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A New Long-life Trimedlure Dispenser for Mediterranean Fruit Fly *Ceratitis capitata* (Wiedemann)

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ABSTRACT. New agricultural techniques are attempting to reduce the application of synthesized pesticides and replace them with new environmentally friendly methods like mass trapping, mating disruption or chemosterilization techniques. All these methods are based on the release of a lure for insect attraction or confusion. The success of the method chosen depends on the quality of attractant emission from the dispenser. Currently used dispensers with a polymeric matrix and new dispensers with mesoporous inorganic materials were evaluated to obtain more efficient emission kinetics. In this study, the selected pest was the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) and the lure used was trimedlure (TML). A field study comparing insect catches with attractant release values was made for validating dispensers. As a result we have demonstrated that the lifetime of mesoporous dispensers is clearly greater than the polymeric plug. Furthermore, a high dependence of the attractant release rate on temperature with polymeric dispensers compared to mesoporous dispensers was assessed.

KEY WORDS: Pheromone, trimedlure, fruit fly, *Ceratitis capitata*, dispenser.

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) is one of the most destructive fruit flies (White et al. 1992) with a wide host range. It is recorded as attacking over 300 species of plants worldwide in 67 different families (Liquido et al. 1991) including pomefruit, stonefruit, citrus, soft fruit and coffee. More than sixty countries, including USA, Japan and most of the European countries, have Mediterranean fruit fly quarantine regulations to prevent new introductions.

In order to monitor *C. capitata* populations or detect possible intrusions two kinds of attractants are used: food lures and sex attractants. Food lures consist of proteinaceous solutions or ammonium salts originated in protein fermentation, such as putrescine, tertiary ammines and ammonia. The mixture of ammonium acetate and trimethylamine has resulted in a very efficient female attractant (Heath et al. 1997, Epsky et al. 1999, Heath et al. 2004), improving protein hydrolyzates. On the other hand, some studies have identified several compounds of the male natural pheromone (Jang et al. 1994, Cosse et al. 1995, Light et al. 1999) but none of the tested blends have obtained a high activity over females. The first semiochemical that showed good efficacy in males attraction was trimedlure (TML) (Beroza et al. 1964) and later ceralure (Demilo et al. 1994) showed a higher efficacy (Warthen et al. 1998) probably due to its high degree of steric resemblance with (+)-alpha-copaene (Casana-Giner et al. 2002). Currently the most frequently lure used in monitoring *C. capitata* populations is TML (Israely and Oman 2005), although the type of dispenser used varies depending on the country pest management strategies and the type of trap used (IAEA-FAO 2005). This lack of uniformity results in variable *C. capitata* population indexes that prevent comparable studies from being made throughout the world. TML is very efficient for the detection of the earliest *C. capitata* males in the increasing spring population (Miranda et al. 2001) and has been used for over 25 years. Although ceralure B1 is more attractive than TML, future refinements in synthesis and costs of this compound, and

also increased availability and testing will be needed before any final evaluation in the field can be carried out (Jang et al. 2003).

Trimedlure is a synthetic attractant consisting of a mixture of eight isomers of the tert-butyl esters of 4- and 5-chloro-2-methylcyclohexanecarboxylic acids (Ripley and Hepburn 1935). The four *trans* isomers constitute 90-95% of TML, one of them, the isomer designated as "C" (Mcgovern and Beroza 1966), is the most abundant (35-44%) and it is the most attractive (Mcgovern and Beroza 1966, Mcgovern et al. 1987, Mcgovern and Beroza 1966, Mcgovern and Cunningham 1988). TML is one of the attractants used to detect introduction of *C. capitata* in pest-free areas. In these countries, a network of traps is used to monitor any accidental invasion of the *C. capitata*. In countries where this pest is already established, monitoring traps with TML are used to follow up *C. capitata* population and decide on the best moment for insecticide treatment. Moreover, TML can be used as an attractant in lure and kill methods, chemosterilization or infective methods (Castillo et al. 2000, Navarro-Llopis et al. 2004).

