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Spinosad bait treatments as alternative to malathion to control the Mediterranean fruit fly, *Ceratitis capitata*, (Diptera: Tephritidae) in the Mediterranean Basin

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SYNOPSIS

Current *Ceratitis capitata* (Wiedemann) control in Spain is based primarily on applications of organophosphate insecticides, especially malathion, mixed with protein baits. In Spain, one of the current research lines is focused on finding more environmentally friendly insecticides. One such alternative is the insecticide spinosad. We compared two formulations and various concentrations of spinosad bait treatments (10% Spintor Cebo® and 0.05, 0.1 and 0.15% Spintor 480 SC® + 0.5% Nu-lure) under laboratory conditions and found all treatments to be effective in causing high mortality of *C. capitata* adults within 6 days of exposure to 0-, 3-, or 6-day-old residues. Field trials demonstrated that 5% Spintor Cebo®, 0.1% Spintor 480 SC® + 0.5% Nu-lure, and 0.5% Malafin 50® + 0.5% Nu-lure reduced *C. capitata* adults similarly and protected fruit from *C. capitata* damage to a similar extent. Both spinosad treatments will be promising as a replacement for malathion in bait sprays.

Key Words: chemical treatment, spray, medfly, IPM, citrus.

Introduction

The Mediterranean fruit fly or medfly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is one of the most devastating fruit pests worldwide. Current medfly control in Spain is based primarily on applications of organophosphate insecticides, especially malathion, mixed with protein baits. The intensity of insecticide treatments with malathion against *C. capitata* has resulted in the development of resistant populations.[1] Moreover, the use of malathion is controversial because of human health concerns[2, 3] and the harmful effects it has on beneficial insects.[4, 5] In recent years, emphasis has been placed on implementing safer environmental measures to control the medfly in Spain. To this end, we are testing insecticides that are more environmentally friendly than malathion.

One of the insecticides considered is spinosad.[6, 7] Compared to malathion, this insecticide has a better environmental profile and is less toxic to natural enemies.[5, 8, 9] At present, a spinosad bait treatment (Spintor Cebo®, named GF-120® in the Americas, Dow AgroSciencies, Indianapolis, IN), containing spinosad and a mix of sugars, water and attractants, [10] is successfully being used to control different tephritid pests worldwide.[11, 12]

Initial applications of Spintor Cebo® in Spain showed promising results in controlling the medfly.[13] Nevertheless, complaints concerning spot damage on fruit due to the proliferation of sooty mold on spraying droplets have been reported, particularly in orchards where sooty mold was already present, usually associated to honeydew segregated by homopteran citrus pests. Additionally, it has been postulated that the sugar contained in the bait part of the formulation could be also responsible for this spot damage. One way to avoid this problem could be to mix the insecticide spinosad (Spintor 480 SC®) with the standard proteinaceous baits registered for citrus; however, no information regarding the concentrations and method of application is available for this mixture.

In this paper, first we compare the efficacy of two formulations of spinosad (Spintor Cebo® and Spintor 480 SC® combined with proteinaceious bait) and various concentrations of Spintor 480 SC®, in the laboratory. In a further step, we test the efficacy of Spintor Cebo® and Spintor 480 SC® plus proteinaceous bait under commercial field conditions by comparing them against malathion bait treatment.

Materials AND Methods

1. Laboratory

1.1. Medfly rearing. Adults used originated from a laboratory colony maintained at the IVIA For each experiment, a cohort of even-aged adults, less than 24 hours old were used in all assays

1.2. Bait treatments. Three experiments were conducted varying the day of exposure of *C. capitata* adults to the residues of spinosad and bait: Experiment 1: fresh residues, Experiment 2: 3-day-old residues and Experiment 3: 6-day-old residues. Five treatments were assayed for each experiment: 1) 10% Spintor Cebo® Fruit Fly Bait (Dow Agrosciencies), 2) 0.05% Spintor 480 SC® (Dow Agrosciencies) mixed with proteinaceous bait 0.5% Nu-lure (Miller Chemical and Fertilizer Co., Hanover, PA), 3) 0.1% Spintor 480 SC® and 0.5% Nu-lure, 4) 0.15% Spintor 480 SC® and 0.5% Nu-lure, and 5) control with mineral water.

