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Data Descriptor

Collection and Processing of Behavioural Data of the Olive Fruit Fly, *Bactrocera oleae*, When Exposed to Olive Twigs Treated with Different Commercial Products

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Abstract: The need for the development of sustainable control methods of herbivorous insects implies that new molecules are proposed on the market. Among the different effects the new products may have on the target species, the alteration of insect oviposition behaviour might be considered. At the scope, parallel simple behavioural assays can be conducted in arena. Freely available software can be used to track observed events, but they often need intensive customization to the specific experimental design. Hence, integrating such software with, e.g., R environment, can provide a much more effective protocol development for data collection and analysis. Here we present a dataset and protocol for processing data of the oviposition behaviour of the olive fruit fly, *Bactrocera oleae*, when exposed to olive twigs treated with different commercial products. Treatments were rock powder, propolis, a mixture of rock powder and propolis, copper oxychloride, copper sulphate, and water as the experimental control. JWatcher was used to simultaneously collect data from 12 arena assays and ad-hoc developed R code was used to process raw data for data analyses. The procedure described here is novel and represents a valuable and transferable protocol to analyse observational events in *B. oleae*, as well as other biological systems.

Dataset: https://zenodo.org/badge/DOI/10.5281/zenodo.6695805.svg

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Keywords: deterrence; insect behaviour; JWatcher; observational events; *Olea europaea*; RStudio; Tephritidae

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1. Summary

- Nowadays the need for sustainable products against herbivorous insects, such as *Bactrocera oleae*, is of paramount importance.
- The evaluation of the effect of these new products on insect behaviour can be conducted in the laboratory, using, e.g., arena bioassays.
- Through the novel integration of different free software, it was possible to simultaneously collect data of 12 *B. oleae* females' behaviour.
- Here, we present ad-hoc developed R script that was used to process raw data obtained by JWatcher.

In the area where it has established, the olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), is the major pest of the cultivated olive tree, *Olea europaea* L. [1]. For a long time, control methods have relied essentially on organophosphate insecticides, mainly used as cover sprays [2]. Because of the side effects on the environment and human health, the

use of different control products is now recommended [3]. Additionally, it is known that the populations of *B. oleae* may exhibit genetic diversity with consequently different levels of susceptibility to pesticides, which requires alternative control methods [4–6]. This phenomenon is indeed very common in worldwide distributed herbivore species, with genetic variability among populations [7–9]. Directive 2009/128/EC promotes integrated pest management (IPM) strategies or organic farming control methods for pest control. Products exhibiting an oviposition deterrence effect on *B. oleae* can be a valid alternative [10,11]. Even if field experiments can give an assessment of the efficacy of such formulations in real agricultural conditions, they hardly explain the underlying mechanisms and product functioning [4,12]. In insect ecology and herbivore pest control, the values of laboratory experiments to predict real field scenarios have been evaluated several times [13–16]. To fully understand the activity of new commercially available products, laboratory assays on *B. oleae* ovipositing behaviour are hence needed. Insect oviposition behaviour and deterrence can be commonly explored using clip cages or plant cages [17], one-way olfactometer [18], two-ways or four-way olfactometers [19,20], and open or closed arena bioassays [21,22]. Such techniques can provide information on insect–insecticide or insect–plant–insecticide interplay [23]. The acquisition of the behavioural data of animals often employs commercial software. However, their usage is often limited by the need to set-up the software well in advance before data collection. More recently, open-source or freely available devices and software may provide customizable solutions for researchers, especially when such tools are provided with source codes [24–27]. Concerning observational data, JWatcher can provide an easy-to-use tool that, after integration with, e.g., R environment, can be easily adapted by users to produce a structured dataset for downstream analysis [28]. In the main article by Daher et al., [29], propolis and rock powder based commercial formulation and the traditional copper formulations were assessed as oviposition deterrents against *B. oleae*. The insect behaviour was manually recorded using JWatcher 1.0, first. Then data processing was conducted using an ad-hoc script realized within R statistical environment [30]. The output resulting from the integration of the two software produced a structured dataset. The results of the analyses are fully reported in the main research article [29] and revealed a lower preference of *B. oleae* females for olive twigs sprayed with rock powder and propolis compared to the experimental control [29]. The procedure reported here would be useful to further explore the mechanisms of the most promising products against *B. oleae*, as well as the possible non-target effects on beneficial insects occurring in olive plantations (e.g., ladybird and ground beetle predators [31,32]), which are still unknown.

