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Control of *Bactrocera oleae* by low environmental impact methods: NPC methodology to evaluate the efficacy of lure-and-kill method and copper hydroxide treatments

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Abstract

The NonParametric Combination (NPC) methodology is proposed to verify the influence of the lure-and-kill method, using Eco-traps (Vioryl SA), on the distribution of *Bactrocera oleae* (Rossi) (Diptera Tephritidae) infestation in olive groves, and the integration of this method with copper hydroxide sprayings. This approach does not require the verification of any rigid assumption as many parametric methods do. Results of field trials indicate a strict relationship among the efficiency of the lure-and-kill method on time, dimension of the field, and population density of the olive fly. Moreover, the lure-and-kill method alone seems insufficient to provide an efficient control of the olive fly population in small olive groves. However, when this method is integrated with two copper hydroxide sprayings, the infestation of drupes is maintained below the damage threshold.

Key words: *Bactrocera oleae*, lure-and-kill, copper hydroxide, NonParametric Combination analysis, NPC.

Introduction

The olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera Tephritidae) is considered the most noxious pest by olive growers in the Mediterranean countries (Neuenschwander and Michelakis, 1978; Kapatos and Fletcher, 1983). Until today, chemical control, using organophosphorous and pyrethroid insecticides, has been the mainly adopted method, even if its dangerous effect on humans and the environment is well known (Murphy, 1986). The wide spreading of organically managed olive groves in the last two decades [over 250,000 hectares in the European countries in 2007, and about half of them (109,992 hectares) in Italy (Eurostat, 2007)], directed the pest control strategy towards low environmental impact control methods. In organically managed Sicilian olive groves no sprayings and early-picking, from the second half of October, is the main adopted control method. This strategy gives good results when hot and dry summers cause a delay in *B. oleae* population increase (after the third week of September). However, humid and not very hot summers frequently allow an early infestation of drupes (starting from the last week of August), causing losses of yield and damaging the oil quality. Other control methods, such as the lure-and-kill, copper and kaolin sprayings are adopted by some organic growers, but very few field data have been available, up to now (Belcari and Bobbio, 1999; Tsolakis and Ragusa, 2002; Broumas *et al*., 2002; Sour and Makee, 2004; Caleca *et al*., 2007).

In the lure-and-kill method a great number of traps (up to one/tree) are set in the field before the drupe becomes susceptible for olive fly oviposition. Each traps (Eco–Trap type, Vioryl. S.A., Athens, Greece) consists in light-green-coloured paper envelope containing ammonium bicarbonate salt, a powerful attractant for both sexes, and the surface of the envelope is covered with deltamethrin. A pheromone (1,7-dioxaspiro [5.5] undecane) dispenser is also fastened externally (Broumas *et al*., 2002). This method reduces the olive fly infestations significantly when the dimension of olive groves is large (Broumas *et al*., 2002; Petacchi *et al*., 2003), but no data are available when it is applied in small groves. It should be mentioned that in most Mediterranean countries olive grove surfaces are very fragmented, i.e. 994,000 hectares of olive groves for 909,000 olive growers in Italy in 1995 (INEA; 1998).

Copper products showed an inhibitory action towards the symbionts of the olive fly (Tzanakakis, 1984; Belcari and Bobbio, 1999), but also an oviposition deterrence and a repellent effect, both in laboratory and field (Prophetou-Athanasiadou *et al*., 1991; Tsolakis and Ragusa, 2002). Unfortunately, copper sprayings alone do not offer adequate protection when the population density is high (Tsolakis and Ragusa, 2002).

No data are available, up to now, on the integration of the above mentioned methods in the Mediterranean area.

The aim of this paper is to evaluate the distribution of olive fly infestation in one hectare of olive grove where the lure-and-kill method was adopted, and to compare the infestation trend to another close field, where both the lure-and-kill method and copper hydroxide sprayings were applied. A nonparametric permutation analysis, based on NonParametric Combination (NPC) methodology, is proposed. This statistical approach could be particularly appropriate in agriculture, where the comparison between two or more groups of treatments is usually performed over a certain period of time. The analysis of such data is often done using classical parametric methods, although the set of all parametric assumptions and conditions are neither verified nor satisfied. This is probably due to the large availability of parametric methods in comparison to the non parametric methods: i.e., before the implementation of NPC methodology, the use of parametric methods in multivariate problems was almost unavoidable (Corain and Salmaso, 2004). It is worthwhile noting that a non parametric approach frees researchers from the verification of stringent model assumption and in many experimental situations an inferential efficiency returns, that is the same as a parametric one.

