Exploring the potential of conservation biological control of Phthorimaea absoluta: arthropod predators in tomato greenhouses of NW-Portugal – A preliminary study

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Exploring the Potential of Conservation Biological Control of *Phthorimaea absoluta*: Arthropod Predators in Tomato Greenhouses of NW-Portugal – A Preliminary Study

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Abstract (in English):

Conservation biological control, using pest natural control by predators and parasitoids, should be considered as an alternative to intensive pesticide use, especially when managing potentially pesticide-resistant pests such as *Phthorimaea absoluta* Meyrick, 1917, one of the most threatful tomato pests. To achieve this, suitable habitat conditions should be guaranteed to increase natural enemies' populations. At the NW-Portugal, an important tomato production region, these conditions may be provided by the semi-natural habitats existing on the greenhouses' edge. Although *P. absoluta* has a wide range of natural enemies in the Mediterranean, their occurrence and abundance on this region needs to be investigated. This preliminary study served as a proof of concept of the conservation biological control potential, by inventorying the arthropod predators in three commercial tomato greenhouses on NW-Portugal, characterizing their communities and understanding their ability to cross-over between crop-edge semi-natural habitats and the crop, over time. This study was performed on May-August 2023, on three semi-natural habitat fragments around each greenhouse. Outside the greenhouses, monthly sampling of predators was carried out. Inside the greenhouses, the predators were sampled by visual observation and beating technique in the most peripheral rows of the greenhouses. To evaluate the biodiversity within the fragments and within the greenhouses we performed a species-rank curve and calculated a Simpson index (1-λ) and $\beta_{SØR}$ biodiversity index to determine the biodiversity over time. In addition, monthly data on the pest population density on plants over time were collected. We were able to count a total 221 individuals on the greenhouses and 199 on the fragments, belonging to 12 different families, with only seven of them occurring on the greenhouses. The Simpson index between the greenhouses and the fragments for each month showed that the greenhouses had a smaller evenness (0.37 ± 0.17), when compared to the fragments (0.80 ± 0.04), with Miridae being the dominant family on the greenhouses. Anthocoridae and Syrphidae are the second and the third most abundant families on the greenhouses, and the first and the third most abundant families on the fragments. Although the preliminary nature of this study, we may state that it provides an insight into the biodiversity of arthropod predators that can be found, being most of them described as predators of *P. absoluta* on the literature. This presents an opportunity to promote conservation biological as control practices on tomato production on NW-Portugal.

Abstract (in Portuguese or Castellan):

A gestão de pragas através da proteção biológica de conservação (limitação natural das pragas pela ação de predadores e parasitoides) deve ser considerada como uma alternativa à utilização intensiva de pesticidas, especialmente no controlo de pragas potencialmente resistentes a inseticidas, como é o caso de *Phthorimaea absoluta* Meyrick, 1917, uma das pragas-chave do tomate. Para tal, devem ser garantidos habitats adequados ao aumento das populações dos inimigos naturais. No noroeste de Portugal, uma importante região de produção de tomate, estas condições podem ser proporcionadas pelos habitats seminaturais existentes na orla das estufas. Embora *P. absoluta* tenha vários inimigos naturais no Mediterrâneo, a sua ocorrência e abundância nesta região necessita de ser investigada. Este estudo preliminar serviu como uma prova de conceito do potencial da proteção biológica de conservação, através da inventariação dos artrópodes predadores em três estufas comerciais de tomate, caracterizando as suas comunidades e a sua capacidade de transitar entre os habitats seminaturais da orla da cultura e a cultura em si, ao longo do tempo. Este estudo foi realizado de maio a agosto de 2023, em três fragmentos de habitat seminatural em redor de cada estufa. No exterior, foi efetuada uma amostragem mensal dos predadores, no interior das estufas (linhas mais periféricas); estes foram amostrados por observação visual e técnica das pancadas. Para avaliar a biodiversidade nos fragmentos e dentro das estufas foi desenhada uma curva de classificação de espécies e calculados os índices de Simpson (1-λ) e βSØR para determinar a biodiversidade ao longo do tempo. Além disso, foram recolhidos dados mensais de densidade da praga. Foi possível contar um total de 221 indivíduos nas estufas e 199 nos fragmentos, pertencentes a 12 famílias, sendo que apenas sete ocorreram nas estufas. O índice de Simpson calculado para cada mês, mostra que as estufas possuem pouca equitabilidade (0,37 ± 0,17), quando comparadas com os fragmentos (0,80 ± 0,04), sendo Miridae a família dominante nas estufas. Anthocoridae e Syrphidae são a segunda e a terceira famílias mais abundantes nas estufas, e a primeira e a terceira famílias mais abundantes nos fragmentos. Apesar do caráter preliminar deste estudo, este mostrou um retrato da biodiversidade de artrópodes predadores, sendo a maioria deles predadores de *P. absoluta* descritos na literatura, o que constitui uma oportunidade para a promoção da proteção biológica de conservação em produção de tomate no noroeste de Portugal.