2. Data Description

The data related to laboratory assays in [29] and the R script for data processing and analysis are deposited in a public repository (available at https://zenodo.org/badge/DOI/10.5281/zenodo.6695805.svg (accessed on 1 April 2022)). The file entitled "output.csv" corresponds to the combined outputs of all JWatcher observational bioassays. Specifically, the first column "experiment" reports the sequential number of each observational assay, with each assay consisting in a simultaneous observation of 12 arenas. The second column "time_old" reports the time (format: hh:mm:ss:ms) at which a given event was recorded. The third column "key" reports the keyboard key that is related to a given female position for each of the 12 arenas. Such keys were a-priori associated to a given event. Using a QWERTY keyboard for each set of experiments, the key "q" was pressed whenever the insect of the first arena was present in the external area of the arena, while the key "1" was pressed when the insect entered the central area. Other keyboard key combinations for the remaining arenas were "w" and "2" (2nd arena), "e" and "3" (3rd arena), and so on.

The file entitled "list.csv" corresponds to the list of the individual insects that were tested at the different experimental treatments and control. The first column "experiment" reports the sequential number of each assay, and the numbering corresponds to the first column of the file "output.csv". The second column "arena" corresponds to the 12 arenas.

The third column "ind" indicates the sequential number of the tested *B. oleae* females. The fourth column "tr" describes the five different experimental treatments and the control.

The two files "output.csv" and "list.csv" are used by the file "R_script.html". This file contains the R code used to decipher the keys associated to the different behaviours and to conduct downstream explanatory data analysis (e.g., Figure 1) and regression analysis (e.g., Figure 2), which are fully reported in [29].

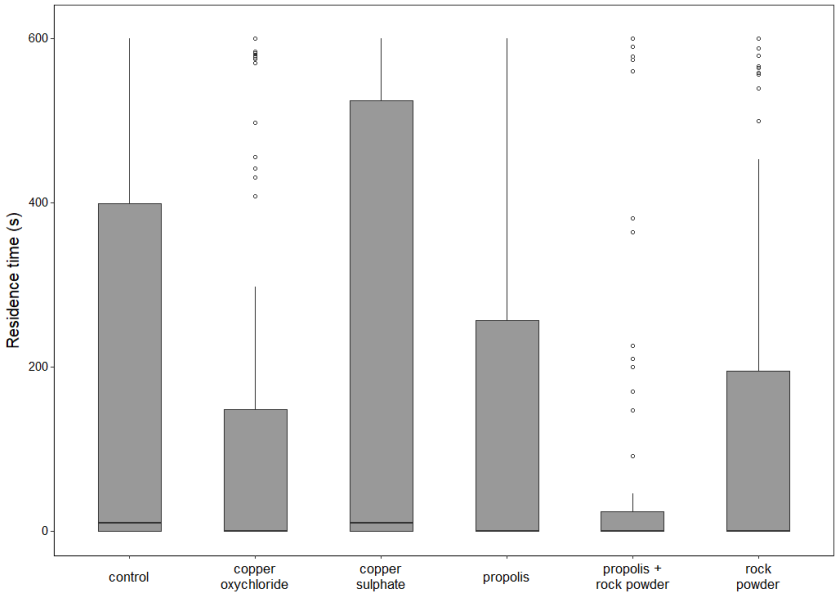

Figure 1. Box plot of residence time (s) of *Bactrocera oleae* in the central area of a Petri dish arena. Each arena contained an olive twig subjected to one of the following treatments: rock powder, propolis, a mixture of rock powder and propolis, copper oxychloride, copper sulphate. Control consisted of water.

