

Response of olive fruit fly *Bactrocera oleae* to various attractant combinations, in orchards of Crete

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Abstract

McPhail traps loaded with various attractant solutions (ammonium sulphate, Entomela 75%, Entomela 50%, Dacus bait 100, sexual pheromone of olive fruit fly *Bactrocera oleae*) combined also with registered plant protection products (lambda-cyhalothrin, alpha-cypermethrin as well as dimethoate) were tested for their attractiveness which was indicated as captures of flies in McPhail traps during three summer year periods. All the tested proteins were significantly more attractive than ammonium salts which are broadly used in Greece for monitoring of pest population while the sex pheromone did not seem to have any impact. The mean number of captured adults of *B. oleae* recorded at McPhail traps which were filled with attractant solution, were at least two times fold higher than when an insecticide was added to it. Thus the application of such attractant combinations in the management of the pest, is discussed.

Key words: captures, McPhail, monitoring, baits, plant protection products.

Introduction

Bactrocera oleae (Rossi) (Diptera Tephritidae) is considered the most serious pest in many of the areas of the Mediterranean basin, affecting the quality and quantity of oil and table olives (Manousis and Moore, 1987). It has been one of the most devastating olive pests for more than 2000 years (Hepdurgun *et al.*, 2009) because it is able to reproduce and develop throughout the year provided that, temperature and humidity are favourable and host fruit is available (Tzanakakis, 2003). Specifically, in Crete island (Southern part of Greece) more than 5 generations have been recorded annually (Varikou unpublished data). Infestation of olive fruit by the larvae causes premature fruit drop and reduces fruit quality for both table olive and olive oil production (Michelakis and Neuenschwander, 1983). In table olives, the presence of a few larvae can lead to rejection of an entire crop. Some infestation can be tolerated in olive oil production. However, the presence of larvae and associated microorganisms raises oil acidity and thereby reduces the quality of the oil. Untreated, olive fruit fly may infest more than 90% of olive fruit (Sharaf, 1980; Kapatos and Fletcher, 1984).

In the Mediterranean and sub Saharan Africa, the olive fruit fly is attacked by a number of parasitoid species. The best-known is a braconid wasp, *Psytalia concolor* (Szepliget), which was introduced into Italy from Africa in 1914 (Clausen, 1978). *P. concolor* was later introduced to France and Greece, and most recently in California. It is a synovigenic koinobiont larval-pupal endoparasitoid able to attack at least fourteen Tephritidae species at different wild/and or cultivated plants, including pests of great economic importance such as *Ceratitis capitata* (Wiedemann) and *B. oleae* (Benelli and Canale, 2012). This species is believed to be relatively ineffective as a biological control agent in Europe. One reason for its poor performance may be a lack of synchronization between the life cycles of the parasitoid and fly (Clausen, 1978). Olive fruit fly larvae

are typically unavailable for parasitism when female *P. concolor* emerge in the spring.

The used control method against the olive fruit fly are bait spray applications; Bait sprays consist of a 2% of a bait mixed with registered insecticides applied either by air or ground sprays, have been used for many years against the olive fruit fly (Nadel, 1966; Manousis and Moore, 1987). Insecticides are usually combined with the attractant of protein hydrolysate to form a bait spray (Roessler, 1989); practical details are given by Bateman (1982). Bait sprays work on mainly female tephritid fruit flies which are strongly attracted to a protein source from which ammonia emanates, and ingest a lethal dose of insecticide together with the protein. Bait sprays have the advantage over cover sprays in that they can be applied as a spot treatment (≤ 300 ml of spraying solution/tree) so that the flies are attracted to the insecticide and there is a minimal impact on natural enemies. Attraction to the protein allows the use of spot sprays, rather than broadcast applications of insecticides. In Greece four to seven treatments only by ground may be required; especially in years and in regions such as Crete, that are favorable to the pest (Varikou *et al.*, 2013).

