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Ants as Predators of the Egg Parasitoid *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae) Applied for Biological Control of the Olive Moth, *Prays oleae* (Lepidoptera: Plutellidae) in Portugal

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The detrimental effect of predators on Trichogramma cacoeciae *March. releases to control the olive moth,* Prays oleae *Bern., in the Trás-os-Montes region (Northeast of Portugal), was evaluated during three releases against the flower generation of the pest in 2002. At 1 and 3 h and at 1, 3, 7 and 14 days after each release, 30* Trichogramma *releasing cards were examined in the field and predators were collected and identified. Furthermore, at 1, 3, 7 and 14 days after each release, the percentage of egg predation on the cards was also determined. Formicidae were the most abundant group of predators at 99.1% of the total individuals collected. Ten species were identified,* Camponotus aethiops, C. lateralis, C. piceus, C. truncatus, Crematogaster auberti, C. scutellaris, Lasius niger, Leptotorax angustulus, Plagiolephis pygmaea *and* Tapinoma nigerrimum. T. nigerrimum *was the most abundant species, both in total numbers as well as in the number of occupied cards. One day after release, the percentage of predation was 24.0% in the first release, 59.4% in the second and 38.0% in the third. Three days after release, the percentage of predation varied from 60.2 to 83.4% during the three release events. Seven days after release, the percentage of predation increased to 97.8% of the total eggs on cards.*

Keywords: inundative releases, predation, ants, *Trichogramma,* olive moth, biological control

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INTRODUCTION

The olive tree has an important socio-economic role in Portugal. Olive groves are distributed practically throughout the national territory occupying about 340,000 ha, with 38 million olive trees (Anonymous, 1996; Castro *et al*., 1997). Trás-os-Montes (Northeast of Portugal) is the second most important production area; the olive growing area is around 65,000 ha which corresponds to 39% of the olive oil production, 20% of olive trees and 22% of the area covered by olive trees in Portugal (Monteiro, 1999).

In the Mediterranean region there are many insect pests which attack olive trees. In Trás-os-Montes, the olive moth, *Prays oleae* Bern (Lepidoptera: Plutellidae), is the major olive pest (Bento, 1999). This insect is a microlepidopteran with three generations a year, feeding on different vegetative structures of the olive tree. The first generation, the phyllophagous or leaf generation, has the longest development period (September/October–March), during which the larva shelters in leafmines. It then emerges and begins a new generation, the antophagous or flower generation (March–May) feeding on the floral buttons. The following generation, the carpophagous or fruit generation, penetrates the fruit and feeds on the soft gelatinous tissue of the fruit (Bento, 1999; Morris *et al*., 2002). These last two generations affect the productivity of the olive tree and cause serious losses, sometimes accounting for more than 50% of the potential olive production (Bento, 1999).

Various methods can be used to control the olive moth. Insecticides such as dimethoate and phention, as well as formulations of *Bacillus thuringiensis* Berliner are currently used in Portugal (Gonçalves & Teixeira, 1999). However, in organic olive production, only *B. thuringiensis* can be used as a pesticide (Council Regulation (EEC) no. 2092191 of 24 June 1991; Lopez-Villalta, 1999). Biocontrol agents like chrysopids were also applied for the control of eggs and larval stages of *P. oleae* with varying success (Bento, 1999; Bento *et al*., 1999). In research programmes for the biological control of *P. oleae*, egg parasitoids of the genus *Trichogramma* (*Trichogramma cacoeciae* March., *T. dendrolimi* Mats., *T. embryophagum* Hasting, *T. euproctitis* Gir., *T. pretiosum* Pil.) were tested (Pelekassis, 1962; Stavraki, 1982; Stavraki, 1985; Jervis *et al*., 1992; Bento *et al*., 1998; Bento, 1999). Results were of relative success with parasitism of *P. oleae* eggs reaching 46.2% (Bento *et al*., 1998).