Materials and methods

Experimental fields

The experiments were carried out in an organically managed olive farm located in a hill (253 m a.s.l.), 8 km distant from the coast (37°58'58.56"N - 13°02'13.22"E), in the province of Palermo (Sicily, Italy), during 2004 and 2005. Two olive groves of the "*Biancolilla*" variety, cultivated for oil production, were taken into account. Field A was 10,200 m^2, with 14-year-old trees and a mean canopy diameter of 3.3m, 6m inter-row x 4m inter-tree (17 rows x 25 trees), and field B was 10,350 m^2, 6m x 6m (16 rows x 18 trees) of 40-year-old trees with a mean canopy diameter of 4.5m. The orientation of rows was North-South in Field A and NorthWest/SouthEast in Field B. An uncultivated strip divided the two considered fields (figure 1). Within 500 m radius other olive groves or single olive trees or *Olea europaea* L. subsp. *europaea* var. *sylvestris* (Mill.) trees were present.

Assessment of fruit infestation by *B. oleae*

The spatial distribution of infestation by *B. oleae* was determined only in field A. Trees surveyed were located along the sides of 4 concentric squares spaced 12 m from each other (figure 1). One plant every three on the row, in alternate rows, was taken into account. In total 81 plants, corresponding to 19% of the total number of trees, were surveyed. This scheme represents a cluster sampling where the population has been divided into 81 groups of six equispaced grids (plants) and one of these grids has been sampled (figure 1). We decided to adopt this sampling scheme because it is more powerful than a complete randomized one with equal run size, as it minimizes the sampling variability.

The effect of integrating traps with copper sprayings was determined in field B. The same experimental design (when possible, as several plants were missing), was applied also in this field (42 plants corresponding to 19% of the total) (figure 1). We considered the two fields comparable from the infestation point of view, because similar infestation trends were registered in these olive groves in previous years (Tsolakis *et al.*, 1999; Tsolakis and Ragusa, 2002). On the contrary we avoided to compare the two fields from the spatial distribution of *B. oleae* point of view, because of the different number of plants and planting space. Samplings were weekly done from the first week of August until harvest (October 20th in 2004 and November 8th in 2005). Five drupes/plant/week were randomly sampled at a height of 1.5-1.8 m. Fruits were examined the same day of the sampling under a stereomicroscope. When more instars were present in the same drupe, the most advanced one was taken into account. Two categories of infestation were taken into account:

a) Active infestation: alive eggs and larvae without exit holes;

b) Total infestation: active infestation + alive and dead pupae, and drupes in presence of exit holes also in absence of larvae and/or pupae.

Passive infestation (total infestation minus active infestation) was evaluated at harvest time for both fields. For the present work, a prudential threshold of 25% of passive infestation is defined, as olives presenting passive infestation under 30% still give good quality oil (Tsolakis *et al.*, 1999).

Traps and sprayings

The commercial traps Eco-trap® (VIORYL SA, Greece) containing food and sex attractants for both females and males of olive fly and having its cover impregnated with deltamethrin, were used (Broumas *et al.*, 2002). In each field during the first week of August, 1 trap/tree was installed. 421 traps in field A and 223 traps in field B were totally installed.