Accordingly, development of alternative methods of chemical, improved management technology attempts to control the olive fruit fly by luring them into killing devices, in almost every tree of an orchard (called as mass trapping) were initiated in 1960's. In recent years, mass trapping has become an important management tool in protecting the beneficial fauna in the ecosystem (Mazomenos *et al.*, 2002; Ragoussis, 2002; Lentini *et al.*, 2003; Caleca *et al.*, 2007). It represents a preventive control measure, which is based on attraction and killing of olive fruit fly adults in order to avoid infestation. The main advantage of mass trapping method is the exclusion from chemicals of the whole tree canopy, with the installation of a trap on each tree. The traps could be filled by different type of attractants (proteins, ammonia, pheromone) and treated by insecticide, or they could be filled with an attractant-insecticide solution

(Haniotakis *et al.*, 1983; Barclay and Haniotakis, 1991; Bjeliš, 2006). Numerous researches have been carried out on the trap type and trap solution (registered insecticide and attractant solution), number of the traps per hectare and trapping duration, attractiveness of trap solution etc. resulted in the development of a better attract and kill system based on the idea of bait sprays. It has been referred a better efficacy of mass trapping method compared to bait sprays (Broumas and Haniotakis, 1987; Delrio and Lentini, 1993; Broumas *et al.*, 1998; Bjeliš, 2006), but more expensive; the cost of the mass trapping method in regular olive orchards, which require a trap density of one trap per two trees and no complementary bait sprays, is approximately 30% higher than the cost of the bait spray method (Broumas *et al.*, 2002).

Nowadays, electronic techniques such as an innovative, integrated agro-environmental Location-Aware System suitable for the ground spray applications of the olive fruit fly was used and evaluated for its effectiveness in order to reduce the duration of sprayings, minimizing their cost and the possibility of cancelling of a spraying application due to meteorological conditions (Pontikakos *et al.*, 2012).

Concerning monitoring of olive fly population, the glass McPhail trap baited with 2% ammonium sulphate solution or hydrolyzed protein has been used for over 50 years in several Mediterranean countries and they are commonly used in Greece for monitoring the olive fruit fly (Rössler, 1989; Katsoyannos, 1992; Haniotakis, 2005); protein hydrolysates have been proved to be more attractive (Orphanidis *et al.*, 1958; Prokopy and Economopoulos, 1975) than ammonium salts (Propheetou *et al.*, 2003). Despite this, McPhail traps are usually loaded with this latter attractant during the whole period (summer and autumn) in Greek olive groves (Katsoyannos and Kouloussis, 2001).

At all the above mentioned methods of *B. oleae* management, the attractants are major components of monitoring and control of the pest. However, only a few comparative studies among these attractants have been done before concerning their attractiveness in monitoring olive fruit fly population, their attractant duration, their combination with insecticides (especially pyrethroids) or with others, their efficacy in bait sprays and mass trapping systems while no study concerning the used concentrations.

In this study, results obtained from the evaluation of various food and sex attractants are reported in order to improve monitoring olive fruit fly populations in olive orchards. Also, bait spraying solutions applied in McPhail traps were tested in order to improve the method of bait spray by ground applications as well as mass trapping with a goal to develop environmentally safe method of pest control.

Materials and methods

Experimental area

All field experiments were conducted on the island of Crete (southern part of Greece) on a 1.1 ha olive grove (cv. 'Koroneiki' with small fruit, an olive oil producing

variety) located in Nerokourou village (35°28'36.18"N 24°02'33.25"E 53 m a.s.l., 5 km NE of Chania), during summer period of 2003, 2010 and 2012. The olive trees were 25-30 years old, 7-10 m tall and 6-7 m apart; the tree density was about 200 trees/ha. The grove was not irrigated. During 2003, 2010 and 2012, the mean olive fruit production/tree at the experimental orchard was estimated about 40, 70 and 60% respectively. No chemical treatment was applied during the experiment trial periods.

Efficacy of various food attractants

During 2003 (23/7-19/11/03) olive fruit fly attractants of *B. oleae* were tested in McPhail traps: hydrolyzed proteins such as 'E50' (Entomela 50, 17% w/w urea and percentage of protein equal to 50% w/w, Stavrakis, Viotia, Greece), 'E75' (Entomela 75, 25% w/w urea and percentage of protein equal to 75% w/w, Stavrakis, Viotia, Greece), 'Db' (Dacus bait 100, consists of 4% w/w ammonium salts, 3-4% w/w of free L- Amino acids and protein equal to 55% w/w, EVYP LLP, Thessaloniki, Greece) all in liquid form, and 'A' (ammonium sulphate) in 2% formulation separately or in combinations with the 'Sp' (Sex pheromone of the olive fruit fly 1,7-dioxaspiro [5.5] undecane). The pheromone dispenser of Eco-Trap (Vioryl. S.A., Athens, Greece) loaded with 50 mg of spiroacetal was attached to each trap (Mazomenos *et al.*, 2002).

