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High Quality Extra Virgin Olive Oil from Olives Attacked by the Olive Fruit Fly, ***Bactrocera Oleae*** (Rossi) (Diptera Tephritidae): Which Is the Tolerable Limit? Data from Experimental 'Nocellara Del Belice' and 'Cerasuola' Olive Groves in Sicily

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The infestation due to the olive fruit fly, ***Bactrocera oleae*** (Rossi), the key pest in most of world olive groves, has been monitored in six years, from 2004 to 2008 and in 2014, in eight coastal Sicilian olive groves consisting of 'Cerasuola' and 'Nocellara del Belice' cultivars. Infestation was recorded following the classical sampling method based on olive collection and dissection, in order to count live olive fruit fly instars (eggs, larvae and pupae) and exit holes. Four different infestation indexes, all of them calculated using data recorded at harvest, have been used to assess their relationship with the main three quality parameters of the olive oil obtained from the same olive samples processed within 24 hours by quality oil mills: free acidity (% oleic acid), peroxides (mEq O_2 /kg of oil) and total phenols (mg·kg^{-1} oil). Each year and in each olive grove up to five plots were differently treated with different products allowed in organic agriculture, stopping sprays at least one month before harvest, obtaining different infestation levels. In this study a total of 43 theses were tested, 10 of them involving 'Cerasuola' and 33 'Nocellara del Belice'.
The two indexes based on the exit holes produced by mature larvae in olives resulted strictly and positively correlated to the lowering of oil quality. Moreover, among the two infestation indexes based on the occurrence of exit holes at harvest, percentage of olives with exit holes and No. of exit holes per 100 olives, the latter is more sensitive in presence of high infestation levels. No significant relation between infestation indexes and total phenols resulted in our analyses.
Nevertheless, when olives are harvested since the end of October to mid-November, and processed by quality oil mills, olives bearing up to 45 % of exit holes produced high quality extra virgin olive oil. Furthermore, all olives bearing up to 62 % of exit holes still produced extra virgin olive oil. Our results, in spite of the widespread prejudices involving an overestimation of damages due to low olive fly infestation, are very close to recent studies on correlation between olive fly attacks and olive oil quality. Late harvesting can lead to worse results at the same olive fly exit holes levels, confirming that timing and quality procedures of harvesting and oil extraction are important almost as much as olive fly control.
Keywords: olive fruit fly, infestation levels, exit holes, free acidity, peroxides values, total phenols, high quality threshold.

1. Introduction

The olive fruit fly, ***Bactrocera oleae*** (Rossi), a Tephritidae monophagous on the fruits of different subspecies of ***Olea europaea***, is the key pest in most of world olive groves and its harmfulness has long been documented in

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Mediterranean area. Assessing the effect of ***B. oleae*** damage on qualitative characteristic of oils could improve management strategies of the fly.
Current limits of free acidity and peroxides values (PV) for extra virgin olive oil (EVOO) are 0.8 % of oleic acid and 20 mEq O_2/kg of oil, respectively (EU 1989/2003 modifying the ECC 2568/91; E.U. Off. J. Eur. Communities, 2003). Although it is well documented that the phenolic content and oxidative stability decrease in oils obtained from fruits damaged by ***B. oleae*** attack (Angerosa et al., 1992; Evangelisti et al., 1994; Gómez-Caravaca et al., 2008; Pereira et al., 2004; Tamendjari et al., 2009), the effects of ***B. oleae*** damage on qualitative characteristics of oils were difficult to quantify because of the many sources of sample variability (cultivar, orchard location, cultural practices, processing technology, in Gómez-Caravaca et al., 2008). For instance, the high variability of samples led Gómez-Caravaca et al. (2008) to conclude that phenolic content was not a good indicator of ***B. oleae*** effects on oil quality.
Servili et al. (2013) suggested some limits for high quality extra virgin olive oil, among them free acidity (% oleic acid ≤ 0.3), peroxides values (≤ 12 (meq di O_2/kg) and total phenols (≥ 200 mg/kg).
Few studies related ***B. oleae*** infestation at harvest to oil quality: Neuenschwander and Michelakis (1978) calculated that infestation levels up to 30 % ***B. oleae*** exits at harvest do not reduce the value of the oil; similarly, Tsolakis et al. (1999, 2011) stated that olives presenting ***B. oleae*** passive infestation (corresponding to percentage of olives with exit holes) under 30 % still give good quality oil.
More recently Gucci et al. (2012) studied the relationship between ***B. oleae*** damage and oil quality, prudentially concluding that thresholds of active infestation currently used in integrated pest management of olive orchard are probably too restrictive.
The aim of this paper is to study the relationships between ***B. oleae*** infestations expressed through different indexes and the oil quality, in order to select the infestation index more significantly related to the three main olive oil quality parameters and to detect the tolerable infestation at harvest for high quality oil production.

