Crop Protection

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Effect of Spray Volume of Two Organophosphate Pesticides on Coverage and on Mortality of California Red Scale *Aonidiella aurantii* Maskell

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Abstract

A trial under laboratory conditions was carried out to study the possibility of decreasing the delivered dose of organophosphate insecticides without affecting their efficacy against California red scale (CRS), *Aonidiella aurantii* (Maskell), by reducing the volume of water used whilst maintaining the concentration of pesticide. In order to establish an optimal application volume, the coverage and the efficacy against different stages of development of CRS of two commercial organophosphate pesticides (chlorpyrifos 750 g kg^{-1} WG [Dursban® 75 WG] and chlorpyrifos-methyl 224 g l^{-1} EC [Reldan® E]) at four volumes of water (1, 2, 3 and 4 ml) were compared. Results showed that in general Chlorpyrifos-methyl-based product (CMBP), provided greater coverage and impact size, but did not achieve the highest efficacy. The minimum deposited volume (1.01 μl/cm^2) produced 11% coverage with Chlorpyrifos-based product (CBP) and 22% with CMBP, reaching the highest efficacies (around 89-95%) against the youngest stages (N1 and N2 stages) with both products. To attain similar efficacy with males (prepupal and pupa stages), twice the volume (2.03 μl/cm^2) of the CBP was required, producing 28% coverage. The CMBP required higher deposited volumes (3.41 μl/cm^2) and higher coverage (51%). The maximum efficacy in the control of adult females (third instar and gravid female stages) was 70% with both pesticides. This level was attained with 3.41 μl/cm^2 of CBP (36% coverage) and 4.72 μl/cm^2 of CMBP (62% coverage). The research shows that greater coverage does not result in greater efficacy, so it would be possible to reduce the spray volume in field conditions. Further research will be carried out in order to check these results under real field conditions

Keywords: efficacy; deposition; chlorpyrifos; chlorpyrifos-methyl; bioassay.

1. Introduction

California red scale (CRS), *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae), is one of the most damaging scale insects in citrus crops worldwide and is considered the main citrus pest in Spain. The most common economic harm caused by CRS to citrus is due to its presence on fruits, downgrading them and making marketing difficult even when CRS populations are low. Severe infestations can also reduce tree vigour and yield (Alfaro et al., 2003; Vanaclocha et al., 2009; Walker et al., 1991).

Organophosphate insecticides have been used worldwide to control CRS in citrus producing regions (Carman, 1977; Grout and Richards, 1992; Levitin and Cohen, 1998; Martínez Hervás et al., 2005). Among them, chlorpyrifos has been particularly widely used due to its high efficacy (Bailey and Morse, 1991). It is well known, however, that their application can have significant adverse environmental effects (FAO, 2008). Moreover, during the last few years some problems with residues of organophosphate insecticides after harvest (especially chlorpyrifos) started to occur in the citrus industry, a problem associated with the repetitive and excessive use of these active materials (Coscollá, 2003, 2007).

A way to restrict contamination by pesticides goes through the reduction of the amount of active ingredient (a.i.) delivered per unit area of cultivation. This could be achieved by 1) decreasing the concentration of a.i. in the solution whilst maintaining the water volume, or 2) decreasing the water volume applied whilst maintaining the recommended concentration.

Reduction of the concentration does not seem advisable, since concentration is one of the parameters that have to be declared and tested in the legal registration of a commercial insecticide (minimal concentration to achieve a certain guaranteed efficacy).

Nowadays, organophosphate field treatments against CRS use high volumes of water to maximize the probability of reaching the insects on the tree canopy. Nevertheless, it is necessary to know how far these volumes can be reduced without affecting the efficacy of applications.

