Bait station devices can improve mass trapping performance for the control of the Mediterranean fruit fly Vicente Navarro-Llopis*, Jaime Primo, Sandra Vacas Centro de Ecología Química Agrícola - Instituto Agroforestal del Mediterráneo (CEQA-IAM). Universitat Politècnica de València, edificio 6C, 5ª planta. Cmno de Vera s/n 46022 Valencia (Spain) *Corresponding author. Tel.: +34 963879058; fax: +34 963879059. *E-mail address*: vinallo@ceqa.upv.es Running title: Bait station efficacy assessment

Pest Management Science

Abstract

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- 2 BACKGROUND: The use of traps and other attract-and-kill devices in pest management
- 3 strategies to reduce Mediterranean fruit fly populations has proven efficient. Nevertheless,
- 4 many farmers are concerned about the effect of these devices on the trees where they are
- 5 hung. Direct field observations have revealed that fruit damage is higher in trees with traps
- 6 than in trees without them. This work evaluates the efficacy of different types of attract-and-
- 7 kill devices to protect fruit of the single tree where the device is placed in.
- 8 RESULTS: Results suggested that trees with traps had, at least, the same fruit damage than
- 9 trees without them. When traps were baited with protein hyrolizate, fruit damage was even
- higher than in trees without traps. However, fruit damage is significantly diminished when
- efficient bait station devices are used.
- 12 CONCLUSION: Although mass trapping is able to control fruit fly populations as a control
- method, trees with some type of traps and baits are more susceptible to fly puncture.
- However, bait station devices reduce fruit damage in the single trees where they are hung. As
- a conclusion bait station resulted more efficient in fruit protection as fruit flies are affected as
- soon as they contact the device. Some recommendations for use of the different attract-and-
- kill devices are discussed.

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Key words: *Ceratitis capitata*; attract and kill; trap, lure and kill

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1. INTRODUCTION

Attract-and-kill devices refer to all kind of devices employed to draw insects to a killing agent. Mass trapping is probably the first technique employed to reduce Mediterranean fruit fly, Ceratitis capitata (Wiedemann), populations by means of a particular attract-and-kill device that retain the flies. The concept of attract and kill is wider, including the so called lure-and-kill devices or bait station, in which the insect is subjected to a killing agent that effectively eliminates it from the population after a brief exposure. Definition of all these terms is included in Table 1 extracted from Navarro-Llopis et al.² In order to avoid misunderstanding in this terminology, hereafter we are going to use mass trapping for all the devices that retain flies, bait stations for all the devices in which flies do not need to go into a trap to be affected by a toxicant and attract and kill (A&K) to refer to both mass trapping and bait stations. All the A&K traps or devices can be used alone or combined with other techniques to reduce fruit fly populations in Area-Wide Programs. Mass trapping has formed part of strategies combined with field sanitation, protein bait sprays, male annihilation and augmentative parasitoids releases in pest management programs that have been deployed in Hawaii,³ Australia⁴ or Spain.⁵ The main weakness of mass trapping is cost because the sum of the price of the trap; for C. capitata attractants and insecticide or drown solution exceed 3 euros per device, equating to over 150 euros/ha.⁶ Moreover, in places with high population densities, this method should be sometimes supported by insecticide sprays, that further increases the final treatment cost. Replacing mass trapping with bait stations may lead to significant savings for growers and recent studies have demonstrated that efficacy of bait stations devices is at least the same as for mass trapping. Yet sometimes farmers are dissatisfied with this method because no dead flies are observed, unlike regular traps. Nevertheless, cost is not the only reason to prefer bait stations to mass trapping. Another reason is device saturation, which sometimes occurs in traps. For this reason, bait stations are preferable to traps to avoid fruit flies from reproducing in isolated hosts or backyards where traps are not frequently serviced, or when fruit fly populations are large enough to overwhelm traps. Another advantage of bait stations is that flies do not need to enter the trap to be affected, they only need to land on the device to become affected. This is a great advantage as a high percentage of flies attracted to a bait station land directly on it and die before causing fruit damage. Conversely with mass trapping, many of the flies attracted land on the trap but do not enter the trap⁸, going back and forth from traps to fruit and leaves. As a result, damage level could become higher in those trees with traps. Several authors have demonstrated that mass trapping strategies with protein-baited traps reduces fruit damage. ^{5, 6, 9-11} However, it has never been studied whether a protein-baited trap reduces fruit damage in the tree where it is hung. In this study, we evaluated the effect of four different types of A&K devices on fruit damage caused by Mediterranean fruit fly to the trees where devices are hung as it is important to know if it is better to place the traps in the most fruitful trees or in non-productive trees. These trees where selected within clementine and persimmon orchards treated with 50 A&K devices per ha. The insecticidal activity of the most efficient bait station tested in field was also studied in the laboratory to verify that the major part of the flies attracted to the device were effectively affected.

