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Ground cover management affects parasitism of *Prays oleae* (Bernard)

María Villa [a,b], Sónia A.P. Santos [a], António Mexia [b], Albino Bento [a], José Alberto Pereira [a,*]

[a] School of Agriculture, Polytechnic Institute of Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[b] School of Agriculture, Lisbon University, Tapada da Ajuda, 1349-017 Lisbon, Portugal

HIGHLIGHTS

- Ground cover management did not influence the emergence rate of *Prays oleae*.
- Spontaneous ground covers favored the overall parasitism and *Ageniaspis fuscicollis*.
- Herbicide application negatively affected the overall parasitism and *A. fuscicollis*.
- *Elasmus flabellatus* was not affected by the ground cover management.
- Surrounding vegetation areas may be important for maintaining parasitoids in the olive grove.

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ABSTRACT

Spontaneous ground covers comprise ecological infrastructures that may provide food, alternative hosts and shelter for parasitoids in olive groves, thus contributing to biological control of pests. This study investigated the effects of herbicide application, tillage, and conservation of spontaneous ground covers on parasitism of the anthophagous generation of the olive moth, *Prays oleae* (Bernard). The study was performed in northeast Portugal in 2011 and 2013 in 14 and 15 olive groves, respectively, with different management types. Generalized Estimating Equations (GEE) were used to analyze olive moth emergence, overall parasitism rate, relative abundance of parasitoid species, and total parasitism of olive moth larvae. *Ageniaspis fuscicollis* (Dalman) accounted for the majority of the parasitism, followed by *Elasmus flabellatus* (Fonscolombe). In both years, ground cover management type did not influence the emergence rate of *P. oleae*. However, overall parasitism rate, emergence of *A. fuscicollis*, and the number of *A. fuscicollis* emerging per olive moth larvae varied among years. In 2011, the latter response variables were significantly higher in groves with spontaneous ground cover than in those treated with herbicide, indicating a negative effect of herbicides on parasitoids. Although tilled groves obtained higher values for these variables in 2013, parasitism rates were generally very low. In sum, the management of ground covers seemed to influence the overall rate of *P. oleae* parasitism in some years, but longer-term experiments are needed to clarify this trend.

1. Introduction

Habitat management through the establishment and maintenance of ecological infrastructure, such as diversified ground cover, is a strategy of conservation biological control that aims to conserve or manipulate the environment in order to enhance the effectiveness of natural enemies (Landis et al., 2000; Boller et al., 2004). Because nectar and pollen are essential food for many adult parasitoids (Jervis et al., 1993; Vattala et al., 2006), flowers can promote the abundance and longevity of parasitoids as well as increase parasitism rates (Díaz et al., 2012). However, apart from providing shelter and alternative hosts for generalist parasitoids (Landis et al., 2000), flowers may also benefit pests (Baggen and Gurr, 1998; Lavandero et al., 2006).

Olive groves have relevant economic, social and landscape importance in the Mediterranean area and the olive moth, *Prays oleae* (Bernard) (Lepidoptera: Praydidae), is one of the most important olive pests. *P. oleae* has three generations per year: the phyllophagous generation feeds on olive leaves from October to April, the anthophagous generation feeds on floral buttons from April to June, and the carpophagous generation penetrates the fruit and feeds on the stone from June to October. The carpophagous generation causes the most damage to the crop (Bento et al., 2001). Several generalist and specialist parasitoid wasps, such as *Ageniaspis fuscicollis* (Dalman) (Hymenoptera: Encyrtidae),

* Corresponding author.
E-mail address: jpereira@ipb.pt (J.A. Pereira).

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Chelonus elaeaphilus Silvestri (Hymenoptera: Braconidae) and *Elasmus flabellatus* (Fonscolombe) (Hymenoptera: Eulophidae), attack the olive moth (Bento et al., 1998; Herz et al., 2005).

In perennial agroecosystems, spontaneous vegetation can be removed through either tillage or herbicide application. Soil erosion and pollution are two consequences of these practices that could influence parasitoid communities (Vanwalleghem et al., 2011; Egan et al., 2014). Previous studies in olive groves showed that spiders, parasitoids and the predatory heteropteran *Deraeocoris punctum* (Rambur) were positively influenced by ground covers when compared with tilled groves (Lousão et al., 2007; Herz et al., 2005; Cárdenas et al., 2012; Rodríguez et al., 2012; Paredes et al., 2013a). However, results obtained for olive pests were inconsistent. Paredes et al. (2013b) found that areas of herbaceous and woody vegetation near olive crops, and smaller patches of woody vegetation within olive groves, were associated with reduced abundance of two olive pests, *P. oleae* and *Euphyllura olivina* (Costa), but inter-row ground covers had no effect on these pests. A long term analysis at a regional scale performed by Paredes et al. (2015) showed that ground covers did not influence the abundance of *Bactrocera oleae* (Rossi), *P. oleae*, *E. olivina* and *Saissetia oleae* (Olivier). Both local factors, such as the intensity of pesticide application or micro-climatic features, and larger-scale factors, such as landscape diversity or patch size, can affect pest abundance in olive groves (Rodríguez et al., 2009; Boccaccio and Petacchi, 2009; Ortega and Pascual, 2014).