Keywords: biodiversity; natural enemies; pest control; tomato pinworm.

Introduction

Biological control, naturally provided by arthropod natural enemies (NEs), predators and parasitoids, is an important ecosystem service that supports the agrosystems and represents a pest control method known as conservation biological control. Nonetheless this service is strongly influenced by the availability of natural and semi-natural habitats at different spatial scales, that can shelter the NEs population throughout their life cycle (Wäckers & van Rijn, 2012). However, habitat simplification, with the loss of spontaneous vegetation due to intensive agriculture, reduces the associated arthropod fauna and promotes insecticide use, which may lead to an intensification of pest pressure (Balzan et al., 2016). To understand the viability of conservation biological control on intensive horticultural systems, several studies have been carried out in the last two decades on NEs' communities, their distribution in the landscape and within the crop, and how they relate to cultural practices. Aviron et al. (2016) found that different species of mirids (one of the main groups of predators of horticultural pests) respond to landscape heterogeneity at different spatial scales. Chaplin-Kramer et al. (2011) also found that generalist groups of NEs respond positively to landscape complexity at different spatial scales, while specialist groups show the same response, but only at a small scale (such as the crop edge). Tscharntke et al. (2008) also state that the process of spillover of NEs from fragments of semi-natural habitats to crops depends on the species richness of each fragment. Such a control method should be seriously considered to control potentially pesticide resistant pests such as *Phthorimaea absoluta* Meyrick, 1917. *P. absoluta* is one of the most important tomato pests and can cause yield losses of up to 100% (Aguiar, 2011). In Mediterranean tomato production, it requires the application of several treatments throughout the cycle (Urbaneja et al., 2012), which has led to an increase in population resistance to the main active ingredients (Giorgini et al., 2019). However, measures such as the promotion of NEs' populations have been shown to be effective in reducing pest populations (Urbaneja et al., 2012; Arnó et al., 2018).

P. absoluta is now known to have a large number of NEs, many of which are endemic to the Mediterranean and inhabit agricultural ecosystems (Ferracini et al., 2019). In the Mediterranean region, the boundaries of agricultural fields with rich assemblages of wild plants and floral resources have led to more complex communities of NEs, which are essential for the control of *P. absoluta* (Pérez-Hedo et al., 2017). However, the NEs present in the agricultural ecosystem, and their ability to move between habitats need to be further characterised. In order to strengthen the promotion of conservation biological control, and given the importance of the Entre Douro e Minho region (EDM) (Northwest of Portugal) for the production of tomatoes in greenhouses and the smallholder nature of this region, it is a perfect case study to characterise NEs' communities and their link with crop-edge semi-natural habitats, allowing the development of control strategies that capitalise on the ecosystem services. For this region, the population dynamics of NEs at the local level are not known, nor are the arthropod communities that compose these edge habitats and that move between the greenhouse and the surrounding vegetation.

This preliminary study will serve as a proof of concept of the potential of biological control, by identifying and inventorying the arthropods predators in three commercial tomato greenhouses on EDM, characterizing their communities and understanding their ability to cross-over between crop-edge semi-natural habitats and the crop, over time.

