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# Characterization of the performance of pheromone traps as a basis for the management of the Citrus Mealybug *Planococcus citri* (Risso) in Citrus groves

**Abstract** - We have studied the effect of basic trap parameters and trap activation on the efficiency of capture of the males of the Citrus Mealybug *Planococcus citri* (Hemiptera: Pseudococcidae). The number of males caught by pheromone baited sticky traps was significantly affected by both size and type of trap. Generally plate traps caught more males than delta traps, and large traps caught more males than did the small ones. Significant dose response to sex pheromone in the range of 25-800mg was observed as high as 100mg. Overdose repellence was not observed even when lures containing 800 mg of the pheromone were used. Three types of rubber septa were tested: American, French and Israeli dispensers. The tested doses were 200, 400, and 800mg and the dispensers were subjected to ageing under ambient conditions for 1,8,15, 22 and 29 days. The tested dispensers displayed a similar rate of release of the pheromone in the laboratory. However, these findings did not coincide with the level of male catch in the orchard. Traps baited with the French dispensers captured significantly fewer males than traps baited with the Israeli and American ones. There were no significant differences between the tested three dosages. The catches, using the American dispensers, were uniform during the four weeks of ageing. This pattern of male catch is expected to last longer if lures containing 400mg or higher loads are to be used. Significantly, more males were caught by the traps suspended inside the trees than by those suspended between the trees or by those put further away from the study plot. The capture by the former traps did not differ from the capture by traps installed on the outer canopy or inside the canopy of Chlorpyrifos-treated trees. These findings suggest that the contribution of individual trees to the level of the catch of traps is insignificant. It is apparent that the male mealybugs fly toward the tree crown and only then start looking for the pheromone source. The results suggest that the above-mentioned American dispensers fit both monitoring and mass trapping. High and continuous catches of males can be achieved using big plate traps baited with at least 200mg of the pheromone and suspended inside the tree crown.

**Key words**: *Planococcus citri*, trap, dispenser, monitoring, attraction.

## INTRODUCTION

The Citrus Mealybug *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) is a major pest of citrus in many citrus growing areas. Biological control is ineffective under the Mediterranean climate (e.g. Mendel *et al.*, 1999). Naturally enough, the reduction of the mealybug population by application of organophosphates is not advisable; their use has led in Israel to a rapid development of resistance to these compounds. It has therefore been suggested that in order to improve the mealybug management emphasis should be placed on improving pheromone the traps design and application, in order to optimize monitoring and lay the basis for mass trapping of males or for mating disruption. Traps baited with sex pheromone reflect the seasonal fluctuations of scale insects (e.g. Hoyet *et al.*, 1983, Gardner *et al.*, 1983; Rice & Moreno, 1969; 1970; Mendel *et al.*, 1997). The study of the effect of basic trap parameters and trap activation on the efficiency of the male capture is a direct consequence. Due to the complex within-tree distribution and the multivoltine development, direct sampling of the mealybug in the citrus grove is tedious. Therefore, indirect sampling of the population by estimation of the density according to the level of male capture, using pheromone traps, may be as practical as it is inexpensive and serve as a simple sampling tool.

Earlier studies revealed that male flight remains at a low level between March and mid May (Franco *et al.*, 2001). It is suggested that "male vacuum" in the citrus mealybug population in the spring, when the population of the scale is still at a low level, may slow down the buildup of the mealybug population. Interfering with the mealybug reproduction by mass trapping of male may result in preventing heavy infestation of the fruitlets. The sex pheromone of the citrus mealybug was already identified in 1981 (Bierl-Leonhardt *et al.,* 1981). However, little has been done to further the adoption of the pheromone traps as a management tool. Nothing is known about the effect of the trap position on the level of male capture. Nor the distance of male attraction to the traps has been studied. Due to practical reasons, the pheromone traps in citrus grove are suspended within the tree canopy. Fruits inside the crown are more infested than those on the periphery (Mendel & Gross, unpublished). In this context, the contribution of individual trees to male catch deserves to be investigated.

The major objectives of the study were therefore to examine the trapping intensity of the males: (1) by determining the effect of position of the trap, as related to the tree canopy, on the capture. (2) by selecting the most effective trap design for handling and mass trapping, as well as for the interaction of type of trap with pheromone concentration; (3) by evaluating of the potency and longevity of three types of dispensers when impregnated with the female sex pheromone.

