosus as predator on pepper and cotton fields. Even more comparable to ours were the laboratory results obtained by Baez et al. (2004) and Chow et al. (2008) with O. insidiosus, who detected the lowest capture success with adult thrips in Petri-dish arenas and regardless of the time and the density assessed. These findings suggest that although Orius spp. may not have an actual preference among life stages, the mobility of adult thrips may affect their vulnerability to predation.

According to our results, the large-sized O. laevigatus strain BIG30 is expected to be more efficient at hunting larval and adult WFT than commercial or wild populations of average size. It has been postulated a scaling of functional response parameters with predator body size (Ball et al. 2015), since a larger predator relative to their prey encounters and captures resources at a higher rate and requires less time for digestion. According to Sabelis (1992), most generalist predators select their prey according to their relative size. This would explain the differences in handling times we obtained between the O. laevigatus strains when fed on both thrips life-stages. BIG30 required half of the handling time needed by Agrobio or Koppert for WFT larvae, and more importantly, up to four-fold less time for adult thrips. To estimate the effectiveness of a predator in relation to its prey, handling time is thought to be a key parameter because it shows how long a predator takes to capture, subdue, kill and digest a single prey item (Atlihan et al. 2010). Lower handling times in BIG30 reveal that O. laevigatus from this population were more active and spent more time searching and feeding, whereas commercial females needed more time for non-searching activities such as resting between hunting periods.

Although the superior predation capacity on both thrips instars of the large-sized strain BIG30 is remarkable, the enhanced functional response on WFT adults is especially important for biological control. BIG30 obtained a maximum prey intake \((T/T_h)\) on adult thrips almost as high as that obtained by the smaller populations on thrips larvae. In addition, BIG30 increased prey consumption as the density of adult thrips increased without reaching a clear plateau. A good killing capacity on adult thrips, in addition to that on larvae, allows that the proportion of the thrips’ life span amenable to effective biological control expands, adding to the 4.4 days of 1st and 2nd larvae, 28.8 days of average adult longevity at 25 ºC (Vangansbeke et al. 2016). The wider prey range of the genetically larger strain BIG30 is particularly relevant for biological control programs where there is a need to lower the pest level below a threshold immediately after the introduction of the predator. Especially when the time for suppression of the pest population must be short, these predation characteristics are essential to determine the initial predator–prey ratio (Sabelis and van Rijn 1997; van den Meiracker and Sabelis 1999).

Vangansbeke et al. (2019) recently managed to increase the size of a predatory mite, Amblyseius californicus (Garman and McGregor) (Acari: Phytoseiidae), through a low-temperature breeding process. This improvement in size led to a higher ability to subdue larger prey, such as 2nd instar F. occidentalis larvae, which it was originally barely able to prey on. This characteristic was also signaled as very beneficial for biological control. However, unlike the genetically improved O. laevigatus strain, the larger size was phenotypic rather than genetic, and demonstrated only a limited transgenerational effect.

In contrast with thrips, for the aphid prey the increase in predator size did not lead to a change in either the curve shape or the functional response parameters. Besides prey size, other factors such as prey defense tactics are important to consider in prey selection and attack by a predator (De Clercq and Degheele 1994; Eubanks and Denno 2000). In this sense, Butler and O’Neil (2007) and Desneux et al. (2006) recorded defensive mechanisms of the soybean aphid, A. glycines, against O. insidiosus, which were more obvious and effective than those observed for F. occidentalis. In the same line, Henaut et al. (2000) detected that all contacts made by O. majusculus along the side of the aphid’s body had the result of the predator easily being dislodged by the aphid’s kicking response with their hind legs. These might be the origin of the relatively low predation rates and high handling times obtained for M. persicae nymphs in both O. laevigatus populations assayed.

On the other hand, regarding the other artificially selected O. laevigatus strain 2POL-11, we also did not find trade-offs in predation rates on its natural prey linked to our breeding process to achieve a better fitness feeding on pollen. In effect, attack rates and handling times on larval and adult thrips were similar (or slightly better) to those obtained in the commercial and wild populations. Moreover, the predation capacity on thrips was maintained even showing a smaller body size, suggesting that although body size is a key trait, as above discussed, other characteristics play a significant role.

However, the main feature that this selected strain has gained through the breeding process is the superior ability to hunt and feed on alternative prey, the juvenile stages of M. persicae. From their semi-field experiments, Bouagga et al. (2018) reported that combined releases of O. laevigatus and A. swirskii effectively controlled F. occidentalis and B. tabaci populations, while that of M. persicae went...
completely out of control, resulting in the collapse of all the plants. In the same line, some failures when facing suboptimal prey as whiteflies (Arno et al. 2008) and especially aphids (Baez et al. 2004; Messelink et al. 2011; Messelink and Janssen 2014; Wang et al. 2014; Bouagga et al. 2018) have been reported in field and greenhouse crops. In our experiments, though, a significantly higher predation rate was registered in 2POL-11 when feeding on M. persicae, especially at high prey densities, thus suggesting the possibility to switch between thrips and other pest species presumably concuring in the sweet pepper crop. In this context, Alvarado et al. (1997) and Hassanpour et al. (2020) reported a mean number of 17–38 aphids eaten per O. laevigatus female from total number of 64 preys at 25 °C. However, in this case first and second instar A. gossypiium individuals were used as prey, which are barely half the size of M. persicae second-instar nymphs (Sampaio et al. 2008), the aphid species tested here. Indeed, when Veiga et al. (2014) assessed the functional response of O. insidiosus on fourth-stage nymphs from two different strains of A. gossypiium, they found a maximum predation rate of only 13–14 aphids per female. More concretely, O. thripoborus and O. naivashae were reported preying only 3–5 M. persicae nymphs per day (Bonte et al. 2015), values clearly below to those obtained in our trials for 2POL-11.