From a sustainability perspective, studies are needed to establish the management practices that most favor the biological control of pests. The objective of the present study was to determine the effect of different management practices (conservation of spontaneous ground cover, tillage, or herbicide application) on the parasitoid species emerging from *P. oleae*. In particular, we hypothesized that farming practices would influence: (i) olive moth emergence rate (ii) parasitoid community composition and (iii) the overall rate of parasitism.

2. Material and methods

2.1. Study sites and sampling design

The studied groves were located in Bragança District in northeastern Portugal (Fig. 1). Fifteen groves with different ground cover management practices were selected in 2011 (six tilled olive groves, five with spontaneous ground cover, and four with herbicide application) and 14 were selected in 2013 (five tilled groves, five with spontaneous ground cover, and four with herbicide application). A heterogeneous distribution of the plots according to the different management practices was used as criteria when choosing the groves to avoid spatial clustering of management types and thus results that might be more related to grove proximity than management practices. The minimum distance among plots was 300 m (from the center of the grove) and the maximum was 65 km. The mean area of these groves was about 2 ha; none were irrigated and no insecticides were applied during the anthophagous generation of the olive moth. According to farmers' information, 2 l/ha of the herbicide glyphosate (Roundup Ultra®, Bayer, aqueous solution with 360 g/l of glyphosate) was sprayed in the plantation row, in herbicide treatment groves, at the end of April. The distance between trees varied from seven to nine meters and the age of trees varied from 18 to 80 years. In 2012, sampling was not possible due to low population levels of olive moth region-wide, probably caused by extreme drought and abnormally high temperatures during the anthophagous generation.

To ensure a heterogeneous distribution of samples within each grove, 10 olive trees were randomly selected at the end of May and 20 olive moth larvae were hand-collected from each tree at a height of 1.5–1.7 m by walking around the tree canopy, for a total of 200 larvae from each grove. In the laboratory, larvae were isolated in plastic tubes (6.0 cm height × 1.0 cm in diameter) and held in a climatic-controlled chamber set to 21 °C and a 16:8 (L:D) day length until emergence. Adult olive moth and parasitoid emergence in each tube was recorded, as well as dead/non-emerged larvae. Parasitoids were identified to species and sexed.

2.2. Data analyses

Since the larvae within each grove probably experienced similar conditions, the values obtained with groves are not assumed to be independent, i.e., spatial autocorrelation exists between these samples (see Zuur et al., 2009). One method available for dealing with such interdependency among samples is the Generalized Estimating Equation (GEE). An advantage of GEEs is that they can cope with misspecifications of the entire distribution and require only the main structure. Thus, correct inferences about regression coefficients are possible even if variances and correlations are erroneously specified (Ziegler and Vens, 2010). In the present study, GEEs were used to analyze the data after model validation. The explanatory variable, X_{is}, was ground cover management with three levels: tillage (T), groves with spontaneous ground cover (S) and groves treated with herbicides (H). Binary response variables were adult moth emergence, overall parasitoid emergence, and most abundant parasitoid species, with values of 1 for success and 0 for failure. The variance structure was of binomial type and the relationship between the conditional mean and the systematic component was logit link, therefore,

$$E(Y_{is}|X_{is}) = e^{\alpha+\beta 1Xis}/1 + e^{\alpha+\beta 1Xis}$$

or

$$E(Y_{is}|X_{is}) = \pi_{is} \text{ and var } (Y_{is}|X_{is}) = \pi_{is} \times (1 - \pi_{is}),$$

where Y_{is} the value of response variable where i = 1,...,200 larvae and s the grove and π_{is} the probability of success of the response variable (Zuur et al., 2009). Exchangeable correlation structure was used because correlation between two observations from the same grove is expected. The scale parameter was fixed to 1 because binary data cannot be overdispersed.

Because the numbers of parasitoids emerging from moth larvae (separately analyzed for the most abundant parasitoid species) are count data, the variance structure was Poisson type and the relationship between the conditional mean and the systematic component was log link, therefore,

$$E(Y_{is}|X_{is}) = e^{\alpha+\beta 1Xis}$$

or

$$E(Y_{is}|X_{is}) = \mu_{is} \text{ and var}(Y_{is}|X_{is}) = \phi \times v(\mu_{is})$$

where v() is the variance function and ϕ the scale parameter. Exchangeable correlation structure also was used in this case.

Data analyses were performed using the *geeglm* function from "*geepack*" package (Højsgaard et al., 2006) in R software (R Core Team, 2014) and the *anova* function from "*stats*" package was applied to test for differences between management treatments, followed by pairwise comparison with the *lsmeans* function from "*lsmeans*" package (Lenth and Hervé, 2015).

Model validation for binary dependent variables was performed using the heat map plot and heat map statistics in the "*heatmapFit*" package (Esarey et al., 2014). In the heat map plot, predicted probabilities are plotted versus within-sample empirical frequencies (obtained by nonparametric smoothing) and a heat map line is drawn. Then one-tailed p-value is obtained by comparing the original heat map line with its parametrically bootstrapped