Egg parasitoids of the genus *Trichogramma* are important natural enemies of Lepidopteran pests. They naturally occur world-wide and are used in augmentative biological control in a large number of countries (Li, 1994; Cagán *et al*., 1998). *Trichogramma* spp. are being mass-reared to control pests on corn, sugar-cane, rice, cotton, soybean, sugar-beet, vegetables and pine (Hassan *et al.,* 1988). Targeted pests include the sugarcane borers, *Diatraea indigenella* and *D. saccharalis* (Gómez *et al*., 1998), the European corn borer *Ostrinia nubilalis* (Bigler, 1986), the cotton bollworm, *Helicoverpa zea* and the tobacco budworm, *Heliothis virescens* (Knutson, 1998), the tomato fruit worm *Helicoverpa armigera* (Abbas, 1998), the codling moth *Cydia pomonella* (Mills *et al*., 2000) and the summer fruit tortix moth *Adoxophyes orana* (Hassan *et al*., 1988).

Several authors reported that predation in the field on parasitized eggs on releasing units may reduce the effectiveness of releases (Smith, 1994; Bento *et al*., 1998; Gómez *et al*., 1998). However, the direct impact of predators on *Trichogramma* releases was not well documented. According to Gómez *et al*. (1998), ants were important predators of *Sitotroga cerealella* eggs, parasitized by *T. exiguum,* and significantly reduced the control of sugarcane borers. Similarly, Suh *et al*. (2000) attributed, in part, the poor performance of *T. exiguum* in cotton to increased predation on the released parasitized eggs. In previous studies dealing with the control of the olive moth by inundative releases of egg parasitoids in Portugal, it was observed that the efficacy of the released wasps (*T. cacoeciae* and *T. dendrolimi*) suffered from predation (Bento *et al*., 1998; Bento, 1999). But there was little information about the responsible predators, the temporal pattern of their attack, and the degree of egg predation in the olive agroecosystem. Thus, the aim of the present work was to identify clearly the different groups of predators that may affect *Trichogramma* releases in olive groves, and to determine percentages of potential egg predation following their releases.

MATERIAL AND METHODS

The study was conducted in an olive grove, situated at Romeu-Mirandela in Trás-os-Montes, Northeast of Portugal. The grove covers an area of 3 ha with a planting density of 10 × 10 m. The trees were of medium size and older than 60 years. Since 1993, the production has followed organic guidelines: the soil was ploughed twice a year and fertilized with organic material. Pruning was made every 3 years. No irrigation or phytosanitary treatments were done. The most important cultivars were Cobrançosa and Verdeal Transmontana.

We estimated a potential effect of predation on releasing units with parasitized eggs of *Sitotroga cerealella* Olivier during inundative releases of *T. cacoeciae* against the flower generation of *P. oleae* in 2002. These releases were performed within an international research programme for the development of control methods for the olive moth (EU contract: ICA4-CT-2001-10004). For proper timing of treatments, the flight activity of *P. oleae* was monitored by Delta pheromone traps (INRA, Bioprox, Grasse-France) baited monthly with the female sex pheromone ((*Z*)-7-tetradecenal-1-al (Agrisense, Cardiff, UK)) of the olive moth. Six traps (2 traps ha^{-1}) were installed about 50 m from each other at a height of 2.0 m on olive trees and were checked weekly for captures of males. Adults were first caught on 17 April, indicating the start of the flower generation. We conducted three inundative releases of *T. cacoeciae* on 23 and 26 April, and May 9, to synchronize treatments with the start of the egg-laying period of the olive moth (Figure 1).

The releasing units were folded cardboard cards ('TrichoKarte', AMW-Nützlinge, Pfungstadt, Germany), each with about 3,000 *S. cerealella* eggs parasitized by *T. cacoeciae,* covering a surface of about 6 cm^2. The cards had two small slits of about 1 cm to allow the emerged parasitoids to leave. However, previous experience showed that these holes may also allow predators to enter the cards.