## MATERIALS AND METHODS

### *Trap design*

Four types of traps were examined: delta and plate, each of two sizes 30x30 cm and 15x15 cm, the size indicating the dimension of the trapping surface (the sticky plate). Each type of trap was tested with the following pheromone concentrations: 0,

25. 50, 100, 200, 400 and 800 mg. The experiments were conducted at 'En Harod Me'uhad located in the center of the Yizre'el Valley. Traps were activated in two citrus orchards, one planted with Star-Ruby (a red grapefruit variety *Citrus paradisi)* and the other planted with Sweetie (*Citrus paradisi* x *Citrus grandis*). In each plot traps were activated in mid July for one week and then replaced with new traps for another week. The traps were arranged in 5 blocks per plot (orchard). Each block contained a single replicate of all combinations of trap type and pheromone concentration. The traps were randomly distributed within each block and period. They were suspended inside the crown of a developed tree at about 1.2m above ground. Fresh dispensers were used during each period.

*Lures*

Three types of rubber septa (dispensers) were tested: American "gray" dispensers (West Co. Pennsylvania, USA), French "red" dispenser (Bioprox, Grasse, France) and Israeli "white" dispensers Yogev ltd, Rishon LeZion, Israel. Test doses were 200, 400, and 800mg of pheromone per septum. Dispensers impregnated with each dose were subjected to aging under ambient conditions for 1,8,15, 22 and 29 days. Each treatment (type of dispenser x dose x age) was replicated 6 times.

The lures were deployed in delta traps 15x15 cm. The traps were activated from 22 to 29, June 1999, in a Star-Ruby plot in 'En Harod Me'uhad. They were arranged in 6 blocks per plot. Each block contained a single replicate of all combinations of dispenser type, dose and age. Trap suspension was as mentioned earlier.

*Release rate measurements of pheromone from the tested dispensers*

The release rate of the pheromone from the above mentioned dispensers as a function of aging was evaluated under controlled laboratory conditions. Dispenser loadings were 200, 400 and 800 mg pheromone. The aging was carried out in a 2 meter long and 60 cm across wind tunnel, at a temperature of 25° ± 1°C and a wind speed of ca. 0.5 m/sec. The lures were placed on a wire grid in the center of the tunnel. They were periodically removed for release rate measurements and then put back into the tunnel.

The release rates of the pheromone from the aged lures were measured at static conditions in closed glass vials sealed with GC rubber septa. The released pheromone was analyzed by capillary GC applying the "solid phase microextraction method" (SPME). The trapped volatiles were quantitatively desorbed by heat (200-250°C) in the GC inlet.

*The effect of the surrounding male population on the male catch by pheromone traps inside the tree crown*

The experiment was conducted in two grapefruit orchards in western part of the Yizre'el Valley at Alonim and in the eastern part of the Valley at Mizra. We selected four groups of trees in each orchard. Each group consisted of six consecutive trees in a row, with a distance of 100-150 m between the groups. Two groups of trees in each orchard were covered by 50 mesh screen for two weeks, in May and June 1997. Delta sticky traps baited with 50mg pheromone were suspended inside the crown of two trees of each of the tree groups. The numbers of the males caught in traps that were suspended in the netted and non-netted trees for each plot and period were compared.

*Trap position*

Traps baited with lures impregnated with 50mg sex pheromone were used for the test. The study was conducted in the Izra'el Valley, 'En Harod Me'uhad in a Star Ruby plot. The traps were exposed for one week in July 1999. They were suspended in different positions in relation to the tree crown: inside the canopy, outside the canopy, 0.2 m from the tree crown, between trees, 2.5 m from the tree crown, 8, 23 and 35 meter away from the plot edge. For controls non-baited traps were activated inside the canopy and baited traps inside the canopy of a tree that had been treated with chlorpyrifos about a week prior the trap activation.

*Data analysis*

Analysis of the results was conducted using SAS general linear Models procedure. The dependant variable was SQ+0.5 of the number of trapped males. Differences between means were test by SNK procedure.

## RESULTS

*Trap design*

The number of males of the citrus mealybug caught by pheromone baited sticky traps was significantly affected by both size and type of the trap. Generally, plate traps caught more males than delta traps, and large traps caught more males than small ones. Significant response to sex pheromone at the range of 25-800mg was observed as high as 100mg. Overdose repellence was not observed even when lures containing 800 mg of the pheromone had been used. Excluding the non-baited traps, the range of the average male catch was 245.5-397.1 and 87.7-171.5, in the Star-Ruby and the Sweetie plot, respectively (Fig. 1).