Some studies have been conducted to analyze the effect of the sprayed volume in the efficacy of pesticides but against other pests and/or using other products. McCoy et al. (1989) did not find differences in control efficacy by changing sprayed volumes of different acaricides against *Phyllocoptruta oleivora* Ashmead (Acari: Eriophyidae). In the same way, no differences were found in the control of *Coccus pseudomagnoliarum* Kuwana (Hemiptera: Coccidae) with petroleum derived spray oils by Chueca et al. (2009). Conversely, other authors found differences in efficacy dependent on the sprayed volume at constant concentration: Beattie et al. (2002) for applications of a mineral oil against soft and armoured scales, Grout and Stephen (1993) for applications of a mineral oil against CRS, and Cunningham and Harden (1999) for methidathion applications against this same pest.

Spray volume is the volume of spray solution that is emitted from the nozzles of the sprayer per unit ground area, but it should be remarked that the important factor as far as efficacy is concerned is which part of this volume reaches the target and how it distributes on the surface of the plant. It is widely known that physicochemical properties of formulations affect droplet size spectrum (Bouse et al., 1990; Haq et al., 1983; Yates et al., 1983) and thus deposition pattern (Salyani, 1988; Spillman, 1984; Zabkiewicz, 2007).

Because of the above-mentioned facts, this study was carried out to determine the optimal volume of solution to be applied for controlling different stages of CRS when using two organophosphate pesticide products containing two different active ingredients registered for citrus against this pest: chlorpyrifos and chlorpyrifos-methyl. Three parameters that describe spray deposition (coverage, average size of impacts and number of impacts per unit area) were studied in order to determine how different applied volumes change these distributions and affect the efficacy of the treatments. The final goal was to determine the relationships between the deposition pattern and the efficacy on CRS control.

2. Materials and Methods

Two experiments were conducted to study the effect of spray volume on (1) deposition, and (2) efficacy against different stages of CRS under laboratory conditions. In both experiments, two commercial organophosphate insecticides were used: Dursban® 75 WG (a.i.: chlorpyrifos 750 g kg^{-1} WG) (Dow AgroSciences Ibérica, Madrid, Spain) and Reldan® E (a.i.: chlorpyrifos-methyl 224g l^{-1} EC) (Dow AgroSciences Ibérica, Madrid, Spain). Both products were used at maximum label concentration, 1.25 g/l for Chlorpyrifos-based product (CBP) and 4 ml/l for Chlorpyrifos-methyl-based product (CMBP). In the efficacy study, a control treatment with water was included. The spray volumes used for both experiments were 1, 2, 3 and 4 ml. This last volume was the greatest spray volume tested because, at higher volumes, the droplets coalesced producing a surface of liquid that ran off from the target surface. These volumes were used since, in preliminary tests, we obtained with them a wide range of coverage levels that could be achieved in field treatments.

These volumes were applied with a Potter Spray Tower (Burkard Scientific, Uxbridge, United Kingdom), fitted with its finest nozzle (internal diameter: 0.762 mm) (Potter, 1952). The pressure was fixed at 0.1 MPa. It was calibrated before each experiment, since the volume of the solution that reached the base of the tower was very low compared with that sprayed up the tower. The amount of solution deposited per unit area (µl/cm^2) on the base of the tower for each applied volume was estimated by a series of tests carried out as follows: Different volumes of water were sprayed to Petri dishes of known surface area. The Petri dishes were weighed before and after application using an analytical balance (XR 205 SM-DR, Precisa Instruments Ltd., Dietikon, Switzerland). The time between spraying and weighting was very short (around two seconds); therefore, evaporation was so small that we decided to disregard it. Five replicates per volume tested were performed. The average increase of weight produced by the deposition of the droplets of the solution per unit area was calculated. From these data, the amount of a.i. per unit area for each organophosphate pesticide treatment was estimated (Table 1).

2.1. Study of deposition.

To study the effect of the spray volume on deposition, four spray volumes with three solutions (water, CBP and CMBP) were compared.