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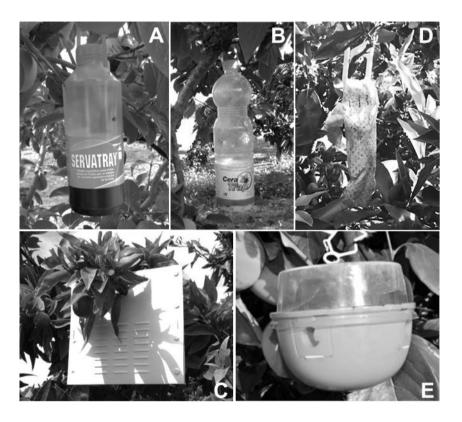
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2. MATERIALS AND METHODS

2.1 Attract-and-kill devices

- A&K approaches were tested using two types of mass trapping devices: (1) protein bait in two
- 25 types of bottle traps, Servatray[®] (Servalesa SL, Valencia, Spain) (Fig. 1A) and Cera Trap[®]

(Bioberica SA, Barcelona, Spain) (Fig. 1B), (2) traps with a synthetic attractant containing ammonium acetate and trimethylamine in a Decis[®] trap (Bayer CropScience, Valencia, Spain) with a DDVP (dichlorvos) tablet as insecticide (Fig. 1E), and two types of bait stations: (3) the Magnet[®] MED bait station (Suterra Europe Biocontrol SL, Valencia, Spain) (Fig. 1C) which consists of a 16x18 cm laminated device coated with deltamethrin and baited with a BioLure® Unipack dispenser placed inside the device to release the attractants (ammonium acetate, trimethylamine and putrescine (ATPu) as described by Navarro-Llopis et al.,⁵) through the holes located in both sides of the device, and (4) hand-made bait station containing the synthetic lure ATPu in a 12x7 cm micro-perforated paper bag impregnated with 200 mg of α-cypermethrin (Fig. 1D).



2.2 Field testing of the effect of A&K devices on fruit damage of single trees

Trials were conducted in 2011 and 2012 in clementine (*Citrus reticulata* Blanco) and persimmon (*Diospyros kaki* L.f.) orchards as they are very sensitive host species. All the trials

were conducted in the Autonomous Community of Valencia (Eastern Spain, Mediterranean coast). The number of plots assayed with each A&K device is detailed in Table 2. All plots were between 0.6 and 1.5 ha and were treated with 50 devices per ha, placed at a height of 1.5 m on the south face of trees. Persimmon orchards were located in Carlet and Alcudia (Valencia) (39° 18' N, 0° 56' W), whereas citrus groves were in the municipalities of Gandía (38° 96'84'' N, 0° 26' 52''W), Torres-Torres (39°73'23''N 0°34'89'' W) and Sagunto (39° 66'52''N, 0° 28'93''W). Each device type was tested at least in four different plots in two different years (except for Decis® trap which was only available in 2012). Traps and bait stations were placed in fields 1 month before fruit ripening began and remained in field until harvesting finished 3-4 months later).

