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Particle Films Combined with Propolis Have Positive Effects in Reducing *Bactrocera oleae* Attacks on Olive Fruits

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Abstract: The olive fruit fly, *Bactrocera oleae*, is a major pest of olive trees in several areas of the world. Testing novel preventive methods against *B. oleae* infestations is paramount. The use of particle film in eluding *B. oleae* and avoiding oviposition is one of the main strategies adopted by olive growers; however, choices are often limited to kaolin. Under field conditions, we tested the efficacy of novel compounds, including particle films, for their effectiveness as oviposition deterrents against *B. oleae*. The trial was conducted from July to October 2021 in an olive orchard located in central Italy. One olive variety, Borgiona, was selected and sprayed with propolis, rock powder, kaolin, the mixture of propolis and rock powder, the mixture of propolis and kaolin and water (control). Laboratory analyses were conducted to study the effects of the treatments on the fruit maturity index. As per the field trial, the rock powder and propolis mixture caused a reduction of *B. oleae* infestation with respect to the control (water), similarly to kaolin. Moreover, the mixture of kaolin and propolis exhibited the best results among all treatments. When mixed with propolis, particle films showed higher protection from *B. oleae* than when applied alone, suggesting a synergistic effect, and demonstrating an interesting role of propolis as an adjuvant. No delay in fruit maturity was noticed. Our results indicate that the tested products have the potential to be incorporated into management programs of *B. oleae*, although possible side effects on olive physiology require additional investigations.

Keywords: *Olea europaea*; olive fruit fly; Tephritidae; preventive methods; particle film; deterrence

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1. Introduction

Cultivated olive *Olea europaea* L. (Oleaceae) is prone to many insect pests, resulting in economic losses of up to 15% of production [1]. One of the major threats to olive production is the olive fruit fly *Bactrocera oleae* Rossi (Diptera: Tephritidae), a pest of *Olea* spp. in many countries of Europe, Asia and Africa, accidentally introduced in North America as well (https://www.cabi.org/isc/datasheet/17689#todistribution (accessed on 27 January 2023)). Annual losses caused by the olive fruit fly account to more than USD 1 billion in the Mediterranean basin [2]. Considerable damage is caused at both quantitative and qualitative levels. When olive fruits reach the pit-hardening stage, mated *B. oleae* females start ovipositing in the olive mesocarp. The larva develops and feeds in the mesocarp [3,4], injuring the fruit and eventually leading to premature fruit drop or to a notable reduction in oil quality [5,6].

In the ongoing scenario of global warming, climate change may have a negative effect on olive pest dynamics [7], as well as an impact on pesticide use and pesticide behavior in the environment [8]. The increasing temperature, especially in autumn, could extend the oviposition window of *B. oleae* and lead to excessive yield loss [9]. Therefore, it is important to merge successful prediction models [10] with a wide range of control methods to adjust to such events, especially when it comes to programming control strategies [9].

Additionally, with the goal of reducing the side effects of insecticide-based pest control, the adoption of more sustainable practices and products is becoming mandatory. Recently, many neurotoxic insecticides (e.g., Imidacloprid) have been withdrawn from the market because of their lethal and sub-lethal effects against beneficial organisms [10,11]. In addition, dimethoate, a highly efficient cytotropic organophosphate used for decades against the larval stages of the olive fruit fly, is currently banned in the European Union (EU) because of its toxic effects (Commission Implementing Regulation (EU) 2019/1090 of 26 June 2019). Safer substances are now available, with particle-film technology being one of the few alternatives accepted in organic agriculture for managing *B. oleae* [12]. A common strategy of ovipositing females is to search for suitable hosts using antennal [13], tarsal [14] and visual receptors [15,16]. The oviposition decision depends on different chemoreceptors present on the antennae, the maxillary palps and the ovipositor [17,18]. Therefore, the presence of unsuitable tactile and olfactory stimuli could impact oviposition, as is generally the case of tephritids that depend on chemical stimuli for host location and acceptance [19–21]. Visual stimuli may also play a crucial role, as foliage color affects host location from a distance [14,22]. In this regard, white color is the least attractive to a female olive fruit fly [15]. Indeed, control measures also contemplate the disruption of fly attraction to olive using kaolin. Kaolin is a clay product capable of producing particle films when applied on surfaces, used as a deterrent to oviposition thanks to its reflective white coating on tree foliage [23]. Additionally, the adhesion of kaolin microscopic particles to the insect body affects movement, feeding and oviposition [24] and leads to rejection of the fruit as a suitable host [25]. However, other types of particle films are available for pest control [26].