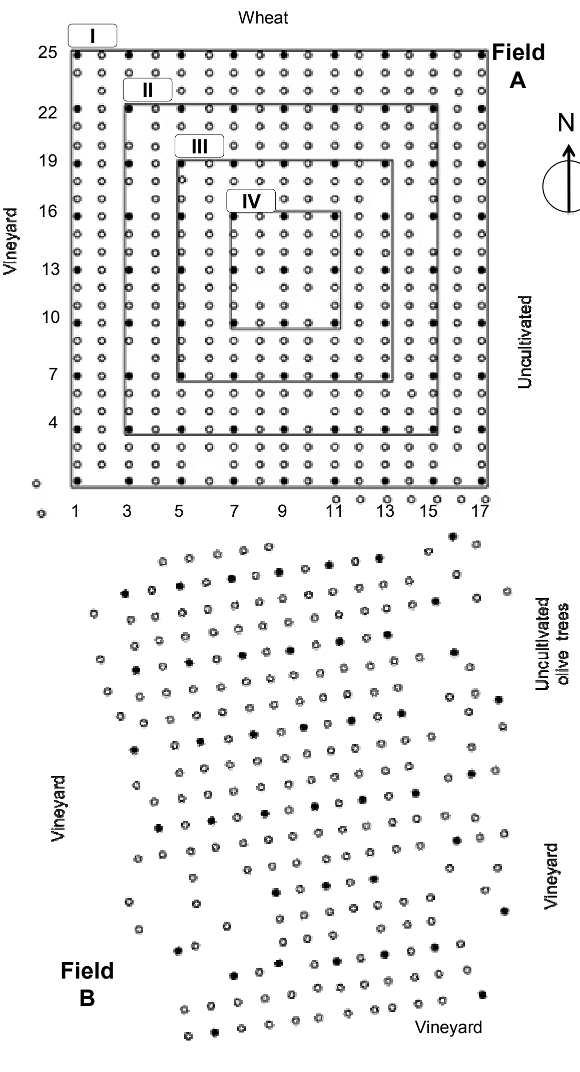

Figure 1. Schematic map of the two fields and experimental design. Circles represent olive trees. Black circles represent the surveyed trees. Latin numbers indicate the four squares of field A.

Copper sprayings were done only in field (B) on September 9th, and October 5th, 2004 and on September 10th, and October 13th, 2005. As copper is mainly reported as repellent, the first spraying was applied after the first eggs were laid and favourable climatic conditions were registered (temperature less than 30 °C and relative humidity more than 60%). The second spraying was done about one month later, in order to maintain an adequate presence of the matter during the whole experimental period. Copper Hydroxide (Copper idro 40 WDG®, SARIAF SA Italy) at the dose of 300 g/100 l of water, was applied and about 1,000 l/hectare of solution were sprayed (corresponding to 1.2 kg of active ingredient/hectare).

Statistical approach

A systematic analysis process was adopted (Cox and Snell, 1981; Chatfield, 1985). Geostatistical Analysis is usually considered a more appropriated method to evaluate the spatial distribution (Rossi *et al*., 1992; Ifoulis and Savopoulou-Soultani, 2006). But the specific objectives of our study were to proof a stated hypothesis rather than to create a geostatistical model. We adopted non parametric methods able to test hypotheses on data collected in successive time instants (Higgins, 2004). The permutation test for repeated measurements was accounted for this requirement (Pesarin, 2001). If measurements on a variable of interest (the presence of infestation in our case) are repeated for a number of times (the dates of sampling) on the same units (the sampled tree), the repeated observations may be viewed as a multivariate variables (these variables also called as response profiles). It is then possible to test the hypothesis that observed response profiles have or have not the same underlying distribution (see the "*d*" symbol in the formula 1) with respect to a treatment, where a treatment could be a group of plants subjected to the same treatment: in our case the insecticide spraying or, a group belonging to the same concentric square.

The general hypothesis can be formally written as in equation 1, against equation 2. Where L is equal to the number of treatment levels, k is the number of sampling dates, X_j is the vector of observations belonging to the j-th group (j-th treatment level).

As it can be observed from the equations 1 and 2, the general null hypothesis can be decomposed into k sub-hypothesis according to time. This decomposition corresponds to the so called time-to-time analysis (Celant *et al.*, 2000). Moreover, the general null hypothesis H_0 (multivariate) is true if all the H_{0t} (univariate) are jointly true. Conversely, the alternative general hypothesis is true if at least one sub-alternatives hypothesis is true.

Complex real problem with repeated measures can be then solved by applying the NPC approach (Corain and Salmaso, 2004). The key idea of this approach is to perform k partial univariate nonparametric tests and then to combine them, through an appropriate combining function, in a global multivariate test (Pesarin, 2001). *p*-values of partial and combined tests are estimated using the approach described in Pesarin (2001) and implemented in the NPC test 2.0.