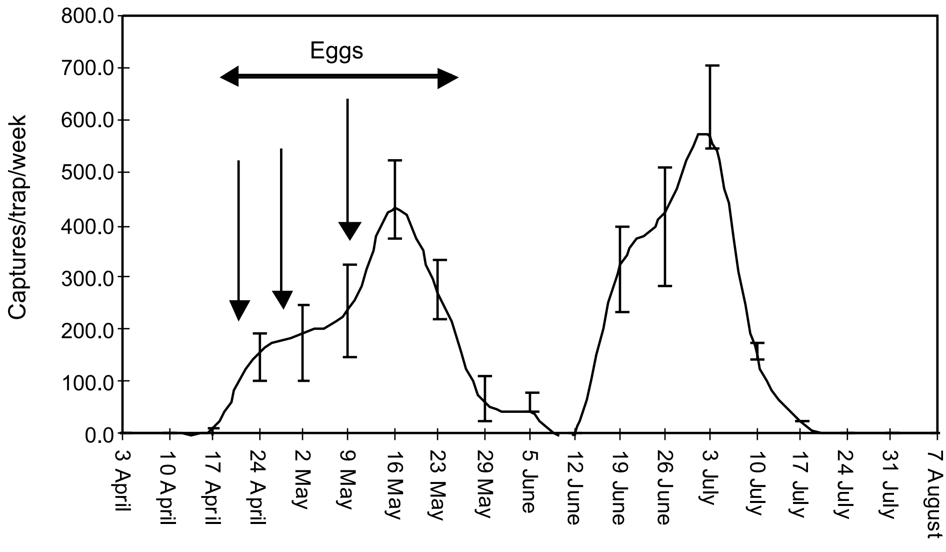

FIGURE 1. Flight of *Prays oleae* Bern in the olive grove at Mirandela in 2002. Arrows indicate the dates of *Trichogramma* releases. The oviposition period of *P. oleae* is also shown.

To apply about 900,000 wasps per ha, we placed three cards in each tree. The units were placed on the interior terminal branches of the tree to protect the parasitized eggs from varying temperature conditions at the outer area of the canopy.

For monitoring predator abundance and their impact on the releasing units, we collected 30 previously placed cards on randomly selected trees at specific time intervals after each release (1 and 3 h; 1 and 3 days; 1 and 2 weeks). The cards were opened and the arthropods found on each card were collected and kept separately in a plastic bag. In the laboratory, we identified the predators to species level, if possible. We determined the percentage of predation on the sampled cards by calculating the percentage of area on the card without eggs in comparison to the previously covered part. This area corresponds to the eggs predated or removed by the predators. The data for the different exposure times in the field were compared by analysis of variance (ANOVA). Data were tested for homogeneity of variance and means were separated by a Tukey test, using the SYSTAT software (SYSTAT version 10.0, SPSS Inc., 2000).

RESULTS

The predators, collected from the *Trichogramma* releasing cards, belonged to the orders Araneae, Heteroptera, and the families Formicidae, Forficulidae and Coccinellidae, respectively. The ants represented more than 99% of all specimens collected, followed by the Forficulidae. Coccinellidae, Heteroptera and Aranea were rare (Tables 1–3).

Ants belonged to seven genera and 10 species, namely *Camponotus aethiops* (Latreille, 1798), *C. lateralis* (Olivier, 1792), *C. piceus* (Leach, 1825), *C. truncatus* (Spinola, 1808), *Crematogaster auberti* (Emery, 1809), *C. scutellaris* (Olivier, 1792), *Lasius niger* (Linnaeus, 1758), *Leptotorax angustulus* (Nylander, 1856), *Plagiolephis pygmaea* (Latreille, 1794) and *Tapinoma nigerrimum* (Nylander, 1856) (Table 4).

In the first release, 108 arthropods (all ants) were found after 1 h on seven cards (Table 1). In the second release, 277 ants were observed in 12 cards (Table 2), and in the third, 320 arthropods on 15 cards during this short time period (Table 3). In all cases, the ant species *T. nigerrimum* was the most abundant predator species.

In all experiments, the number of predators as well as the number of occupied cards increased as time progressed. On the third release (9 May), 1621 predators, the maximum number collected, were recorded 3 h after the release. Across all three releases, 40–60% of the cards were occupied within predators within 3 h (Tables 1–3).

The highest number of predators was collected 1 day after release in the first experiment. In the second and third releases, the number of predators was lower than before, but the number of occupied cards still increased. During this time period, 60–70% of the cards were occupied in all experiments. Three days after release, the number of cards detected by predators was still increasing, although the number of predators per card began to fall. Seven days after release, the earwing *Forficula auricularia* appeared in greater numbers, whereas the number of ants were reduced. Fourteen days after release, the number of predators was generally low in all experiments.

