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IPM trials on attract-and-kill mixtures against the olive fly *Bactrocera oleae* (Diptera Tephritidae)

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Abstract

The key insect pest of the olive grove is the olive fly, *Bactrocera oleae* (Gmelin) because it affects the quantitative and qualitative production of olive oil. In order to first attract and then kill *B. oleae* adults before egg laying, thus limiting the infestation and avoiding treatments on the whole olive grove, we tested a mixture of the female sexual pheromone of the olive fly (1.7 dioxaspiro-5.5 undecane), the protein hydrolisate Buminal, and the insecticide Deltamethrin. We also tested different doses of the female sexual pheromone (1999: 1.212 ml/hl water and 2.424 ml/hl water; 2000: 2.424 ml/hl water and 4.848 ml/hl water). Both in 1999 and in 2000, treatments were applied when the gravity index Z exceeded the threshold level Z > 0.10. Irrespective of the general infestation level (high or low), the mixture successfully attracted and killed *B. oleae* adults. In both years, the mixture containing the pheromone at a dose of 2.424 ml/hl was the most effective.

Key words: *Bactrocera oleae*, IPM, olive, female sexual pheromone, Buminal, Deltamethrin, yellow sticky trap.

Introduction

The olive grove is an agroecosystem with integrated and biological control methods in constant evolution, and new integrated pest management (IPM) strategies aim at a reduced, targeted and, therefore, more selective use of agrochemicals.

The key phytophagous pest of the olive grove, *Bactrocera oleae* (Gmelin), shows the following characteristics: strict monophagism, direct association to areas with olive cultivation, likely disruption of the quantitative and qualitative production of olive oil (Del Rio, 1979; Michelakis and Neuenschwander, 1983; Belcari *et al.*, 1989; Ochando and Reyes, 2000). Traditional control techniques, usually used in Italy and other European countries, are based on agrochemical treatments, which are applied to the whole olive grove, against larval and adult stages of *B. oleae*. However, these treatments may also affect the useful entomofauna, and the olive oil quality due to the presence of residues (Spanedda and Terrosi, 2002a; 2002b).

In 1999 and 2000, in olive groves of Northern Lazio, the efficiency of a different *B. oleae* control method was investigated. We verified whether attract-and-kill treatments (mixtures including the sexual pheromone, a food attractant, and an insecticide) applied only to 6% of the trees within the olive grove, would attract and kill the adults before egg laying.

Materials and methods

The research was carried out at the specialized olive grove of the "Sugarella" Agriculture Farm, property of the Supreme Military Order of Malta in the town area of Canino (Viterbo, Italy) during the two-year period 1999-2000. The olive grove extends over a surface of approximately 14 ha, and has an altitude of 250 m above sea level. To the South and East it is surrounded by pastures, while to the North and West it borders with seed crop fields. It has a 6 x 6 tree spacing plantation layout with 35-40 year old trees. 'Canino' (87%) is the main cultivar; the other cultivars are 'Leccino', 'Frantoio', 'Maurino' and 'Pendolino' (13%). The experimental area was subdivided into three plots (plots A, B, and C). Plots A and B consisted each of approximately 185 trees, while plot C included approximately 300 trees.

In each plot, 12 plants of the main cultivar 'Canino' were selected and then exposed to the different treatments (see below). Both in 1999 and 2000, treatments were applied when the gravity index Z (Pucci, 1993) exceeded the threshold value Z > 0.10. This index allows an estimate of the development of *B. oleae* infestations (Pucci *et al*., 1979; Pucci, 1993) and is defined as:

$$Z = 0.039 (Fm - 9.7) - 0.186 (Tm - 22.1)$$

where:

- Fm is the mean number of females per yellow sticky trap, captured weekly;
- Tm is the mean of the daily mean temperatures recorded in that week.

Every year and in each plot, adults were monitored with three yellow traps placed on trees other than those exposed to treatments within the different plots. Traps were 15 x 20 cm in size, limed with Temocid glue (Kollant company), and oriented South. Traps were inspected weekly, and the number and sex of captured adults was recorded.

Once the threshold level Z > 0.10 was exceeded, the twelve target trees of plots A and B were treated with a mixture of the female sexual pheromone 1.7 dioxaspiro-5.5 undecane, the protein hydrolisate Buminal, and the insecticide Deltamethrin, diluted in 10 l of water. In both years and plots, Buminal was applied at a dose of 1,000 g/hl, and Deltamethrin at a dose of 1.67 mg/hl, but the doses of the female sexual pheromone varied

between years and plots (1999: 1.212 ml/hl in plot A, and 2.424 ml/hl in plot B; 2000: 4.848 ml/hl in plot A and 2.424 ml/hl in plot B). The twelve trees of plot C were treated with an equal volume of water, thus acting as control.