The release rate of the TML dispensers, their lifetime, the isomer composition of TML and the trap used are the main factors that affected the efficacy of the monitoring program. In any case, the trap type and TML isomer composition are well known factors, but the kinetic of dispensers could be largely optimized. There are some references in the literature about the lifetime of different dispensers. An effective lifetime of about 2-4 weeks was obtained in cotton-wick dispensers (Rice et al. 1984). The use of a polymeric dispenser increases effectiveness due to slower evaporation. A polymeric plug with 4 g TML further extended the duration of effectiveness to 12 weeks or longer (Leonhardt et al. 1989).

In other methods such as chemosterilization (Navarro-Llopis et al. 2004) or mass-trapping or lure and kill, traps are not replaced during the whole year, and

replacing dispensers would involve an unacceptable extra labour cost. In such cases, a nine-month to one-year lifetime dispenser would be required.

Another important characteristic of the dispenser is its capacity to release at a constant rate, from the beginning to the end of the dispenser's lifetime. Previous work demonstrates that zeolites are a suitable material for manufacturing dispensers because they are porous materials that retain substances depending on their polarity and size. Moreover, zeolite's chemical structure and properties can be adapted to release substances at an adequate release rate over a long period of time (Munoz-Pallares et al. 2001). In addition, zeolite, as a dispenser support, is more environmental friendly than polymeric plugs. This is particularly important for the mating disruption technique, where hundreds of dispensers per hectare that will remain in the field after the treatment are used.

In order to adjust the retention of semiochemicals in a porous material we can modify five main factors: the porous size, hydrophilic/hydrophobic balance depending of the Si/Al proportion, the nature of compensating cation, the specific surface of the material and the nature and strength of acid groups (Munoz-Pallares et al. 2001). In this way, we can design a specific dispenser for each semiochemical and, for TML, pore dimension and the presence or absence of acid sites are the main factors. Other studies agree with Munoz-Pallares showing that total surface area and micropore structure are the most significant factors in retarding evaporation from a porous dispenser (Stipanovic et al. 2004). According to this, TML release rate would theoretically be less dependent on temperature in porous than in polymeric materials (Leonhardt et al. 1989, Bradley et al. 1995).

In this work, our aim was to evaluate the efficacy of a polymeric standard plug in comparison with a new mesoporous dispenser. The efficacy of each dispenser is measured in field trials as *C. capitata* catches, and these were correlated with their TML

release rate. The study provides data on the threshold of TML emission which obtained the minimum required efficacy. Using this threshold, a lifetime can be given for each type of dispenser.

Material and Methods

Materials: Two controlled release dispensers, Aralure® (polymeric plug) and EPAlure (mesoporous dispenser), were obtained from Agrisense (Pontypridd, UK) and Ecología y Protección Agrícola (Valencia, Spain), respectively. Aralure and Epalure dispensers were cylindrical. Aralure was, 17.4 mm height and 14.1 mm diameter, weighing 2.8 grams without a theoretical load of TML provided. Epalure was, 13.6 mm height and 26 mm diameter, weighing 10 grams with a theoretical load of 1.2 g of TML. Insect kill strip, DDVP Dichlorvos 20% w/w, was obtained from Econex (Murcia, Spain) and Tephri-trap® traps (Katsoyannos et al. 1999) were obtained from Utiplas (Madrid, Spain).

Release Rate Measurement: The release rate of each type of dispenser was obtained by residual amount analysis. The release rate was calculated by subtracting the quantity of TML at two continuous aging dates. For this purpose 30 dispensers of each type were placed in Tephri-traps in the field. Three replicates of polymeric plug (PP) and mesoporous dispenser (MD) were taken to the laboratory at different aging times (0, 15, 30, 60, 90, 120, 150, 180 and 210 days) for Gas Chromatography (GC) analyses of residual TML content. Each dispenser was individually extracted in Soxhlet with 50 ml dichloromethane (CH_2Cl_2) during 5 hours. All TML samples were analyzed as described previously by Leonhardt (Leonhardt et al. 1982) using an internal standard (50 µl of dodecane). The TML recovery with this methodology was 98% for PP and 99% for MD. Each dispenser extraction was injected three times. The average of these

injection results were considered as a single data for each dispenser sample and each aging date. Three replications of each dispenser sample for each aging date were made.