T. nigerrimum was the most abundant ant recovered in this study and represented 83.3% of all collected, followed by *C. scutellaris* with 13.2% (Table 4). *C. auberti* and *C. lateralis* appeared in significant numbers. All other species occurred only sporadically (Table 4).

One day after releases, predation varied between 24 and 59% (Table 5). There were significant increases in predation with time in all releases ($F = 14.004$, df $= 3$, 116, $P < 0.001$ in the first; $F = 6.524$, df $= 3$, 116, $P < 0.001$ in the second; and $F = 31.700$, df $= 3$, 116, $P < 0.001$ in the third).

Predation increased rapidly until day 3, then began to decrease (Table 5). In the first and second releases, no significant differences were observed in the predation between 3 days after release and 7 as well as 14 days after release. In the third release, there were significant

TABLE 1. Number of arthropods found on cards (*N*) and number of cards occupied by each taxon (Occup)[a] at several periods after the release of *Trichogramma cacoeciae* on 23 April 2002 at Romeu-Mirandela, Portugal

	Period after releasing											
	1 h		3 h		1 day		3 days		7 days		14 days	
Taxon	*N*	Occup	*N*	Occup	*N*	Occup	*N*	Occup	*N*	Occup	*N*	Occup
Hymenoptera/Formicidae												
Camponotus aethiops	0	0	5	1	0	1	0	0	0	0	0	0
Camponotus lateralis	26	1	8	1	7	3	0	0	0	0	0	0
Camponotus piceus	0	0	6	4	2	2	1	1	2	1	0	0
Camponotus truncatus	0	0	0	0	1	1	0	0	0	0	0	0
Crematogaster scutellaris	32	4	57	5	133	4	31	7	7	4	0	0
Plagiolephis pygmaea	0	0	0	0	1	1	0	0	0	0	0	0
Tapinoma nigerrimum	50	3	41	2	219	4	73	9	0	0	2	1
Dermaptera												
Forficula auricularia	0	0	0	0	3	3	6	6	23	18	23	15
Aranea (not identified)	0	0	0	0	0	0	1	1	0	0	0	0
Total	108	7	117	12	367	15	112	18	32	21	25	16

[a]Cumulative number on 30 releasing cards.

TABLE 2. Number of arthropods found on cards (*N*) and number of cards occupied by each taxon (Occup)[a] at several periods after the release of *Trichogramma cacoeciae* on 26 April 2002 at Romeu-Mirandela, Portugal

	Period after releasing											
	1 h		3 h		1 day		3 days		7 days		14 days	
Taxon	*N*	Occup	*N*	Occup	*N*	Occup	*N*	Occup	*N*	Occup	*N*	Occup
Hymenoptera/Formicidae												
Camponotus aethiops	0	0	2	2	11	7	1	1	0	0	1	1
Camponotus piceus	13	1	3	3	0	0	2	2	0	0	1	1
Crematogaster auberti	0	0	1	1	6	2	1	1	0	0	0	0
Crematogaster scutellaris	3	1	0	0	6	1	7	3	0	0	11	1
Plagiolephis pygmaea	0	0	5	1	0	0	0	0	1	1	0	0
Tapinoma nigerrimum	261	11	337	11	99	9	110	8	125	3	0	0
Dermaptera												
Forficula auricularia	0	0	0	0	2	2	8	7	6	6	1	1
Coleoptera/Coccinellidae	0	0	0	0	0	0	1	1	1	1	0	0
Total	277	12	349	14	124	19	130	21	133	9	14	3

[a]Cumulative number on 30 releasing cards.