Two hypotheses were investigated. The first concerns the influence of the lure-and-kill method on the distribution of the infestation: i.e. traps slow down the advance of infestation creating an edge effect, so that the infestation level is higher in the external zone of the field than in the internal one. This hypothesis was tested in field A where only the lure-and-kill method was used (hypothesis $H_{1,0}$), analyzing the differences between the two external squares (I+II) and those of the two internal squares (III+IV) (figure 1). Multivariate variables were created according to the sampling dates. Formally see equations 3 and 4.

The second hypothesis assumes that the integration of the lure-and-kill method and copper hydroxide sprayings, reduces the percentage of active and total infestation (hypothesis $H_{2,0}$). To test this hypothesis, the infestation levels in field A (lure-and-kill method alone) and those in field B (lure-and-kill method + copper hydroxide sprayings) were compared using the same NPC approach. Formally see equations 5, 6, 7 and 8.

The C sample test with repeated measure option of NPC Test software (version 2.0) was used. The default settings of this software were left (automatic seed for random number generation and number of conditional Monte Carlo simulation equal to 10,000). A nonparametric *F*-test was chosen as the most suitable statistical test for partial univariate test, while the combination test

Equations 1-8.

$$H_0 : \left\{ \mathbf{X_1} \overset{d}{=} \ldots \overset{d}{=} \mathbf{X_L} \right\} = \left\{ X_1(t) \overset{d}{=} \ldots \overset{d}{=} X_L(t),\ \ t = 1,\ldots,k \right\} = \left\{ \bigcap_{t=1}^{k} \left[X_1(t) \overset{d}{=} \ldots \overset{d}{=} X_L(t) \right] \right\} = \left\{ \bigcap_{t=1}^{k} H_{0t} \right\} \quad \textbf{(1)}$$

$$H_1 :\ \ \left\{ \bigcup_t \left[H_{0t} \text{ not true} \right] \right\} = \left\{ \bigcup_t H_{1t} \right\} \quad \textbf{(2)}$$

$$H_{1,0} : \left\{ X_{\mathbf{I+II}}(t) \overset{d}{=} X_{\mathbf{III+IV}}(t),\ \ t = 1,\ldots,k \right\} \quad \textbf{(3)}$$

$$H_{1,1} : \left\{ X_{\mathbf{I+II}}(t) \overset{d}{>} X_{\mathbf{III+IV}}(t),\ \ t = 1,\ldots,k \right\} \quad \textbf{(4)}$$

$$H_{2',0} : \left\{ X_{\textbf{active A}}(t) \overset{d}{=} X_{\textbf{active B}}(t),\ \ t = 1,\ldots,k \right\} \quad \textbf{(5)}$$

$$H_{2'',0} : \left\{ X_{\textbf{total A}}(t) \overset{d}{=} X_{\textbf{total B}}(t),\ \ t = 1,\ldots,k \right\} \quad \textbf{(6)}$$

$$H_{2',1} : \left\{ X_{\textbf{active A}}(t) \overset{d}{>} X_{\textbf{active B}}(t),\ \ t = 1,\ldots,k \right\} \quad \textbf{(7)}$$

$$H_{2'',1} : \left\{ X_{\textbf{total A}}(t) \overset{d}{>} X_{\textbf{total B}}(t),\ \ t = 1,\ldots,k \right\} \quad \textbf{(8)}$$

was performed using all available combining functions (Fisher, Liptak, Tippett, Direct) (Salmaso and Solari, 2005). The test giving the most conservative result (higher p-value) was then chosen, according to the strategy proposed by Pesarin (2001).

Results and discussion

Statistical approach

Table 1 report the main statistics for active and total percentage of the infestation in fields A and B from the moment the infestation significantly appeared. From this table, it is evident that at the beginning of the infestation, the large proportion of slightly infested or non infested trees in both fields is reflected in the distribution of a strong positive skew. With the increasing of the infestation across the field A, the distribution of infested trees is less skewed and it approaches a normal distribution. At the end of the observation period, the infestation level was high everywhere and the resulting distribution of infested trees in this field became highly negative skew. This trend is less marked in field B, where the increase of infested trees was slow and the infestation was maintained at low levels up to the end of the observation period. The different shape of these distributions along the time confirms, once again, that in many practical situations, the assumptions regarding the validity of the parametric method, such as normality, are not satisfied. A high variability of the infestation distribution along time and space is typical for phytophagous insects in cultivated fields. It depends on different variables such as the intrinsic characteristics of the species, the availability of food, the climatic conditions, the density of natural enemies etc. It is worthwhile noticing that, even if parametric approaches require the verification of a set of rigorous distributional assumptions, they are still preferred to non parametric approaches in many applications. A possible explanation for such trend is that many analysts consider parametric methods more inferentially efficient than non parametric ones. Data are often transformed to be normalized, and standard methodologies are then applied. For instance, in this case a power transformation might be applied, with an exponent varying from [1/3;2] according to the different degree of

Table 1. Summary statistics for the active and total percentage of infestation in fields protected by lure-and-kill method (A), and lure-and-kill method plus copper hydroxide treatments (B).