The extracts from dispensers were analyzed using a Clarus 500 GC (Perkin Elmer Instruments, Shelton, Connecticut) with FID detector. The GC system was equipped with 30 m x 0.25 mm i.d. x 0.25 μm, ZB-5 capillary column (Phenomenex, Torrance, CA). The oven was programmed from 120 (held for 2 min) to 240 ºC at 20ºC min^{-1}; carrier gas, helium; flow rate, 50 ml/min; split ratio 32.3:1; injection temperature, 250 ºC; detection temperature 300ºC. The injection volume was 1 μl.

All of the TML isomers were included in two peaks (5.73 and 5.85 min retention times) whose areas were summed for estimation of TML contents.

The release rates of two TML dispensers were measured using the formula:

$$RR_{[(n+1)]} = [(RC_n) - (RC_{n+1})] / [(T_{n+1}) - (T_n)]$$

RR= TML release rate (mg / day) ; RC = TML residual content (mg) ; T =aging days (days); n= aging period (1,2,….,n).

The data were then plotted to depict the TML release rate versus time.

Temperature effect: To detect the relationship between dispenser average release rates and temperature, a laboratory assay and an evaluation of release rates with field temperatures were made. Both types of dispensers were aged at three controlled temperatures (25, 40 and 50ºC) during seven days in an oven (model Incubig, P Selecta, Spain). The TML content of dispensers was measured at the beginning and end of the aging assay, obtaining a release rate at 25, 40 and 50ºC in laboratory conditions. This test was replicated three times. The release rate was correlated with the monthly average temperature in the field assay. The residual content was obtained by GC and the release rate was calculated in the same way as the release rate for dispensers in the field.

TML isomer release: The isomer composition of the two dispensers was determined at the beginning of the study and at the end of dispenser aging time by GC.

The chemical analyses were made using three dispensers without aging, and three aged dispensers of November (end of assay). Isomer emission was calculated from initial and final isomer content. All release rates were calculated according to release rate formula.

Isomers of TML dispensers were analyzed according to Leonhardt (Leonhardt et al. 1982). The analysis was made on a Clarus 500 GC (Perkin Elmer Instruments, Shelton, Connecticut) with FID detector. In this analysis the GC system was equipped with 60 m x 0.25 mm i.d. x 0.25 μm, ZB-Wax capillary column (Phenomenex, Torrance, CA). The oven was programmed from 100 (held for 1 min) to 215 ºC at 5ºC min^{-1}; carrier gas, helium; flow rate, 50 ml/min; split ratio 32.3:1; injection temperature, 225 ºC; detection temperature 275ºC. The injection volume was 1 μl.

Attraction field experiments: The trial was conducted in a *Citrus sinensis* (L.) Osbeck orange orchard, in Valencia, Spain. The field trial was run from April 1, 2004 to November 3, 2004. Figure 1 shows the temperatures and relative humidity during the experiments. The maximum average temperature was in August (26.2ºC), whereas the maximum relative humidity value corresponded to September (71.3 %). The temperatures values varied between 9ºC and 37ºC and the relative humidity was close to 60% during the entire experiment. Dispensers were placed in Tephri traps and hanged in trial field. Traps were separated 50m to avoid direct interaction between them. One DDVP (renewed bi-monthly) was placed in each trap. Three traps were hung in each block, with three blocks in the trial field. The three traps in each block contained a MD, a PP and a reference polymeric plug (RPP). RPP was renewed bi-monthly in order to have a reference plug with the maximum efficacy. MD versus PP was therefore compared, and both versus an RPP. Traps were maintained in the field for eight months, with the trap position randomized within blocks. Fly catches were counted every week and traps in the same block were rotated clockwise.

Statistical analysis: The release rates measured in the laboratory temperature assay were subjected to ANOVA and the differences between release values depending on temperature was determined by LSD intervals at the P = 0.05 level**.**

The possible differences between isomer C emission for two dispensers were subjected to ANOVA and the differences between dispensers was determined by LSD intervals at the P = 0.05 level.

