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Breeding and conservation of the parasitoid *Psyttalia concolor* (Hymenoptera: Braconidae) to control the olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae) and protect olive crops

Kamila GACEM,[1*] Farid MEZERDI[2]

[1] Department of Agronomic Science, Faculty of Exact Sciences and Natural and Life Sciences, University of Biskra, Laboratory of Ecosystem Diversity and Agricultural Production System Dynamics in Arid Zones (DEDSPAZA), Algeria
e-mail: kamila.gacem@univ-biskra.dz*; ORCID: https://orcid.org/0000-0002-0800-110X

[2] Department of Agronomic Science, Faculty of Exact Sciences and Natural and Life Sciences, University of Biskra, Laboratory Promotion of Innovation in Agriculture in Arid Regions (PIARA), Algeria
e-mail: f.mezerdi@univ-biskra.dz; ORCID: https://orcid.org/0000-0002-4388-2520

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Abstract. Our work involves the biological control of the olive fly *Bactrocera oleae*, using the endoparasitoid *Psyttalia concolor*, which develops within the olive fly larvae. We studied the development stages of the host, and the emergence of adults in parasitoid rearing and discussed the contribution of this information to optimize production. The biological control methods require a significant investment in optimizing insect rearing. Our study describes breeding procedures for the parasitoid and its host *Bactrocera oleae*. This is still insufficient to reduce the pest and minimize its damage, achieving integrated management without applying other control techniques or using pesticide treatments.

Keywords: pest, damage, factor, olive, host, pesticide

1. Introduction

The olive fruit fly *Bactrocera oleae* is the main pest of the olive tree and one of the factors hampering olive production. The damage caused by this pest is extensive and varied, resulting in premature fruit drop, direct destruction of the pulp by the larvae, and reduced olive oil quality due to increased acidity [1]. Integrated pest management is defined as the process of controlling harmful organisms using a set of methods that meet economic, ecological, and ecotoxicological

requirements, giving priority to actions that promote the natural control of crop pests and respecting economic intervention thresholds [2]. Optimal integration of olive fruit fly control techniques cannot be achieved without an understanding of their population biology and in particular their dynamics [3]. Until now, most information on the population fluctuations of these insects has been based on adult trapping. However, these data are only of relative importance and reliability, as the adults caught represent just a fraction of the population [4]. The olive fruit fly is a harmful pest, present throughout the Mediterranean countries, and efforts have been made to specify the main parameters to assess the damage caused by the pest [5]. The olive fruit fly and its parasitoids have been studied only slightly in Algeria despite their significant impact on olive products. Its parasitoids are divided into four species of Chalcidian ectoparasites with a wide distribution range and one Braconidae endoparasite originally from North Africa only. However, the most common ectoparasitic ones are *Eupelmus urozonus* and *Pnigalio mediterraneus*. It intrudes preferentially to parasitize third-stage larvae. Biological control also includes using other organisms or their products to prevent and minimize damage to plant production caused by pests [6]. At present, biological control is the method most favoured in research programmes, given its economic and agro-environmental benefits in maintaining a bio-ecological balance [7]. Most parasitoids prefer the third larval stage of *B. oleae* as a host; *Psyttalia (Opius) concolor* can parasitize all larval stages; *Eupelmus urozonus* can also lay eggs inside pupae [8]. Biological control uses living organisms to reduce the population levels of harmful organisms. The latter are responsible for many biological control successes and play an important role in natural ecosystems [9]. Biological control involves the use of an auxiliary parasitoid, predator, or pathogen to manage a targeted pest species. In our case, we are talking about classical biological control, as we have introduced a parasitoid to control a targeted pest species. *Psyttalia concolor* was the ideal parasitoid for the olive fruit fly in the Biskra region, due to its well-studied biology and parasitism. It is an important natural enemy against Tephritidae and is an endoparasitoid species, meaning that females lay their eggs inside their host, which continues to live throughout the first part of the parasitoid larvae development. It is a solitary parasitoid, with a single larva able at best to develop in a host. As with any biological control programme aimed at introducing an auxiliary organism into a new territory, several measures were required to comply with phytosanitary standards and risks. From the long series of trials and errors that make up the history of biological control, remarkable successes and promising achievements have been made in economic, environmental, and agricultural terms, particularly following the successful use of various biological control agents [10].

In biological control, the parasitoid must reduce the pest population to an acceptable level and maintain a consistently low density to prevent future pest

reproduction. *Psyttalia concolor* has been introduced in many parts of the world to control the olive fruit fly *Bactrocera oleae*. In its native range (North Africa and the Near East), *Opius concolor* can generate parasitism rates of up to 60% on the olive fruit fly. Attempts were made to use this parasitoid as a biological control agent against *B. oleae*, but without real success, the challenges stemming from the difficulty of effectively reproducing this parasite in natural and controlled environments and the unpredictability of the results of this biological control. Thus, achieving high parasitism rates of *Psyttalia concolor* remains a goal that needs intensive research [11].