Four pairs of contiguous trees were selected per field, one with an A&K device and the other without any device. One week before harvesting, fruit damage was assessed in each pair of trees by visually inspecting all the fruit of each tree or up to a maximum 200 fruits. Fruit was considered damaged when *C. capitata* eggs or larvae were found during assessment. For this purpose, fruits were inspected with a 6x Linen Tester Magnifier.

2.3 Laboratory evaluation of insecticidal activity

The insecticidal activity of the Magnet[®] MED devices was tested in laboratory conditions. To ensure the contact between flies and devices, flies were chilled at 5°C for 7 min just before the start of the test. As the activity of chilled flies is very low, they can remain on the device for at least 15 sec before taking flight. This time was determined after some field observations in which more than 90% of the flies stayed at least 15 sec on the device after landing on it.

One Magnet[®] MED device was placed inside each metallic cage (30x30x30 cm), and then 20 previously chilled flies (10 males and 10 females) were released over them. Devices were removed from the cage after 15 sec of contact, and flies remained inside the cages, supplied

with water and food, for 24 h. The mortality of flies was observed 1, 3 and 24 h after removing the devices. Flies were considered to be dead if they did not respond to contact with a brush, although some of them were only knocked down. This fact was verified when mortality was checked 24 h after the test. These trials were performed with new and 100 day-aged devices to study the loss of efficacy after the exposure of the devices during this period in the field. Climate conditions during ageing were provided by the nearby climate station -average daily temperature:22.9-26.6°C, average monthly maximum temperature:27.2-31.2°C, average minimum temperature: 18.8-22.2°C, average relative humidity:62.3-68.8%, and 12 rainy days (39 mm total in 100 days) with total rain per day between 0.2 and 16 mm/day. This

2.4 Field evaluation of knock down effect

study was replicated 4 times for each aging time.

In order to evaluate the consequences of the knock down effect in the field, another test was conducted in one of the Sagunto citrus groves. For this purpose, 10 dead flies were placed on the soil of the orchard and predation was evaluated after 45 and 90 min. The remaining fruit fly bodies were counted and the predators carrying or feeding on the dead flies were identified. This trial was repeated 5 times in 5 different plots.

2.5 Statistical analyses

The field trial design was performed to obtain pairs of data in order to compare fruit damage in trees with vs. without A&K. A paired data t-test (at P < 0.05) was performed with the fruit damage percentages. Statistical analyses were performed using the Statgraphics Plus 5.1 package (Statpoint Technologies, Warrenton, VA).

3. RESULTS

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3.1 Effect of A&K	devices on trill	to appendi	cingle treec	n nergimman	nrchards
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- Regarding bottle devices containing protein bait (Servatray® or Cera Trap®), three of the four
- 4 plots inspected in 2011 showed fruit damage. Thus, the data of only 14 pairs of trees in 2011
- 5 and of the four plots (20 pairs of trees) in 2012 were included. Although the average fruit
- damage on trees with bottle was 70% higher than in trees without them, differences were not
- statistically significant (t = 1.91, P = 0.063; see Table 2). There was no significant difference
- 8 in damaged fruit in trees with and without the Decis[®] (t = 0.09, P = 0.92; Table 2).
- 9 The last device tested in persimmon was Magnet® MED, which was evaluated in 12 different
- plots in 2012. There were significant differences in the paired data t-test (t = 1.99 and P =
- 11 0.05) between trees with Magnet® MED and without it(Table 2). In this case the average of
- punctured fruits was almost double in trees without the device. Hence, the Magnet® MED
- device was able to reduce fruit damage in the tree where it was placed in.