To sample and collect the adults that died because of the treatment, three of the twelve trees in each plot were provided with white plastic nets (12 x 6 m) underneath the canopy. The adults collected in the nets were counted and sexed daily.

In each year and plot, olive samples consisting of around 200 olives each were collected weekly at random from trees not exposed to treatments. The samples were then transferred to the laboratory, dissected, and the different infestation stages, i.e. eggs, first, second, and third instar larvae (L1, L2, and L3), abandoned galleries, were recorded. For each treatment and year, we also counted the number of infested and not infested olives.

Finally, at harvest, in each plot and year, 3 kg of olives were collected from trees adjacent to the treated ones, and olive oil samples were obtained by pressure extraction. These samples were then analyzed to assess for the qualitative parameters degree of acidity (% of oleic acid per 100g of oil) and number of peroxides.

Statistical analysis

The number of dead *B. oleae* adults (males + females) collected in the nets was compared across treatments using an univariate one-way analysis of variance (ANOVA). Data were rank-transformed, because enumeration data are non-parametric (Helsel and Hirsch, 1995). Orthogonal contrasts were constructed to compare combinations of means in the ANOVA analysis. The ANOVA was also applied to two measures of oil quality (i.e., number of peroxide, degree of acidity).

The numbers of infested and not infested olives were compared across treatments applying χ^2 statistics to 3 x 2 contingency tables. The 3 x 2 contingency tables were then disaggregated into 2 x 2 contingency tables, and pair-wise comparisons between treatments were made using χ^2 statistics corrected according to Yates. The data of each year were analysed separately.

Results

1999

After the treatment (8 October), the climatic conditions (decrease in temperature) generally favoured an increase of the infestation (figures 1 and 2). In plot A, until 21 September less than 5% of the sampled olives were infested mostly by eggs and first instar larvae (figure 1A). Thereafter, the infestation increased. It reached a peak on 12 October, followed by a slight fall, and then it increased again consistently. At harvest, 90% of the sampled olives were infested. The development over time of the infestation in plot B was similar to that in plot A (figure 1B). However, in plot B the infestation fall after the peak of 12 October was much stronger than in plot A, and, at the end of the testing period (at harvest), the percentage of infestation was around 60%. In

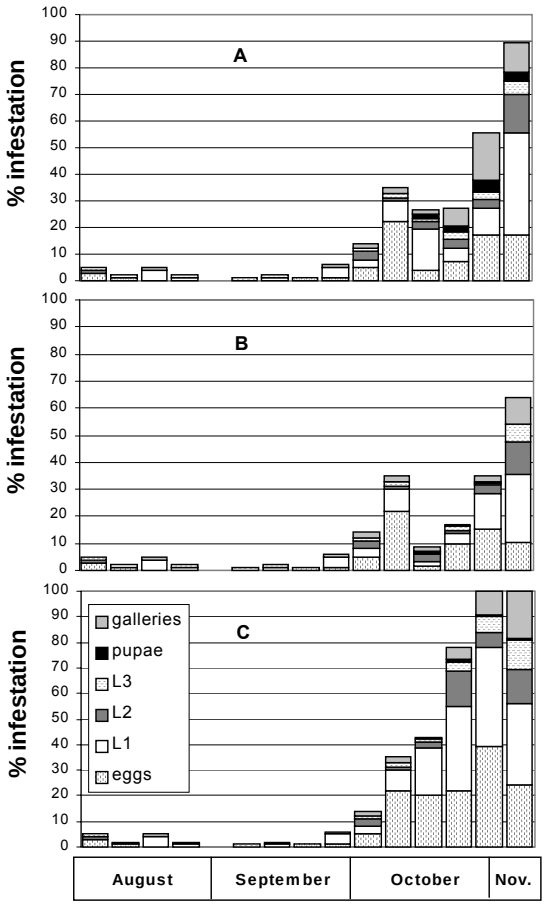

Figure 1. Olive Infestation trends of plots A, B and C (control) during 1999.