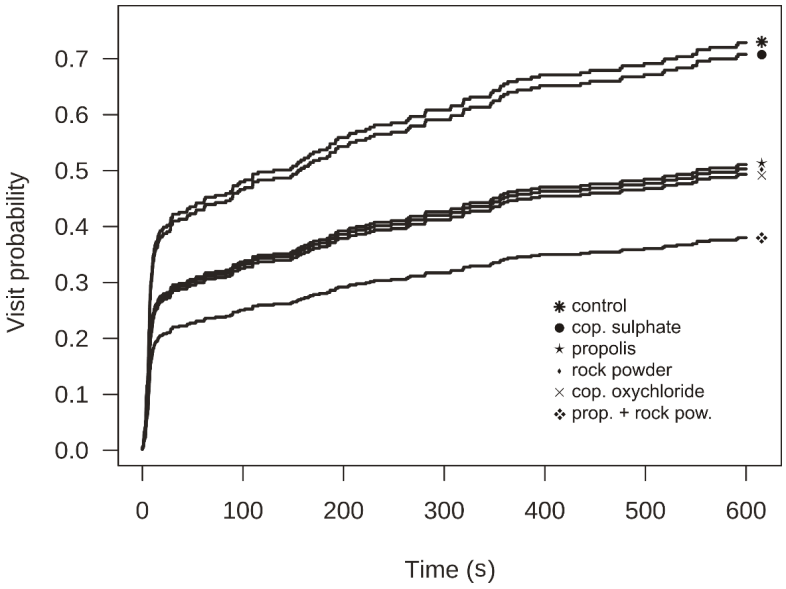

Figure 2. Cumulative *Bactrocera oleae* probability to visit the central area of a Petri dish arena containing a treated olive twig, as estimated by Cox proportional hazards model. Treatments consisted of rock powder, propolis, a mixture of rock powder and propolis, copper oxychloride, and copper sulphate. Control consisted of water. The legend and symbols were added using Corel Draw X3.

3. Methods

3.1. Insects, Olive Twigs Treatments, and Arena Preparation

Female of *B. oleae* for bioassays were obtained and maintained as described in [29]. Treatments consisted of rock powder (commercial name "Polvere di Roccia", CIFO Srl, San Giorgio di Piano, BO, Italy), propolis (commercial name "Propolis", CIFO Srl), a mixture of propolis and rock powder, copper oxychloride (commercial name "Cupravit Blu 35 WG", Bayer Cropscience S.r.l.), and copper sulphate (Poltiglia Caffaro 20 DF NEW-Sumitomo Chemical Italia s.r.l.). Details of the different product concentrations are reported in [29,33]. Water was used as the experimental control. Olive twigs of Frantoio cv. holding two fully developed and green olive fruits (average weight: 1.59 g $\pm$ 0.08) and a leaf were sprayed with 2 mL of each tested formulation using a 20 mL atomizer bottle (Shenzhen Jiawang Trading Co., Ltd., Shenzhen, China). Twigs were air-dried for 30 min. Preparation of experimental arena is detailed described in [29]. Each arena consisted of a Petri dish (15 cm diam $\times$ 1.5 cm) positioned upside-down on a filter paper. A treated olive twig was placed in the central part of the arena, that was delimited by a 5 cm diam. circle drawn using a fine-tip pencil.

3.2. Laboratory Bioassays and Data Analysis

For each arena, a *B. oleae* mated female was released below the Petri dish, in the external area of the arena. Females were allowed to acclimatize for two min. Hence, the observations conducted during this time frame were eventually trimmed from the final dataset (see "R_script.html" file, available at https://zenodo.org/badge/DOI/10.5281/zenodo.6695805.svg (accessed on 1 April 2022)). Each *B. oleae* was observed for additionally 10 min. Its behaviour was manually recorded using JWatcher 1.0, which logs the specific time at which keyboard keys are digited. In detail, two operators observed *B. oleae* and, using different couples of keys, continuously recorded whether each insect was inside or outside the central area of the arena. Twelve females were concurrently observed using 24 keyboard keys (see R script for coding). Ninety-two females were evaluated for each treatment. Bioassays were performed in a laboratory room kept at 25 °C ($\pm$1) and 55% R.H.

Through the R script provided, a structured dataset was eventually produced. The residence time, i.e., the time spent by each *B. oleae* female in the central area of the arena, was calculated and reported as box plot (Figure 1). Moreover, the time to visit, i.e., the time period from the start of the bioassay to the first entrance to the central area, was evaluated using the Cox proportional hazard model, and reported as cumulative visit probability [34,35]. The following packages were used: "knitr" [36], "ggplot2" [37], and "survival" [34].

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behavioural observations; "list.csv": details of the treatments used on the different experiments; "R_script.html": R script used to process raw data and downstream analyses.

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