Methodology

This study was carried out between May and August 2023, in three commercial tomato greenhouses located in the EDM region, Northwest Portugal. We performed a fragmented sampling, selecting nine fragments (three semi-natural habitat fragments around each greenhouse) with the same type of semi-natural habitat, which we classified as spontaneous meadows composed of a wide variety of herbaceous ruderal plants. In addition, all greenhouses were standard plastic tunnels with side windows, which were opened throughout the survey. All the greenhouses were on Integrated Pest Management and similar cultural practices were applied. The nine fragments were left with spontaneous herbaceous vegetation throughout the study, with no herbicide applications or vegetation cutting.
Outside the greenhouses, monthly sampling of arthropod predators was carried out using 10 net sweeps through five transects within each fragment, selecting a 100 m^2 sampling plots adjacent to the margin of the greenhouse (spill-over area). The procedure was repeated in the nine selected fragments. Inside the greenhouses, the presence of the predators was recorded by visual observation of 30 tomato plants per greenhouse (5 min/plant) and by collecting individuals using the tapping technique (15 taps/plant basal section + 15 taps/plant medium section) in the most peripheral rows of the greenhouse and near the sampled fragments. The specimens collected were preserved in 96% ethanol and were counted and classified later in the laboratory using a binocular magnifying glass. Whenever possible, the specimens were classified down to species level; however, in order to standardise the analysis, we stratified the data and analysed it by taxonomic family. In each family only the predators' genera were counted. To evaluate the biodiversity within the fragment habitat and within the greenhouse we performed a species rank curve base on the families' relative abundances and calculated a Simpson index (1-λ) to determine the biodiversity evenness over time, using the monthly data on the occurrence and abundance of each predator's family. Also, to measure the families' substitution between the fragment and the greenhouse, we calculated $\beta_{SØR}$ biodiversity index, by characterizing the predators' community composition.
In addition, monthly data on the population of the pest over time were collected by monthly surveying and counting larvae to adults found on 30 randomly selected tomato plants in each of the greenhouses.

Results and Discussion

Combining the sampling methods applied and the data collect on the abundance and occurrence of arthropod predators in the three greenhouses and in the nine fragments thorough the four months of surveys, we were able to count a total of 420 individuals (greenhouses: 221 individuals; fragments: 199 individuals) belonging to 12 different families, with only seven of them occurring on the greenhouse. When analysing the abundance distribution between the 12 families on the fragments and the seven families on the greenhouse in a rank curve (Figure 1), it is notorious that the highest percentage of relative abundance is held by one family on the greenhouses (82%), while on the fragments the relative abundance is more evenly distributed between the families (24%-1%), that indicates higher evenness.

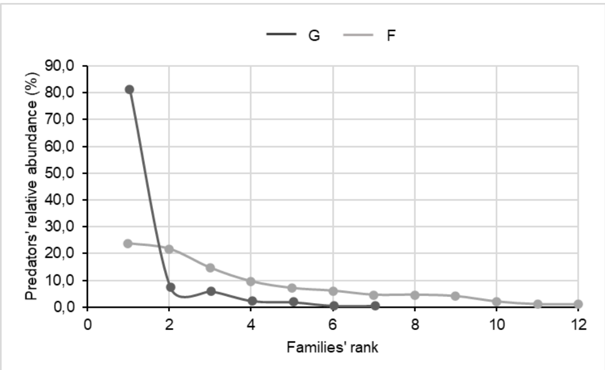

Figure 1 – Families rank of the surveyed arthropod predators based on their relative abundance on the greenhouses (G) and on the fragments (F).

Although the biodiversity slightly fluctuates over the months (Table 1), by observing the Simpson index between the greenhouses and the fragments for each month, one can observe that greenhouses show a small evenness (1-λ) (0.37 ± 0.17), when compared to the fragments (0.80 ± 0.04), being the Miridae the most dominant family on the greenhouse, with a total of 181 individuals counted.

Table 1 – Number of arthropod predators and pest larvae surveyed each month inside de greenhouses (G) and on the fragments (F), with the respective Simpson biodiversity index (1- λ) calculated per site, per month.

		May		**Jun**		**Jul**		**Aug**	
Order	**Family**	**G**	**F**	**G**	**F**	**G**	**F**	**G**	**F**
Number of predators									
	Carabidae	0	5	0	1	1	2	0	1
Coleoptera	Coccinellidae	1	9	0	4	0	21	0	10
	Staphylinidae	0	0	0	0	0	1	0	1
	Anthocoridae	4	7	0	20	2	2	10	18
Heteroptera	Geocoridae	0	0	0	0	0	0	0	8
	Miridae	59	1	27	7	16	3	79	3
	Nabidae	0	1	0	7	0	4	0	7
	Chrysopidae	0	3	1	2	4	2	0	5
Neuroptera	Coniopterygidae	0	2	0	0	0	0	0	0
	Hemerobiidae	0	4	0	0	0	0	0	0
Diptera	Cecidomyiidae	0	3	1	2	3	3	0	1
	Syrphidae	2	15	5	6	2	8	4	0
Total		*66*	*50*	*34*	*49*	*28*	*46*	*93*	*54*
Simpson's index	1-λ	0.20	0.85	0.36	0.78	0.65	0.76	0.27	0.82
Number of pest larvae									
		121	-	143	-	239	-	3513	-

