

Are surrounding patches barriers for *Prays oleae* (Bernard) in the olive agroecosystem?

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ABSTRACT

The increasing interest in organic products leads to a need of finding strategies for pest control free of pesticides. Conservation biological control, through habitat management, seeks to manage the environment in order to achieve pest reductions. In this context, the surrounding habitats of agroecosystems can be management in order to impede pest dispersion and for that is crucial to understand how landscape connectivity affects pest's species. Thus, we studied the capability of *Prays oleae* (Bernard) to disperse through non-crop patches composed by woody and herbaceous vegetation. For that the flight activity of *P. oleae* was monitored in olive groves, and surrounding scrubland and herbaceous patches from the end of March to December of 2012 and 2013. Generalized Linear Models were used to analyze the abundance of *P. oleae* in the different patches and locations. *P. oleae* captures varied between years, likely because of a strong relation with the weather conditions. For the first time, landscape connectivity aspects were identified for *P. oleae*, being that was clearly able to disperse over scrublands and herbaceous patches. This study provides new data that contributes to the knowledge about *P. oleae* dynamic under adverse weather conditions and discloses new queries about the *P. oleae* dispersion and movement between patches.

KEYWORDS: *olive grove, olive moth, connectivity, conservation biological control, scrubland, herbaceous patches*

INTRODUCTION

Organic production has increased from 11 million hectares in the year 1999 to 43.1 million hectares in the year 2013, and the organic market size from 15.1 billion euro in 1999 to 54 billion euro in 2013 (IFOAM, 2014). Therefore, the organic products consumption, free of synthetic pesticides, is increasing. However, conventional agriculture uses pesticides for pest control. This makes necessary to find alternative strategies for a sustainable agriculture. The biological control is one of the alternatives and is applied through several approaches, among them the conservation biological control (CBC). CBC is defined as *the modification of the environment or existing practices to protect and enhance specific natural enemies of other organisms to reduce the effect of*

pest (Eilenberg et al. 2001) and is accomplished by: (i) reducing the use of pesticides (Gurr et al. 2002); (ii) habitat manipulation to create ecological infrastructures that provide resources to natural enemies and enhance their performance and effectiveness (Gurr et al. 2002; Landis et al. 2000). The ecological infrastructures can be located outside or inside of the crops (Boller et al. 2004) and can be constituted by anything that support the pest reduction. However, crop and non-crop habitat may contain characteristics that reduce but also that enhance pest and studies about proper management strategies are crucial for sustainable agriculture.

The landscape connectivity has been defined as “the degree to which the landscape facilitates or impede movements among resource patches” (Taylor et al. 1993) and constitute an interesting characteristic that may be managed to improve conservation biological control. Tischendorf et al. (2000) suggested that species habitat, scale at which the species response to landscape structure, how the species responds to the different elements of a landscape (including movement pattern, mortality risk on landscape elements and reaction to boundaries) must be analyzed to determine landscape is facilitating or impeding movement among resource patches. However, Mitchell et al. 2013 detected that few studies directly measure organism movement.

The olive tree (*Olea europaea* L.) is a widespread crop in Mediterranean areas that is cultivated in 42 countries (FAOSTAT, 2014) and has an important social-economic and landscape impact, but pests can cause significant losses and reduce profits of the growers (Arambourg 1986; Ramos et al. 1998). In Tras-os-Montes region (northeast of Portugal), the olive moth *Prays oleae* (Bernard), is one of the most important pests of the olive tree (Bento et al. 2001). It has three generations a year and their larval stages feed on different organs of the olive tree. Eggs of the anthrophagous generation are laid on floral buds and, after hatching, larvae feed on the flowers. The flight period of adults occurs at the end of spring, laying the eggs on the olive calyx and larvae of the carpophagous generation, bore into the olive stone

and feed on the seed. At the end of summer and beginning of autumn, adults emerge and lay the eggs of the phyllophagous generation on the olive leaves. Larvae of the phyllophagous generation dig galleries and feed on leaves, where they overwinter till the beginning of spring (Arambourg, 1986).