Date*	Variable	Field (A)			Field (B)		
		Mean	S.E.	Skewness	Mean	S.E.	Skewness
26 Aug 04	Active	0.49	0.347	6.2	-	-	-
	Total	**0.49**	0.347	6.2	**0.48**	0.476	6.5
2 Sep 04	Active	2.22	0.703	2.5	0.48	0.476	6.5
	Total	**2.47**	0.736	2.3	**1.43**	0.804	3.5
9 Sep 04	Active	7.41	1.549	1.8	0.95	0.665	4.4
	Total	**13.09**	1.993	1.4	**1.43**	0.804	3.5
20 Sep 04	Active	21.98	2.667	0.7	4.29	1.452	2.2
	Total	**34.32**	3.374	0.4	**5.71**	1.709	1.9
27 Sep 04	Active	23.95	2.906	0.8	12.86	1.906	0.4
	Total	**41.23**	3.838	0.1	**13.81**	1.986	0.4
5 Oct 04	Active	21.48	2.694	1.3	6.19	1.736	1.7
	Total	**46.91**	3.603	0.0	**9.52**	2.181	1.2
11 Oct 04	Active	29.63	3.164	0.9	11.43	2.171	0.8
	Total	**63.95**	3.608	−0.4	**19.52**	3.441	1.6
18 Oct 04	Active	31.36	3.219	0.8	8.10	1.811	1.2
	Total	**90.37**	2.111	−2.2	**23.81**	2.990	0.1
19 Sep 05	Active	-	-	-	-	-	-
	Total	**0.74**	0.422	5.0	**0.95**	0.952	7.0
27 Sep 05	Active	1.23	0.538	3.7	1.90	0.919	2.9
	Total	**1.48**	0.586	3.3	**2.86**	1.093	2.1
8 Oct 05	Active	6.42	1.441	2.1	4.29	1.604	2.5
	Total	**9.63**	1.761	1.5	**6.19**	2.207	2.4
12 Oct 05	Active	3.21	0.893	2.5	0.95	0.665	4.4
	Total	**6.67**	1.571	2.7	**6.19**	2.207	2.4
18 Oct 05	Active	2.47	0.888	3.4	5.24	1.811	2.2
	Total	**7.16**	1.696	2.2	**10.00**	2.912	1.9
26 Oct 05	Active	40.49	3.022	0.0	5.24	1.533	1.7
	Total	**53.09**	3.165	−0.3	**10.48**	2.572	1.7
1 Nov 05	Active	39.01	3.477	0.3	6.67	2.007	1.8
	Total	**51.60**	3.386	−0.2	**13.33**	2.945	1.3
7 Nov 05	Active	48.89	3.258	0.4	7.62	1.797	1.3
	Total	**90.12**	1.615	−1.3	**16.19**	2.830	1.2

*Until 26 August 2004 and 19 September 2005 there was no infestation.

asymmetry. This makes the analysis difficult. The NPC approach does not require a preliminary treatment of data nor a verification of distributional assumptions.

The simplicity of non parametric methods and their statistical properties together with the availability of statistical packages implementing them, turn in favour of the applicability of such methods in many complex real situations, where distributional assumption cannot be preliminarily verified.

