Control in Citrus Fruit Crops
IOBC/wprs Bulletin Vol. 38, 2008
pp. 67-74

Biological efficacy of two organophosphate insecticides against California red scale (*Aonidiella aurantii* Maskell) related to deposition parameters under laboratory conditions

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Abstract: California red scale (*Aonidiella aurantii* Maskell) is a major economic pest of citrus in many countries. It is mostly controlled by organophosphate insecticides. The present work is aimed at adjusting an optimal dosage of two insecticides, in order to reduce the presence of residues on the fruit while assuring their efficacy. The paper establishes, under laboratory conditions, the relationship between the deposition characteristics of these insecticides and their efficacy. It takes into account the influence of the development stage of the scale to build curves of expected mortality against the amount of active ingredient deposition. The results demonstrate the importance of applying the insecticides in the early stages of the pest and shows that the amount of active ingredient has to be doubled or even quadruplicated when treating the adult phases (pre-pupae, young female, adult female) with respect to the amounts required for the young ones (L1 and L2).

Key words: *Aonidiella aurantii*, organophosphate, coverage, efficacy

Introduction

California red scale (*Aonidiella aurantii* Maskell) is the most harmful pest in Spanish citrus growing regions, as well as in many other countries, being especially well established in almost all citrus areas of its Mediterranean coast (Durbá Cabrelles et al., 2006). Its management includes insecticide treatments, being chlorpyrifos, methidathion, pyriproxyfen, buprofezin and mineral oils the most used in Spain (M.A. Martínez Hervás et al., 2005). The use of pesticides represents a serious problem for the environment and their application is also dangerous for the field workers, who need special protections for the application. Inadequate dosage also induces resistance on the pest. However, very little research has been done to study the optimal dosage in order to decrease the quantity of applied products.

The aim of the present work is to relate the deposition of two organophosphate insecticides with the expected mortality of the California red scale in its different development stages. This is the first step to establish the dose and time of application for an optimal control of the pest, with the intention of reducing the presence of residues of insecticides while assuring their efficacy.

Therefore, in this work it has been sought to determine, on the one hand, the characteristics of the depositions of the products when applying different volumes and, on the other hand, the efficacy obtained on the mortality of California red scale in different development phases. Finally, the characteristics of the deposits have been related to their efficacy.

Material and methods

Product application

The pesticides were applied by means of a Potter precision laboratory spray tower (C. Potter, 1952) (Figure 1). It consists of a metallic central tube, with has a small deposit on the top, connected to a pneumatic nozzle. At the bottom of the tube there is a platform where the specimen that receives the spray is situated. It performs a pneumatic spray and in all the trials 1 bar of air pressure was used.

In previous experiences using this device, it was realized that an important part of the sprayed volume did not reach the target, because it evaporated or adhered to the tube walls. In order to know the actual volume that reaches the target a preliminary trial was performed to calibrate the procedure. In this experiment 5 plastic Petri dishes were dried and weighed (Precision scales, XR 205 SM-DR) for each tested volume (500 µl, 1000 µl, 2000 µl, 3000 µl and 4000 µl). Then, they were sprayed with the corresponding volumes of water and weighed again, thus obtaining the weight of the deposited spray by calculating the differences. Finally, this data was converted to volume and expressed in percentage of the total volume that had been added to the tower deposit. This is what was called the recovery percentage.

Figure 1. Potter tower

Figure 2. Lighting system

Deposition studies

Square, white PVC collectors (4x4 cm), which have drop retention behaviour similar to that of the citrus leaves (G. Mercader et al., 1995), were used as artificial targets. They were sprayed with water, adding 1% p/p of an iron quelate (Sequestrene 138 Fe G-100, Syngenta) as a red colouring agent, to generate a high contrast with the background, necessary for the subsequent image analysis. An experimental design of one factor, the volume (µl) with 5 levels (500, 1000, 2000, 3000 and 4000) and 5 replicates, was performed.

These collectors were then photographed with a digital camera (Canon PowerShot A70) under a lighting system (Figure 2) composed by an aluminium hood, which was the support, and two circular fluorescent lamps (Philips, TLE 22W/54 and TLE 32W/54). The photographs were taken with a resolution of 2 pixels per mm, and compressed to JPG format. The camera was set in a Polaroid MP-4 Land Camera support at a vertical distance of 27.5 cm over the samples, to make all the photographs in similar conditions.

