Introductions of the African parasitoid *Psyttalia lounsburyi* in South of France for classical biological control of *Bactrocera oleae*

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Abstract: *Psyttalia lounsburyi* is an African parasitoid of the olive fruit fly *Bactrocera oleae*. Its introduction in France has been made with two different objectives: i) controlling the olive fruit fly in France, and ii) testing how intraspecific hybridization affects the demographic success of small introduced populations. For this, we introduced two parental strains of *P. lounsburyi* originating from either Kenya or South Africa, and a hybrid strain resulting from their admixture. In this paper, we report the first years of research and progress toward our two objectives. In 2006 and 2007, intensive field surveys were carried out to locate 60 suitable release sites in South of France. During fall 2007, before the introduction of *P. lounsburyi*, a first set of olive samples was collected to assess the density of *B. oleae* and the diversity of indigenous natural enemies. The year 2008 was dedicated to parasitoid mass production, release, and a second set of sampling. In summer, a total of about 43,000 individuals *P. lounsburyi* were introduced in the 60 sites. Individuals of the genus *Psyttalia* were found in some samples, suggesting the ability of the released parasitoids to locate *B. oleae* in French olive trees and to complete their preimaginal development into these hosts. However, molecular identification of the recaptured individuals needs to be done to confirm these results. More sampling is also necessary to test the ability of African *P. lounsburyi* to overwinter and establish in South of France.

Key words: biological control, hybridization, invasion, *Bactrocera oleae*, *Psyttalia lounsburyi*

Introduction

The introduction of the parasitoid *Psyttalia lounsburyi* (Hymenoptera: Braconidae) in South of France fills two different aims. The agronomic aim of this project is to assess the adaptability and efficacy of a new biological control agent against the olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae) in South of France. This applied facet of the project is of primary importance since the olive fruit fly is the main pest in oleiculture (Daane & Johnson 2010). Its control still mainly relies on conventional control strategies which are incompatible with an organic production. Moreover, resistances organophosphate insecticide has been recently observed (Skouras, *et al.* 2007). With regard to this context, the introduction of different allopatric strains of *P. lounsburyi* represents a new hope for the olive producers.

From a more fundamental scientific perspective, the primary introduction of exotic parasitoids is an opportunity to test hypotheses derived from invasion biology. The underlying idea is that classical biological control is nothing else than a planned biological invasion. To date, only a few pioneer experimental studies have capitalized on biological control introductions (Fauvergue *et al.* 2007, Fauvergue & Hopper 2009, Grevstad 1999, Memmott *et al.* 2005). The present study is another example. Here, the hypothesis under scrutiny is the positive effect of hybridization on invasions success (i.e., population establishment, growth and spatial spread). Such a hypothesis is supported by recurrent observations suggesting that multiple introductions of exotic species into novel environments and subsequent genetic
admixture do promote invasion success (Facon et al. 2005, Kolbe et al. 2004, Schierenbeck & Ellstrand 2009). However, observations from fortuitous invasions are only weak evidences for the effect of hybridization. Our use of a manipulative field experiment with replicated introductions is a way to demonstrate the presence or absence of such an effect.

With regard to these two objectives, this paper summarizes the first results on the pre- and post-release field monitoring.

Material and methods

Biology of Psyttalia lounsburyi
The biology of P. lounsburyi has been recently investigated and more details can be found in Daane et al. (2007) and related papers. P. lounsburyi is a koinobiont and solitary endoparasitoid which seems to be quite specialized on second instar larvae of B. oleae. The known distribution of P. lounsburyi is the subsaharan Africa since it has been found in Kenya, Namibia and South Africa. Its introduction in California is currently underway but this species has not been released in Europe or neighbouring countries. Hence, introduction of this parasitoid in France actually reflects the definition of an invasion, i.e., the spread of an organism outside its native range. This introduction was made possible by the transfer by the European Biological Control Laboratory (ARS-USDA, Montpellier, France) of several strains of P. lounsburyi originating from different African countries.

Mass production
The first important step was to produce a great number of parasitoid wasps for the releases in summer 2008. The rearing of Psyttalia lounsburyi has been realised on Ceratitis capitata which can be easily produced on an artificial diet. However, the use of this substitution host is associated to strong pre-imaginal mortality as well as male-biased sex-ratio. In order to circumvent these problems, we increased progressively the mass-rearing during the first six months of the year, with the aim to introduce 1 000 individuals in each of the 60 experimental sites.