2. Materials and Methods

The infestation due to ***B. oleae*** was monitored in six years, from 2004 to 2008 and in 2014, in eight coastal Sicilian olive groves of Trapani and Palermo provinces consisting of 'Cerasuola' and 'Nocellara del Belice' cultivars, which in a recent study on Sicilian olive cultivars resulted among the most susceptible ones (Rizzo et al., 2012). Each year and in each olive grove up to five plots were considered: one untreated, others differently treated with different products allowed in organic agriculture, stopping sprays at least one month before harvest and obtaining different infestation levels. In this study a total of 43 theses were tested, 10 of them involving 'Cerasuola' and 33 'Nocellara del Belice'. Harvest periods were: end of October (27 theses), mid-November (11 theses) and mid-Dicember (5 theses); in these coastal Sicilian olive groves the last harvest period has to be considered as a late, or very late, harvest period.
Samples of 60–100 olives of each cultivar and treatment (10–20 per tree) were randomly collected at harvest on 5-10 trees and examined in the laboratory under a stereomicroscope, first to check for the presence of oviposition punctures and exit holes. Olives have been then dissected in order to count live olive fruit fly instars (eggs, larvae and pupae). Four different infestation indexes have been calculated: % olives with exit holes, No. of exit holes per 100 olives, % olives with harmful infestation (occurrence of 3rd instar larvae, pupae, exit holes in absence of larvae or pupae), % olives with total infestation (presence of live instars plus exit holes in absence of larvae or pupae).
In all experiments, the oil was obtained from at least 100 kg of olives for each different thesis within 24 h from harvest, using quality three-phases oil mills (Pieralisi or Alpha-Laval brand). Quality parameters of oils were measured immediately after oil extraction. The free acidity (% oleic acid) and peroxides values (mEq O_2 /kg of oil) of all 43 oil samples were determined by titration, while the total phenols concentration (mg·kg^{-1} oil) was determined by spectrophotometry only from 28 out of 43 oil samples, in this last case obtained from harvest at the end of October (22 samples) and in mid-November (6 samples).
Quality parameters of oils have been used to perform a regression analysis, after data normalisation by means of a Box-Cox transformation (Minitab Inc., USA), with the aim to assess their relationship with the four different infestation indexes. In the regression analysis the cultivar and harvesting period have been included as categorical factors too.

3. Results

All four ***B. oleae*** infestation indexes tested in our analysis resulted significantly related to free acidity and peroxides values, whereas the regression analysis on phenols did not result significant for any of infestation indexes (Table 1).

Table 1: Results of the regression analyses performed to assess the relationships between three oil quality parameters and B. oleae ***infestation indexes recorded at harvest. R^2 and p-values refer to a different regression analysis for each infestation index.***

	Oil quality parameter					
	Free acidity (n=43)		Peroxides values (n=43)		Phenols (n=28)	
Infestation index	R^2	***p***	R^2	***p***	R^2	***p***
% olives with exit holes	70.98	0.000	57.33	0.000	17.42	N.S.
No. exit holes 100 olives	69.70	0.000	59.80	0.000	12.31	N.S.
% olives with harmful infestation	59.96	0.001	55.12	0.000	13.91	N.S.
% olives with total infestation	57.94	0.004	56.58	0.000	19.84	N.S.