Adults of *P. oleae* from an olive grove are likely to disperse to other olive groves. However, they may feed on non-crop resources that can be provided by the occurring vegetation in the agricultural area and their surroundings that, in addition, may be used as shelter and they must cross non-crop patches in order to disperse to other olive orchards. However despite the importance of *P. oleae* as olive pest, few studies addressed the effect of the olive crop surroundings on its abundance and to our knowledge any study addressed dispersion traits of *P. oleae* such as potential barriers for dispersion. Paredes et al. (2013b), in a study about the effect of non-crop vegetation on the olive psyllid (*Euphyllura olivina*) and *P. oleae*, found that areas of herbaceous vegetation and areas of woody vegetation near olive crops, and smaller patches of woody vegetation within the olive groves, decreased pest abundance.

In this context, the goal of this study was (i) to determine the capability of *P. oleae* to disperse through non-crop patches composed by woody and herbaceous vegetation, i.e., if woody and herbaceous patches surrounding olive orchard would impede *P. oleae* movement, and (ii) to discuss potential implications for biological control.

MATERIALS AND METHODS

Study areas

The study was conducted in Mirandela municipality (northeastern Portugal), during 2012 and 2013 in three olive groves from different localities (Cedães: 41°29'16" N, -7°07'34" W, Paradela: 41°32'8"N, -7°07'29"W, and Guribanos: 41°34'12" N, -7°09'59" W) and two surrounding habitat type (a herbaceous vegetation patch and a scrubland) next to each olive grove (Figure 1). During the experimental years, the olive groves were not tilled and were not sprayed with pesticides. Scrubland patches were composed by three vegetation strata: herbaceous, shrub and tree strata derived from agriculture abandonment. Herbaceous vegetation patches were composed by cereal or grass mixture for livestock food. The areas of the three olive groves were about 2 ha and the surrounding patches (scrubland and herbaceous) 1 ha. The land uses selection (olive groves, scrubland and herbaceous) was based on the most frequent field types occurring in the region.

Experimental design

The flight activity of *P. oleae* in different type of land uses (olive grove, scrubland and herbaceous) and in three locations (Cedães, Guribanos and Paradela) was monitored from the end of March to December of 2012 and 2013. For that, five Delta traps were installed in each olive grove, scrubland and herbaceous patch and separated about 50 m from each other. The delta traps were baited with *P. oleae* sex pheromone ((Z)-7-tetradecenal (Biosani, Palmela, Portugal) in order to simulate pheromones produced by females in the same or in different olive groves. In olive groves and scrublands the traps were hung on trees (at about 2 m height) and in the herbaceous vegetation patches were hung on a T-structure made of wood (at 70 cm height). Captures were recorded on a weekly basis.

Data analyses

Generalized Linear Models (GLMs) were used to analyze the abundance of *P. oleae* in the different patches (olive groves, scrublands and herbaceous vegetation) and locations (Guribanos, Paradela and Cedães). Cumulated counts of *P. oleae* were modeled separately for each generation. The negative binomial distribution was applied for the response variable (abundance of *P. oleae*) to account with overdispersion and *glm.nb* function from the "MASS" package was used (Venables and Ripley, 2002). The Log-link was used between the expected value of the response variable and the systematic part of the model. Overall differences among main effects and interactions were checked using the likelihood-ratio chi square test with *Anova* function from the "car" package (Fox and Weisberg, 2011). Significant differences among interactions were checked using function *testInteractions* from the package "phia" (de Rosario-Martinez, 2015). Bonferroni p-value adjustment was applied. In the cases that mean effects were analyzed (following the "marginality principle", main effects should not be analyzed when non-null-interactions stand out) (de Rosario-Martinez, 2015), a Tukey test for *post hoc* analysis was carried out to detect the differences between treatments using the *glht* function from the "multcomp" package (Hothorn et al. 2008). The models were validated plotting the residuals versus fitted values to assess the homogeneity of the variance and a plot of the residuals versus each covariate in the model and not in the model was used to investigate model misfit (Zuur et al. 2009).

RESULTS

The olive groves were the patches with the highest number of captures in 2012. The herbaceous patches and scrublands in all locations presented low

numbers of captures, barely exceeding 10 individuals per trap. Due to the low captures, the data of 2012 were not statistically analyzed. The olive grove located in Cedães was the patch with the highest number of captures followed by groves in Paradela and Guribanés. In olive groves, the first adults of the phyllophagous generation were captured during April showing a peak in mid-May. Captures of the individuals of the anthophagous generation increased during June with a peak at the end of that month. The number of individuals of the carpophagous generation was very low in all patches.