TABLE 3. Number of arthropods found on cards (N) and number of cards occupied by each taxon (Occup)[a] at several periods after the release of *Trichogramma cacoeciae* on 9 May 2002 at Romeu-Mirandela, Portugal

	Period after releasing											
	1 h		3 h		1 day		3 days		7 days		14 days	
Taxon	*N*	Occup	*N*	Occup	*N*	Occup	*N*	Occup	*N*	Occup	*N*	Occup
Hymenoptera/Formicidae												
Camponotus aethiops	0	0	0	0	5	5	0	0	0	0	0	0
Camponotus lateralis	0	0	0	0	1	1	0	0	0	0	0	0
Camponotus piceus	1	1	0	0	0	0	1	1	0	0	0	0
Camponotus truncatus	0	0	1	1	0	0	0	0	0	0	0	0
Crematogaster auberti	1	1	25	1	10	1	5	1	0	0	0	0
Crematogaster scutellaris	7	1	132	2	114	1	61	2	0	0	0	0
Lasius niger	1	1	0	0	0	0	1	1	0	0	0	0
Leptotorax angustulus	0	0	0	0	0	0	1	1	0	0	0	0
Plagiolephis pygmaea	0	0	1	1	0	0	0	0	0	0	0	0
Tapinoma nigerrimum	308	10	1460	14	675	13	39	10	0	0	0	0
Dermaptera												
Forficula auricularia	0	0	0	0	1	1	0	0	0	0	0	0
Heteroptera/larvae	0	0	0	0	0	0	1	1	0	0	0	0
Total	320	15	1621	18	806	21	109	16	0	0	0	0

[a]Cumulative number on 30 releasing cards.

TABLE 4. Ant species composition, numbers caught and number of occupied cards used in releases of *Trichogramma cacoeciae* in an olive grove at Romeu-Mirandela, Portugal in 2002

	Releasing dates							
	23 April		26 April		9 May		Total	
Species	*N*	Occup	*N*	Occup	*N*	Occup	*N*	Occup
Camponotus aethiops (Latreille, 1798)	5	2	15	11	5	5	25	18
Camponotus lateralis (Olivier, 1792)	41	5	0	0	1	1	42	6
Camponotus piceus (Leach, 1825)	11	8	19	7	2	2	32	17
Camponotus truncatus (Spinola, 1808)	1	1	0	0	1	1	2	2
Crematogaster auberti Emery, 1809	0	0	8	4	41	4	49	8
Crematogaster scutellaris (Olivier, 1792)	260	24	27	6	314	6	601	36
Lasius niger (Linnaeus, 1758)	0	0	0	0	2	2	2	2
Leptotorax angustulus (Nylander, 1856)	0	0	0	0	1	1	1	1
Plagiolephis pygmaea (Latreille, 1794)	1	1	6	2	1	1	8	4
Tapinoma nigerrimum (Nylander, 1856)	385	19	932	42	2 482	47	3 799	108
Total	704	60	1 007	72	2 850	70	4 561	202

TABLE 5. Percentage predation on cards used in releases of *Trichogramma cacoeciae* at several periods after the release at Romeu-Mirandela, Portugal in 2002

	Period after releasing			
Date of release	1 day	3 days	1 week	2 weeks
23/04/02	24.0±33.40% a	60.2±38.90% b	68.8±33.2% b	78.2±33.00% b
26/04/02	59.4±40.80% a	83.4±30.30% b	84.8±32.3% b	93.5±17.40% b
09/05/02	38.8±41.04% a	73.9±36.41% b	97.9±7.48% c	100.0±0.00% c

In the same row, mean values followed by different letters differ significantly ($P < 0.01$).

differences between 3 and 7 days ($F = 12.455$, df = 1, 58, $P < 0.001$) but no significant differences were observed between 7 and 14 days.

DISCUSSION

Predation on parasitized eggs on release cards is considered an important factor influencing the success of *Trichogramma* inundative releases (Smith, 1994; Bento *et al*., 1998; Gómez *et al*., 1998; Suh *et al*., 2000). In the present study, we were able to evaluate the potential risk caused by predators to augmentative biological control of the olive moth and to identify the predator species which were responsible for it. Most of the species found are well known natural enemies in the olive agro-ecosystem (Cabanas *et al*., 1999; Morris *et al.*, 1999a; Ramalho, 2000; Lozano *et al*., 2002).

Araneae, Heteroptera and Coccinellidae were important and abundant arthropods in olive groves (Cabanas *et al*., 1999; Morris *et al*., 1999a) and their action on predation of eggs and larvae of *P. oleae* has been described by Morris *et al*. (1999b, 2000). In the present study, only one specimen of Araneae and Heteroptera and two of Coccinellidae were observed (Tables 1–3). The contribution of these predators to predation on cards was low.