White PVC collectors (4.5 x 4.5 cm) were used as artificial targets whose drop retention behaviour is similar to that of citrus leaves (Mercader et al., 1995). Collectors were sprayed with the corresponding solution; adding 20 mg/l of iron chelate (Sequestrene 138 Fe G-100, Syngenta Agro S.A., Madrid, Spain) as a dye to produce a high drop/background contrast, required for subsequent image analysis. Five replicates for each combination of solution and spray volume were performed.

Sprayed collectors were photographed and images analyzed with specific software (Matrox Inspector, version 2.2, MatroxTM, Dorval, Canada) following the methodology described by

Chueca et al. (2010). The images were taken with 20 pixels/mm resolution. Objects in the image constituted by one single pixel were considered as noise and thus removed. Therefore, impacts less than 50 µm diameter were not necessarily detected. In each image, the program detects all the impacts (deposited droplets produced by the spray over the collector) bigger than $2.5 \cdot 10^{-3}$ mm^2, and then calculates three parameters to describe deposition: coverage (%) (Percentage of the total surface covered by the impacts), mean area of impacts (mm^2) and number of impacts per unit area (No. of impacts/cm^2).

2.2. Study of biological efficacy.

To study the effect of the different spray volumes on the efficacy against CRS, the same four spray volumes of each pesticide solution (CBP and CMBP) were applied on lemons infested with CRS populations in four groups of stages of development. These groups of stages were labelled as follows (each one included the growth stages shown in brackets): N1 (nipple stage and first molt), N2 (second instar and second molt), N3 (third instar and gravid females) and PP (prepupal and pupa males). A control treatment (only water) was also included. Each lemon fruit was used as one replicate. five replicates of each combination of stage, solution and spray volume were performed.

Lemons were infested following the protocol developed by Pina (2006): Briefly, clean lemons were partially covered with wax, leaving a clean surface (arena) of about 16 cm^2, by dipping them in molten paraffin while the long axis of the fruit was held horizontal, so that the level of paraffin slightly surpassed the stem and blossom ends. The paraffin film reduced desiccation and avoided the spreading of CRS individuals. Lemons were big enough relative to the size of the arena so that the arenas could be considered flat. The arenas were kept horizontal during and after spraying.

The infestation procedure was as follows: Each of the waxed lemons was put on the top of black paperboard tubes (10 cm high and 3 cm base diameter) with the unwaxed surface up. On the bottom of each tube, a lemon infested with hundreds of reproducing CRS females, from a colony maintained under laboratory conditions, was put with the unwaxed surface up. Fluorescent lights were placed over this set up to attract crawlers from the infested lemons to the arenas for 24 hours. After crawlers reached the “whitecap” stage, lemons with more than 50 fixed scales were removed and placed in a tray until around 90% of individuals had reached the corresponding stage for each test. This time was around 5 days to reach the N1 stage, 9 days for the N2 and 15 days for the N3 and the PP stages. To guarantee that the corresponding development stage had been reached, the infested lemons were checked before treatment. Trays and colonies were kept in chambers at a temperature of 26 ± 3 ºC, 50 ± 5% relative humidity (RH) and continuous light.

Before being sprayed, about 50 healthy scales per lemon of the corresponding stage were circled with a permanent marker (Lumocolor F width, Staedtler Mars GmbH & Co. KG, Germany). Ten days after treatment, the numbers of dead and live scales from those that have been circled were recorded. N1, N2 and PP scales that had not matured to the next stage were considered dead. N3 scales were considered dead when their body under the shield had a dry, thin and flat appearance. Mortality rates were calculated as the ratio of dead individuals to the total checked individuals (alive and dead).

2.3. Data Analysis.

A two-factorial analysis of variance (MANOVA) was performed for each deposition variable studied (coverage, mean area of impact and number of impacts per square centimetre). The two factors were the “deposited spray volume” and the “pesticide” (CBP, CMBP and water).