Influence of the lure-and-kill method on the distribution of the infestation

The hypothesis $H_{1,0}$ (that traps slow down the advance of infestation creating an edge effect), was tested analyzing the differences among the four concentric squares in which the field A was divided (figure 1). The infestation level, in both years, in the external part of field A (squares I and II), was significantly higher than that registered on trees located in the internal zones (squares III and IV) (figures 2 and 3, table 2). Combined tests, with all combining functions, were significant at $p < 0.05$, confirming a statistical difference among the considered zones of field also overall along the whole observation period (table 3). *B. oleae* can fly more kilometres per day, especially when searching for olive trees (Economopoulos *et al.*, 1978). When the crop is located, the species flies more slowly way, but is still able to cover up to 200m in few days (Fletcher and Economopoulos, 1976). Furthermore, after ovipositing, the olive fly marks the drupe with deterrent substances. In this way other females have to search intact olives to oviposit (Girolami *et al.*, 1981). This behaviour indicates a quick spread of the infestation within an olive grove, but the results here obtained showed a significant edge effect in this field, declining with the increase of both resident and immigrant *B. oleae* population. In this situation, the number of traps related to the small surface is unable to attract and kill a sufficient number of flies and to block the infestation spread. It should be mentioned that the pressure of olive fly population after the half of October is very high in the coastal Sicilian strip, because of the density of cultivated and wild olive trees. These results might indicate the lure-and-kill method as an interesting control method for great surfaces of olive groves, as also suggested by Petacchi *et al.* (2003), although insufficient to control the infestation in small olive groves, when environmental conditions favour the population increase. Delrio (1989) and Caleca *et al.* (2007) report similar results, using the same or other mass trapping methods. It should be mentioned that Viggiani (2001) and Caleca *et al.* (2007), consider this method economically unacceptable for its weak results and for the high cost of the traps.

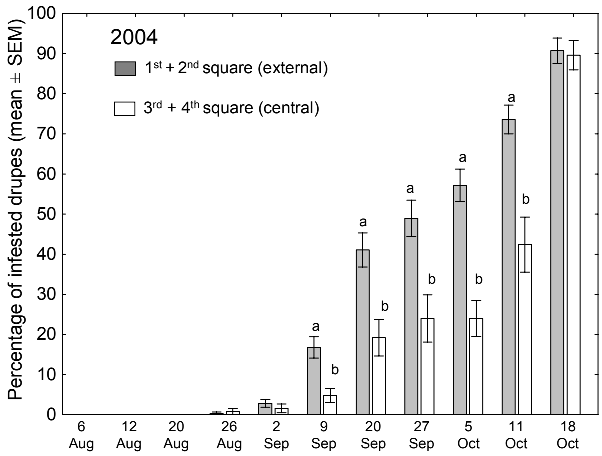

Figure 2. Infestation levels in the external and central zones of field A in 2004. Different letters denote significant differences for $p < 0.05$ (see also table 2).

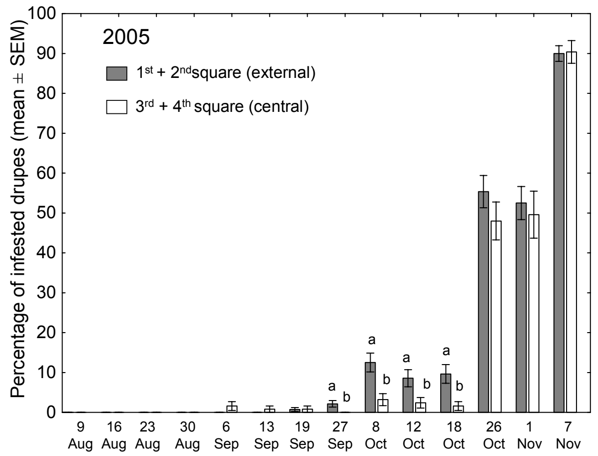

Figure 3. Infestation levels in the external and central zones of field A in 2005. Different letters denote significant differences for $p < 0.05$ (see also table 2).

Table 2. *p*-values of partial tests on infestation level differences among squares and during the infestation period. Asterisks denote the significant test, i.e. hypothesis $H_{1,1}$ is accepted.

2004	$H_{1,1}$	26 Aug	02 Sep	09 Sep	20 Sep	27 Sep	05 Oct	11 Oct	18 Oct
	I+II>III+IV	0.9045	0.3485	0.0021*	0.0012*	0.0002*	0.0012*	0.0001*	0.4450
2005		19 Sep	27 Sep	08 Oct	12 Oct	18 Oct	26 Oct	01 Nov	07 Nov
	I+II>III+IV	0.6278	0.0423*	0.0304*	0.0092*	0.0041*	0.5724	0.5558	0.9833

Table 3. *p*-values of combined tests according to the different combining functions considering the whole observation period. Asterisks denote the significant test, i.e. hypothesis $H_{1,1}$ is accepted.