In 2013, the low number of captures in all patches (zero in most of the sample dates) during the phyllophagous generation did not allow its statistical analysis. During the anthophagous and carpophagous generations, *P. oleae* adults were captured in all patches. First individuals of the anthophagous generation were captured at the beginning of June and reached a peak at the beginning of July in all patches. The individuals of the carpophagous generation started to appear at the end of September and reached a peak at the beginning of October.

Regarding to the captures of the anthophagous generation in 2013, the likelihood ratio test showed that the interaction between environment and location ($\chi^2 = 13.709$, $df = 4$, $P = 0.008$) significantly influenced the abundance of the anthophagous generation of *P. oleae*. The difference found between the number of captures in patches with herbaceous vegetation and scrubland were significantly higher in Paradela than in Guribanés, being in both cases lower in herbaceous patches than in scrublands ($\chi^2 = 9.386$, $df = 1$, $P = 0.020$), but the differences found among environments in the other locations did not significantly differ ($\chi^2 < 6.760$, $df = 1$, $P > 0.084$ in all cases) (Figure 2). Given that the significance of the interaction was due to the significance of only one level of the interaction and that the p-value was not extremely low (0.02), the main effects were also analyzed: the likelihood ratio test showed that the environment ($\chi^2 = 76.999$, $df = 2$, $P < 2.2e-16$) significantly influenced the abundance of the anthophagous generation of *P. oleae* but the location did not ($\chi^2 = 1.138$, $df = 2$, $P = 0.566$). The Tukey test for *post hoc* analysis showed that the captures of the anthophagous generation were significantly higher in olive groves, followed by scrublands and herbaceous vegetation patches.

With regard to the carpophagous generation in 2013 (Figure 2), the likelihood ratio test indicated the interaction between environment and location significantly affected its abundance ($\chi^2 = 32.555$, $df = 4$, $P = 1.473e-06$). The differences between captures were significant: (i) between patches with herbaceous vegetation and with olive groves being significantly

higher in Guribanés than in Cedães ($\chi^2 = 16.329$, $df = 1$, $P = 4.792e-04$) and Paradela ($\chi^2 = 8.071$, $df = 1$, $P = 0.040$) and in all locations higher in olive groves; (ii) between olive orchards and scrublands, being significantly higher in Paradela than in Cedães ($\chi^2 = 17.407$, $df = 1$, $P = 2.716e-4$) and in both locations higher in olive groves; and (iii) between patches with herbaceous vegetation and scrublands, being significantly higher in Guribanés than in Paradela ($\chi^2 = 17.089$, $df = 1$, $P = 3.210e-4$), and in both cases higher in scrublands. The differences found among environments between the other locations were not significant ($\chi^2 < 6.471$, $df = 1$, $P > 0.098$ in all cases). In this case the main effects were not statistically analyzed, because due to the significance of the interaction would be meaningless.

DISCUSSION

Prays oleae captures observed in this study showed some differences in relation to other works (Ramos et al. 1989; Pereira et al. 2004), with a general low number of captures in both years and a nearly absence of the carpophagous generation in 2012 and phyllophagous in 2013.

Weather annual variations strongly affect *P. oleae* dynamics (Gonzales et al. 2015) and accordingly to the Portuguese Sea and Atmosphere Institute, I. P. (IPMA, 2012), during winter, spring and summer 2012, an extreme drought situation ravaged Portugal mainland. The winter was the driest since the first records in 1931, and in February, Mirandela registered 28 days with minimum temperatures equal or lower than 0. During the spring, the drought remained (a bit softened by some precipitation in May) and temperatures in the spring and the summer were higher than the mean. At the end of the autumn the drought was finished in almost all the northern locals. A decrease of *P. oleae* larvae growth has been described under unfavorable weather conditions (Tzanakakis, 2003 and references therein). Moreover, low temperatures in winter increased the mortality of *P. oleae* larvae (Ramos et al. 1978; Kumral, 2005) and high temperature and low relative humidity during the anthophagous and carpophagous generation caused high mortality of eggs and larvae (Civantos, 1998). Also low temperatures would affect to eggs of the phyllophagous generation in autumn (Montiel Bueno, 1981). Therefore, in this study the extreme weather conditions observed in 2012 could have led to an increase of *P. oleae* mortality and/or the larvae dormancy, resulting in the low the number of anthophagous and phyllophagous captures and the nearly absence captures of carpophagous adults. The 2012/2013 winter was colder and drier than the mean (IPMA, 2013), and this together with the