In order to calibrate the images spatially, a ruler was photographed in the same enlargement conditions of the collectors and the number of pixels contained between two marks of the ruler in this image was counted. This gave the scaling factor to convert pixels in µm.

After the calibration, the images were analysed with commercial software (Matrox Inspector v. 2.2, Matrox Electronic Systems Ltd.). The analysis consisted of 4 phases:

- A representative region of the observed deposition on the image was manually selected.
- This region was converted to a 256 grey level image.
- On the latter image, a grey level threshold to separate droplets from background was set by an operator. This person compared visually the segmentation result with the original image until obtaining a satisfactory accuracy in the representation of the spray
- Once the impact of the droplets was isolated, three features were calculated from the image: coverage (percentage of total surface covered by the spray), Feret mean diameter (FMD) of each of the impacts (µm) and number of impacts per square centimetre.

Efficacy trials

Biological efficacy of treatments was estimated based on insect absolute mortality, this defined as the percentage of individuals that did not evolve from the total, since mortality of the control was almost zero.

California red scale infested lemons, reared under a protocol developed by the Entomology Unit of the IVIA, were employed along the experiments (Figure 3).

Figure 3. California red scale infested lemon

The life cycle of the California red scale was divided in four phases, based on the cycle stages described by L.D. Foster et col. (1995), which were supposed to have different sensitivity to the treatments:

- L1, which includes Instar 1 and Molt 1 stages
- L2, which includes Instar 2 and Molt 2 stages
- H, which includes young female, adult female and gravid female stages (H1, H2 and H3)
- PP, which includes prepupae male and pupae male stages

To obtain individuals in a specific phase for the trial the larvae were let evolve during a prefixed number of days since they fixed in the lemon surface. This number was 5 days for L1, 9 days for L2 and 15 days for H and PP.

At least 50 live individuals in each lemon were identified by marking them with a permanent pen (Figure 4) before performing the treatments. Ten days after treatments, the marked individuals that had not evolved were counted to estimate the percentage of mortality, because they were supposed to have died because of the treatment.

Figure 4. Surface of an infested lemon, prepared for its spraying

An experimental design of two factors with 5 replicates was performed: the first factor was the water volume rate, with 5 levels, 4 volumes (µl) (1000, 2000, 3000 and 4000) plus a control; and the second factor was the life phase with the four levels the life cycle of the California red scale had been divided in (L1, L2, H, PP). This experimental design was carried out with two organophosphate insecticides (Product A and Product B), both of them applied at the label maximum concentration, prescribed for the treatment of this pest in citrus.

It is important to remark that the dose of treatment 1 is half of that of the treatment 2, third part of the treatment 3 and fourth part of the treatment 4, which means that different treatments implies a dose increase by means of increasing the volume while the product concentration is always the same.

Statistical analysis

The Analysis of Variance (ANOVA) was employed in its factorial version to study the results of the efficacy trials (two factors: volume and development phase) and in its simple version to evaluate the deposition parameters (one factor: volume). The Shapiro-Wilks test was used over the model residues to test the normality of the data and the Levene test to evaluate their homocedasticity (homogeneity of variances). All these tests were performed with 95% confidence interval. The non-parametric Tukey test was used for the comparison of means, which lowers Type I error.

When some of the assumptions of the ANOVA were violated, the Kruskal-Wallis non-parametric variance analysis was performed. In this case, the Box and Whisker plot with the median confidence intervals was used to compare the means. However, in this work only ANOVA results are presented, because results were identical and interpretation is straightforward.

Results

Deposition studies: Recovery percentage

It is important to remark that the obtained recovery percentages were very low, going from 4.8% to 7.1%. The volume was not statistically significant for this factor (Figure 5), that is to say, the percentage of recovered volume was almost the same whichever the applied volume was, so the volume reaching the target was proportional to the initial volume that is located on the tower deposit. Therefore, the active ingredient dose that later on would be effectively put on the lemons would be proportional to the water volume.

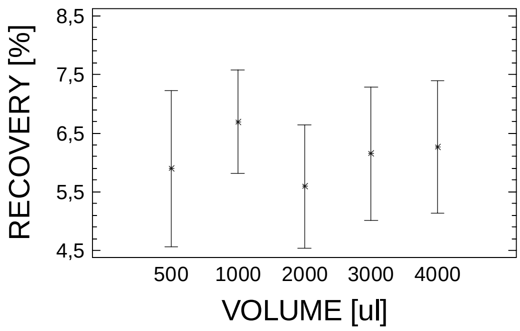

Figure 5. Tukey HSD-Intervals for the recovery percentage

Deposition studies: Deposit characterization

Regarding the coverage percentage, although there was no significant difference between 1000 µl and 2000 µl, the volume was statistically significant, as it can be observed on the Figure 6. The coverage percentage increased proportionally with the volume.