Even though the number of individuals captured inside and outside the greenhouses does not variate much (greenhouses: 55.3 ± 26.1; fragments: 49.75 ± 2.9) among the two sites, when observing the data over time, it is clear that at the greenhouses the counts peaked at May and August. This may be due to the pesticide pressure, which is higher during the fruit development (June and July), and start to decrease at the end of the cycle (August and September), and may constitute a severe disturbance on the predator's populations inside. The capacity of the predators to cross over the greenhouse to the surrounding habitat could be determinant to the survival of this populations in face of such disturbance (Tscharntke et al., 2008). Although the number of mirids did decrease inside the greenhouses, their counts did not largely improve on the fragments. These results are consistent with the analysis performed by Arnó (2021) which found very low populations of predatory mirid bugs on companion herbaceous plants placed near tomato crops (0.5–1.8 m from the external row), even though some of these species are commonly released as biological control agents on horticultural crops in Spain. The ability of the mirids to escape the greenhouse may not be assured and the marginal semi-natural habitats may not be suitable to enhance these populations (Balzan et al., 2016). This may due to their more restrict plant preferences when compared to other zoophytophagous groups such as the Anthocoridae, that due to their smaller body, can feed on a wide variety of floral corollas (Wäckers & van Rijn, 2012). On the other hand, when comparing the number of Miridae and Anthocoridae inside the greenhouse with the observed pest larvae on tomato plants, the pest peak in August corresponds with a higher number of individuals belonging to these two families. The Dicyphini tribe and the *Orius* sp. are often stated important predators of *P. absoluta* and other tomato

pests, with species widely use in biological control such as *Nesidiocoris tenuis* Reuter and *Orius laevigatus* (Fieber) (Desneux et al., 2022), so this association would be expected (Tscharntke et al., 2008).
The Anthocoridae and the Syrphidae are the second and the third most abundant families on the greenhouses, with a total of 16 and 13 individuals counted, and coincidently and respectively the first and the third most abundant families on the fragments (47 and 49 individuals counted), which may indicate a higher ability of these predators to navigate between the two habitat types. Again, Arnó (2021) also found *Orius* sp. and Syrphidae to be the most abundant groups sampled on the herbaceous companion plants near tomato crops. On the contrary, the second and the fourth most abundant families on the fragments, the Coccinellidae and the Nabidae (total of 44 and 19 individuals counted) respectively, where almost inexpressive inside the greenhouse, maybe due to the abundance of more suitable prey on the spontaneous vegetation than on the greenhouse where *P. absoluta* dominates and forces a higher pesticide pressure (Tscharntke et al., 2008; Wäckers & van Rijn, 2012).
The families that colonize the greenhouse are the same that can be found among the fragments, but from the 12 families classified there are five families missing in the greenhouse. We calculate a $\beta_{SØR}$ biodiversity of 0.58 which indicates a loss of nearly half of the biodiversity from the fragments to the greenhouses. Tscharntke et al., 2008 affirm that the β biodiversity between natural habitats and agrosystems are strongly influenced by the intensity of the cultural practices and the connectivity between those habits. On intense horticulture systems, like the ones observed on this study, a high turnover/nesting phenomena is expected when comparing the two sites. Nonetheless it would be necessary a landscape scale study to assess different metrics influencing β biodiversity, such as habitat diversity and connectivity.
Although the high diversity loss from the fragments to the greenhouses, when we cross-referenced the data on the families that occur in the study sites with the data presented by Ferracini et al. (2019), we find that from de families classified on the greenhouses, 99.5% of the individuals were predators of *P. absoluta*, in contrast with the 60.3% of individuals belonging to a referred family on the fragments.

Conclusions

Although the preliminary nature of this study, we may state that it provides an overview of the biodiversity of arthropod predators that can be found even at intensive horticultural productions. Furthermore, it demonstrates the capacity of several groups of predators to move from semi-natural habitats at the edge of the crops to the greenhouse, with many of these groups preying *P. absoluta,* as described on the literature. This presents an opportunity to promote conservation biological control practices on tomato productions in the EDM region, by capitalizing on the existent semi-natural and natural habitat mosaic that connects the agricultural landscape. A deeper understanding of the specific spontaneous flora influencing the biodiversity of arthropod predators, as well as landscape composition, would be essential to assess the need for habitat management to boost pest control ecosystem services and reduce the use of chemical pesticides.

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