A significant interaction was found for both plots between type and dose (Star-Ruby $F_{1,6}$ = 3.66, P=0.0018; Sweetie $F_{1,6}$ = 2.48, P=0.0242) and between size and dose in the Star Ruby plot ($F_{1,6}$ = 2.86, P=0.0105).

Regardless of the effect of trap design, a significant response to dosage was observed as high as 200mg and 100mg in both plots. In different traps exposed in the Star- Ruby plot, a response was observed to a concentration of 200mg but only for 30x30 delta type; no response was observed in the other trap designs (Fig. 2a). Nor

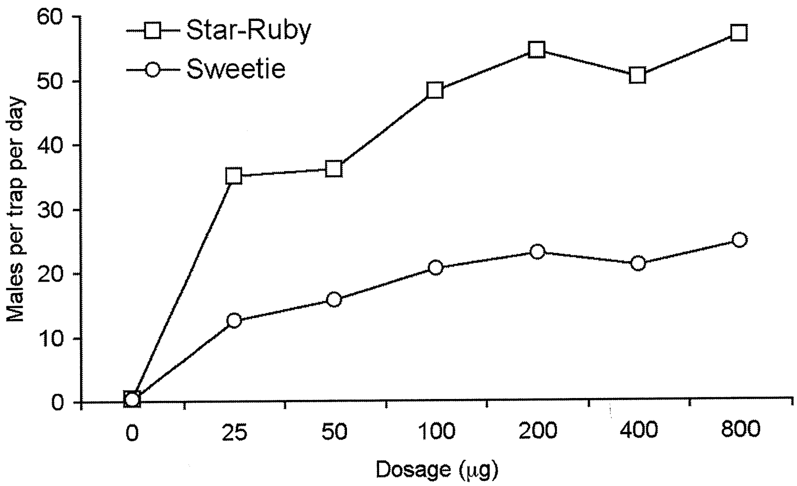


Fig. 1 - Catches of males of *Planococcus citri* by sticky traps (N= 80) in relation to elevated dosages of female sex pheromone.

dose-response was obtained in the Sweetie plot for the any trap design. The highest catches achieved by high pheromone concentrations differed significantly only from catches with the 25mg dosage in the 30x30 plate, 15x15 delta and 15x15 plate trap (Fig. 2b).

In the Star-Ruby plot the 30x30 plate trapped the highest number of males for each pheromone concentration while the 15x15 delta trap obtained the lowest catch. Differences between the above mentioned types for each pheromone concentration were significant. The 15*15 plate trap caught more males than the 30*30 delta trap only at dosages of 25mg and 50mg (Fig. 2a). Except for a single case (200mg), no differences were found in the number of trapped males among the four types of traps in the Sweetie plot (Fig. 2b), with each pheromone concentration.

Significantly more males per $cm^2$ were trapped in the inner zone of both 30x30 types in both study plots for some of the pheromone concentrations. It is interesting to note that in the case of the 30x30 type, regarding most pheromone concentrations, more males were trapped in the inner zone, viz. close to the dispenser.

*Lures*

Effect of dispenser type

There were significant differences in catch between the tested dispensers (Tables 1). For each of the three tested doses, traps baited with the French dispensers captured fewer males than traps baited with the Israeli and American ones. There were no significant differences between male catches in traps loaded with each of three dosages.