The disruption of insect-microbe gut symbiosis through the use of antibacterials offers another opportunity for pest control [27]. The use of copper compounds and propolis affects adult fly fitness, fecundity and mortality as well as the presence of the *B. oleae* main symbiont *Candidatus* Erwinia dacicola (Enterobacteriaceae: Gammaproteobacteria) [28,29]. Propolis is a natural resinous substance collected and transformed by honeybees [30]. Accordingly, investigations on possible applications of propolis as a natural alternative for the control of olive flies are needed [29].

With the increasing efforts to achieve more sustainable and ecosystem-friendly agriculture, novel products are therefore necessary for more flexible control strategies and to overcome the restrictions on the use of many conventional products. In this study, we investigated the effects of a zeolite-based rock powder, kaolin, propolis, propolis + rock powder and propolis + kaolin on *B. oleae* infestations, and on fruit maturation.

2. Materials and Methods

2.1. Field Trial

The field trial was conducted from July to October 2021 in an olive grove located in Spello (Umbria, Italy; geographic coordinates 42°59′26′′ N 12°41′47′′ E). The area under investigation was approximatively 0.5 ha. Plants were 30 years old and belonged to the cultivar Borgiona. The volume of each plant was ~16 m^3, on average. The distance between olive trees was 5 m × 5 m. The minimum distance between the treated olive trees and the orchard edge was 20 m.

Six trees were randomly selected and, on each tree, six treatments were applied on six small branches, each bearing 14 to 25 healthy drupes. Replicates, i.e., branches, were randomly selected in such a way that all treatments featured in all cardinal directions in order to limit the effect of the branch position and the consecutive selectivity effect of the olive fly. Treatments consisted of zeolite-based rock powder (commercial name Polvere di Roccia, CIFO Srl, San Giorgio di Piano, BO, Italy) at 6 kg/ha (600 g/hl concentration), kaolin (Caolino 100%, Biogard) at a dose of 40 kg/ha (4 kg/hl), propolis (commercial name Propoli, CIFO Srl) at a dose of 3 L/ha (300 mL/hl), a mixture of propolis plus rock powder (3 L/ha + 6 kg/ha, respectively), a mixture of propolis plus kaolin (3 L/ha + 40 kg/ha, respectively) and water as control. Using a hand sprayer (2 L capacity), each treatment was sprayed on each replicate until drip to ensure complete coverage. All commercial products were used at dosages within the ranges officially reported on their labels. Rock powder and kaolin were applied as water suspensions. Rock powder is <20 μm (content: 70% chabasite, 2% phillipsite, 5% K-feldspar, 2% biotite, 3% pyroxene and 18% volcanic glass). Propolis is an extract of honeybee propolis (1.2% w/w) in linseed oil solution. During the season, two treatments were applied. The first treatment (29 July) was carried out in correspondence with initial captures of *B. oleae* in pheromone traps, when no olives were attacked yet. The second treatment (20 September) was applied after a period of intense rain episodes that washed away the products from the leaves. Two pheromone traps (Certis, Italy) were positioned at about 80 m distance, covering about 0.5 ha of orchard. Traps were monitored on a weekly basis. Pheromonal lures were replaced every month, according to the manufacturer's instructions. As per the attack evaluation, for each survey, oviposition punctures were recorded at different times (i.e., on 29 July; 19 and 26 August; 2, 13, and 30 September; 14 October 2021) by careful inspection in the field of all olives belonging to treated and control branches [31]. Olives were not detached from branches.

2.2. Physiological Tests in the Laboratory

During the first week of November, olive fruits deriving from all the replicates were separately collected, weighed, assessed for infestation and subjected to further laboratory analyses, as explained below. We visually analyzed the pigmentation index (Jaen index) or maturity index (MI), which refers to the change in fruit color that occurs during ripening [32]. Olive fruits were separated into 8 classes based on the degrees of color achieved (from 0 to 7: with 0 for green olives and 7 for olives with pigmentation of 100% of the epicarp and 100% of the mesocarp). The MI was calculated using the following formula [32]:

$$\sum_{i=0}^{7}(i \times ni)/N$$

where i is the class number, ni is the number of olives belonging to the class and N is the total number of olive fruits in the sample. The classification of olives in classes was based on [33,34]. The Maturity Index was calculated from six replications.

2.3. Statistical Analysis

For field data, the effect of treatments on the percentage of *B. oleae* attacked fruits was evaluated by means of a three-parameter nonlinear mixed effects model (function "nlme" in package "nlme" [35], R environment [36]. Starting values were derived using "medrm" function in the "medrc" package [37]). To account for repeated observations across space (multiple treatments conducted on the same plant) and time (repeated observations on the same branches), the plant identity effect was included as a random term for the inflection point parameter [38,39]. Different variances for treatment levels were allowed to account for heteroscedasticity among treatment levels (function "varIdent" in "nlme" package, [35,39], as confirmed by significant likelihood ratio test (LRT) of comparisons of two nested models (i.e., with and without specific variances for all the treatment levels) [39,40]. Models were validated by graphical inspection of residuals.