Due to variations in captures derived from the variability of the fruit fly population during the bioassay, fly captures were transformed by log (x+1). The transformed data were subjected to ANOVA and the differences between treatments was determined by LSD intervals at the P = 0.05 level. All statistical analysis was performed using the Statgraphics 5.1 package.

Results and discussion

TML release rate: In the field test we observed that MD residual content decreased linearly (Figure 2) from April to the end of August, whereas PP content decreased slowly from April to the end of May, but very quickly during the rest of the time period.

The residual contents of the two dispenser types were used to calculate the release rate for each aging time. When these residual content values were transformed to release rate in mg per day, dividing residual content loss between two aging dates by the number of days, the release rate data plot was obtained (Figure 3). This graph shows that both dispensers released TML throughout the experiment, but in different ways. PP increases emission in June and July, coinciding with average daily temperature increase. While the MD release rate remained more constant from May to early September. The TML release rate of the mesoporous dispenser remained within an interval of 2 to 4 mg

per day in the higher temperature period, whereas the polymeric plug varied from 0.62 mg per day in mid-April to more than 10 mg per day in late June.

Release rates in MD were the highest at the beginning and at the end of the assay. However, in less than one month the MD reach the equilibrium between internal and external emission. The final increase can be explained by the breaking of the structure of MD while the initial increase is produced by a TML release from the external surface of the dispenser. Some breaking of dispensers always occurs at the end of dispenser lifetime, probably due to the empty pores that TML leaves when it is released.

Release rate vs average temperature: Table 1 shows the TML release rate of the two types of dispensers at 25, 40 and 50ºC. TML release increases in the range of 25-40ºC 3.63 times for PP and 0.14 times for MD. Thus, Leonhardt (Leonhardt et al. 1984) obtained an emission rate increase for a polymeric plug of 3 times in the range of 25-35ºC and this value agrees with the value calculated in this study (3.63 times). However, in the range of 25-40ºC, the increase rate for MD is 0.14, significantly lower than for PP ($F = 80.2$; d.f.= 1,4; $p<0.05$). In the other temperature range (40-50ºC), the increase rate for MD (10.62) was significantly lower than the PP increase rate (13.53) ($F = 36.4$; d.f.= 1,4; $p<0.05$). As a conclusion, we can say that MD release rate is more independent of temperature increase when compared to PP dispensers. Moreover, this effect is more pronounced in the range of summer temperatures in the Mediterranean area (maximum temperatures in summer between 35 and 40ºC).

To obtain a relationship between release rate and temperature, a regression analysis was made. Logistic regression showed the best fit to data; Figure 4 shows linear correlation between the logarithm of release rate and the temperature for PP. Mesoporous dispenser release rate did not show this correlation and the emission of TML was independent of the temperature increase. The equations for the log rate of PP

and MD were (94.962*T-100.63) and (54.202*T-29.29), respectively, with T expressed as ºC. The R^2 value for PP was 0.94, while the MD R^2 value was 0.76. On the basis of these values it can be confirmed that there is a positive significant correlation between temperature and release rate for PP.

Field-measured emission rates under natural weather conditions form April to July showed a linear correlation of release rate with an average temperature for the PP dispenser ($R^2 = 0.927$). Contrarily, release rate in MD did not show this correlation ($R^2 = 0.437$) (Figure 5). This fact demonstrates a greater sensitivity of the PP to temperature increases and explains the increase in emission in the warmest months. In addition, this implies an excessive release of TML, leading to a reduction of PP lifetime. This conclusion agrees with preliminary bioassay results reported by Leonhardt (Leonhardt et al. 1984, Leonhardt et al. 1989).