The relationship between two exit holes indexes, expressing their percentage or their number per 100 olives, is shown in Figure 1. When infestation is high, the No. of exit holes per 100 olives seems to express the damage to olives more precisely than their percentage. Nevertheless these two exit holes indexes provided results very close one each other and better than percentages of harmful and total infestation, both in terms of R^2 and significance (Table 1), with a negative correlation with both oil quality parameters (Figures 2-3). As the percentage of exit holes is a more common used infestation index, it has been chosen to show our results in Figures 2-4, in order to easily compare them with those obtained by Gucci et al. (2012).

Regarding the free acidity, the harvest period of mid-December resulted significantly different from other two harvest periods (end of October, mid-November) (Figure 2); this result is probably due to the very humid climate recorded in the last month before harvest that increased the degradation of oil content of the fruit bearing exit holes.

In our analyses the Cultivar variable has not significantly influenced any of tested oil parameters.

Our data show that olives harvested at the end of October and mid-November can produce a high quality EVOO olive oil (limits suggested by Servili et al., 2013) up to 45 % of olive fruit fly exit holes, corresponding to about 75 exit holes per 100 olives.

Free acidity resulted the quality parameter more sensitive to olive fruit fly infestation (Table 1, Figures 2-4). The only four oils resulting above the EVOO level for free acidity had a percentage of exit holes from 68 % to 97 %; moreover, three of them were obtained from olives harvested at mid-December.

Peroxides values of tested oils were almost all below the upper limit of high quality EVOO olive oil (Servili et, 2013), excepting one sample with 91 % exit holes still below the upper limit of EVOO (Figure 3).

The total phenolic content recorded from tested oils, all obtained from olives harvested at the end of October or mid-November, although resulted not significantly correlated to infestation indexes, satisfies the high quality EVOO level (Servili et al., 2013) in 26 samples out of 28 (Figure 4).

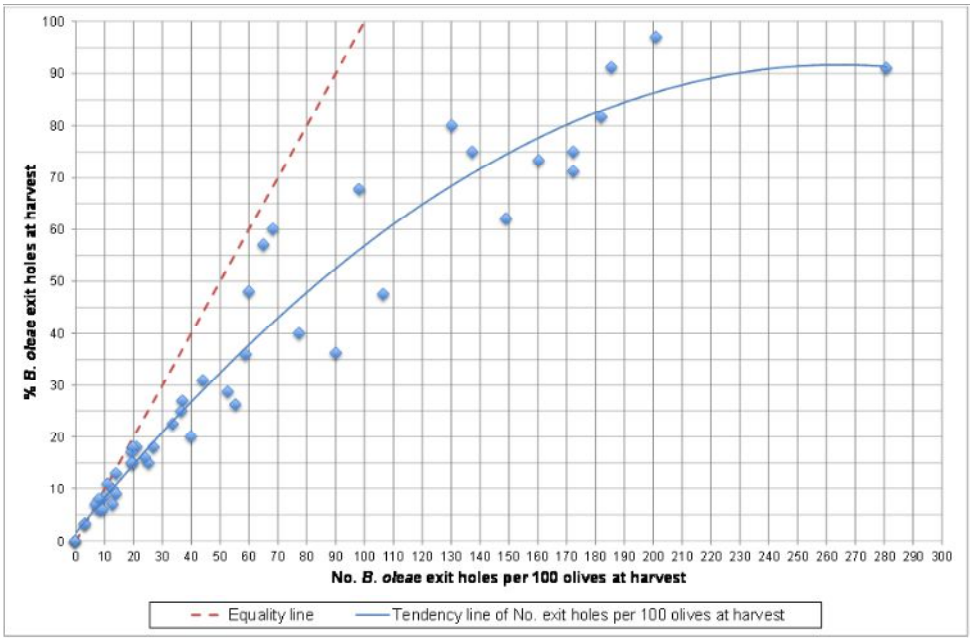

Figure 1: Relationships between the percentage B. oleae exit holes at harvest and No. of B. oleae exit holes per 100 olives at harvest in 43 samples collected in 2004-2014 in coastal Sicilian olive groves.