Efficacy of food attractants mixed with the registered plant protection products

Similarly, during 2010 (20/7-1/11/10) and 2012 (10/7-6/11/12), the efficacy of 'Db' and 'E75' was tested in 2% formulation separately or in combinations with the registered plant protection products at the recommended doses against the olive fruit fly; alpha-cypermethrin (Class: pyrethroid; Fastac 10SC; 300 cc/hl; BASF, Hellas) and lambda-cyhalothrin (Class: pyrethroid; Karate with Zeon technology 10 CS; 125 cc/hl; Syngenta, Hellas) during 2010 while dimethoate (Class: organophosphate; Dimethoate 40EC; 750 cc/hl; Bayer, Hellas) were mixed with 'Db' and 'E75' during 2012. 'A' and Success (Class: Spinosyn; Success; 3.7 lt/hl; Dow AgroSciences SA Ltd; a readymade product which includes in a formulation a new fruit fly bait developed by the company) were also evaluated for their attractiveness in McPhail traps. All treatments were compared to control which was water.

Experimental design

The experimental design used randomized block with three replications. Each block has one representative of each treatment. The traps were 21 m apart inside and between blocks. The traps were installed in the northern part of a tree at a height of about 1.5 meters. Olive fruit fly captures of traps were checked once a week during 2003 and twice a week during 2010 and 2012, during all the trial. After collecting the captured olive fruit flies (females and males) from the traps, these were rotated sequentially in the same block in a clockwise way while the solution was replaced with fresh one. The criterion chosen to proceed with rotation was to avoid the influence of the position of the trap on fly captures.

Data analysis

The number of captured adults of *B. oleae* in McPhail traps was analyzed separately for each year and each trial, using one-way analysis of variance (ANOVA) and means were separated using the Tukey's honestly significant difference (HSD) test. Data analysis and fitted curves were carried out using the statistical package JMP (SAS Institute, 2008).

Results

Efficacy of the tested food attractants

The obtained data from the study indicated that olive fruit fly adults showed different degrees of preference to the different tested food attractants. When 'Db', 'E75' and 'E50' were tested alone for their attractiveness, they captured significantly higher mean number of olive fruit flies than just 'Sp' while 'A' captured significantly lower mean number of olive fruit flies

than 'Db', 'E50' and 'Db+Sp' treatments. Also, the addition of 'Sp' to proteins did not influence significantly their attractiveness ($F = 14.293$; $df = 9,500$; $P < 0.05$) (figure 1).

Efficacy of the tested food attractants mixed with the registered plant protection products

During summer of 2010, the addition of plant protection products in the attractant solution reduced significantly the fly captures in traps (2-5 times less) ($F = 10.753$; $df = 11,356$; $P < 0.05$). All tested baits captured significantly higher mean number of flies than 'A' as well as Success treatment (figure 2).

Similarly during summer of 2012, the addition of plant protection products in the attractant solution reduced (> 2 times) significantly the fly captures in McPhail traps while the tested baits captured significantly higher mean number of flies than 'Db+a-cypte' and significantly lower than the other treatments ($F = 26.94$; $df = 10,1154$; $P < 0.001$) (figure 3).

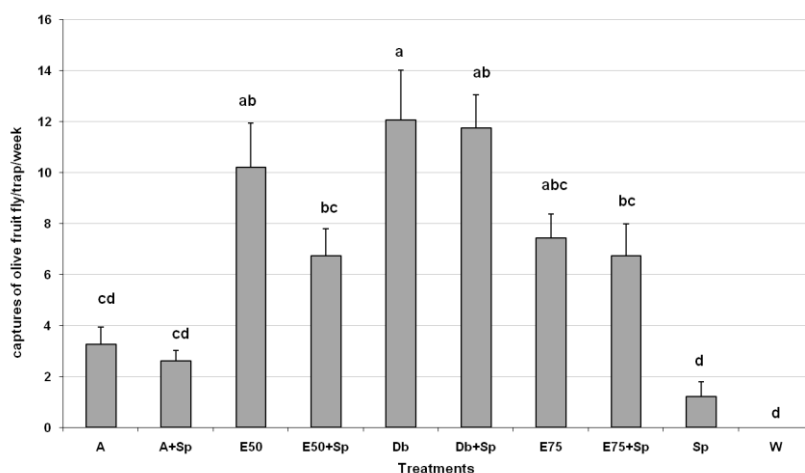


Figure 1. Mean number of captured adults per trap and week for each treatment during summer 2003. Bars with the same letter (s) are not significantly different at $P < 0.05$. Ammonium sulphate 'A', Entomela 75% 'E75', Entomela 50% 'E50', Dacus bait 100 'Db', sexual pheromone 'Sp', water 'W'.