Release rate vs attractiveness: One field test was performed to assess the attractiveness of TML dispensers. Monthly *C. capitata* catches obtained with the two types of dispensers (PP, MD) and reference polymeric plug (RPP) over the whole field trial are shown on Table 2. There were no significant differences ($F = 0.14$; d.f.= 2, 24; $p>0.05$) between dispensers in fruit fly catches during the first three months. However, aged PP was significantly less attractive ($F = 2.31$; d.f.= 2, 42; $p>0.05$) than RPP in the fourth aging month (July), so the lifetime of the plug dispenser was established as ninety days (1 April-2 July). This result agrees with an 84-day lifetime considered in other works (Leonhardt et al. 1989). However, Mediterranean fruit fly catches obtained with RPP and MD did not differ significantly in six months ($F = 13.93$; d.f.=2, 33; $p<0.05$). Data shows that in our field conditions the lifetime of MD (1 April - 14 October) is clearly greater than the PP (1 April-2 July).

Although PPs release 10 mg of TML per day, MDs caught the same number of *C.capitata* while releasing 2 mg of TML per day. There is one possible reason to

explain this effect. PPs release larger quantities of TML at midday (higher temperatures) and much of this TML is lost because tephritids activity is lower during the warm mid day hours (Yee 2002). Meanwhile, the mesoporous TML rate during the day is more constant and efficient (Figure 4 and Figure 5). For this reason, 2 mg TML/day was enough in the MDs to maintain the same effectiveness as PP releasing 10 mg of TML per day.

Table 3 shows the isomer composition and the isomer emission for the two dispensers. The initial content of isomer C was similar in the two formulations (40.88 % and 41.82% for PPs and MDs, respectively), as was the final content (47.85 % for PPs and 46.31 % for MDs). The isomer C emission in the assay, calculated from the initial and final isomer C content, was 365.17 and 331.84 mg of TML for the polymeric plug and the mesoporous dispenser, respectively. So there were no differences between isomer C emission for the two dispensers in this study ($F = 0.12$; d.f.=1,5; $p>0.05$). It was therefore concluded that possible differences in *C. capitata* attraction are not affected by isomer C, since there were no significant differences in isomer C emission between PP and MD.

The new mesoporous dispenser surpasses the effectiveness of the polymeric plug as demonstrated in this study. The new long-life mesoporous dispenser attracts flies during the entire *C. capitata* season in Mediterranean countries with no decrease in effectiveness. MD can therefore be used more economically for monitoring *C. capitata* or control methods, like the chemosterilant method, where long-life dispensers are required to avoid replacement of attractants. Moreover, using MD, fruit fly catches in monitoring programs do not depend on weather conditions or dispenser management protocols related with replacements in each country. As conclusion remark, the new mesoporous dispensers represent an improvement over the old pheromone dispensers since their pheromone diffusion is more prolonged and independent of temperature

increases compared to PP. Moreover, the mesoporous support is benign with respect to the environment, since it is biodegradable, in contrast to the majority of polymeric plugs.

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Table 1. Effect of temperature in laboratory release rate

Temperature (ºC)	Release rate (mg TML/hour)[a]	
	MD	**PP**
25	1.76a	0.33b
40	1.85a	2.60b
50	6.28a	8.24b
Temperature range (ºC)	**Emission rate increase (mg TML/day ºC)[a]**	
	MD	**PP**
25-40	0.14a	3.63b
40-50	10.62a	13.53b

425 [a] Means followed by the same letter within a row are not significantly different ($p > 0.05$; LSD intervals).
PP: Polymeric plug
MD: Mesoporous dispenser

430

Table 2. Insect captures for dispensers evaluated

Treatment	Mean[a] ± SEM male catches[b]				
	Date				
	7 June-29 June	2 July-28 July	4 August-24 August	1 September-29 September	14 October-17 November
PP	43.33 ± 12.8a	113.93 ± 17.02a	36.5 ± 12.35a	2 ± 0.69a	3.22 ± 1.05a
RPP	44.11 ± 10.75a	215.87 ± 31.37b	93.19 ± 29.49b	12.33 ± 2.69b	25.11 ± 4.96c
MD	31.55 ± 10.76a	142.93 ± 24.48ab	62.42 ± 14.66ab	8.25 ± 2.26b	9.49 ± 0.11b

[a] Averages of insect captured as back-calculated from log (x+1) transformed data.