extreme conditions of the previous year probably could have weakened *P. oleae*, leading to the almost absence of phyllophagous generation in 2013. The spring 2013 registered the highest precipitations in the last 50 years, probably causing the observed recovery of *P. oleae* populations. The weather conditions in the summer and autumn 2013 remained close to normal values (IPMA, 2013). In agreement with our results, the high variability in the response of *P. oleae* to the surrounding vegetation was attributed to climatic variability between years (Paredes et al. 2013b).

Results obtained in 2013 indicated that scrubland and, in less degree, herbaceous patches did not act as barriers to the movement of *P. oleae*. Our results suggest that scrublands and herbaceous patches would not affect landscape connectivity for *P. oleae*, i.e., would not impede movements among resource patches, especially during the anthophagous generation, when the most suitable weather conditions occur and *P. oleae* is more likely to disperse. During the carpophagous generation the results depended on the location, suggesting that other factors related to each site characteristics could be involved. However, the capability of *P. oleae* to penetrate non-olive grove patches is positive, negative or null for its dispersion toward other olive groves needs to be clarified since also mortality risk on landscape elements (patches) is an important aspect to be addressed (Tischendorf et al., 2000). *P. oleae* was probably attracted to non-crop patches by the pheromones that potentially and naturally would penetrate non-crop patches from other olive groves. Then, different processes could take place in the non-crop habitats: the vegetation can affect pests directly through different processes such as the disruption of capability to locate suitable host plants, repelling or trapping pests, blocking movements or altering volatile profile of crop plants (Gurr et al., 2017). Additionally, the feeding behavior of *P. oleae* in field is unknown and might be possible that *P. oleae* died by starvation while traveling through enough large non-crop habitats to other olive groves. The vegetation can also act indirectly through increasing the action of natural enemies and *P. oleae* could be attacked by predators or parasitoids in the non-crop habitats. Multiple studies described the increase of the action of natural enemies with herbaceous and wooded habitats as well as landscape patchiness (Bianchi et al. 2006 and reference therein). In the olive agroecosystem, herbaceous and woody vegetation areas near and within olive groves, were found to decrease the abundance of *P. oleae* and *E. olivina* (Paredes et al. 2013b) and Paredes et al. (2013a) found that herbaceous and large woody vegetation adjacent to de crop influence the abundance of natural enemies, being this effect modulated by ground cover. Also Ortega et al. (2016)

found that *B. oleae* captures decreased with the presence of scrubland at 1500 radius.

In conclusion, the variation between years of *P. oleae* captures were strongly related with the weather conditions, being negatively affected by low precipitations along the year, low Winter temperatures and high temperatures in spring and summer. For the first time, landscape connectivity aspects were identified for *P. oleae*, being that was clearly able to disperse over a heterogeneous landscape composed by scrublands and herbaceous patches, fact particularly noticeable in scrubland patches. This study provides new data that contributes to the knowledge about *P. oleae* dynamic under adverse weather conditions and discloses new queries about the *P. oleae* dispersion and movement between patches.

ACKNOWLEDGMENTS

The authors are grateful to the Portuguese Foundation of Science and Technology for financial support through the project EXCL/AGR-PRO/0591/2012 "Olive crop protection in sustainable production under global climatic changes: linking ecological infrastructures to ecosystem functions".

