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To cite this version:
Marion Tiradon, Adrien Bonnet, Hong Do Thi Khanh, Etty Colombel, Maurane Buradino, et al.. Evaluation of a new biological control method against the palm borer, Paysandisia archon using oophagus parasitoids. AFPP – Palm pest mediterranean conference, Jan 2013, Nice, France. hal-02748240

HAL Id: hal-02748240
https://hal.inrae.fr/hal-02748240
Submitted on 3 Jun 2020

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EVALUATION OF A NEW BIOLOGICAL PEST CONTROL METHOD AGAINST THE PALM BORER, PAYSANDISA ARCHON USING OOPHAGUS PARASITOIDS

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SUMMARY
As part of the European project Palm Pprotect (2012-2014, 3 million €), which aims to eradicate the two palm pests: Rhynchophorus ferrugineus and Paysandisia archon, the Mediterranean Forest and Entomology Experimental Unit of INRA PACA intends to develop a new biological pest control method against the palm borer P. archon. The egg stage of this moth is the most accessible. On one hand, a screening of several potentially effective parasitoids of the genus Trichogramma will be performed against the moth. On the other hand, collects of P. archon eggs will be conducted in order to observe if parasitoids, naturally present in the environment, would have succeeded in establishing themselves, particularly through the importation of palm trees in the Mediterranean region. The first results are reported and discussed.

Key words: Trichogramma, Paysandisia archon, biological pest control, oophagous parasitoids, palm tree.

RESUME
En tant que partenaire du projet européen Palm Protect (2012-2014, 3 millions d'euros) qui vise à éradiquer les deux ravageurs des palmiers Rhynchophorus ferrugineus et Paysandisia archon, l'unité Expérimentale Entomologie et Forêt Méditerranéenne INRA PACA propose de développer une nouvelle méthode biologique de contrôle contre le papillon palmivore P. archon. Les œufs sont le stade le plus accessible. D'une part, un criblage de plusieurs parasitoïdes potentiels du genre Trichogramma sera réalisé. D'autre part, une collecte d'œufs de P. archon sera effectuée pour observer si des parasitoïdes naturellement présents dans le milieu ambiant pourraient réussir à s'installer, en particulier via l'importation de palmiers de la région méditerranéenne. Les premiers résultats sont décrits et discutés.

Mots clés : Trichogramma, Paysandisia archon, lutte biologique, parasitoïdes oviphages, palmier.
INTRODUCTION
Since 2009, the palm borer Paysandisia archon (Burmeister, 1880) is classified as a quarantine pest in France (Huguet and Fouilleux, 2010). Native from South America (Argentina, Brazil, Paraguay, Uruguay), it was first reported in France in the regions of Hyères and Toulon and would have been imported via palm trees (Trithrinax campestris) from Argentina in the 1990s (Montagud Alario, 2004). P. archon larvae cause significant damage on palm trees by feeding on the stipe. Moreover, the pest is able to attack different palm species as Chamaerops humilis, Livistona chinensis, L. decipiens, Sabal spp., Phoenix canariensis, P. dactylifera, Trachycarpus fortunei and Washingtonia filifera (Sarto i Monteys, 2005).

Human activities have greatly contributed to the expanding of the pest. In France, the moth has been observed for the first time in the Var region. Now, it can be seen throughout South of France from the “Alpes-Maritimes” to the “Pyrénées-Orientales”. It is also found in many European countries as Italy, Spain, Greece, Slovenia, Switzerland and Cyprus (EPPO, 2011). The lack of controls and vigilance during trade allowed the moth to gain ground. Furthermore, it appears that P. archon can travel large distances by flying. Its biology makes detection and control difficult: larvae remain endophagous throughout their life, the adults do not feed and are active during the day, females do not secrete long range pheromones (Sarto i Monteys et al., 2012), making attractant-based trapping presently impossible. A new research is in progress (CNRS) concerning pheromones secretion by the male butterflies.

Mechanical control methods such as bagging or the use of birdlime have been advised as well as the use of chemicals. Regarding biological pest control, tests were performed and means established. The use of nematodes Steinernema carpocapsae as biopesticide, or products based on the entomopathogenic fungi Beauveria bassiana, are known to control the larvae. To date, no attempt of control using parasitoids or predators have been introduced.

In Argentina, natural enemies such as birds or Ichneumonidae parasites are likely controlling the pest (Sarto i Monteys and Aguilar, 2005).

The INRA PACA MFEU aims to find new natural enemies, particularly oophagous parasitoids.
Tests were set up and collects carried out with two distinct objectives:

➔ In the laboratory, a screening of different Trichogramma strains was performed. Strains with physiological and morphological benefits were previously selected. The strains were then raised for several generations on hosts laying bigger eggs than the host Ephestia kuehniella usually used. Trichogramma produced on big eggs were then tested on P. archon eggs.