[b] Means followed by the same letter within a column are not significantly different ($p > 0.05$; ANOVA followed by LSD).

PP: Polymeric plug

MD: Mesoporous dispenser

RPP: reference polymeric plug (renewed bi-monthly)

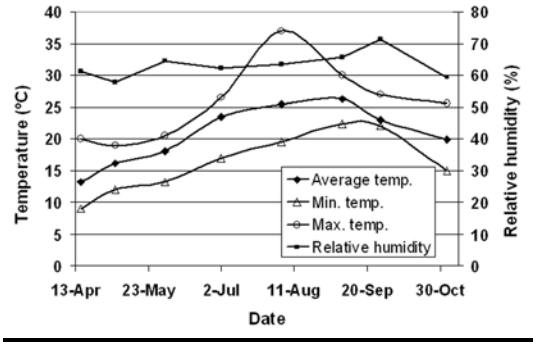
Table 3. Proportion (%) and emission (mg TML isomer) of TML isomers in the two dispensers evaluated

Isomer	Proportion of TML isomers in the two dispensers (%)				Emission (mg TML isomer)[a]	
	PP		MD		PP	MD
	Initial	*Final*	*Initial*	*Final*		
TML-A	27.02	22.53	30.84	28.31	282.30	264.57
TML-B1	8.45	7.83	5.63	5.14	84.76	48.40
TML-B2	21.26	19.42	14.94	13.92	214.50	127.47
TML-C	**40.88**	**47.85**	**41.82**	**46.31**	**365.17a**	**331.84a**
TML-V	0.35	0.28	3	2.39	3.71	26.97
TML-W	1.56	1.56	1.01	1.03	15.13	8.32
TML-X	0.35	0.34	2.2	2.2	3.44	18.26
TML-Y	0.14	0.19	0.56	0.67	1.13	4.27

[a] Means for Isomer C emission followed by the same letter within a row are not significantly different ($p > 0.05$; LSD intervals).
PP: Polymeric plug
MD: Mesoporous dispenser

Figures:

Figure 1. Plot of temperatures, relative humidity versus date.



460

Figure 2. Residual trimedlure contents of dispensers evaluated.

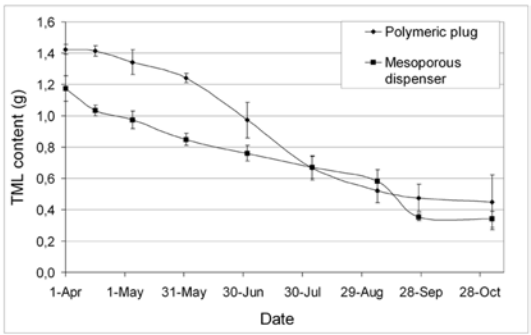
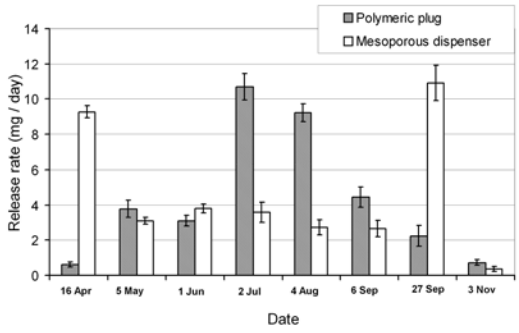
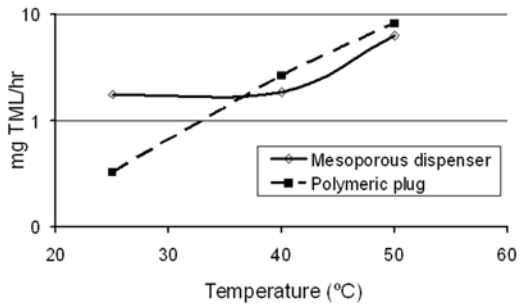


Figure 3. Release rate of dispensers evaluated during field test



465

Figure 4. Laboratory-measured release rates as a function of temperature.



470 **Figure 5. Relationship of average temperature to release rate during first three months.**