Field experiment
To test the effect of hybridization on demographic success, two allopatric strains of P. lounsburyi (parental strains, originating from Kenya and South Africa) and their hybrid strain were used. 60 suitable release sites (20 replicates for each strain) were selected from 233 potential sites according to the following criteria: (a) a minimum of 15km between sites (to prevent dispersal and subsequent lack of independence between sites), (b) the presence of olive flies, (c) the promise that the release sites and surrounding trees will not be treated with insecticides during the four years of experiment.

Packaging and carriage
Because it is not possible to discriminate between unparasitized and parasitized pupae of C. capitata, young parasitoids were released. Wasps were collected by mouth aspiration from emergence cages, and packaged in cardboard tubes (60mm diameter × 22mm long) closed on each side with a piece of tights. The tubes were brought to the fields in cool boxes to minimize thermal stress during travel. Early in the morning or during evening hours, two tubes were hanged and opened in the central tree of each field sites. Insects which had died during transportation were counted. Two independent introductions were made in each site (one in July, one in August) in order to reduce environmental stochasticity and catastrophe events.
**Sampling**
In fall 2007 and fall 2008, samples of 1000 olives were collected in each of the 60 field sites and brought back to the laboratory. Species and number of insects emerging from olives allowed to estimate the relative abundance (number per 1000 olives) of *B. oleae*, indigenous natural enemies, and *P. lounsburyi*. In 2008, yellow sticky traps baited with virgin *P. lounsburyi* females were also placed two weeks in each field site to improve the detectability of introduced parasitoids. A trap consisted of a small cage enclosing five females, surrounded by a yellow plastic sheet folded as a tent, and covered with tangle-foot. Three traps were hanged at the release point of each site during 10 days, time after which they were returned to the laboratory. Captured *Psyttalia* were counted.

**Results and discussion**

**Field sites**
Our 60 experimental field sites were scattered across the entire geographic range of olive trees in France, in regions surrounding the Mediterranean Sea, from the Pyrenees to the Alps (Figures 2 and 3). This exceptionally large scale for a field study resulted in a considerable amount of variation among sites. For some variables, this variation was quantified. Most sites (35/60) relied on organic practices only. However, some (7/60) were sometimes sprayed with pesticides as part of an IPM-type of agriculture, and others (18/60) were sprayed regularly, although of course not within the experimental plot. Most sites were not irrigated (43/60) but some were irrigated with a dripping system (11/60) or with conventional irrigation (6/60). Altitude varied from sea-level to 685m, the average altitude being 247m. Different olive varieties were grown. Each field was characterized by an average olive weight. Olives varied from very small (0.29g/olive) to large (4.4g/olive), with an average weight of 2.3g/olive. Although olive varieties were constant across years, olive weight was not, as suggested by a low correlation coefficient of weight between years (Pearson correlation $r=0.37$, $n=50$, $p=0.04$).

**Olive fruit fly abundance**
The number of flies emerging from olive samples was used as a measure of relative abundance for *Bactrocera oleae* in South of France. There were 123 and 97 flies per 100 olives in 2007 and 2008 respectively (means and 95% confidence limits estimated from log-transformed data). The variation of olive fruit fly abundance among sites is illustrated on Figure 1. The distribution of the number of flies per 100 olives is characteristic of count data, with most sites having low abundances (0 to 30 flies/100 olives), and few having much higher abundances (up to more than 100 flies/100 olives). The correlation across years was not significant (Spearman rank correlation, $r=0.21$, $n=57$, $p=0.12$) suggesting that a large amount of variation in fly abundance may not be explained by intrinsic characteristics of field sites.
Figure 1. Frequency distribution of the number of olive fruit flies *Bactrocera oleae* per 100 olives in South of France across two successive years, 2007 and 2008. In each of the 60 field sites, olive fruit fly abundance was estimated as the number of individual flies emerging from a total of about 1000 sampled olives, and standardized for 100 olives.

In order to analyze olive fruit fly abundance more thoroughly, we fitted a generalized linear model with a Poisson distribution and a log-link function to the number of flies per olive. We first fitted a maximal model with all possible explanatory variables (professional or amateur, insect pest and weed management, irrigation practice, olive weight, year, altitude, longitude, latitude), and then searched the most parsimonious model using a backward model selection procedure. The most parsimonious model revealed three explanatory variables for olive fruit fly abundance: altitude and longitude both had a clear negative effect (likelihood ratio tests for altitude: $G^2=11.1; \ df=1, \ 112; \ p=0.0012$; longitude: $G^2=4.5; \ df=1, \ 112; \ p=0.036$). There were fewer flies at higher altitude and in the eastern regions of the French olive geographic range (Figure 2). Surprisingly, pest management practices had only a marginally significant effect ($G^2=2.92; \ df=2, \ 112; \ p=0.058$), with chemically treated plots having slightly lower abundances than plots relying on biological control only.