The use of integrated pest management (IPM) has often produced good results; however, the extension of these methods is still limited due to the complexity of the biological control agents [12].

Depending on the method of use, there are three main methods of biological control: introduction, augmentation, and conservation [13].

2. Materials and methods

We sampled olive fruits infested with the larvae and pupae of the olive fruit fly *Bactrocera oleae* in the olive grove.

Breeding

The preservation and transport of fruit found in the olive orchards required the use of plastic bags and plastic Petri dishes for the collection of olive fruit fly larvae and pupae. A qualitative census of larvae and pupae requires meticulous, repeated checks on as many olive trees as possible during the study period. It took much work to follow a well-defined sampling method. In addition, it took some more work to find the larvae and pupae present in the infested olive fruit or in the soil. For this reason, all olive fruits containing larvae collected from olive trees on different dates were taken into consideration for the calculation of emergence rates, sex ratio, and parasitism.

We put olives at room temperature in the laboratory. After we sanitized them with a diluted bleach disinfectant solution and dried them with sterile paper to avoid the appearance of fungi, we put olives in a clear plastic box on a net screen large enough to allow larvae and pupae to pass. Providing favourable conditions for olive fruit fly reproduction is necessary for their development. Larvae and pupae were collected along with the olive fruits on which they were found. These were then placed in Petri dishes; the date of collection and place of harvest were noted. The boxes reserved for the larvae and pupae were covered with a piece of tulle, which did not hinder the breathing of the parasitoids inside the pupae; the emerged *Bactrocera oleae* adults and parasitoids were then identified under a binocular

magnifying glass and a digital microscope. Pupae that had not yet emerged were left in Petri dishes for up to 20 days, sufficient time for the emergence of olive fruit fly pupae that had not died or diapaused. We counted the pupae every second day. After a fortnight, adult emergence is monitored daily. We assessed the rate of parasitism and the nature of the parasite by counting flies and emerging parasites. Reproduction also enabled us to determine the duration of pupal development on different dates. Based on the number of adults emerging, we can estimate pupal survival rates, as well as fly sex ratios.

Host production

We conducted rearing experiments under stable conditions of temperature (22°C ± 2°C) and relative humidity, around 55%, with a 16-hour lighting period.

Bactrocera oleae rearing was relatively straightforward and consisted of three stages. The 10 boxes were filled with a dish containing around 30 larvae and pupae. Small boxes of nutrient medium and a drinking trough were also added for future adults. Five to six days after adult emergence, olive flies started laying eggs in olive fruits, which we used as larval feeders to maintain normal conditions in the laboratory. The first larvae generally hatched 48 hours later. 8 days after their creation, the boxes were opened and placed in another, larger box with a covered bottom to facilitate the pupation of larvae, and the tulle to facilitate aeration. After ten days, we recovered the pupae.

Rearing of the parasitoids

Psyttalia concolor is a Hymenoptera of the Braconidae family. The adult is a small, tawny-coloured insect, almost 3.5 mm long, with darker pigmentation on the long antennae. The rearing experiment for *Psyttalia concolor* was conducted at the rearing laboratory temperature. The breeding room was equipped with transparent plastic boxes. The boxes used were disinfected to avoid the risk of contamination by fungi or the multiplication of undesirable insects.

At the same time, *Bactrocera oleae* was being reared to provide larvae for the parasitoids to use as hosts. For this purpose, olive fruits were collected in various regions of Biskra and incubated. Individuals emerging from these fruits constituted the first generation of laboratory rearing. After 25 days, *Bactrocera oleae* larvae were collected using the same procedure as for rearing the parasitoids.

We placed *Bactrocera oleae* larvae in the boxes containing the adult parasitoids. The pupae resulting from the pupal phase of the third instar larvae were collected using a soft brush and placed on a rearing medium until the adults' emergence. Once the adults had emerged, the parasitoids were transferred to new boxes for a new development cycle. We collected infected olive fruits containing the larvae

of *Bactrocera oleae*, which were then placed in other rearing boxes for larval pupation, with muslin lids, and we placed them under the same rearing conditions as mentioned before.

Data acquisition

Our work was carried out at the laboratory, enabling us to monitor the evolution of adults, as well as that of parasites. The results of rearing carried out in the laboratory, which yielded pupae and adults, enabled us to follow the complete development cycle of *B. oleae*. To compare the use of different parasite densities on olive fruit fly populations, we used ANOVA analysis of variance at $\alpha = 0.05$ significance level. This test was performed in Excel 2019.