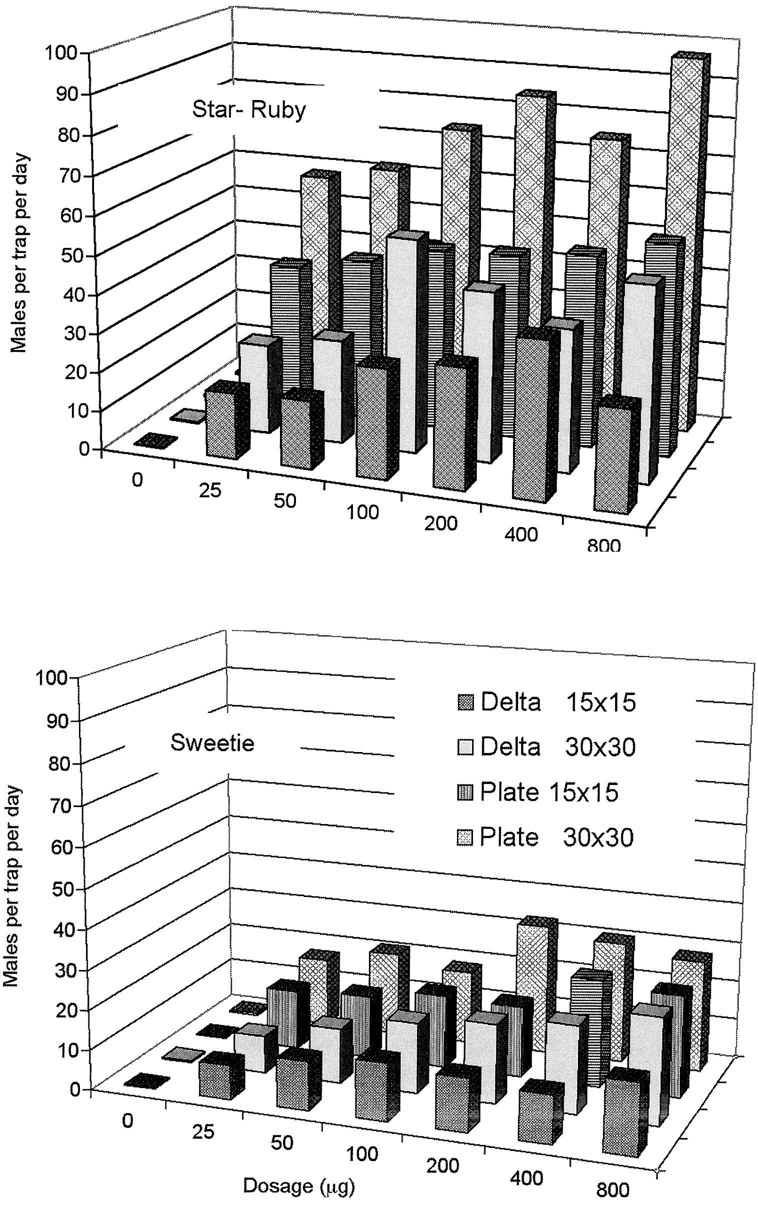


Fig. 2 - The effects of trap design and pheromone load on the level of capture of males of the Citrus Mealybug in Star-Ruby (a) and Sweetie (b) orchards.

*Table 1 - General linear model procedure for testing the male catches.*

| Source | DF | F value | Pr > F |
|---|---|---|---|
| Dispenser | 2 | 5.97 | 0.0030 |
| Dose | 4 | 21.74 | 0.0001 |
| Dispenser * Dose | 6 | 0.39 | 0.8869 |

In general, there was no great difference between the three tested dispensers in their male capture (Fig. 3).

The rate of release of the pheromone in the laboratory as measured by SPME for the tested dispensers did not agree with the level of male catch in the orchard. Traps baited with the French dispensers captured significantly fewer males than traps baited with the Israeli and American ones. There was no significant difference between the tested three dosages. The catches using American dispensers did not vary during the four weeks of ageing. Each type of dispenser behaved differently with regard to lure longevity. There was no significant difference between the three dosages for most of the aged lures of male capture regardless the dispensers used.

Male capture using fresh (1-day aged) lures was significantly higher than male capture using aged ones as regard the French and Israeli dispensers. When using Israeli dispensers male capture differed significantly between the three dosages. However when using with the fresh dispensers, captures differed significantly for the higher dosages only 4-week aged lures alone. There was no significant difference of male captures in the dosages between ages. However, among the three types of dispensers