In order to study the efficacy of the treatments, Dunnett’s test (Dunnett, 1985) was used to compare N1, N2, N3 and PP mortality of control (only water) and organophosphate treatments for each spray volume. When significant differences were found, efficacies of the two products were calculated using the Schneider-Orelli formula (Püntener, 1981). These efficacies were the dependent variable in a two factor MANOVA performed for each pesticide. In these tests, the factors were “deposited spray volume” (µl solution/cm^2) and “CRS stage” (N1, N2, N3 and PP).

Both in the study of deposition and in the study of biological efficacy, the ANOVA assumption of normal distribution of residues was assessed by the Shapiro-Wilk’s test (Shapiro and Wilk, 1965) and the assumption of homocedasticity by the Levene´s test (Levene, 1960). After MANOVA in both studies, Fisher's LSD test (Fisher, 1935) was used for mean comparisons. All tests were considered at the 95% confidence level and were carried out with Statgraphics® Plus version 5.1 (STSC Inc., 1987).

3. Results

3.1. Study of deposition.

The interaction between the factors, deposited spray volume and product, was significant for coverage (F = 2.51; df = 6, 75; P = 0.0306), mean area of the impacts (F = 1738.82; df = 6, 72; P < 0.0001) and number of impacts per unit area (F = 4.13; df = 6, 70; P = 0.0016). The increase of spray volume deposited caused an increase of coverage (%) of the three solutions tested (Fig. 1A). The greatest coverage values were obtained with CMBP for all deposited volumes (from around 22% at 1.01µl/cm^2 to around 62% at 4.72 µl/cm^2). CBP produced the lowest coverage values for all spray volumes, except for 2.03 µl/cm^2 where it produced coverage similar to that of water (from around 11% at 1.01µl/cm^2 to around 43% at 4.72 µl/cm^2). The differences of coverage between both pesticides were around 10-20%. The values for water were between those of the two pesticide treatments.

No significant differences of mean area of impacts (mm^2) were found between water and CBP for all deposited volumes, except for the highest (4.72 µl/cm^2), in which CBP produced larger mean area of impacts than water (Fig. 1B). For the lowest spray volume of 1.01 µl/cm^2, no significant differences of mean area of impact were found among the three solutions. However, CMBP produced significantly greater values of mean area of impacts than water and CBP for volumes deposited starting from 2.03 µl/cm^2. These values ranged from 3.68 mm^2 at 3.41 µl/cm^2 to 19.94 mm^2 at 4.72 µl/cm^2.

An increase in deposited spray volume caused a decrease in number of impacts per square centimetre for the three solutions tested (Fig. 1C). This was because the droplets joined on the target, thus increasing the size of the impacts. Water produced the greatest number of impacts per square centimetre for all spray volumes (around 770 impacts/cm^2 at 1.01 µl/cm^2 and around 270 impacts/cm^2 at 4.72 µl/cm^2). The curve produced by CBP decreased approximately in parallel to that of water treatment, but with a difference of 200-300 impacts per square centimetre at each volume, with lower values for CBP. CMBP produced the lowest number of impacts per square centimetres at all volumes (170 impacts/cm^2 at 1.01 µl/cm^2 and 10 impacts/cm^2 at 4.72 µl/cm^2). There were no significant differences in the number of impacts per square centimetre between the spray volumes of 1.01 and 2.03 µl/cm^2 for any of the tested solutions. For water and CBP solutions, significant differences were found between these volumes and 3.41 µl/cm^2 and between this and the highest volume (4.72 µl/cm^2). For CMBP, there were no significant differences between the two highest volumes, but there were between these volumes and the lowest (1.01 µl/cm^2).

3.2. Study of biological efficacy.

N1, N2, N3 and PP mortalities produced by both pesticide solutions differed significantly from the water control (Dunnett test, P<0.05). The mortality percentages for water controls were 12.50% (SE=3.49%) for N1, 8.42% (SE=1.24%) for N2, 0.8% (SE=0.8%) for N3 and 7.94% (SE=3.59%) for PP.