The total number of olives in a branch at a given sampling date was sometimes lower than at the previous survey. This was because, often, fruits drop during the season as a consequence of factors such as *B. oleae* attack [3,6,41,42], but other causes cannot be disregarded. Hence, dropped fruits were not counted in the analysis. LRT was used to evaluate differences between treatment levels [43].

The effect of the different treatments on the maturation index was analyzed by means of linear model. The difference of each treatment against control was evaluated by Tukey method for multiple comparisons (function "emmeans" in package "emmeans") [44].

3. Results

Field Experiment and Maturity Index

The percentage of olives attacked by *B. oleae*, i.e., showing at least one oviposition puncture, differed among experimental treatments and control (Figure 1, Table S1). At the end of the trial, two different groups were clearly distinguishable (LRT = 42.01, d.f. = 12, $p < 0.0001$). One group consisted of control, propolis and rock powder and exhibited a final attack above 20%. Olives in the control group exhibited a similar attack to those treated with propolis (LRT = 0.28, d.f. = 3, $p = 0.963$), but a higher attack compared to those treated with rock powder (LRT = 8.50, d.f. = 3, $p = 0.037$). Attacks on olives treated with these two latter treatments were similar (LRT = 6.61, d.f. = 3, $p = 0.085$). The second group consisted of kaolin, rock powder and propolis mixture or kaolin and propolis mixture and exhibited a final attack lower than 10%. The kaolin and propolis mixture was the most effective in reducing *B. oleae* oviposition, compared to the kaolin alone (LRT = 9.48, d.f. = 3, $p = 0.024$) or rock powder and propolis mixture (LRT = 22.92, d.f. = 3, $p < 0.0001$). Attacks on branches where these two latter treatments were applied did not differ (LRT = 5.16, d.f. = 3, $p = 0.161$).

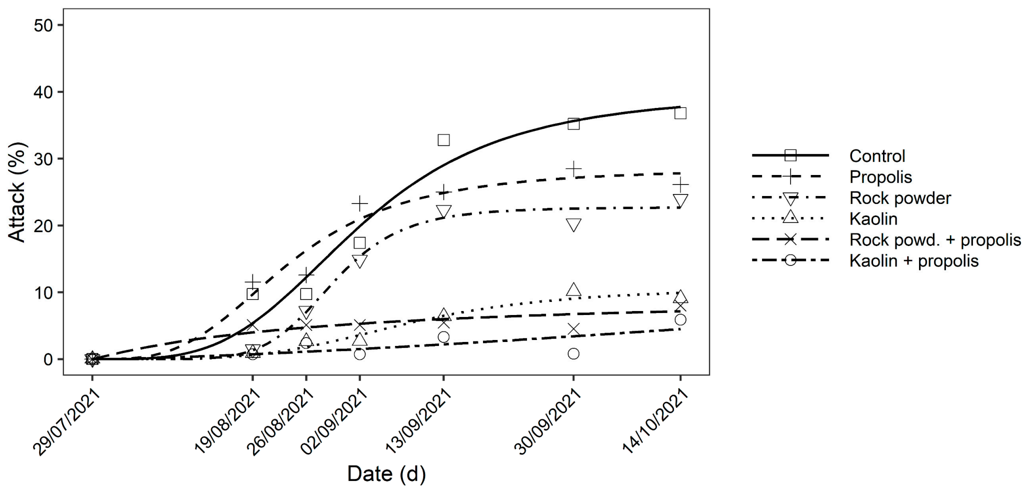

Figure 1. Seasonal trends of olive fruits of the cultivar Borgiona attacked by *Bactrocera oleae* in treated and control branches. Symbols represent observed mean percentage of raw data. Lines represent predictions from the fitted model. Data were analyzed by means of three-parameter nonlinear mixed effects model. Likelihood ratio test was used to evaluate differences among treatments and control ($p \leq 0.05$). Significant differences were detected among curves, except for control vs. propolis and for kaolin vs. rock powder and propolis mixture.

Considering the effect of treatments on fruit maturation, the maturity index was similar for the different treatments (Table 1).

Table 1. Effect of treatments on maturity index of olives of the cultivar Borgiona (all mean ± SE). No differences were detected among treatments according to linear model followed by multiple comparisons procedure.

Treatments	Maturity Index (0–7)
control	2.05 ± 0.239
propolis	1.84 ± 0.212
rock powder	1.81 ± 0.248
kaolin	1.62 ± 0.244
rock powder + propolis	1.53 ± 0.138
kaolin + propolis	1.30 ± 0.049

4. Discussion

In the attempt to grant a sustainable management of olive orchard infestations, prevention is mostly needed over curative solutions [45]. In this study, we tested the efficacy of two environmentally low-impact commercial products, propolis and rock powder, in preventing *B. oleae* oviposition in the field and evaluated possible side effects on fruit maturation.