	$H_{1,1}$	Fisher	Liptak	Tippett	Direct
2004	I+II>III+IV	0.00014985*	0.00024992*	0.00034996*	0.00024995*
2005	I+II>III+IV	0.00044995*	0.00884911*	0.01744026*	0.01334867*

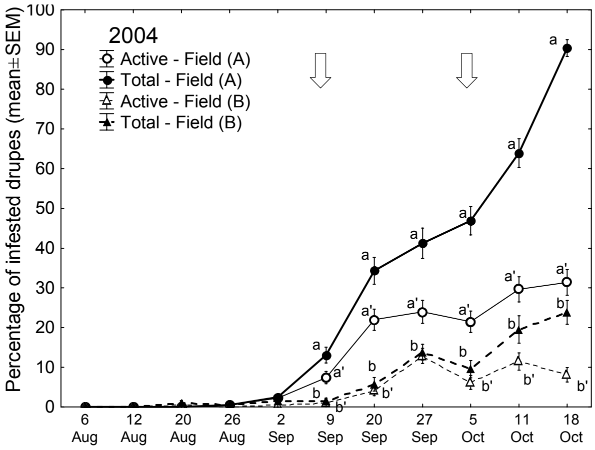

Figure 4. Trend of active and total infestation of drupes in the two experimental fields in 2004. Arrows indicate the copper hydroxide sprayings in the field B. Different letters denote significant differences for $p < 0.05$ (see also table 4).

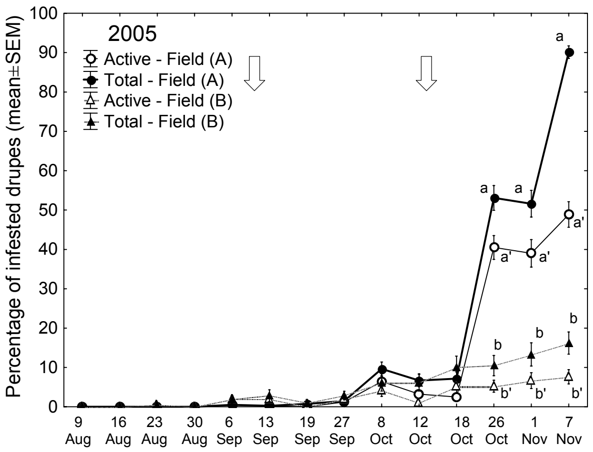

Figure 5. Trend of active and total infestation of drupes in the two experimental fields in 2005. Arrows indicate the copper hydroxide sprayings in the field B. Different letters denote significant differences for $p < 0.05$ (see also table 4).

Integration of the lure-and-kill method with copper treatments

Results related to the integration of lure-and-kill method and copper hydroxide sprayings, are reported in figures 4 and 5. Infestation level in field (A) during 2004 constantly increased with a peak after six weeks (90.37 ± 2.11% of infested drupes), while it was maintained at low levels (under 10%) until October, 18th in 2005, and reached the same infestation level as in the previous year (90.12 ± 1.62%) after three weeks. On the contrary, in field (B), infestation slowly increased reaching a similar peak in both years (23.81 ± 2.99% and 16.19 ± 2.83% in 2004 and 2005 respectively). Partial tests confirm that, for the period ranging from September, 9th to the end of the observation period for the year 2004, and from October, 26th to the end of the observation period for the year 2005, the infestation level in field A was higher than in field B (table 4). Combined tests, considering the whole window time, were significant ($p < 0.05$), confirming that the active and total infestation level was generally lower in field (B) (table 5).

As far as the passive infestation at harvest time is concerned, it was quite high in field (A) (59.01% and 41.23% in 2004 and 2005 respectively), while statistically lower percentages were registered in field (B) in both years (15.71% and 8.57% in 2004 and 2005 respectively) (table 6).

Table 6. Percentage of passive infestation at the harvest time in the two fields in 2004 and 2005.

	2004 Mean ± S.E.	2005 Mean ± S.E.
Lure-and-kill method (Field A)	59.01±3.582	41.23±3.431
Lure-and-kill method + Copper Hydroxide sprayings (Field B)	15.71±2.105	8.57±2.375
	$p < 0.0001$	$p < 0.0001$

Table 4. *p*-values of partial test on differences between active and total infestation into the two fields (A and B) and throughout the infestation period. Asterisks denote significant differences for $p < 0.05$.

