Intensive Biological Control in Spanish Greenhouses: Problems of the Success

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Abstract**—**Currently, biological control programs in greenhouse crops involve the use, at the same time, several natural enemies during the crop cycle. Also, large number of plant species grown in greenhouses, among them, the used cultivars are also wide. However, the cultivar effects on entomophagous species efficacy (predators and parasitoids) have been scarcely studied. A new method had been developed, using the factitious prey or host *Ephestia kuehniella*. It allow us to evaluate, under greenhouse or controlled conditions (semi-field), the cultivar effects on the entomophagous species effectiveness. The work was carried out in greenhouse tomato crop. It has been found the biological and ecological activities of predatory species (*Nesidiocoris tenuis*) and egg-parasitoid (*Trichogramma achaeae*) can be well represented with the use of the factitious prey or host; being better in the former than the latter. The data found in the trial are shown and discussed. The developed method could be applied to evaluate new plant materials before making available to farmers as commercial varieties, at low costs and easy use.

Keywords**—**Cultivar Effects, Efficiency, Predators, Parasitoids.

I. Introduction

PEST control in greenhouse crops in northern Europe has shown an important evolution within the last 30 years through replacing chemical control by biological control, mainly because of pest resistance to insecticides [1]. The same has recently been reported in greenhouse crops in Spain [2], [3]. The causes have been the same: excessive use of chemical control [4] and pest resistance levels to insecticides [2]. In contrast with the northern countries, this change has been quick and surprising in southern Spain, as the total greenhouse area has passed from 1,400 biologically-controlled hectares in 2007 to 26,372 Ha in 2014**.**

Biological control programs in greenhouse crops, such as those in Spain, several species of natural enemies (predators and parasitoids) are used at the same and/or different times throughout the crop cycle to control different pests using different release methods [3]. They have been mainly based on the use of *Eretmocerus mundus*, *Nesidiocoris tenuis*, and *Amblyseius swirskii* to control sweetpotato whitefly, *Bemisia tabaci*; inundative releases of *Trichogramma achaeae* to control the tomato pinworm, *Tuta absoluta*; inoculative releases of *Diglyphus isaea* help to control other pest species with lower incidence such as leafminers, *Liriomyza bryoniae* and *L. trifolii*; to control aphids, mainly the potato aphid, *Macrosiphum euphorbiae*, releases of *Aphidius ervi* are used; while *Phytoseiulus persimilis* have been released to control the two spotted spider mite (*Tetranychus urticae*) and other species in early pest infestation hot spots [3]. The current trend about biological control in greenhouse crops in Spain demands the use of predatory species instead of parasitoids to control the pest species, as well as omnivorous species rather than strictly zoophagous ones. A reason is a high incidence of several pest species and a high-pressure of them from the beginning of the crops, which means the early establishment of the natural enemies is also compulsory [3]. Refuge plants have been recently developed for this purpose as well as the so-called biopropagation or pretransplanted release of omnivorous predators releasing of adult specimens in nurseries, so plants already carry the eggs of the natural enemies when transplanted into the greenhouses, which contributes to guarantee an early colonization of crops [3], [5].

Today, two important aspects are presented in greenhouses such as: a.- The effect of intraguild predation among used natural enemies because of the intensity of biological control, as mentioned above, and b.- the effects of different cultivars, also quite broad, on such intensive use of natural enemies; all in the efficiency of biological control in this situation.

The use of more than one natural enemy in augmentative biological control programs can lead to direct and indirect interactions such as apparent competition, intraguild predation, and resource competition [6]. These interactions may impact the overall efficiency of these biological control agents [7]. Interest in these interactions has resulted in a remarkable number of researches, both theoretical and experimental. But, studies show the impact of interspecific interactions on biological control are still rather rare [6]−[9].

Another important aspect, about biological control programs are the effects of cultivar on entomophagous activity and effectiveness, as pointed out by [10]-[15]. Likewise, the complexity of this issue has also been highlighted [16]. As mentioned above for the case of intraguild predation, also few works have studied the influence of cultivars on the effectiveness of biological control in greenhouse crops, except for some works such as [17], [18].

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II. Objectives

A. Problems Addressed by the Study

- Biological control programs in greenhouse crops include joint use of different entomophagous (predator and parasitoid species) at the same time.
- Commercial cultivars used, for each botanical species, in greenhouse crops is important, with a significant addition of new ones every year.
- Works carried out and published on the effects of crop varieties in entomophagous activities and efficiencies is low or almost null.
- There is no a method to assess the effects of the currently available cultivars or the new plant materials, before marketing, in natural enemies.
- Today for greenhouse crops − where several entomophagous are applied − it would not be possible to introduce a new commercial cultivar not be compatible with these entomophagous species.