3. Results and discussions

We placed the larvae and pupae emerging from olive fruits in boxes for 8 days to be used in our experiments and to maintain rearing until the emergence of adults. After this period, we sequentially counted the adult olive fruit flies and the parasitoids. *Psyttalia concolor* (Braconidae: Opiinae) emerged from pupae where adults were found. This parasitoid mainly attacks the *Bactrocera oleae* species of Tephritidae present in a wide range of olive varieties. In our case, in all samples, the emerged *Psyttalia concolor* individuals were accompanied only by the *B. oleae* fly. This species may be the dominant current host of the parasitoids in Biskra (Algeria).

Adult emergence of the parasitoid Psyttalia concolor *and the olive fly* Bactrocera oleae *(*figs 1–7*).*

Figure 1. Adult female *Bactrocera oleae* laying an egg under the fruit epidermis

Figure 2. Parasitized L3 larva of *Bactrocera oleae* (left); L3 larva and prenymph of unparasitized *Bactrocera oleae* (right)

Figure 3. Olive fly pupae *Bactrocera oleae*

Figure 4. Adult emergence of *Bactrocera oleae*

Figure 5. Adult *Bactrocera oleae* – female (left), male (right)

Figure 6. Adult *Psyttalia concolor* – female (right), male (left)

Figure 7. Parasitized L3 larva of *Bactrocera oleae* and *Psyttalia concolor* adults

The olive fruit fly larvae were collected from infected olive fruits and deposited on a stretched muslin, usually for 24 hours. The larvae were transferred to a small plastic box, which was placed in a larger, aerated box for pupation. The first olive fruit fly adults (from non-parasitized larvae) emerged from the pupae after around 11 days, with the first *P. concolor* emerging after 10 to 11 days. Under controlled conditions, only 90 larvae were parasitized out of a total of 300 olive fly larvae and pupae, which is 30%. Moreover, according to our observations, a period of 10 days corresponds to the time required for the first appearance of *Bactrocera oleae* and *Psyttalia concolor* adults.

Figure 8 shows the emergence of the parasitoid and olive fly adults. The conditions applied were: temperature – 22°C ± 2°C; relative humidity – around 55%; lighting period – 16 hours.

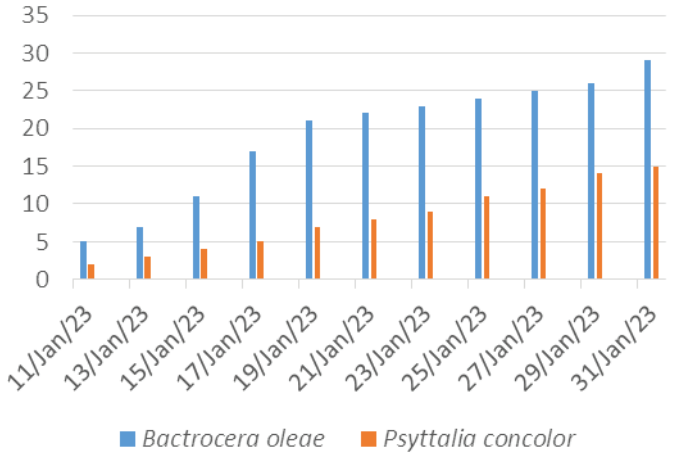

Figure 8. Adult emergence of the parasitoid *Psyttalia concolor* and of the olive fly *Bactrocera oleae*

P. concolor females prefer the L3 larval stages, pre-pupae, and pupae of *Bactrocera oleae* for oviposition. All the stages presented to the females were larvae, prepupae, and pupae, which were large and easily distinguishable to the naked eye. These stages were reached 10 days after oviposition under rearing conditions. Parasitoids were reared under the same rearing conditions as above, on *Bactrocera oleae* larvae, which were introduced into plastic boxes containing fruit infested with olive fly larvae at L3 stage. The boxes were placed in a laboratory at 22 °C, and three days later the parasitoids were removed and introduced into other boxes containing host larvae. The boxes were kept under the same rearing conditions until adult emergence – a generation of *P. concolor* developed in around 10 to 20 days. Parasitoid species were used immediately or introduced into rearing boxes

until they were used for experiments on reproductive success and the biological control test.

Anova: Single factor

To test the time effect and the interaction between this factor and the variation in total adult emergence, we analysed variance (ANOVA). The results of the probability test are shown in *Table 1*.