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3.2 Effect of A&K devices on fruit damage of single trees in citrus groves

- The trial carried out in Gandía (2011) produced no fruit damage, even though fruit remained
- in the field to over-ripen for 1 month after harvesting. Therefore, Table 2 provides only the
- Torres-Torres data with 23 pairs of values. The hand-made bait station was unable to prevent
- fruit damage. The use of this hand-made device resulted in nearly 3 times more punctured
- 20 fruit than the average damage obtained in trees without bait stations. This difference was
- significant (t = 2.27, P = 0.03) and therefore this device was ruled out and was not tested
- 22 again in 2012.
- 23 Magnet[®] MED was tested in Sagunto in two citrus plots with four pairs in 2011 and in two
- other citrus plots with four pairs in 2012. Differences were not significant in the paired t-test

- 1 (t = 0.81, P = 0.43), although the average fruit damage in trees with Magnet[®] MED was 30%
- 2 less than in trees without them (Table 2).

- 4 3.3 Evaluation of insecticide activity in laboratory
- 5 Three hours after 15 sec Magnet® MED contact, 92.5±1.6 % of the flies still remained
- 6 knocked down. However, mortality assessed after 24 h was only 37.5±12.1 %. This indicates
- 7 that not all the flies landing on the device are effectively killed and only become knocked
- 8 down for several hours. Mortality was not significantly different between 1 and 3 hours after
- 9 exposure, but mortality after 24 h was significantly lower due to fly recovery after initial
- 10 knockdown (F=76.84, df=2,27, P<0.001).
- Regard loss of activity with ageing, no significant differences in mortality were obtained
- when 100 days-aged devices were compared to new ones (F=0.89, df=1,26, P=0.353).

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3.4 Evaluation of knock down effect in the field

- Only 46 ± 17 % of flies (average \pm SE) from the initial quantity left on the soil of the orchard
- remained in the same place after 45 min, and only 16±9 % after 90 min. The main predator
- species identified carrying the dead flies were ants (Lasius niger (L.) and Pheidole pallidula
- 18 (Nylander)) and yellow-jackets (Vespula germanica (Fabricius)).

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4. DISCUSSION AND CONCLUSIONS

- 21 The literature contains several works explaining how fruit flies are attracted to traps and the
- 22 percentage of flies entering in relation to all the flies attracted to them. Aluja et al. 7 reported
- 23 that only 31% of the Anastrepha flies landing in glass McPhail traps, baited with torula
- 24 pellets, were effectively caught (drowned in liquid bait). This efficacy result was worse in
- certain species; e.g., only 10.3% of *Anastrepha obliqua* (Macquart) were retained in Multilure

traps baited with Nulure or Biolure, 12 whereas 20.8% of A. ludens (Loew) were retained. 1 Similar results were obtained with C. capitata in Jackson traps baited with trimedlure. 13 Yet 2 despite trimedlure being a powerful attractant for males, only 18.6% of males approaching 3 traps were initially caught. Although other traps available in the market can double the 4 efficacy of Multilure or glass McPhail traps, 14 the number of Mediterranean fruit flies 5 effectively caught in traps would be below 40-50% of all the flies landing on traps. 6 Accordingly, the efficacy of bait stations can increase if no trap entry is required. This may be 7 8 the main advantage of bait stations as compared to mass trapping systems. While less than 9 50% of flies landing on traps are effectively trapped, a good bait station design can eliminate 10 90% of the flies landing on this device. However, results of laboratory trials showed that many flies were only knocked down after contact with the lure and kill device and not 11 12 effectively killed. Nevertheless, this study demonstrates that about 85% of the knocked down 13 insects are predated in field in the first 90 min and therefore mortality after device contact is expected to exceed 90%. 14 15 In the Integrated Pest Management program conducted in Spain, the combination of 16 insecticide bait sprays, SIT, mass trapping, chemosterilization, cultural practices and control of backyards and isolated hosts was the strategy adopted to reduce C. capitata populations.⁴ 17 18 To treat isolated hosts or backyard orchards, which can be hotspot for fruit fly populations, the local government placed more than 50,000 traps per year to reduce the effect of these 19 20 uncontrolled hosts. Unfortunately, some of these traps were overloaded with captures, thus efficacy was poor. Direct observations of some isolated trap-treated trees have demonstrated 21 22 that fruit damage exceeded 90%, which motivated the study of the effect of these bait station systems in the tree where they are hung. 23 24 Results herein suggest that protein-baited traps do not protect the fruit of the tree where the trap is hung, and damage can be even higher. More Mediterranean fruit flies females can be 25