B. Questions and Hypothesis Tested

- Is it possible to develop a method for evaluating the effects of cultivars in the activity of entomophagous in greenhouse crops?
- Is it possible to replace the pest species, which are often difficult rearing and at high costs for some alternative species?
- Are the results representative, using alternative prey or hosts, to evaluate the effects of cultivars in biological control programs?

C. How Findings are Intended to be Used

Develop a method, at low cost and easy, that may allow assessment of the effects of new plant material before being placed on the market as commercial cultivar, avoiding compatibility problems with the natural enemies currently used in biological control programs.

D. Objectives of Study

a. This work is the first step in developing a method to assess the plant cultivar effects in the efficiency of biological control agents in greenhouse crops.
b. The specific aim was to evaluate the entomophagous activity, predatory and/or parasitoid species, in the pest species compared with the factitious species: *Ephestia kuehniella* (Lep.: *Pyralidae*).
c. The work has been carried out with the pest species: *Tuta absoluta* and two entomophagous species: the egg-parasitoid *T. achaeae* and the predatory species *N. tenuis* in greenhouse tomato.

III. Material and Methods

A. Colonies

T. achaeae and *T. absoluta* used in the trial came from populations collected at Fuencaliente, La Palma Island, Spain (28º 28' 43" N, 17º 51' 42" W) and Mazarron, Murcia, Spain (two sites: 37º 32' 36" N, 1º 22' 28" W and 37° 33' 51.64" N, 1° 23' 51" W), respectively. *T. achaeae* was reared at the Agricultural Entomology Laboratory at the University of Almeria (Spain) for 24 generations, and *T. absoluta* was reared in the same laboratory for 12 generation before starting the trial. *T. achaeae* was reared on the factitious host *E. kuehniella* in plastic containers (1 l), following the methodology used by [19]. A piece of cardboard (13 x 10.5 cm) to which 12,500 host eggs were attached was placed in each container, and parasitoid adults at a ratio of 1:4 were introduced. The pest was reared according to the methodology used by [17]: Oviposition chambers (132-ml cylinders) constructed with mesh (#1.5 mm) were used, and a tomato leaf was placed around the sidewalls of the chamber as a substrate for female oviposition. Rearing was done in plastic containers (12 l) where the larvae were fed until pupation. About 6-7 tomato leaves were renewed weekly. In both cases, rearing was carried out at 25±1°C, at 60-80% RH, and under 16:8 hours of light/darkness.

The other insects used in the trial were commercially available; *N. tenuis* adults were supplied as Nesicontrol ® (Agrobio, Almeria, Spain). *E. kuehniella* eggs used were of two types: frozen (Ephescontrol®, Agrobio, Almeria, Spain) or irradiated (Biotop, Valbonne, France). The first were stored at -20 °C and the latter at -7 °C until use.

B. Experimental Design and Procedure

The trial was carried out from March to July in a 260 m^2 experimental greenhouse at the Experimental Station of the Cajamar Foundation (La Mojonera, Almeria, Spain). Tomato seedling (cultivar: Vernal®, Enza Zaden, Almeria, Spain) were transplanted at a density of 0.45 m^2/plant in soil with gravel-sand mulch on 4 March. In the greenhouse, the release rate for *N. tenuis*, once time, was 2.3 adults/m^2. Instead, *T. achaeae* was released at a dosage of 50 adults/m^2 every week during crop cycle. In both cases, the first release of natural enemies was carried out on 7 March (7 days after transplanted, DAT). Irrigation and fertilization were carried out according to commercial practices for integrated production in Andalusia, Spain [20].

The assay was arranged in a completely randomized design, with only one factor (at four levels or treatments) and 18 replications per treatment. Four different types (treatments) of host prey were used: a.- non-parasitized irradiated eggs of *E. kuehniella*, b.- parasitized irradiated egg of *E. kuehniella*, c.- non-parasitized eggs of *T. absoluta*, and d.- parasitized eggs of *T. absoluta*. When parasitized eggs were used, they were exposed to parasitism by *T. achaeae*, under laboratory conditions for 24 hours, before use in the greenhouse trial; the method of parasitization was similar to that described above.

Because there was no pest infestation − natural or artificial − in the tomato crop to avoid the existence of extra intraguild prey; frozen *E. kuehniella* eggs were used as food for the last nymphal stages and adults of *N. tenuis*; 25 g of eggs were released on the apical part of each plant of the greenhouse that had previously been sprayed with distilled water. This procedure was repeated fortnightly.