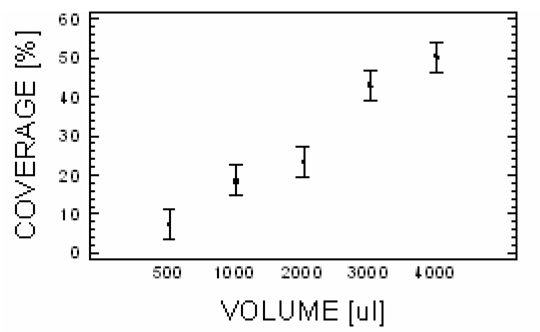

Figure 6. Tukey-HSD Intervals for coverage percentage

It was also noticed that when the volume was higher than 1000 µl, the impact size increased too, and the number of impacts decreased (Figure 7), which seemed to happen because as the volume increased, the impacts aggregate and give rise to larger impacts.

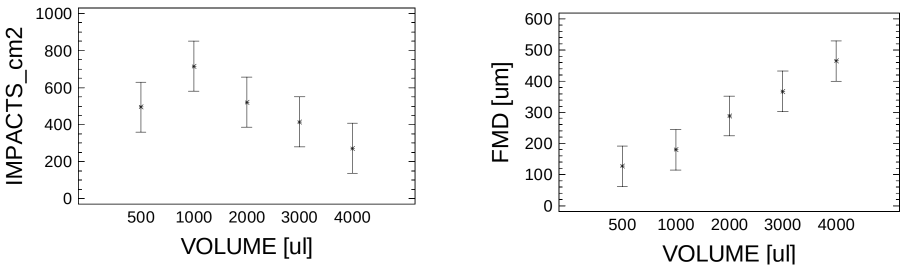

Figure 7. Tukey-HSD Intervals for the number of impacts per square centimetre and FMD (µm)

Efficacy trials

First of all, the quantity of sprayed active ingredient with each volume (µg a.i./cm^2) was estimated on the basis of the recovery percentage, the employed concentration and the content of active ingredient in each product (Table 1).

Table 1. Estimation of active ingredient sprayed (µg a.i./cm^2) (Average ± Standard Error)

TREATMENTS	**µg a.i. A/cm^2**	**µg a.i. B/cm^2**
1000 µl	0.88 ± 0.10	0.99 ± 0.10
2000 µl	1.64 ± 0.18	2.17 ± 0.21
3000 µl	2.82 ± 0.25	3.47 ± 0.11
4000 µl	3.82 ± 0.28	4.74 ± 0.23
Control	Control	Control

Regarding the mortality, significant differences between the control and the other treatments and among the different phases of the scale were observed (Figure 8).

L1 and L2 phases were the most sensitive since they presented the highest mortality (96%) and because high mortality levels were achieved even with the minimal volume, which implied both the minimal coverage and the minimal quantity of active ingredient (0.88 µg a.i. A/cm^2 and 0.99 µg a.i. B/cm^2).

PP phase was less sensitive. Its mortality increased between treatments of 1000 and 2000 µl (from 0.88 to 1.64 µg a.i./cm^2) with Product A, reaching 30% of coverage, and between 1000 and 3000 µl (from 0.99 to 3.47 µg a.i./cm^2) with Product B, reaching almost 40% of coverage, with 83% of mortality in the first case and 98% in the second one. For higher volumes, its mortality did not increase significantly.

The H phase was the least sensitive, showing the lowest mortality levels. The increase of volume up to 2000 µl (1.64 µg a.m. A/cm^2 and 2.17 µg a.m. B/cm^2), which implied an increase of coverage up to 30%, did not result in a significant increase of mortality. It only increased when the volume was raised up to 3000 µl (2.82 µg a.i. A/cm^2 and 3.47 µg a.i. B/cm^2), with almost 40% of coverage, going the mortality up to 65% with Product A and 60% with Product B. From there on its mortality did not change even if the volume increased up to 4000 µl (3.82 µg a.i. A/cm^2 and 4.74 µg a.i. B/cm^2) and therefore the coverage up to 43%.