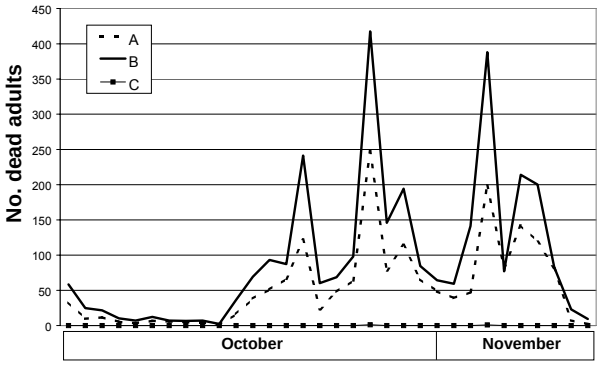

Figure 2. Average adults dead number, after treatment during 1999.

the control plot C, the percentage of infested olives constantly increased from 21 September on, and at harvest 100% of the sampled olives were infested. In the treated plots A and B, in the days ensuing the treatment (October 8), the mean number of dead adults found in the nets was very low, but it increased towards mid-end October peaking in 122.0 dead adults on 23 October and 248.3 on 27 October in plot A, and 214.3 on 23 October and 417.7 on 27 October in plot B (figure 2) respectively. On 3 November, a peak of 198.7 dead adults in plot A

Table 1. Number of dead *B. oleae* adults: comparison between treatments in 1999 and 2000 (results of the non-parametric analysis of variance).

source of variation	deviance	df	variance	F	p
		year 1999			
Total	60	8			
Treatment (Control, A, B)	54	2	27	27	0.001
Control *vs* (A+B)	40.5	1	40.5	40.5	0.001
A *vs* B	13.5	1	13.5	13.5	0.010
Error	6	6	1		
		year 2000			
Total	60	8			
Treatment (Control, A, B)	48.67	2	24.33	12.88	0.007
Control *vs* (A+B)	40.5	1	40.5	21.44	0.004
B *vs* A	8.17	1	8.17	8.17	0.083
Error	11.33	6	1.89		

and 388.0 dead adults in plot B was reached. As expected, mortality in the control plot C was minimal. Dead male and female *B. oleae* adults were pooled for statistical analysis, because in each plot, the sex ratio over time was always around 1:1. As shown in table 1, significant effects of A and B emerged when comparing the number of dead adults in the different plots (p=0.001). Moreover, treatment B performed significantly better than A (p=0.010) in reducing the number of insects. Similar results (p≈0.000) are given when comparing the effects of different treatments on the infestation (table 2). The total number of dead adults collected in the plots exposed to the different treatments was 5,330 (2,771 females and 2,599 males) in plot A, 9,007 (4,637 females and 4,370 males) in plot B and 3 (2 females and 1 male) in plot C. Both the degree of acidity (0.54 plot A, 0.53 plot B and 0.59 plot C) and the number of peroxides (8 plot A, 9 plot B and 10 plot C) show no statistically significant differences (p>0.05).

2000

Treatments were carried out on 10 October. However, temperatures remained high until 26 October (max. 40 °C, min. 22 °C), and this caused a break in the development of the population (figures 3 and 4). In plot A, the infestation became evident starting from 10 October. Infestation percentage reached a peak of 44% on 31 October, and 23% at harvest (figure 3A). In plot B as well (figure 3B), the infestation became evident from 10 October on. The first peak, with an infestation of 6%, was reached on 17 October, and a second peak, with 20% infestation, was reached on 31 October. At harvest (14 November) infestation was 24%. In the control plot C (figure 3C), the infestation began a week in advance compared to the other plots, and a maximum of 58% was reached on 31 October. At harvest (14 November) infestation was 48%. In the treated plots, two peaks in the number of dead *B. oleae* adults were recorded. The first peak occurred on 14 October, with a mean of 61.3 dead adults in plot A and 66.3 in plot B. The second peak occurred on 20 October, with a mean 15.0 dead adults in plot A and 34.44 in plot B. Dead male and female *B. oleae* adults were pooled for statistical analysis, because in each plot, the sex ratio over time was always

Table 2. Number of infested and not infested olives: comparisons between treatments in 1999 and 2000 (results of the χ^2 test).

treatment	infestation		total
	presence	absence	
	year 1999		
Control	91	79	170
A	96	329	425
B	19	426	445
total	206	834	1040
	χ^2=191.409 p≈0.000		
Control	91	79	170
A	96	329	425
total	187	408	595
	χ^2=53,516 p≈0.000		
Control	91	79	170
B	19	426	445
total	110	505	615
	χ^2=203.228 p≈0.000		
A	96	329	425
B	19	426	445
total	115	755	870
	χ^2=63.593 p≈0.000		
	year 2000		
Control	26	155	181
B	6	258	264
A	19	199	218
total	51	612	663
	χ^2=22.590 p≈0.000		
Control	26	155	181
B	6	258	264
total	32	413	445
	χ^2=21.748 p≈0.000		
Control	26	155	181
A	19	199	218
total	45	354	399
	χ^2=2.615 p≈0.106		
B	6	258	264
A	19	199	218
total	25	457	482
	χ^2=8.811 p=0.003		