C. Sampling

To evaluate the activity of predation / parasitization of both natural enemies, as well as intraguild predation, was used the sentinel method [21]. To do this 18 plants uniformly distributed in the greenhouse were chosen. In each, 4 cardboard were placed 10 cm from the apex of the plant (the preferred plant section for oviposition of *T. absoluta* adults [22] and for localization of *N. tenuis* nymphs and adults [23], carrying, each card, 10 eggs of a single type of aforementioned eggs, randomly selected in 4 positions: Northeast, Northwest, Southeast and Southwest. The cards with eggs were left exposed for 4 days in the greenhouse. This procedure was performed twice during the crop cycle at 55 and 132 DAT. Later, the cards were collected, labelled, and transported in an icebox to the laboratory and then examined under a stereoscopic microscope to determine whether eggs had hatched or had been killed by predators. Eggs not in these categories were individually isolated, into glass vials, and incubated during two weeks at 25±1ºC and 60-80% RH to assess *T. achaeae* parasitism.

D. Data Analysis

Data are expressed as mean values and standard errors. To avoid overestimation of parasitism ratios, the following equation was used for parasitism calculation [17]:

$$\%P = \left(\frac{b_p}{b_e}\right) \cdot 100; b_i = \frac{A_i}{T_i}$$

where $\%P$ is the actual parasitism ratio, b_p is the total number of parasitized eggs, b_e is the total number of eggs entering this stage on each day, A_i is the area under the state frequency curve (total collection number), and T_i is days of development time.The percentage of killed eggs by *N. tenuis* or parasitized by *T. achaeae* was subjected to univariate general linear model (GLM), prior to analysis the data were transformed using arc-sine square-root. All analyses were done with the statistical software package IBM SPSS version 21 [24].

IV. Results and Discussion

Fig. 1 shows the average percentage of eggs of both prey species *E. kuehniella* and *T. absoluta* (nonparasitized or parasitized by *T. achaeae*) at two sampling dates (55 and 132 DAT). GLM analysis showed the type of prey eggs had no significant effect on the percentage of killed prey by *N. tenuis* at 55 and 132 DAT (F = 0.453, df = 3, P = 0.716, and F = 0.576, df = 3, P = 0.633, respectively). According to these data the predatory species seems not to discriminate between different types of eggs, feeding on all of them, at both dates. The only difference remarked is that predation rates were higher at 55 DAT that at 132 DAT.

The found values − in the same trial − of parasitism by *T. achaeae* in eggs of factitious host *E. kuehniella*, and pest host *T. absoluta*, are shown Fig. 2. In this case, the statistical analysis (MLG) showed significant effect of the species prey at two sampling date 55 and 132 DAT (F = 8.256, df = 1, P < 0.01, and F = 9.716, df = 1, P < 0.01, respectively). Parasitoid adult females showed a host preference; parasitizing more pest host eggs (*T. absoluta*) that factitious host eggs (*E. kuehniella*), at both sampling dates. Also, as pointed out above for the predatory species, the percentage of parasitism was higher in the first sampling date than in the second one.

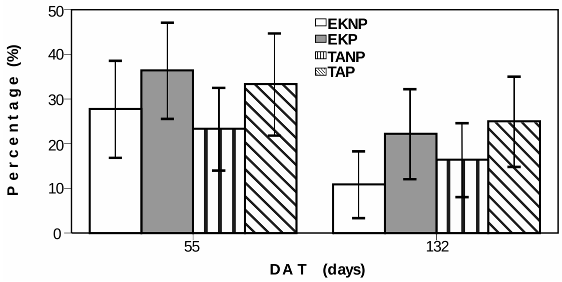

Fig. 1 Average percentage (±SE) of four type of prey eggs (EKNP = non-parasitized eggs of *E. kuehniella*, EKP = parasitized eggs of *E. kuehniella*, TANP = non-parasitized eggs of *T. absoluta*, and TAP = parasitized eggs of *T. absoluta*) killed by *N. tenuis* in a greenhouse tomato crop, where this predatory species and the parasitoid *T. achaeae* had been released

The predatory species *N. tenuis* did not have shown a prey preference in the both type of offered egg prey: *T. absoluta* or *E. kuehniella*, according to the found data in the trial. This may be due, in part because *N. tenuis*, first with a palaeotropical distribution, which was introduced in Europe [27], [28], is an omnivorous species that can feed on both plant [25] and prey [25], [26]. Besides, about their predatory activity the species has a wide range of prey species: Aphids, Whiteflies, Lepidoptera, etc. [29]-[31]. This varied diet has probably originated mechanisms of food selection should not be selective, and this could explain the results.