1.3. Methodology. To assay the mortality of the treatments tested in these experiments, the extended-laboratory method was used.[14)] Five droplets of 5 μl of each corresponding treatment were randomly distributed on each orange leaf (*Citrus sinensis* (L) Osb. var. 'Navelina') using a micropipette. The petiole of each leaf was placed in an Eppendorf tube containing a nutritive solution to keep the leaf turgid during the experiments[15)] and was sealed with plasticine. The treated leaf was transferred into a plastic cage (15 x 7 x 10 cm depth) with a hermetic lid having a mesh area of 12 x 8 cm for ventilation.

In the fresh residue experiment, once droplets were dried, ten adults per replicate were introduced. In the 3- and 6-day residue experiments, until used, treated leaves were left undisturbed in a climatic chamber at 25 ± 2ºC, 60 ± 10% RH and a photoperiod of 16:8 h (L:D). In each cage, water was offered *ad libitum* in an Eppendorf tube sealed with cotton, and sucrose was supplied in small plastic vials. Ten replicates per treatment were considered in all assays. In all experiments, mortality was evaluated daily until day 6 after introduction of the adults, and percentage mortality, corrected for control mortality, was then calculated.[16)]

2. Field

2.1. Treatments. Two field experiments were conducted to compare the efficacy of two spinosad treatments with standard malathion treatment under commercial conditions against the medfly. Two orchards with different agronomic characteristics located in Llíria (UTM X706741 Y4400206; Z 330 m altitude) (Valencia, Spain) were selected. Each one was divided into three plots of approximately 1 ha each, corresponding to the three different treatments tested. Treatments included: 1) 0.5% Malafin 50® (Agrodan, SA, Madrid, SP) mixed with 0.5% Nu-lure proteinaceous bait. A volume of 80 l/ha was used and 200 g/ha of active ingredient malathion was applied, 2) 5% Spintor Cebo® Fruit Fly Bait. A volume of 30 l/ha was used and 0.24 g/ha of the active ingredient spinosad was applied as recommended by the label and 3) 0.1% Spintor 480 SC® mixed with 0.5% Nu-lure. A volume of 80 l/ha was used and 3.84 g/ha of the active ingredient spinosad was applied.

For each treatment, eight applications were made, starting on 14 September and ending on 5 November when citrus fruits were susceptible to medfly. The product was sprayed only on the south side of the trees, the side that was more exposed to sunlight.

Malathion treatments were applied with a conventional, 2000 l air blaster sprayer, at 4 bar and a tractor speed of 3.5 km/h. Spinosad treatments were applied using a special prototype sprayer developed at the IVIA for bait treatments. This machine is a hydraulic sprayer which includes electronics that can be programmed by the user to spray

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intermittently at pre-defined intervals, providing the possibility to spot a little on the target trees and to adjust the volume sprayed depending on the vegetative characteristics of the orchard.[17)] In all treatments, Teejet AI11003VS air-induced nozzles were employed. These nozzles are used to obtain large droplets and are also recommended to apply Spintor Cebo®.[13)] Two nozzles were needed in 80 l/ha applications, while only one in 30 l/ha applications.

2.2. Medfly capture. Medfly capture assessment was based on servicing traps weekly. In each 1 ha plot, six Tephri-traps® (Sorygar, S.L.. Madrid, SP) were randomly placed. Traps were baited with the synthetic food bait Tri-pack® (5 gm a.i. ammonium acetate, 50 mg a. i. putrescine, and 2.50 gm a. i. trimethylamine) (Kenogard SA, Barcelona, SP). Traps also included a tablet of diclorvos [0.5 gm a.i. dimethyl 2,2-dichloroethenyl phosphate (DDVP) per tablet] (Biagro, SL, Valencia, SP) as insecticide, which was replaced every 6 weeks. Traps were placed in all plots on 1 July 2004 and serviced weekly until 25 November 2004, four weeks after the last treatment. Trapped flies were counted weekly.