captured with synthetic attractants (blend of trimethylamine, ammonium acetate and 1 putrescine) than with protein bait, ¹⁵ demonstrating that synthetic attractants are more powerful 2 than protein baits. This difference can explain why fruit damage does not increase when 3 4 synthetic attractants are used as they compete with fruit as attractants. Accordingly, efficient 5 bait stations tested in this work are able to reduce fruit damage of a single tree by 1.5-2 times, as compared to trees without devices. 6 The main attribute of bait stations is their capability to kill or knock down flies in less time as 7 they do not need to enter into the traps. Our results have shown that flies touching the 8 Magnet® MED device for only 15 seconds were knocked down, even when devices were field 9 10 aged for 100 days. However, this effect was not observed for field-aged hand-made bait stations during the same short period. This agrees with the results of the present field trials as 11 12 the insecticide effect diminished in the hand-made bait stations, probably due to the 13 unprotected insecticide formulation against rain or sunshine. Consequently, flies effectively attracted to trees are not killed by the hand-made device, which results in 3-4 times more 14 15 damage than in trees without attractants. Moreover, a larger surface for flies to land on may improve bait station efficacy, as observed in Magnet® MED if compared to hand-made 16 17 devices. 18 The findings of this study have practical conclusions for mass trapping strategies. While protein-baited traps should be placed in trees with less fruit, bait stations devices can be 19 20 placed in trees with more fruit as they are able to prevent the damage. Likewise, proteinbaited traps should not be recommended to protect fruits of isolated trees. 21 22 Another strategy that can be carried out when mass trapping is applied combined with bait sprays is to emphasize the chemical treatments in the trees with protein baited traps as the 23 24 flies remain more time around this tress. This crop trapping strategy can increase efficacy of mass trapping when the used attractants are less efficient. 25

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Table 1. Classification and commercially available products of attract and kill.

		Types	Mode of action	Description	Example
Attract and kill	Mass trapping	Wet traps	Flies drown in	Liquid baits (protein hydrol. or ammonium salts)	Ceratrap®, Olipe, Servatray®
			liquid	Dry attractants + water	Multilure® baited with Biolure+water
		Dry traps	Sticky traps	Dry attractant	Delta trap
			Insecticide	Inhalation (DDVP)	Mosquisan®+ Biolure®
				Contact insecticide	Decis®
		Lure&Infect	Contact contamination		Fungi
	Bait Stations	Lure&Kill	Contact insecticion	M3®, Vioril® Magnet® MED MAT	
			Ingestion insection	SPLAT (Anamed®) EPALure&kill®	
		Lure&Sterilize	Ingestion steriliz	Adress®	

Table 2. Fruit damage in trees with and without the A&K traps and devices

Crop	#	#	A&K device]	No device	
Стор	plots	pairs	Type ^a	N^b	% of damage ^c	N^{b}	% of damage ^c	
Persimmon	8	34	PBT	5328	0.34 a	5473	0.20 a	
Persimmon	4	15	DT	1663	0.60 a	2100	0.58 a	
Persimmon	10	42	MM	5469	0.37 a	8390	0.68 b	
Clementine	6	23	HLK	3815	4.79 b	3641	1.73 a	
Clementine	6	24	MM	4158	0.26 a	4388	0.37 a	

^a Type of device: PBT (Protein-baited traps), DT (Decis[®] trap baited with dispensers of ammonium and amine salts ,and DDVP), MM (Magnet[®] MED), HLK (Hand-made L&K device baited with dispensers of ammonium and amine salts, coated with cypermethrin)

^b N: number of inspected fruit in all inspected trees

^c Values with the same letters in a row are not significantly different (paired data t-test, P > 0.05).