2004	26/08	02/09	09/09	20/09	27/09	05/10	11/10	18/10
Active A > Active B	0.430	0.086	0.000*	0.000*	0.005*	0.000*	0.000*	0.000*
Total A > Total B	0.729	0.152	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
2005	19/09	27/09	08/10	12/10	18/10	26/10	01/11	07/11
Active A > Active B	0.999	0.912	0.456	0.265	0.951	0.000*	0.000*	0.000*
Total A > Total B	0.999	0.938	0.152	0.569	0.878	0.000*	0.000*	0.000*

Table 5. *p*-values of combined test according to the different combining functions in 2004 and 2005. Hypotheses $H_{2',1}$ and $H_{2'',1}$ are accepted in all tests.

		Fisher	Liptak	Tippett	Direct
2004	$H_{2',1}$ Active A > Active B	0.00014998	0.00014998	0.00024997	0.00014998
	$H_{2'',1}$ Total A > Total B	0.00014998	0.00014998	0.00014998	0.00014998
2005	$H_{2',1}$ Active A > Active B	0.00014998	0.00034996	0.00024997	0.00014998
	$H_{2'',1}$ Total A > Total B	0.00014998	0.00054994	0.00034996	0.00014998

Olive fly females can start egg laying in July; however, due to the hot and dry Sicilian summers, the oviposition activity usually is delayed at the end of August/beginning of September. Infestation of drupes generally reaches high levels (over 50% of drupes) during the second half of October (Tsolakis *et al.*, 1999; Tsolakis and Ragusa, 2002; Caleca and Rizzo, 2006). Weather conditions in this period (temperatures of 17 - 27 °C and ≈70% RH) are optimal for the olive fly postembryonic development, which is completed in about 10 days. When the exit hole is made, the drupe starts to degenerate both because of pulp oxidation and of the microorganisms introduced through the larvae's exit hole. Only after this moment drupes should be considered damaged (passive infestation), as drupes with *B. oleae* eggs or larvae and without exit holes still give a good quality oil (Longo and Parlati, 1993; Tsolakis *et al.*, 1999). On the other hand, in Sicily drupes reach an adequate content of oil from an economic point of view (up to 15%), after the first week of October. Thus any delay in the spreading of the infestation, increases both the quantity and the quality of oil yield. Our results indicate an effective control of *B. oleae* infestation, in both years, when the lure-and-kill method was integrated with copper sprayings. As a matter of fact the integration of the above methods guaranteed a very low passive infestation level at the harvest time due to its negative influence on the infestation spread. Copper sprayings alone showed a significant reduction of olive fly infestation in various environmental situations (Belcari and Bobbio, 1999; Tsolakis and Ragusa, 2002), but, according to the latter authors, this method alone cannot compete with synthetic chemicals. Copper is a heavy metal that may cause various ecological disorders due to its accumulation in the soil, so that regulation for its use is applied in many European countries (Commission Regulation EC No. 473/2002). However, the quantity used for the treatments in our experiments (2.4 kg of copper/hectare for 2 sprayings/year) was lower than the limit fixed by the above mentioned guideline (6 kg of copper/hectare/year).

As far as the passive infestation is concerned, it was under the damage threshold in both years in field B, while it was quite higher in field A, where only the lure-and-kill method was adopted. On the other hand it should be mentioned that Tsolakis *et al.* (1999) reported also acceptable quality oil (acidity 0.44%, peroxides 0.01158 ppm, rancimat test 6.2 h) obtained from olives with passive infestation levels similar to that registered in field A.

Conclusions

The NPC methodology frees the analyst from the evaluation of a set of statistical properties, and consequently allows focusing on the entomological results. As far as the lure-and-kill method is concerned, it seems to be an inappropriate control method for small surfaced olive groves in coastal Mediterranean areas. However, the edge effect noticed in the present work indicates it as a suitable method for large olive groves. Further studies are needed to confirm our results in other Mediterranean countries. Finally, it can be affirmed that the integration of two weak control methods in small surfaces gave a good control method of the tephritid's population, especially in situations where synthetic chemicals are forbidden.

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