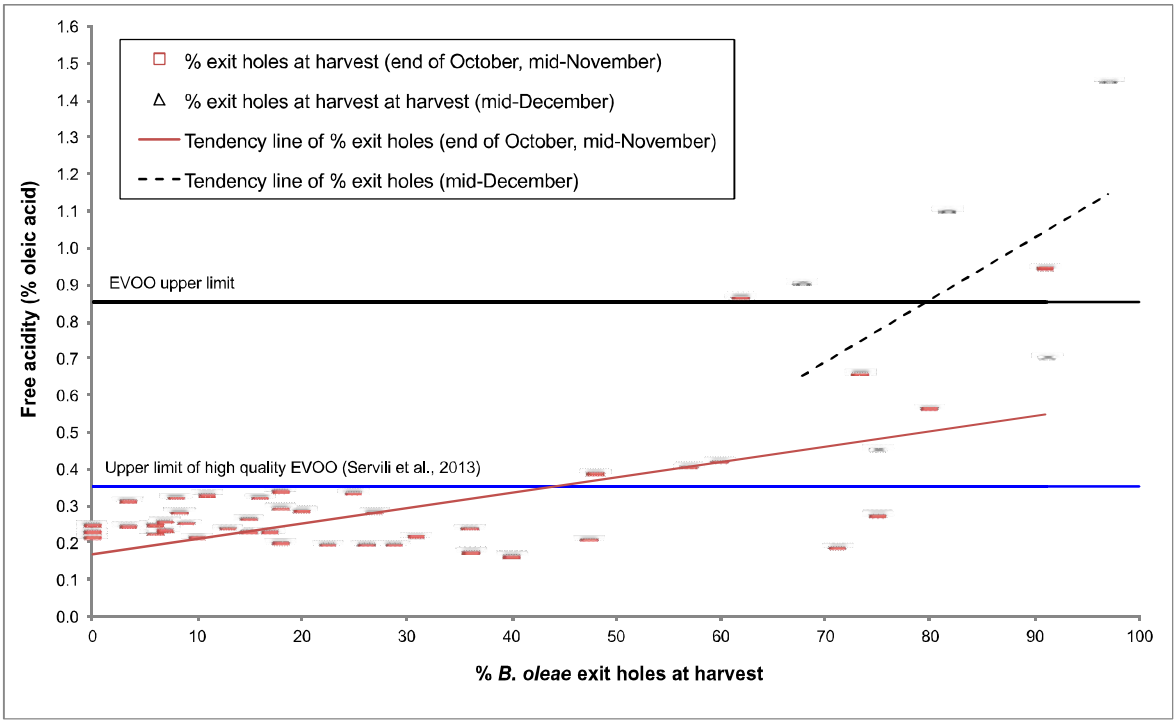

Figure 2: Correlation between the percentage of B. oleae exit holes at harvest and free acidity of olive oil; the regression analysis is highly significant (see Table 1).