➔ In the field, collects of P. archon eggs have started in order to look for native oophagous parasitoids which would naturally parasitized eggs of this butterfly. The oldest sites attacked will be preferred. The aim is to raise those parasitoids on large lepidopteran eggs and then, to study their potential parasitism on P. archon eggs.

This article informs the first results obtained by INRA PACA during the first year of Palm Protect program.
MATERIAL AND METHODS

REARING OF TRICHORAGRAMMA

Selected strains

Nineteen strains of trichogramma belonging to 9 species and taken from the INRA PACA collection (Figure 1) were selected on biological criteria that could facilitate their potential on big eggs of *P. archon*:

- Strains fertility, or their ability to parasitize a large number of eggs.
- Originated host from which the strains have been collected before rearing on *E. kuehniella* eggs. Strains from biggest host eggs were selected.
- Others benefits such as large ovipositor (which could help to penetrate chorion of big eggs more easily), or the ability to parasitize on high vegetation layers, have also been taken into account.

The different selected strains are shown in Table 1. Those strains, initially reared on *Ephestia kuehniella* eggs, were reared during several generations on *Philosamia ricini* (Drury) (Lep., Saturniidae), and *Bombyx mori* (L.) (Lep., Bombycidae) eggs, in order to accustom the parasitoids on larger eggs and stronger chorion.

Table 1: Species / strains of trichogramma initially selected. Espèces / souches de trichogrammes pré-sélectionnées

<table>
<thead>
<tr>
<th>Specie / strain</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichogramma chilonis</em> SB</td>
<td>Ishii</td>
</tr>
<tr>
<td><em>Trichogramma chilonis</em> Th</td>
<td>Ishii</td>
</tr>
<tr>
<td><em>Trichogramma chilonis</em> Ca</td>
<td>Ishii</td>
</tr>
<tr>
<td><em>Trichogramma chilonis</em> P</td>
<td>Ishii</td>
</tr>
<tr>
<td><em>Trichogramma chilonis</em> C</td>
<td>Ishii</td>
</tr>
<tr>
<td><em>Trichogramma cordubensis</em> Pt</td>
<td>Vargas &amp; Cabello</td>
</tr>
<tr>
<td><em>Trichogramma cordubensis</em> Et</td>
<td>Vargas &amp; Cabello</td>
</tr>
<tr>
<td><em>Trichogramma cordubensis</em> Ct</td>
<td>Vargas &amp; Cabello</td>
</tr>
<tr>
<td><em>Trichogramma pretiosum U</em></td>
<td>Riley</td>
</tr>
<tr>
<td><em>Trichogramma pretiosum HA</em></td>
<td>Riley</td>
</tr>
<tr>
<td><em>Trichogramma pretiosum HB</em></td>
<td>Riley</td>
</tr>
<tr>
<td><em>Trichogramma dendrolimi</em> I</td>
<td>Matsumura</td>
</tr>
<tr>
<td><em>Trichogramma dendrolimi</em> L</td>
<td>Matsumura</td>
</tr>
<tr>
<td><em>Trichogramma buesi C</em></td>
<td>Voegelé</td>
</tr>
<tr>
<td><em>Trichogramma exiguan U</em></td>
<td>Pinto &amp; Platner</td>
</tr>
<tr>
<td><em>Trichogramma embryophagum It</em></td>
<td>Hartig</td>
</tr>
<tr>
<td><em>Trichogramma embryophagum I</em></td>
<td>Hartig</td>
</tr>
<tr>
<td><em>Trichogramma euproctidis A</em></td>
<td>Girault</td>
</tr>
<tr>
<td><em>Trichogramma daumaleae C</em></td>
<td>Dugast &amp; Voegelé</td>
</tr>
</tbody>
</table>
Tests on alternative new host *P. ricini* and *B. mori*

The rearing of *Philosamia ricini* (Figure 2) was undertaken, in order to provide big eggs necessary for trichogramma rearing. The first larvae were ordered at the OPIE association. The breeding was then maintained on privet leaves over several generations, in order to have available adults and eggs for maintaining trichogramma rearing. Tubes containing 60 eggs of *P. ricini* for about 30 trichogramma were used. The eggs renewal was performed by migration of the emerging trichogramma in a new tube (7.5 × 1.2 cm) with honey, for each generation of each strain. In the same way, a *B. mori* rearing was undertaken and the selected trichogramma were bred on those eggs.