Table 1. Analysis of variance with two classification criteria at a 5% threshold for total adult emergences

SUMMARY

Groups	Count	Sum	Average	Variance
Bactrocera oleae	11	210	19.09091	64.69091
Psyttalia concolor	11	90	8.181818	19.76364

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between Groups	654.5455	1	654.5455	15.50054	0.000815	4.351244
Within Groups	844.5455	20	42.22727			
Total	1499.091	21				

The analysis revealed a significant variation: P value being equal to 0.000815 is significant, the same as in the case of other values below the selected threshold for significance ($P < 0.05$); this value is less than $P < 0.001$, which implies high significance.

The biological control programme has been successful with Braconidae parasitoids, which was also introduced in several countries – where recent surveys show that the parasitoid achieves over 20% of parasitism on *B. oleae* in fruits collected in the olive grove. However, the success of this parasitoid is limited to some cases, while in others it has yet to be established or yielded only low parasitism in the target species. Recent studies have reported over 50% of parasitism of *B. oleae* when reared on artificial feed [14]. The data obtained on the biology and behaviour of the olive fly have enabled us to develop an effective control strategy.

Our bred parasitoids may explain up to 30% of parasitism. However, the first individuals were reared in laboratory on *B. oleae*. Thus, host adaptation is not a barrier for the individuals. Climatic factors play a role in determining parasitoid development, longevity, and parasitism rates.

Adaptation to these factors has also been listed for the selection of a potential biological control agent, and temperature is often considered to be the most important in the acclimatization of a reared parasitoid. A temperature range

of 20–25 °C is appropriate and gives rise to a higher rate of parasitism, which corresponds to the optimal temperature for the development and survival of its host *B. oleae*. In addition to climatic conditions, the physiological state of a parasitoid can modify its host selection behaviour and thus its effectiveness as a biological control agent [15].

As far as the acclimatization of *Psyttalia concolor* in the laboratory is concerned, climatic conditions in the Biskra region are generally favourable for its multiplication. The abundance of *B. oleae* should encourage its rapid expansion. Many researchers reported that *Psyttalia concolor* mainly attacks *Bactrocera oleae*, capable of further parasitizing some non-host fruit fly species. However, the host range of *Psyttalia concolor* appears to be limited to the Tephritidae family. 24-hour-old *Psyttalia concolor* females are already able to contain an average of 18 mature eggs [16]. Supplied with food, they can have up to a hundred mature eggs in their ovaries four days after emergence [17]. Moreover, parasitoids are released 8 to 10 days after emergence, mainly in olive orchards, where fruit infestation by *B. oleae* is high.

In the years to come, the installation and multiplication of this parasitoid will need to be monitored to determine its full Tephritidae host range in Biskra and its real impact on population density. Several results are expected from this future study, including the parasitoid's dispersal capacity around release zones, altitude, temperature, and rare rainfall. It would also be interesting to observe whether the parasitoid competes with other Opiinae already present in the area.

The Braconidae endoparasite *Psyttalia concolor* has been the subject of numerous studies, given its vast distribution in the Mediterranean basin, which overlaps with the southern part of its host; it covers northern Africa.

[18] began breeding *Opius concolor* as early as 1958 and conducted several experiments in 1968, ten years later, which led him to confirm that the early releases of this parasitoid would yield spectacular results. Oviposition on *B. oleae* involves all larval stages, but the female prefers the third stage [19]. The development of pre-imaginal stages is inhibited at temperatures below 15 °C, and survival rate is reduced at low relative humidity. Older larvae are resistant to cold and can withstand temperatures of 0°C for several days. Parasitoid emergence depends on host availability, density, and age [20].

Biological treatments with *P. concolor* have reduced *B oleae* populations, but without being satisfactory [21]. For this to be successful, releases must be repeated regularly. In addition, the parasitoid attacks the third larval stage, which has already caused damage to the olives [22].

[23] emphasize the need for high humidity levels for the successful rearing of *P. concolor*, while [24] confirms the importance of the availability of unharvested olives for olive fly and parasitoid populations.

4. Conclusions

This study represents a critical first step in our knowledge of Braconidae parasitism. The low olive fruit fly densities we observed are due to parasitism by *Psyttalia concolor*. The low number of individuals obtained is likely related to the limited number used during breeding campaigns. However, with its presence already detected in larval hosts, its reproduction and involvement in the control of invasive *B. oleae* species looks promising in the coming years.

The introduction of *Psyttalia concolor* into olive groves makes it possible to effectively control *Bactrocera oleae* populations and reduce the use of chemical pesticides. This environmentally-friendly approach has many advantages, particularly in terms of sustainability and biodiversity protection. It is interesting to know to what extent *P. concolor* is having an impact on the Tephritidae family in Biskra, and it is important to monitor its spread to identify all of its host species and its impact on the density of other species. It will also be crucial to monitor parasitism rates in all regions, according to host species. Finally, it will be necessary to reinforce the phytosanitary surveillance system to minimize the risk of invasion by other pests.

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