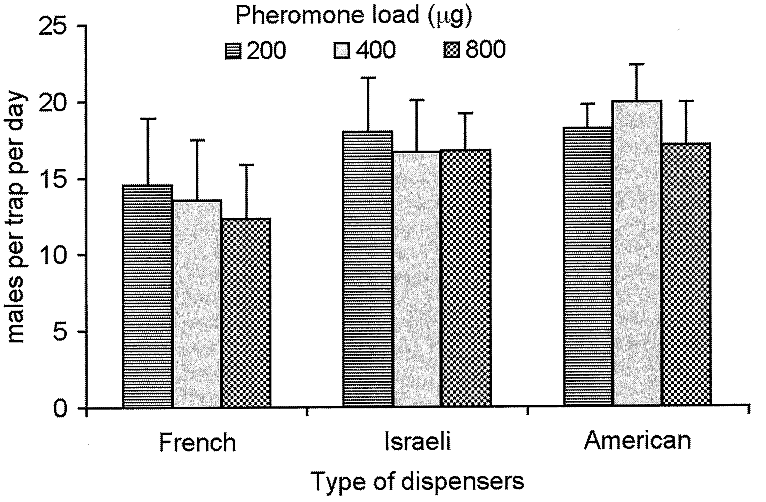


Fig. 3 - Comparison of male catches of the Citrus Mealybug by traps baited with different type of dispensers (means were calculated regardless of the lure age).

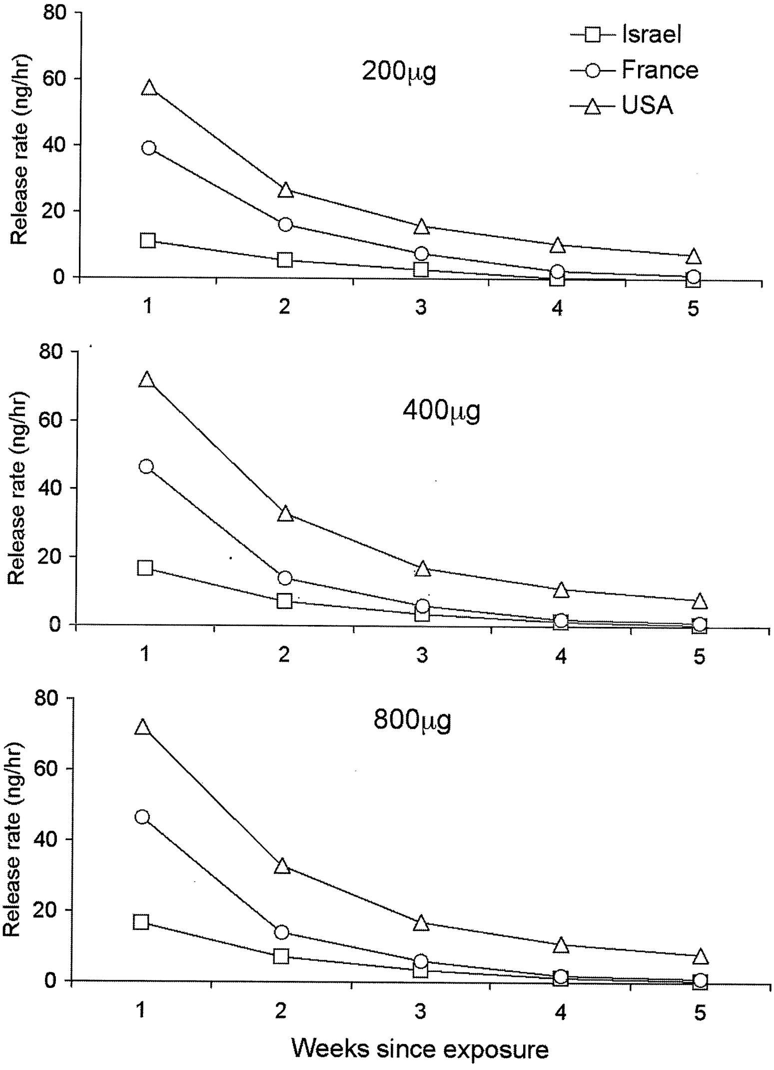


Fig. 4 - Release rate (ng/hr) of the pheromone of the Citrus Mealybug from three types of dispensers impregnated with 200, 400 and 800 mg, as measured in static atmosphere by SPME method.

the "American" type displayed approximately an even capture particularly when compared with the rather great changes in male capture when using French and Israeli lures of different ageing period.