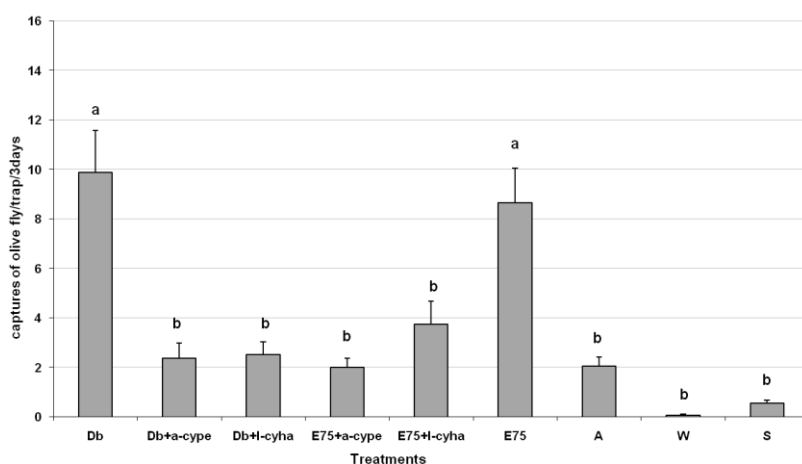


Figure 2. Mean number of captured adults per trap and 3 day period for each treatment during summer 2010. Bars with the same letter (s) are not significantly different at $P < 0.05$. Water 'W', Ammonium sulphate 'A', Entomela 75% 'E75', Dacus bait 100 'Db', lambda-cyhalothrin 'l-cyha', alpha-cypermethrin 'a-cypte', Success 'S'.

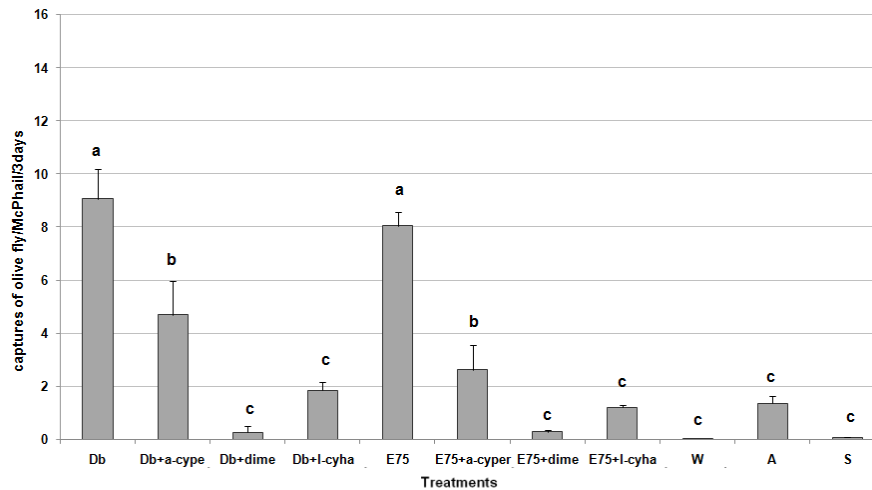


Figure 3. Mean number of captured adults per trap and 3 day period for each treatment during summer 2012. Bars with the same letter (s) are not significantly different at $P < 0.05$. Water ‘W’, Ammonium sulphate ‘A’, Entomela 75% ‘E75’, Dacus bait 100 ‘Db’, lambda-cyhalothrin ‘l-cyha’, alpha-cypermethrin ‘a-cypr’, Dimethoate ‘dime’, Success ‘S’.