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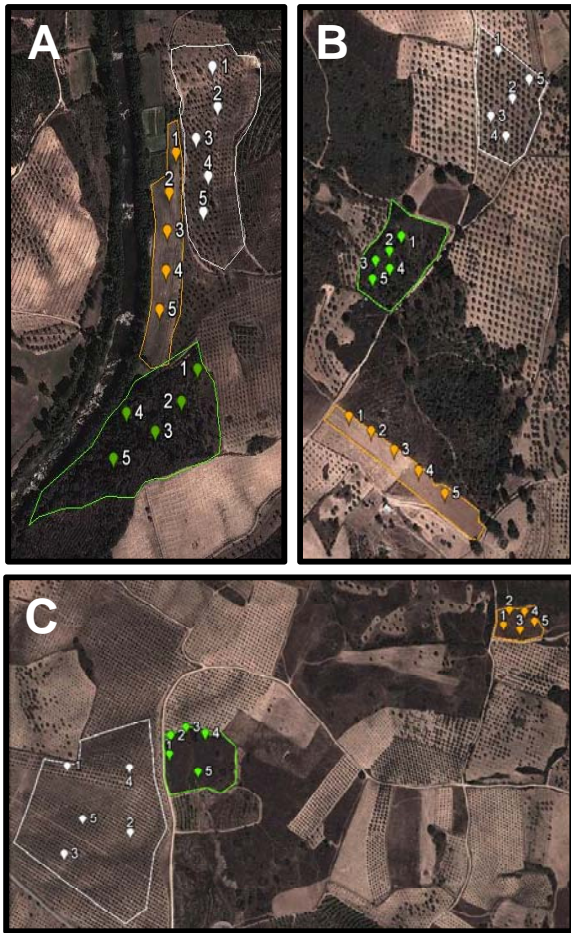


Figure 1. Study sites (A: Guribanes; B: Paradelas, and C: Cedães). Olive orchards are indicated in white, herbaceous vegetation patches in orange and scrubland patches in green. Numbers represent the Delta traps location. Images © 2015 Google.

Environment*Location effect plots

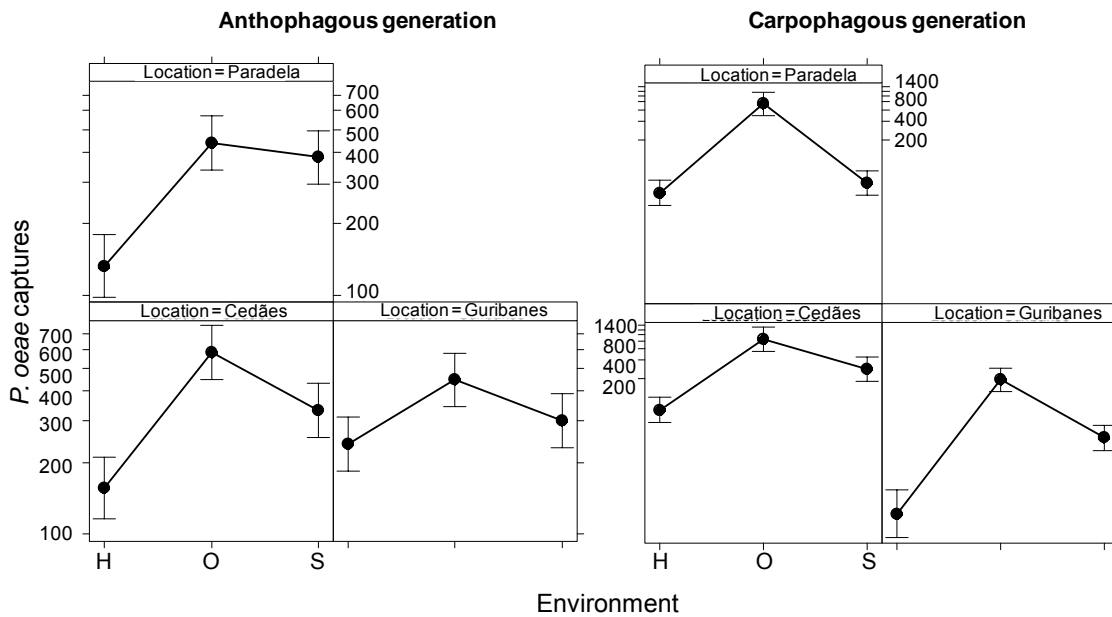


Figure 2. Means (points) and standard errors (bars) obtained from GLMs of cumulated captures by delta trap for the anthrophagous and carpophagous generation of *Prays oleae*. H: patches with herbaceous vegetation, O: olive orchards, S: scrublands.