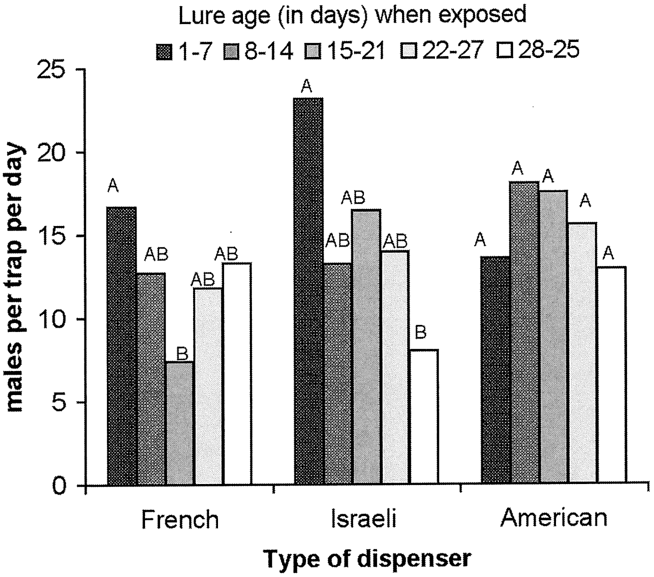


Fig. 5 - Capture of males of the citrus mealybug in relation to dispenser ageing (200mg load).

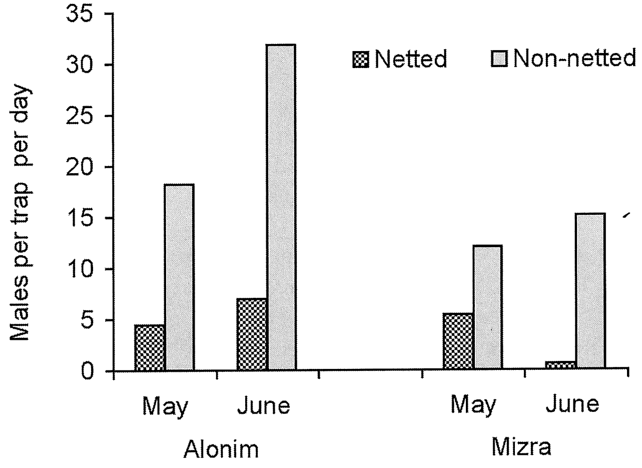


Fig. 6 - Catches of males of *Planococcus citri* as affected by netting. The traps baited with 50mg pheromone were exposed inside the tree canopy for two weeks.

*Study of the effect of the surrounding male population on the male catch by pheromone traps*

In both plots and trapping period (Fig. 6) many more males were caught by the traps suspended in non-netted trees as compared with those suspended in netted trees.

*Table 2 - Mean capture (n=10) of males of* Planococcus citri *in relation to trap position on citrus tree canopy. (Pheromone load = 50µg).*

| **Trap position** | **Males per trap** |
|---|---|
| Inside the canopy | 287.8 a* |
| Outside the canopy | 358.9 a |
| Between trees | 35.9 c |
| Non-baited trap inside the canopy | 4.8 d |
| Inside the canopy of the Chlorpyrifos-treated tree | 247.9 ab |
| 8 meter away from the plot | 116.4 B |
| 23 meter away from the plot | 38.2 c |
| 35 meter away from the plot | 82.2 bc |

* Means with the same letter are not significantly different

*Trap position*

Significantly, more males were caught by the traps suspended inside the trees than those suspended between trees or set away from the study plot. The capture by the former traps did not differ a great deal from capture by traps installed either on the outer canopy or inside the canopy of Chlorpyrifos-treated trees. Significantly, more males were caught by the traps suspended inside the trees as compared with those suspended between trees or set away from the study plot. The capture by the former traps did not significantly differ from capture by traps installed either on the outer canopy inside the canopy of Chlorpyrifos-treated trees (Table 2).

## DISCUSSION

The physical structure of the trap has a major effect on the trapping magnitude (Ring *et al.* 1984, Howse *et al.* 1998). In the present, study we showed that the number of males of the citrus mealybug, caught by pheromone sticky traps was affected significantly by both size and type of trap. Generally plate traps caught more males than delta traps, and large traps caught more males than the small ones. This pattern was true for all tested pheromone concentrations in the Star Ruby orchard where the male density was relatively high, but not in the Sweetie orchard with a relatively low male density. The results showed a weak dose-response in both plots to the high pheromone amounts. The significant interaction between trap size and pheromone dosage, obtained only in the Star-Ruby plot, further emphasizes the effect of the size of the trap on trapping efficiency when the mealybug population is high. Among the most efficient trap types in the highly infested plot no dose-response was obtained for various dosages. These results suggest that the weak dose-response obtained in the study may be due to the short longevity of lures of low pheromone concentrations, also because of the low trapping efficiency of the delta traps.