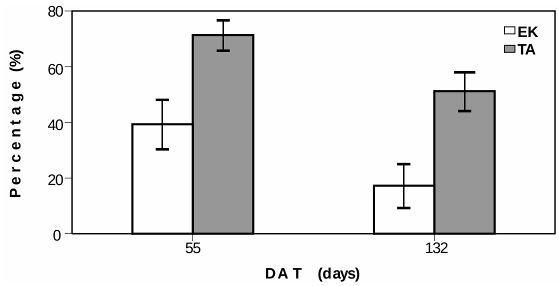

Fig. 2 Mean (±SE) number of *T. achaeae* parasitism in *E. kuehniella* (EK) and *T. absoluta* eggs (TA) in a greenhouse tomato crop, where the parasitoid species and predator *N. tenuis* releases were completed

Likewise this predator showed no preference between nonparasitized and parasitized prey. Reference [9] shows that the most common result is that predators do not distinguish between parasitized and nonparasitized individuals early in the parasitoid's development. This pattern is consistent with the consumption behavior that *N. tenuis* hast shown (Fig. 1). The literature contains several examples where predator species show no preferences between nonparasitized and parasitized eggs by *Trichogramma*. These include *Orius insidiosus* [32], *Chrysoperla carnea* [33], and *Coleomegilla maculata* [34]. However, other studies have reported predator preference for nonparasitized eggs, for example, *Xylocoris flavipes* [35] or *Podisus maculiventris* [36].

On the other hand, *Trichogramma* are generally considered to be generalists [37]. *Trichogramma* not have a physiological host specificity, and 250 species of *Trichogramma* and *Trichogrammatoidea* have been reared for more than 180 generations in eggs of factitious host *E. kuehniella* [38]. Although some species have clear host preferences [37], for example: *T. nubilale* prefers *Ostrinia nubilalis* eggs [39]. *T. chilonis* and *T. exiguum* prefer *Corcyra cephalonica* eggs [40]. As well, *Helicoverpa armigera* eggs, offered to different species of *Trichogramma* were parasitized more by *T. evanescens*, *Trichogramma* sp. [38], and *T. cordubensis* [19]. These preferences could explain the results we found for the lower parasitism in eggs of the factitious host *E. kuehniella* in relations to the natural host species *T. absoluta*.

Perhaps the method used is not the most suitable for the parasitoid species; although this method has been indicated as a standard method in evaluating side effects of pesticides in *Trichogramma* species [41]. However, this method is demonstrated not suitable for the case of *T. achaeae* in evaluating side effects of pesticides in field trials [42]. Thus, we developed another, assessing the activity of the parasitoid by the number of adults captured in chromatic traps [42].

Finally, it should be noted on the results (Figs. 1 and 2) the rates of predation or parasitism varied overtime, so as plant development is larger theses percentages are reduced. The crop development, with its matching increased to the search area which the entomophagous species need to cover, for example, the incidence of parasitism of *T. pretiosum* has been related to plant development [43], and similar results have been reported for other species of the same genus [44]; although this effect may be reduced with high host densities [45]. The same has been cited for *N. tenuis* [17].

V. Conclusion

From the found results in the trial, it may point out the use of sentinel eggs of the factitious host *E. kuehniella* would be a good method for evaluating predatory activity in greenhouse crops. This may allow their application in studies of the effects of experimental new plant material and allow an estimation of their activity in assays premarketing assessment of them. It is noted that today in Europe greenhouse crops, as mentioned above in the Introduction, the control of arthropod pests is performed using almost only natural enemies. Thus, any seed companies can be disclosed to commercializing new cultivars that do not adapt to current biological control programs.

By contrast, the found results of the use of *E. kuehniella* sentinel eggs not fully reflect the activity of the other entomofagous *T. achaea*. So, in the first sampling the percentage of parasitism was 1.8 times higher in natural host eggs that in the factitious host, and almost 3 times higher in the last sampling. It could choose between three possible solutions: a.- Using different methods (for example: chromatic traps pointed out above; but has not been yet evaluated in cultivar assays). b.- Using, at the same time, both methods: sentinel eggs and chromatic traps. c.- Using sentinel eggs versus a control (or check), previously settled as standard.

The developed method could allow, at low cost and in an easy way, evaluating the effects of cultivars on the activity of both natural enemies, both in semi-field, greenhouse, and open air crop conditions.

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