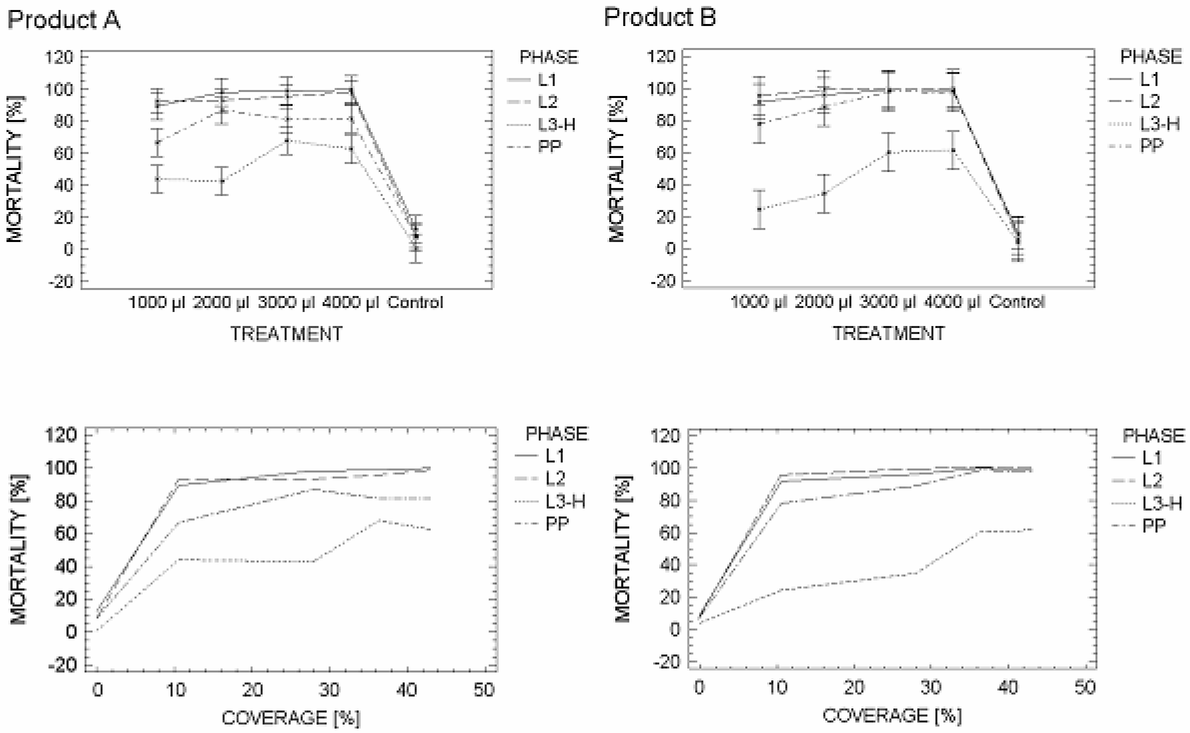

Figure 8. Mortality of California red scale based on the treatment and coverage percentage in each development phase

Conclusions

Regarding the obtained depositions, first of all it was determined that the applied doses (µg a.i./cm^2) were proportional to the volume. On the other hand, it was observed that, when applying 1000 µl or more, the more volume is applied, the more coverage is obtained, by means of increasing the impact size and decreasing the number of impacts, which could be because the adjacent impacts aggregate.

Regarding the biological efficacy, the highest mortality was obtained for L1 and L2 when applying 0.88 µg a.i. A/cm^2 and 0.99 µg a.i. B/cm^2. Their mortality did not increase although the dose was increased by means of increasing the volume, that is to say, the increase of active ingredient, coverage and impact size did not produce significant changes.

PP phase presented lower mortality and a slight increase was observed when raising the quantity of active ingredient up to 1.64 µg a.i. A/ cm^2 and 3.47 µg a.i. B/cm^2. The mortality remained around 85-90% for higher doses, but it is not known if the maximum was reached.

The H phase was the least sensitive, showing the lowest mortality levels. The highest level reached by this stage was 60-65%.

To obtain levels of efficacy in the PP phase similar to those of the young phases, depositions of the active ingredient should be doubled, whereas depositions with the quadruple of the active ingredient only would raise the mortality of the H phase up to 65%.

Hence, this work demonstrated the importance of performing field treatments when the pest is in its first phases of development, since later on treatment is not going to be as effective as it could be.

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