For mass trapping of large-size insects, like beetles and moths, funnel traps have been successfully set up. When the target insects are minute, like the males of scale insects, sticky traps are preferred (Rice & Moreno, 1969; 1970; Mendel *et al.*,1997). Our results suggest that the 30x30 plate trap is most appropriate for the mass trapping of males of the citrus mealybug. Lures containing 800 mg of the sex pheromone may serve for this purpose since overdose repellence has not been observed. We suggest using 15x15 delta traps for monitoring of males of the mealybug. These are easy to handle and seem to be more sensitive than the small plate traps; the latter baited with every pheromone concentration, caught more males than the former.

Absence of repellence, when high pheromone dosages were applied, was also figured out by the distribution of the trapped males among the sticky plate zones. The similarity in the male density among inner and marginal zones in small traps, as well as for most treatments of the 30x30 delta traps, indicated that males were trapped in close proximity to dispensers loaded with 800mg pheromone. On the contrary, results obtained with most treatments using 30x30 plate traps revealed that male density was significantly higher in the inner surface of the sticky plate, viz., near the dispenser. However some overdose response was obtained when the dispensers were studied (see below).

*Dispensers*

A significant decrease of male catch with higher pheromone dose was obtained using traps baited with the fresh Israeli and French dispensers, suggesting a light effect of overdose when 400 and 800mg were employed. However, the differences were small. This pattern of decrease was not observed for the American dispensers, even when fresh dispensers impregnated with 1600mg had been used. The results suggest that the American dispensers should be selected for monitoring and mass trapping. The catches, using the latter type of dispensers, were consisted during the four weeks of ageing. Furthermore, there was no improvement of male capture for that particular dispenser with doses higher than 200mg for all tested aged lures, in relation of our findings of the trap design tests.

The typical male capture pattern of the American dispenser is also evident by the lack of significant correlation between male capture and pheromone release rate, as measured. We found that, for each type of the tested dispensers, a similar rate of release of the pheromone in the laboratory did not result with a corresponding level of male catch. It is therefore suggested that each type of dispenser behaves differently under ambient air temperature and contributes in the orchard to the discrepancy between male capture and release rate. The results suggest that the above-mentioned American dispensers are fit for both monitoring and mass trapping. High and uninterrupted catches of males can be achieved using big plate traps baited with at least 200mg of the pheromone suspended inside the tree crown. This pattern of male catch is expected to expand if lures containing 400mg or higher amount of the pheromone are to be used.

*Trap position*

The importance of trap position has been revealed in many studies (Youm *et al.*, 1997). For example, in apple orchards traps are suspended at the sites preferred by the pest on the trees (Riedl *et al.*, 1979). Capture of the males of the citrus mealybug was affected significantly by the position of the trap. The results suggest that most of the trapped males originated from other trees in the grove. It is obvious that the trapped males reached the plot from relatively long distances (inside the plot) rather than from neighboring trees. This was conclusively proved by the level of the male catch recorded for traps suspended inside the canopy of Chlorpyrifos-treated trees that were practically free of mealybugs.

The remarkable difference of male catch between traps installed inside the canopy (287.8) and those suspended on a wire between trees (35.9) confirms our conclusions that males fly toward the tree crown and only then they start looking for the pheromone source. It is apparent that the male mealybugs tend to look for females inside the crown. Traps deployed further away outside the plot trapped a similar and even a greater number of males than traps suspended between trees. These data provide some insight about the effective area of trapping. This flight behavior may discourage to use of trap pheromone to estimate the mealybug densities between the subplots in the orchard based on males capture. The high male capture in traps placed inside Chlorpyrifos suggests an insignificant contribution of individual trees to the level of catch of traps exposed inside the tree crown.

Our results suggest that most of the males that were trapped by sticky traps came from other trees in the grove. Hence, arrivals in the vicinity of the trap from longer distances rather than from neighboring trees may provide the general pattern of scale distribution in the grove. It seems that when attracted to the trap, the males fly into the tree crown and only then also they approach the trap. It is suggested that the male mealybugs tend to look for the female inside the crown. During summer, most of the citrus mealybug population occurs on fruits inside the crown.

## ACKNOWLEDGEMENTS

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