2.3. Fruit infested by medfly. One week after the last application, at harvest time, the percentage of fruit infested by medfly was estimated. In each plot, 30 trees were randomly selected and 10 fruits per tree from different areas of the canopy were checked for medlfy oviposition.

2.4. Insecticide Residue Levels. Insecticide residue levels were determined in a clementine orchard. Two different tests were carried out. The first was aimed at determining the maximum residue level of the fruit that directly received treatment (worst case scenario). In each treatment, 72 fruits were randomly collected from 24 trees and were divided into three batches of 24 fruits [approximately 2 kg/batch]. The second test was aimed at determining the maximum residue level obtained under commercial conditions. In this case, 12 trees were randomly selected in each plot and 6 fruits from different parts of the canopy were randomly chosen, thus obtaining again three batches of 24 fruits. Fruit were harvested and

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sent to the official accredited laboratory of the Conselleria de Agricultura Pesca y Alimentación located at Burjasot (Valencia) for analysis.

3. Data Analysis

Laboratory data comparing the percentage mortality of medfly adults were subjected to one-way variance analysis (ANOVA). The least significant difference (LSD) multiple range test was used for mean separation at P > 0.05. If necessary, percentage mortality data were transformed using arcsine [square root (x)] prior to analysis to meet the assumption of normality.

As the initial number of medfly captures per treatment was not uniform, we standardized the number of medflies found in each plot by subtracting the mean medfly capture during the time period considered and dividing the result by the same mean, thus obtaining a percentage of increase/decrease of the number of medfly captures. In this analysis, we compared the data after insecticide applications had begun. The comparison of treatments was based on linear regression between the above-mentioned percentages. We assumed that treatments were equally effective if regression coefficients were not significantly different from 1 and the intercept was not significantly different from 0, in both cases with $P < 0.01$.

Results

1. Laboratory

All spinosad treatments and all residue periods (0, 3 and 6 days after leaves treated) were toxic to *C. capitata* adults (Table 1). For all three exposure periods, after the second day of evaluation, statistical differences were found between spinosad bait treatments compared to the control. Furthermore, during days 3-6 of mortality evaluations, no significant differences were found between the four spinosad treatments. Corrected mortality of medflies reached values higher than 90% after the third day of exposition and by day 6, mortality reached values close to 100%.

2. Field

2.1. Medfly captures. The number of medflies trapped per day (FTD) were quite different in the two orchards (Fig. 1). In the clementine orchard (Fig. 1A), the FTD varied between 0 and 9.3 whereas in the case of the orange orchard (Fig. 1B), the FTD ranged between 9 and 80 before treatments (end of the summer), and between 0 and 14 after treatments.

In both experiments, a similar trend was observed for the three treatments tested. Table 2 shows the results of linear regression analysis comparing the treatments. Significant correlations were found between the treatments (R^2 ranging from 0.8 to 0.9). In all cases, the intercept (a) had a value not statistically different from 0 (P = 0.47 to 1.00). Moreover, in all cases, the regression coefficients were significantly different from 0 ($P < 0.0001$) and close to 1 (low standard errors). For these two reasons, it was concluded that the effect of the treatments in each plot was not statistically different.

2.2. Fruit infested by medfly. In Malafin 50® plots, no fruits infested by medfly were found in the clementine orchard and 0.33% infested fruit was found in the orange orchard. In Spintor 480 SC® plots, this percentage was 0% and 0.66%, respectively, whereas in Spintor Cebo® plots, the percentage of infested fruit was 0.33 and 0.66%, respectively.

2.3. Insecticide Residue Levels. No detectable residues of spinosad (detectable limit = 0.02 mg/kg) were found on fruit in treatments with Spintor 480SC and Spintor Cebo® both in samples directly sprayed and in those randomly taken from all areas of the tree. In contrast, residues of 0.2 ppm of malathion were found in samples directly exposed to Malafin 50® and residues of 0.08 ppm in the commercial sample.