It is to be expected that the ability to detect, capture and handle a certain type of prey (in this case aphids) varies among individuals within a population, making it susceptible to parallel selection during our breeding process for increased pollen tolerance. After all, individuals have to cope with a wide range of resources that may vary in quality, quantity and distribution (Bolnick et al. 2003). Selection for individuals with a stylet that is able to suck pollen more efficiently may lead to a greater ability to subdue and feed on aphids. In any case, the nature of the superior functional response on aphids observed in 2POL-11 remains to be revealed, opening up new research opportunities in this field.

Since quality differs among prey species (Eubanks and Denno 1999), alternative prey may nutritionally supplement each other and together form a better diet than each prey separately (Evans et al. 1999). Although a generalist insect as O. laevigatus shows preference for thrips as superior prey, when this is not sufficiently abundant, inferior prey such as M. persicae would be included in the diet. This broad diet may enable our strain to maintain a more stable presence in agrosystems by switching among prey types as these vary in abundance over time (Wang et al. 2014), which is a valuable feature for biological control, whether for augmentative release or conservation. Through the utilization of alternative prey resources, our predators can increase their density early in the season (Butler and O’Neil 2007), before pests arrive, and later switch to feeding on the main pest (Settle et al. 1996).

On this same basis, the greater ability to take profit from aphids by the selected insects opens up the possibility to use aphid-banker-plant systems to promote the long-term establishment of our anthocorid predator in the crop likewise they are already well implemented to favor parasitoids installation (Yano 2006; van Driesche et al. 2008). This strategy, along with pollen supplementation, would allow not only survival but also reproduction and thus multiplication of the released populations.

Finally, the overall high predation rates in our study may be caused by the relatively simple searching environment. Reports by Isenhour and Yeargan (1981) support this idea, since they found a lower predation rate when thrips were offered to O. insidiosus on larger (65 cm²) than smaller (3.8 cm²) leaf surfaces. In our study, the arena consisted of small-scale setups such as plastic vials, which may have little resemblance to those experiments measured in natural conditions. In fact, this small experimental arena accelerated the search efficiency of the predatory bugs allowing them to repeatedly attack prey that initially escaped (Rehman et al. 2020). Then these values are questionable to generate good representations of average natural predation levels and should be interpreted with care because of the poor chance for thrips to avoid predation (Kareiva 1990). However, Baez et al. (2004) found that almost all predation (87–100%) occurred within the flower, hence in a small natural area. Besides, the anthocorids in the present study killed prey without entirely consuming it, although this is a common behaviour in anthocorids and has been suggested to increase their effectiveness as biological control agents (Isenhour and Yeargan 1981; de Clercq and Degheelee 1994). Be that as it may, we believe our results are consistent enough to compare the functional response among different populations tested in the same conditions, and have value as a first step in estimating their predatory capacity but recommend further studies being conducted, especially trials in real field conditions.

In conclusion, despite the selective breeding process, the selected strains did not show trade-offs in predation rates and functional response parameters either on larval or adult thrips. Moreover, O. laevigatus body size was significantly related to functional response parameters, so that the larger body size strain showed a markedly superior predation capacity, both on thrips larvae and especially on adult thrips. Therefore, the larger body size of the selected strain may increase its effectiveness as biological control agent, especially in situations where a short pest suppression time is required. On the other hand, the selected strain for better fitness on pollen, far from showing trade-offs in predatory capacity as a result of our breeding process, presents enhanced features as a generalist predator compared to commercial populations. The functional response obtained in presence of their natural prey, F. occidentalis, is similar or even superior in the selected strain. Furthermore, wider
range of prey was observed, according to the improved performance of the selected strain as predator of aphids. This attribute, together with the superior performance with a diet based exclusively on pollen, makes the enhanced strain less dependent on the density of their main prey, offering a series of agronomic and industrial advantages.

Author’s contribution

PB conceived research. JEM and PB designed experiments. PB, JEM, VB, ARS and DC collected and prepared material. JEM, ARS and VB conducted experiments and collected data. PB supervised experiments. JEM analyzed data. JEM and PB wrote the manuscript. All authors read and approved the manuscript.

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Data availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest

The authors declare an agreement with the bio-producer Agrobio to commercialize the pollen-tolerant strain of Orius laevigatus.

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