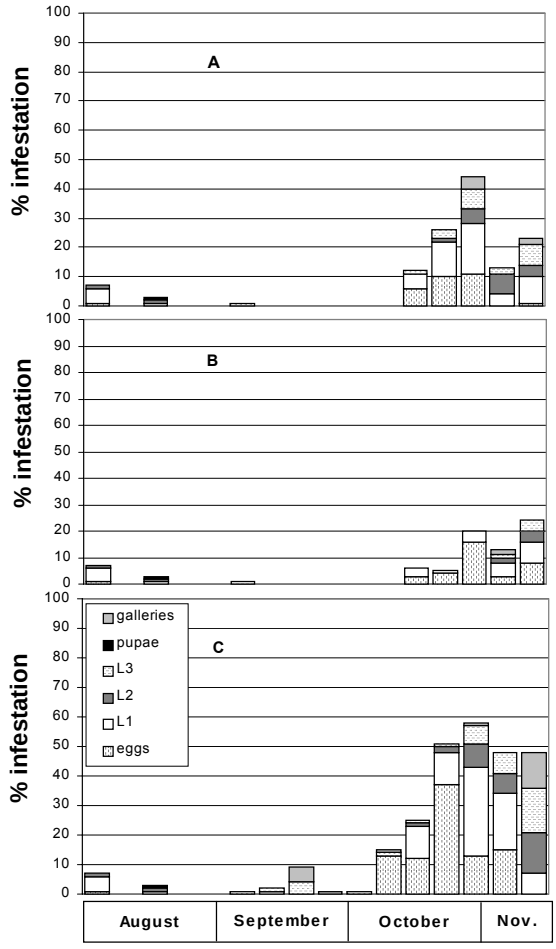

Figure 3. Olive infestation trends of plots A, B and C (control) during 2000.

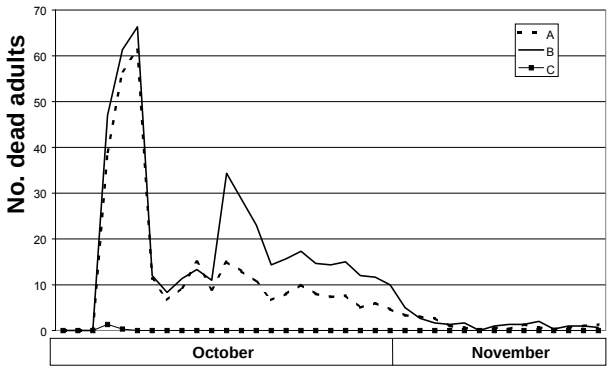

Figure 4. Average adults dead number, after treatment during 2000.

around 1:1 as underlined for 1999. The beneficial effect of treatments A and B in reducing the insect number (table 1) was confirmed for the year 2000 ($p=0.007$) with no difference between A and B ($p=0.083$ for the contrast A *vs* B). As regards the infestation (table 2), treatment B produced a significant result both versus the control plot ($p\approx0.000$) and the treatment A ($p=0.003$). On the contrary, treatment A did not show any significant effect when compared to the control plot ($p=0.106$). The total number of dead adults collected in the plots exposed to the different treatments was 1,039 (550 females and 489 males) in plot A, 1,433 (803 females and 630 males) in plot B and 4 (3 females and 1 male) in plot C. Both the degree of acidity (0.59 plot A, 0.60 plot B and 0.68 plot C) and the number of peroxides (7 plot A, 8 plot B and 9 plot C) show no statistically significant differences ($p>0.05$) as underlined for 1999.

Discussion and conclusion

Compared to 1999, year 2000 was a light year for the olive fly in the study-site, probably because of different climatic trends (e.g., larger rainfall amounts occurred in 1999, data not shown). In particular, in 1999 infestation of the control plot was registered at 100% of damaged olives; the treatment at a dose of 1.212 ml/hl of pheromone determined a reduction in the infestation by almost 10%, whereas the treatment with a dose of pheromone equivalent to 2.424 ml/hl allowed for a reduction of 44%. In 2000 the percentage of infestation reported on the control plot was 48% and in the two plots treated with two doses of sexual pheromone (2.424 ml/hl and 4.848 ml/hl) there was a reduction of infestation by 50%. The qualitative analysis of the oils obtained from the three plots and for the two years have not shown any statistically significant differences. This result is determined by the milling times of the olives (in 12 hours) as already underlined in previous works (Pucci and Dominici, 1982). The statistical processing of the data relating to both dead adults after the treatment (table 1) and infestation (table 2) indicates a clear effect of treatments in both high- and low-infestation years; in any case, the pheromone dose of 2.424 ml/hl gave the best results. This result induces us to continue our research, increasing the number of plants per hectare to be treated and, above all, anticipating the intervention period with the aim of reaching even more satisfying results.

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