2.4. Presence of sooty mold. In the orange orchard sprayed with Spintor Cebo® a nonquantified proliferation of sooty mold was detected on droplets sprayed on fruits whereas in the other treatments, this phenomenon was not detected.

Discussion

The three concentrations of Spintor 480 SC® bait tested under laboratory conditions yielded similar mortality levels of medfly adults and no statistical differences were found among these treatments and standard Spintor Cebo®. For this reason, and as a first step, the intermediate concentration of 0.1% Spintor 480 SC® was selected to perform field trials where insignificant differences among 0.1% Spintor 480 SC®, 5% Spintor Cebo® and Malafin 50® were found. Although the pest was noticeable in the orchards, the percentage of infested fruit was very low in each treatment area; therefore, we concluded that all treatments adequately controlled the medfly.

One of the advantages of spinosad replacement of malathion is that provides a better toxicological profile. Under our experiment conditions, no spinosad residues were detected on fruit. Malathion residues were detected, although at concentrations below the maximum residue levels allowed on harvested fruit in Spain (2 ppm).

In the orange orchard sprayed with Spintor Cebo®, sooty mold damage on the fruit was associated with honeydew production by the citrus mealybug (*Planococcus citri* (Risso) (Homoptera: Pseudococcidae). No sooty mold was observed in the other two bait treatments, suggesting the advantage of Spintor 480 SC® plus Nu-lure treatment over Spintor Cebo®.

Currently, to control medfly in citrus, the Spintor Cebo® label recommends foliar application of an aqueous mixture of 1-1.5 l of Spintor Cebo® in a volume of 20-30 l/ha, with a droplet size of approximately 4-6 mm in diameter.[13)] These conditions are difficult to attain with the current air blast sprayers available in the market, and a hand-pumped back-pack sprayer with an adjustable nozzle is required, which increases the time and cost of the applications. Our tests recognized that the prototype sprayer developed at IVIA is a satisfactory alternative to automatically apply bait treatments, because the machine produced spray patterns that resulted in minimal medfly damage.[17] We hope to increase farmers' convenience and profit in the near future with our results.

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References

1) F. Ortego, C. Magaña, P. Hernández-Crespo and P. Castañera: *Phytoma España* **173**, 63-66 (2005).

2) P. Flessel, P.J.E. Quintana and K. Hooper: *Environ. Mol. Mutagen* **22**, 7-17 (1993).

3) M.A. Marty, S.V. Dawson, M.A. Bradman M.E. Harnly and M.J.J. Dibartolomeis: *Exp. Anal. Environ. Epidemiol. 4*, 65-81 (1994).

4) K.M. Daane, D.D. Dahlstena and S.H. Dreastadt SH: *Environ. Entomol.* **19**,1130-1134 (1990).

5) A. Urbaneja, O. Dembilio, D. Tortosa, E. Viñuela and P. Castañera: *Phytoma España* **160**, 28-40 (2004).

6) A. Adan, P. del Estal, F. Budia, M. González and E. Viñuela: *Pestic. Sci.* **48**, 261-268 (1996).

7) T.C. Sparks, G.D. Crouse and G. Durst: *Pest Manag. Sci.* **57**, 896-905 (2001).

8) T. Williams, J. Valle and E. Viñuela: *Bio. Sci. & Tech.* **13**, 459-475 (2003).

9) J.D. Stark, R. Vargas and N. Miller: *J. Econ. Entomol.* **97**, 911-915 (2004).

10) http://www.cdms.net/ldat/ld62A001.pdf

11) R.E. Burns, D.L. Harris, D.S. Moreno and J.E. Eger: *Fla. Entomol.* **84**, 672-678 (2001).

12) R.J. Prokopy, N.W. Miller, J.C. Piñero, J.D. Barry, L.C. Tran, L. Oride and R.I. Vargas: *J. Econ. Entomol.* **96**, 1485-1493 (2003).

13) P. Vergoulas and M. Torné: *Phytoma España* **153**, 134-138 (2003).