Discussion and conclusions

Ammonium salts have long been used as lure in fruit fly traps although it has been reported as a weak attractant (Ripley and Hepburn, 1929; McPhail, 1939; Gow, 1954) compared to proteins (Prokopy and Economopoulos, 1975; Haniotakis, 2005). However in Greek orchards, ammonium salts is still used in McPhail for monitoring the olive fruit fly population during the whole period (summer and autumn) though it has been proved, that its attractivity was significantly reduced compared to all the tested protein hydrolysates during both summer and autumn conditions; the mean number of captured adults of *B. oleae* were more than two times fold higher in all the tested proteins than in the ammonium solution. Generally among the food-based attractants, protein-hydrolysates are highly attractive to female Dacinae (Metcalf, 1990) as well as a variety of other fruit flies (Steiner *et al.*, 1961), because they require a source of protein to complete egg maturation; this requirement is probably the main cause for the strong attraction of females towards decomposing proteinaceous substances, first observed by McPhail (1939). Since ammonia, is emitted from decomposing proteins, it was assumed that attraction of fruit flies to protein baits is related to the release of ammonia. Mazar *et al.* (1987) suggested that *C. capitata* response to protein baits is affected not only by ammonia but by other volatiles as well.

In addition, researches have been carried out providing a direct comparison of McPhail captures baited with ammonium salts or proteins, and the actual olive fruit fly population within an orchard showing that the captures of traps loaded with the former (Varikou *et al.*, 2013) or the latter attractant (Neuenschwander and Michelakis, 1979) are not so validated in the evaluation of the actual population in the field. The use of the ammonium salts in McPhail traps for monitoring the *B. oleae* population must be redefined and the identification of superior protein hydrolysates would be useful for

improving the sensitivity of traps for early detection of the actual fly population in the olive orchard. This is very important in *B. oleae* management especially during autumn months, where the use of such inefficient trophic solutions in monitoring traps may not record the real number of flies and this can be a disaster for olive production.

Concerning fruit fly pheromones, they have been reported to have limited success (Landolt and Averill, 1999) since their action is not understood completely (Kuba, 1991). In this study, the addition of ‘Sp’ of the olive fruit fly in the attractant solution did not increase significantly the olive fruit fly captures. Instead, other research papers report that a combination of traditional trapping systems such as the McPhail trap and sex pheromone-baited traps gives the best population monitoring information for pest management purposes (Montiel, 1989). In addition other traps such as sticky panels baited with food or sex attractants have been calibrated and used in different countries for monitoring populations in cases of various environmental and climatic conditions (Mazomenos *et al.*, 2002; Haniotakis, 2005).

The addition of plant protection products such as pyrethroids (lambda-cyhalothrin, alpha-cypermethrin) or organophosphorous (dimethoate) in the attractant solution reduced the attractiveness of the spraying solution to *B. oleae* adults. The best combination of attractant and insecticide seemed to be all tested proteins (‘E75’ and ‘Db’) plus pyrethroids instead of organophosphorous. There is probably a repellent affect of the tested insecticides to the olive fruit flies when the tested solution is three days old. A mixture of Provesta protein and malathion was reported to be repellent in *C. capitata* (Vargas *et al.*, 2002) or deter feeding (Prokopy *et al.*, 1992). Similarly Moustafa (2009) reported that adding a pesticide to the food attractant preparations obviously reduced the attractiveness of the lures to *C. capitata* and *Bactrocera zonata* (Saunders) adults.

Another disadvantage of the used attractants is their

attractant duration; they do not seem to last more than 3-6 days in traps and more than 3 days in bait spray applications (Varikou unpublished data). It has been referred that aged baits (four-day old) were not as attractive to *C. capitata* as fresh baits (Vargas *et al.*, 2002). In addition, as Broumas *et al.* (1998) referred, the active life of insecticides or pheromone dispensers used in traps for mass trapping, cover the entire period of fruit susceptibility to the pest but the used food attractant does not. The findings of this study provide useful fields for further investigations regarding *B. oleae* management and especially real effectiveness of the spraying solution applied at the olive foliage.

A search for a new attractant, or a new formulation of the present attractants is presently on a way; new born insecticides consists of more 'improved' bait and insecticide in one formula such as Success. Although Success did not perform well in McPhail traps, it has been reported to be very effective in bait spray from the ground applications against *B. oleae* as well as *C. capitata* (Varikou unpublished data). This is due to the accumulation of the bait formulation in large spraying spots (diameter > 7 mm) on the olive foliage which are renewed and release odours to the flies with the morning humidity.

Future IPM research of *B. oleae* should emphasize in the identification of a new protein which will improve the sensitivity of McPhail traps in recording the actual olive fruit fly population, it should be more stable under extreme conditions, attract for a long time, combine well with insecticides in bait sprays and in 'Lure and Kill' devices. At the end, more research is needed in optimization of the protein and toxicants that are included in bait so that non-target effects of broad spectrum contact poisons can be avoided and acceptable crop protection will be determined.

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