Forficula auricularia is a common predator in Portuguese olive groves, but its role was not well known. In the present work, *F. auricularia* appeared in greater numbers, but usually 7 days after the release when a considerable number of wasps had already emerged and predation had been very high. Probably, it was not easy for the earwigs to enter the cards due to their size ($\cong$ 16 mm in length). However, after visits of other smaller predators the holes may have increased in size, thus also allowing the entrance of *F. auricularia.* This predator probably used the cards mainly as a refuge and did not consume many parasitized eggs.

Several ant genera are recognised as important biological agents in agro-ecosystems. This group of arthropods is very common and frequent in olive groves (Arambourg, 1986; De Andrés, 1991; Cabanas *et al*., 1999; Morris *et al*., 1999a; Ramalho, 2000; Lozano *et al*., 2002). They are predators of several olive pests, such as the olive moth, *P. oleae* (Morris *et al*., 1998a; Morris & Campos, 1999; Morris *et al*., 2002; Pereira *et al*., 2002a), the olive psyllid, *Euphyllura olivina* Costa (Pereira *et al*., 2002a) and the olive bark beetle, *Phloeotribus scarabaeoides* Bern. (Russo, 1938; Gonzalez & Campos, 1990).

Ants are known predators of insect eggs (Rossi & Fowler, 2000; Van Mele & Cuc, 2000, 2001; Way *et al*., 1992, 1999). However, their action was not always beneficial. In the olive orchards, they were also considered responsible for destroying biological control agents such as eggs of *Crysoperla carnea* (Stephens) (Morris *et al.*, 1998b). The results of our study clearly showed that ants destroyed parasitized eggs on releasing units for the release of *Trichogramma* wasps, thus risking the efficacy of inundative releases of this egg parasitiod.

The different ant species behaved in different ways. *T. nigerrimum* is known as the most frequent and common ant species in the olive agro-ecosystem in the Northeast of Portugal (Pereira *et al*., 2002a,b). The colonies consist of various interconnected nests and the ants show an aggressive behaviour (Morris *et al*., 1999b). These features can explain the fact that no other ant species were found on cards where *T. nigerrimum* was present. This species also detected *S. cerealella* parasitized eggs on the cards very rapidly, leading to a fast accumulation of this species on the cards shortly after the release (Tables 1–3).

According to Morris *et al.* (2002), *T. nigerrimum* is an important predator of *P. oleae* that can have a significant role in the natural control of this pest. However, the workers of this species are also known to protect the olive scale, *Saissetia oleae* (Oliv.), from parasitoids (Panis, 1981) and to remove eggs of chrysopids (Morris *et al*., 1998b). In the present work, *T. nigerrimum* destroyed the *S. cerealella* eggs parasitized by *Trichogramma*. For these reasons, this ant can negatively interfere with the application of biological control methods, such as inundative releases of *Trichogramma*.

C. scutellaris was the second most abundant ant species. It is a very common omnivorous species in olive groves. Morris *et al*. (1998b) described *C. scutellaris* as a predator of chrysopid eggs. On average, this ant appeared in 6.6% of the observed cards (Table 4), but sometimes also in great numbers (e.g., 1 day after the third release: 114 ants). This species may also have a detrimental effect on the success of *Trichogramma* releases.

The effect of other ant species collected in the present study was much lower, as they appeared in smaller numbers and only on few cards. Parasitoids usually start to emerge from cards 2–7 days after placing them in the field. In the present study, the main predation was observed in the first days after release, thus heavily reducing non-emerged wasps on the cards. Predation occurring after day 7 was less important as most of the eggs had already hatched. This may also explain the decrease in number of predators found on the cards during this time period.

Our results suggest that predators can be an important detrimental factor that should be taken into account when inundative releases of *Trichogramma* in olive groves are planned. Releasing devices for the application of parasitoids in the olive ecosystem should therefore be modified to guarantee protection from predation, especially from ants. To minimize predation or removal of the eggs by predators, especially ants, the holes of the cards should be reduced to less than 3 mm to prevent the entry of *T. nigerrimum*, the most abundant and 'dangerous' predator in our study. The reduction in predation will probably increase the efficacy of *Trichogramma* releases, thus optimizing this method for the biological control of olive pests.

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