14) P. Medina, I. López, F. Budia, A .Adán and E. Viñuela: *IOBC/WPRS Bulletin* **27**, 59-66 (2004).

15) G. Moutous and A. Fos: *Revue de Zoologie Agricole et de Pahtologie Végétale* **1**, 27-28 (1973).

16) W.S. Abbott: *J. Econ. Entomol.* **18**,265-267 (1925).

17) P. Chueca, A. Gutiérrez, J.A. Burgos and E. Moltó. "8th Workshop on spray application techniques in fruit growing", Barcelona, Spain, pp. 169-171, 29 June - 1 July, (2005).

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Table 1. Percentage mortality (mean ± SE) of *Ceratitis capitata* adults on days 1-6 after exposure to spinosad fruit fly baits. Residual effects measured at 0 (fresh residues), 3 and 6 days after spraying.

Exposure	Treatment	% Mortality of medflies a)					
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Fresh residues	Spintor Cebo 10%	18.1 ± 2.5a	59.8 ± 5.1a	82.9 ± 5.6a	93.9 ± 2.7a	95.9 ± 1.7a	95.9 ± 1.7a
	0.05%Spintor 480 SC + 0.5%Nu-lure	14.9 ± 3.1a	55.5 ± 5.8a	80.3 ± 4.6a	92.1 ± 3.3a	98.1 ± 1.3a	98.1 ± 1.3a
	0.1%Spintor 480 SC + 0.5%Nu-lure	10.0 ± 3.3ab	56.0 ± 4.3a	82.0 ± 2.5a	92.0 ± 2.5a	97.0 ± 2.1a	97.0 ± 2.1a
	0.15%Spintor 480 SC + 0.5%Nu-lure	12.1 ± 2.0ab	62.1 ± 3.8a	93.8 ± 2.8a	97.9 ± 1.4a	99.0 ± 1.0a	99.0 ± 1.0a
	Control	4.0 ± 2.2b	4.0 ± 2.2b	4.0 ± 2.2b	5.0 ± 2.7b	10.0 ± 4.2b	16.0 ± 7.2b
3-day residues	Spintor Cebo 10%	32.8 ± 3.7a	62.3 ± 4.0a	85.2 ± 2.7a	91.1 ± 2.8a	95.1 ± 2.2a	95.1 ± 2.2a
	0.05%Spintor 480 SC + 0.5%Nu-lure	13.2 ± 3.3bc	36.6 ± 5.6b	69.3 ± 7.5a	83.7 ± 5.5a	89.7 ± 4.9a	95.9 ± 2.2a
	0.1%Spintor 480 SC + 0.5%Nu-lure	16.0 ± 4.5b	48.0 ± 2.5ab	78.0 ± 5.5a	93.0 ± 2.6a	98.0 ± 1.3a	100.0 ± 0.0a
	0.15%Spintor 480 SC + 0.5%Nu-lure	15.0 ± 3.7b	47.0 ± 7.5ab	82.0 ± 4.4a	96.0 ± 2.7a	96.0 ± 2.7a	100.0 ± 0.0a
	Control	0.0 ± 0.0c	1.0 ± 1.0c	3.0 ± 2.1b	8.9 ± 3.1b	14.8 2.7b	19.6 ± 3.3b
6-day residues	Spintor Cebo 10%	39.7 ± 4.9a	73.9 ± 4.0a	96.3 ± 2.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
	0.05%Spintor 480 SC + 0.5%Nu-lure	22.6 ± 2.7b	49.9 ± 5.1b	84.6 ± 4.8a	92.0 ± 3.9a	93.9 ± 4.0a	98.0 ± 1.3a
	0.1%Spintor 480 SC + 0.5%Nu-lure	32.2 ± 3.7ab	67.8 ± 5.2a	81.4 ± 6.0a	98.0 ± 1.3a	97.0 ± 2.1a	99.0 ± 1.0a
	0.15%Spintor 480 SC + 0.5%Nu-lure	25.5 ± 4.3ab	68.4 ± 3.8a	89.1 ± 3.8a	92.0 ± 3.6a	96.0 ± 2.2a	100.0 ± 0.0a
	Control	0.9 ± 0.9c	0.9 ± 0.9c	0.9 ± 0.9b	6.8 ± 3.6b	16.4 ± 4.9b	21.4 ± 4.4b

a) In each residue period, within each column, mean values followed by a different letter are significantly different (*P* > 0,05, LSD test).