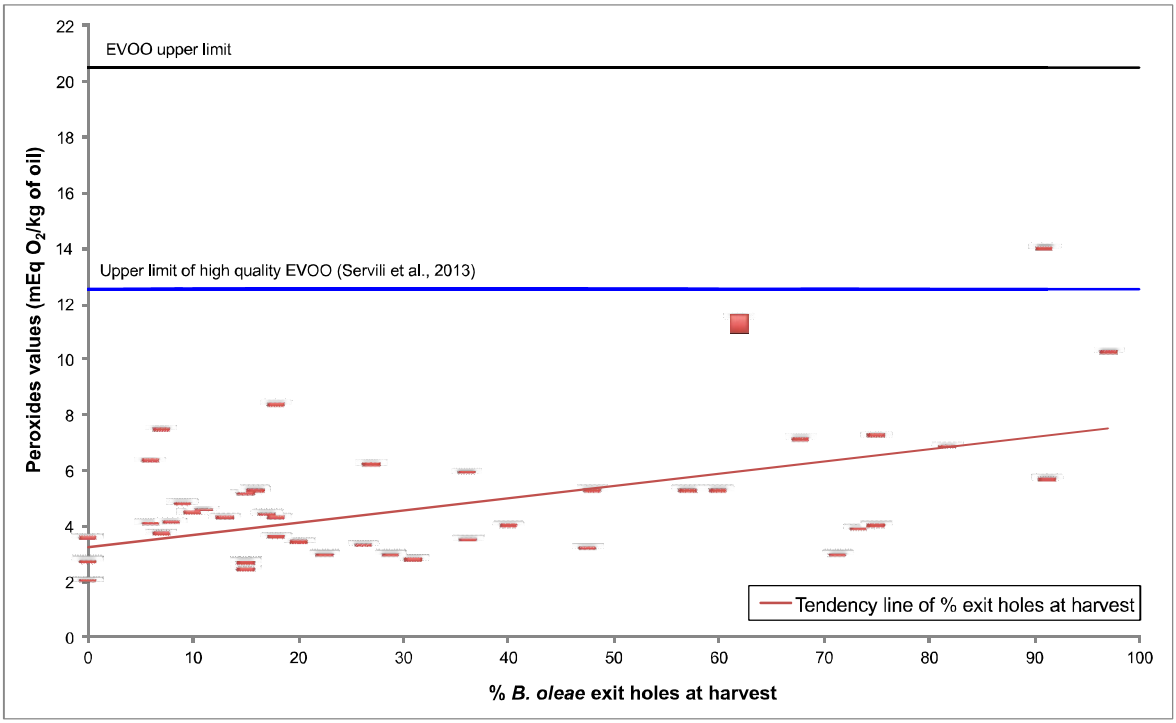

Figure 3: Correlation between the percentage of B. oleae ***exit holes at harvest and peroxides values of olive oil; the regression analysis is highly significant (see Table 1).***