**Native parasitoids**

Three species of native parasitoids were found in our samples: *Pnigalio agraules*, *Eupelmus urozonus* and *Eurytoma martellii*, the most abundant being *P. agraules* (about 80% of the specimens captured). In contrast with the olive fruit fly, native parasitoids were not found in all sites. In 2007, native parasitoids were found in one third of the sites (19/58). In 2008, parasitoids were found in only 10% of the sites (6/59). As for olive fruit fly abundance, we used a generalized linear model to search for variables explaining the presence of native parasitoids. This model was implemented with a binomial distribution and a logit link function. The maximal model was based on the same variables as the one fitted to olive fruit fly data. Model selection revealed three variables affecting the proportion of sites colonized by native parasitoids: year (higher proportion in 2007 than in 2008; $G^2=10.9; \ df=1, \ 112; \ p=0.0009$),
latitude (decreasing proportion with increasing latitude; $G^2=14.2; df=1, 112; p=0.0012$) and olive fly abundance (increasing proportion with increasing abundance; $G^2=11.4 df=1, 112; p=0.0007$).

Figure 2. Spatial distribution of olive fruit fly (*Bactrocera oleae*) abundance in South of France across two successive years, 2007 and 2008. In each of the 60 field sites, olive fruit fly abundance was estimated as the number of individual flies emerging from a total of about 1000 sampled olives, and standardized for 100 olives.
Figure 3. Spatial distribution of native parasitoids of the olive fruit fly *Bactrocera oleae* in South of France across two successive years, 2007 and 2008. In each of the 60 field sites, presence/absence was assessed from the number of individual parasitoids emerging from a total of about 1 000 sampled olives. Three species were found: *Pnigalio agraules*, *Eupelmus urozonus* and *Eurytoma martellii*.

When native parasitoids were present, the parasitism rate (number of emerging parasitoids/number of emerging flies and parasitoids) was highly variable. In most sites colonized by parasitoids (19/25), parasitism rate was lower than 5%. For the six sites with more parasitoids, observed parasitism rates were 10%, 13%, 14%, 41%, 50% and 57%.

**Introduced parasitoids**
A total of 43,300 adult *Psyttalia lounsburyi* were introduced in South of France. Most sites received a total of 300-400 females and 400-500 males in summer 2008 (Figure 3). Sampling in 2007 showed that *P. lounsburyi* was not present in France prior to introduction. In contrast, in 2008, some parasitoids of the genus *Psyttalia* were found in 11 sites. With regard to the introduced strain, *Psyttalia* parasitoids were found in 6, 4 and 1 site where we had respectively released the South African, the hybrid and the Kenyan strain. In sites where we collected some *Psyttalia*, the number of individuals per 1000 olives was very small: <4 individuals (10 sites) and 12 individuals (1 site). Although the collected *Psyttalia* individuals resembled the introduced *P. lounsburyi*, their molecular identification will be done to determine the species.
Figure 4. Distribution of the total number of live male and female *Psyttalia lounsburyi* released per site. Two releases were made in each site, one in July 2008 and the other August 2008, in order to prevent the effects of adverse conditions at the time of release. Each site was allocated randomly to one parasitoid strain: Kenya, South-Africa, or Hybrid, so that each strain was released in 20 replicated sites.

Conclusions

We used introductions of the parasitoid *Psyttalia lounsburyi* as a way to control *Bactrocera oleae* and to test the effect of hybridization on invasion success. This article reports the first steps toward these two aims. We succeeded in finding 60 suitable experimental sites in south of France, in releasing around 700 parasitoids per release site, and in sampling all the experimental sites once a year, in fall 2007 and 2008. Our sampling effort in each site was sufficient to observe native parasitoids as well as some *Psyttalia* sp. The latter might be the offspring of the introduced parasitoids. However, molecular identification of these parasitoids needs to be done properly to remove any ambiguities at the species level. In any case though, the presence of parasitoids among samples nails down the value of our ambitious experimental design. Altogether, we created an experimental situation, based on classical biological control, which could enable us to measure the effect of hybridization on the demographic success of an invasive species.

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