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Table 2. Linear regression coefficients between each treatment in clementine and orange orchards.

Orchard	Treatments	Intercept (a)		Regression coefficient (b)			Correlation factor (R^2)	
		a	*P*	b	± SE	*P*	R^2	*P*
Clementine	Malafin 50 vs Spintor 480SC	0.0514	0.5900	0.9250	0.1127	<0.0001	0.8082	<0.0001
	Malafin 50 vs Spintor Cebo	2E-16	1.0000	0.9595	0.0882	<0.0001	0.8809	<0.0001
	Spintor 480SC vs Spintor Cebo	0.0556	0.4705	0.9257	0.0902	<0.0001	0.8681	<0.0001
Orange	Malafin 50 vs Spintor 480SC	0.064	0.4375	1.0756	0.0875	<0.0001	0.9043	<0.0001
	Malafin 50 vs Spintor Cebo	0.0067	0.9448	1.1214	0.1121	<0.0001	0.8621	<0.0001
	Spintor 480SC vs Spintor Cebo	0.0566	0.5382	0.9815	0.1052	<0.0001	0.8448	<0.0001

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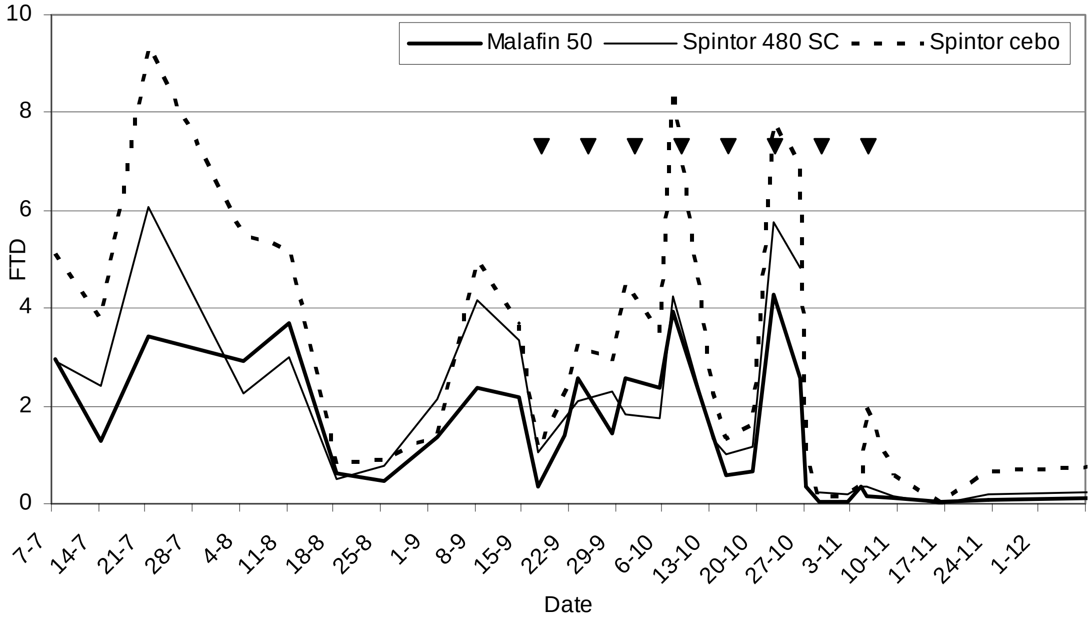
Figure 1. *Ceratitis capitata* captured per trap/per day (FTD) in A) the clementine orchard and B) the orange orchard. Vertical arrows indicate the dates of the insecticide applications.

A)



B)