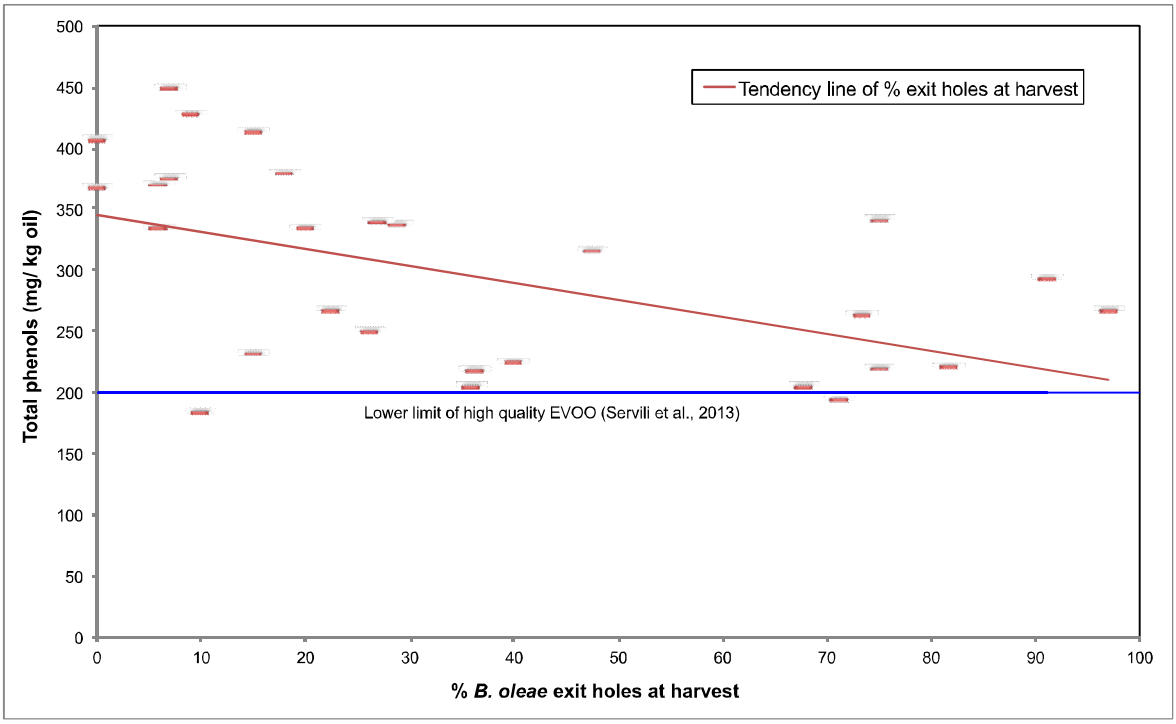

Figure 4: Correlation between the percentage of B. oleae ***exit holes at harvest and total phenolic content of olive oil; the regression analysis resulted not significant (see Table 1).***

4. Conclusions

The infestation indexes recorded at harvest expressed in percentage of ***B. oleae*** exit holes and No. of exit holes per 100 olives are more significantly linked to the increase of free acidity and peroxides values, rather the harmful and total infestation, confirming that olive fruit fly larvae still inside the pulp without any exit holes counted in harmful and total infestation, do not greatly influence the quality of oil.

Our results obtained in six years from 43 samples of eight coastal Sicilian olive groves state that, when the harvest is performed at the end of October or in mid-November, quick oil extraction and quality oil mills are used, olives attacked by the olive fruit fly bearing up to 45 % of exit holes can produce oils fitting high quality EVOO levels (Servili et al, 2013) for free acidity, peroxides values and phenols. Using the same procedures of harvesting, timing and processing, olives up to 80 % of exit holes produced EVOO oils. Late harvesting (mid-December) has worsened oil quality and, also at the same level of ***B.oleae*** exit holes of a correct harvest period, did not produce high quality EVOO, probably due to a very humid last month before harvest influencing the degradation of oil content in olives still on the tree.

Our data, although a different methodology in olive sampling and oil extraction, are very similar to those obtained by Gucci et al. (2012), that prudentially concluded that thresholds of active infestation currently used in integrated pest management of olive orchard are probably too restrictive. These authors (see figures 2 and 3 of their paper) obtained oils with EVOO parameters for free acidity and peroxides values also from olives with 100 % of exit holes, and oils with high quality EVOO parameters for free acidity, peroxides values and total phenols (sensu Servili et al, 2013) from olives up to 40 % of exit holes.

Differently from Gucci et al. (2012), in our study the relation between olive fruit fly exit holes in olives at harvest and the total phenolic content resulted not significant; this result could be linked to the higher sources of variability influencing the phenolic content of oil in our study, carried out in six years and eight different olive groves, in comparison with Gucci et al. (2012).

Finally, in spite of the widespread prejudices involving an overestimation of damages due to low olive fly infestation, a tolerable limit for the production of high quality EVOO can be considered around 40 % (Gucci et al., 2012) or 45 % (this study) of olives with exit holes at harvest, when harvest is performed at the correct moment, and olives are quickly processed by quality oil mills. Based on this findings, an intervention threshold can be specifically fixed, taking into account also the characteristics of each olive farm, the evolution of olive fruit fly infestation and the impact of fruit drop.

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