Despite being limited to only one year of observations, the results demonstrated the efficacy of the rock powder and propolis mixture in reducing *B. oleae* infestation throughout the experiment, with respect to control. These results confirm those reported in a previous study [46]. In laboratory conditions, both propolis and rock powder and their mixture were effective in reducing *B. oleae* attacks, while in open field conditions the most effective control was provided by the mixture [46].

In addition, in the present study, it was seen that the rock powder and propolis mixture had an efficacy similar to that of kaolin. Two major types of particle films, used in crop protection, can be distinguished: kaolin-based and zeolite-based. The rock powder we used is derived from mechanical grinding of volcanic rock and composed of 70% of chabazite (a mineral of the zeolite group) (https://www.cifo.it/prodotto/agricoltura-professionale/polvere-di-roccia-3/ (accessed on 27 January 2023)). Chabazite zeolite is becoming attractive for crop protection, although studies on its effect on olive fly are scarce [26,47]. According to Rotondi et al. [26], both zeolite and kaolin sprays could significantly reduce the olive fly infestation, which is consistent with the results obtained in this study. Therefore, according to the results obtained both in this study and in Daher et al. [46], zeolitic rock powder is a potential alternative product to kaolin.

Another important aspect is the role of propolis. Kaolin and rock powder, when mixed with propolis, performed better than when applied alone. Although propolis did not show efficacy when applied alone in the field, these results, together with those of Daher et al. [46], point to an interesting potential of this product as an adjuvant that enhances the efficacy of particle films for foliar applications. Based on observations in the field, propolis could have provided a longer persistence of rock powder and kaolin on plants, which only allowed two treatment applications throughout the season. We do not know if the effect of the two products is merely additive or there could be a possible synergistic relation between propolis and particle films, perhaps due to propolis's potential role as an antimicrobial [29,48]. In this case, the mixture of rock powder and propolis could act both as a deterrent for the olive fly and as a symbioticide against *Candidatus* E. dacicola [28], a crucial endosymbiont for larval development of *B. oleae* in olives [27]. Therefore, propolis, with its similar properties with copper and which has been used for decades against the olive fly [29], might represent a promising substitute. However, more studies are necessary to better investigate the mechanisms and potential use of propolis in control strategies.

The kaolin and propolis mixture presented the best result with respect to all treatments and control. This is not surprising, since kaolin is largely known for its efficacy against *B. oleae* [49–51], but the higher efficacy obtained when mixed with propolis is of high interest for *B. oleae* management, and its integration in crop protection might also help in increasing the interval between treatment applications and delaying the first insecticide sprays, if any. When considering practical field applications in olive orchards, the inclusion of propolis in the particle film mixture may result in additional costs for farmers. Therefore, future field trials should focus on determining the minimum effective dosage of propolis per hectare when combined with rock powder or kaolin.

Another aspect investigated in this study is the effect of kaolin, rock powder, propolis and the mixtures on olive fruit maturation. Several studies have tackled the positive role that kaolin plays in mitigating abiotic stress such as heat and water stress [52–54]. Furthermore, zeolite-based compounds were reported to have similar effects [26]. From another perspective, foliar applications of kaolin showed negative effects on the physiology of the plant, such as decreasing photosynthesis and stomatal conductance compared with zeolite [26]. According to our findings, no delay in maturation of Borgiona fruits was noticed. Saour and Makee [55] found kaolin to increase the maturation index with respect to untreated trees of "Zeity" cultivar. Further studies are needed to better clarify the impact of kaolin and chabazite powders on olive fruit physiology.

Non-target effects of kaolin-based clays on beneficial insects have sometimes been reported [49,56,57]. Hence, further investigations should be conducted on selected invertebrates occurring in olive plantations (e.g., predatory Coccinellidae and Carabidae, [58,59]) to verify whether noxious effects are likely to occur.

To conclude, in the current field study we show the efficacy of a novel particle film, rock powder, in reducing *B. oleae* infestation in the field, thus acting as a successful deterrent to oviposition. The use of zeolite is increasing in agriculture and, thanks to its properties, it could be an alternative to kaolin [26], consequently requiring further research in the context of olive protection. Additionally, this study highlighted the role of propolis as an adjuvant to foliar particle films. Consequently, both rock powder and propolis are advisable in terms of crop protection agents against *B. oleae*, although possible side effects on olive maturation and on beneficial organisms and biological control need further investigation. Their exploitation could enhance the flexibility and adaptability of control strategies to face the upcoming challenges.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9030397/s1, Table S1: Summary of the statistics associated to a nonlinear mixed effects model for evaluating the effect of control ("intercept") and five different treatments ("treatment") on *Bactrocera oleae* attacks.

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