Tests on the pest *P. archon*

Strains with the best parasitism on big eggs were tested on *Paysandisia archon* eggs in tubes and fed with honey. The pest eggs were regularly provided by our partner CIRAD of Montpellier. Several tests have been done following different modalities showed in Figure 3.

**Strains of Trichogramma**

**Trichogramma reared on:**
- *Ephesia kuehniella*
- *Bombyx mori*
- *Philosamia ricini*

**Number of Trichogramma tested:**
1, 2, 3, 5, 30

**Trichogramma size:**
selection of the biggest

**Age of *P. archon* eggs:**
- Young: 1 to 4 days old
- Old: 5 to 8 days old

**Number of *P. archon* eggs:**
1, 2, 30

**Figure 3** : Modalities of tests on *P. archon* eggs.

In a first time, emergence of trichogramma from *P. archon* eggs proposed to the parasitoids for oviposition was then examined.

**COLLECTS OF OOPHAGUS PARASITOIDS**

First collect on the field were done. The aim was to let incubate the collected eggs until emergence of any oophagous parasitoids. Parasitoids will be then grouped by type and then reared on large eggs.
FIRST RESULTS

TESTS ON BIG EGGS

Rearing results on *Philosamia ricini* eggs

Concerning trichogramma rearing on *P. ricini* eggs, the different strains initially selected all have been raised on this host over several generations. The Trichogramma size has significantly increased in the first generation.

Rearing results on *Bombyx mori* eggs

Breeding of the different trichogramma strains on *B. mori* eggs was also a success. However, although the size of trichogramma from these eggs is greater than those reared on *E. kuehniella*, they remain significantly smaller than those produced on *P. ricini* eggs.

Screening on *Paysandisia archon* eggs

To date, no emergence of trichogramma could have been observed on eggs of *P. archon*. The eggs have hatched, giving a larva, or aborted. Whatever the host, trichogramma females showed no real interest in the moth eggs.

COLLECTS

First results of collects for *P. archon* eggs evidenced difficulty of finding eggs *in situ*. Other collects are nevertheless considered in areas where *P. archon* attacks are denser.

DISCUSSION

Trichogramma are widely used in biological pest control around the world. They are rather generalist parasites capable of parasitising many host eggs with variable shapes and sizes. Indeed, trichogramma are observable on a large number of moth family, they can be found on *Sphingidae, Noctuidae, Tortricidae, Saturniidae*, etc. depending on the species of trichogramma (Consoli, 2010).

The first step of our protocol, which covers trichogramma rearing on *P. ricini* and *B. mori* eggs showed positive results. These parasitoids were able to develop over several generations on these hosts, with a larger size. Females were found interested in these eggs from their first contact with them. In addition, laboratory production has supply fresh eggs that are more attractive for trichogramma.

Unfortunately, in this study, we were unable to demonstrate their ability to parasitize *P. archon* eggs. Several factors come into play in the choice of oviposition and can cause a rejection from the female:

- egg size,
- egg resistance to the ovipositor,
- chemical factors,
- age of the egg: a too old egg reduces the attractiveness of trichogramma and decreases their success of parasitism.
- secretion of toxins (Consoli, 2010).

*P. archon* eggs have a very characteristic shape (rice grain) which differs greatly from the original host of rearing. Moreover, they are very large (about 1.5 mm wide and 4.5 mm long).
Also, the selected alternative hosts for rearing was perhaps not the better choice. Additional assays have to be realized on others lepidopteran host families. Indeed, trichogramma gain experience at the larval stage regarding their host preference. This preference goes toward a host from which they originate (Consoli, 2010) and as there is no native Castniidae in France, phylogenetically or physiologically close families will be used for our next experiments. These assumptions could be the cause of lack of interest of tested trichogramma toward *P. archon* eggs.

**CONCLUSION AND PERSPECTIVES**

To date, parasitism tests on *P. archon* eggs using different trichogramma strains have not yielded positive results. However, the interest to continue the breeding of these parasitoids on other major lepidopteran hosts before testing on *P. archon* eggs remains. Families potentially close of Castniidae will be preferred. An European trichogramma specie (*Trichogramma gicai*), naturally more robust than those we usually harvest will be sought from the spring of 2013. A new campaign for collecting *P. archon* eggs in heavy infested areas is already planned. Similarly, *P. archon* eggs will be placed in different climate and environmental zones, chosen in order to trap any native oophagous parasitoids potentially adapted to this pest eggs. Thus, many new avenues are still developing in the field of oophagous parasitoids.

**ACKNOWLEDGEMENTS**

The present work was supported by the EU under project Number FP7-KBBE-2011-5-289566. We thank the FREDON PACA and LR, the CIRAD of Montpellier for their help and collaboration.

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