The effect of spray volume on the efficacy against CRS depended on the stage for both organophosphate pesticides. The interaction of the two factors, spray volume and stage, was significant for CBP (F = 5.83; df = 9, 82; P < 0.0001) (Fig. 2A) and for CMBP (F = 9.83; df = 9, 78; P < 0.0001) (Fig. 2B). However, trends apparently differed for both pesticides.

Differences of spray volume did not statistically affect efficacy against N1 and N2 stages for both insecticides. For these stages, efficacies ranged between 88-100% whatever the spray volume applied.

The effect of volume was significant when CBP was applied against the PP stage. The efficacy increased from 63% with the volume of 1.01 µl/cm^2 to 85% with 2.03 µl/cm^2, but with the subsequent volumes, the efficacy did not increase. When CMBP was applied against PP, there were significant increases of efficacy, from 76 % with the volume of 1.01 µl/cm^2 to 97% with 3.41 µl/cm^2. No significant increase of efficacy was found with volumes higher than 3.41 µl/cm^2.

The effect of spray volume was also significant on the N3 stage for both pesticides. In the case of CBP, there were no significant differences between 1.01 µl/cm^2 and 2.03 µl/cm^2, with an average efficacy around 45%. However, an increase in volume to 3.41 µl/cm^2 did affect efficacy, which increased significantly to 68%. Despite the rise of volume to 4.72 µl/cm^2, average efficacy did not change significantly. When CMBP was applied, efficacy against N3 increased significantly at every volume from 4% to 68%, except for the highest volume (significant differences between 3.41 µl/cm^2 and 4.72 µl/cm^2 were not found).

4. Discussion and Conclusions

In general, increases in spray volume increased both coverage and mean impact size and reduced the number of impacts. This was due to coalescence of droplets produced when using the Potter Tower. This phenomenon is often observed in other surfaces, with other spraying devices and in field treatments (Chueca et al., 2009; Ebert and Downer, 2006; Salyani and McCoy, 1989).

Clear differences in the relationships between spray volume and deposition pattern of the solutions were found. CMBP produced a greater coverage, larger impacts and a lesser number of impacts per square centimetre than CBP. These results are probably due to differences in the formulation that affect the surface tension of the solution and hence droplet formation and deposition. This is most likely to be the explanation for the differences in the deposition pattern between water and the other solutions. Other authors have also observed how different formulations affect deposition pattern (Akesson and Gibbs, 1990; Akesson et al., 1994; Bouse et al., 1990; Butler-Ellis et al., 1997).

All treatments achieved high efficacies (around 89-95%) against N1 and N2 stages, so it was not possible to find a relationship between deposition and efficacy. The minimum-deposited volume (1.01 $\mu l/cm^2$) of CBP produced 11% coverage and an a.i. deposition of 0.945 mg of chlorpyrifos/ cm^2. CMBP produced greater coverage (22%) but similar deposition of a.i. (0.903 mg of chlorpyrifos-methyl/cm^2). We assume that both products produced the same effect on N1 and N2 stages since, even at these low dosages, enough a.i. has been deposited to kill the insects, no matter how the products spread on the target.

At the PP stage, 2.03 $\mu l/cm^2$ of CBP produced 22% coverage and 80-90% efficacy. In the case of CMBP, higher deposited volumes (3.41 $\mu l/cm^2$) and higher coverage (51%) were required to achieve similar efficacy.

The maximum efficacy on N3 stage with both pesticides was 70%. To reach such a level, it was necessary to deposit 3.41 $\mu l/cm^2$ of CBP (36% coverage) and 4.72 $\mu l/cm^2$ of CMBP (62% coverage). As before, it was observed that more deposition of CMBP than CBP was required to achieve the same effect. This indicated that chlorpyrifos had a higher toxicity.

The results show that N1 and N2 stages are the most sensitive to both products, because maximal efficacies were obtained with the lowest volumes. This conclusion is explained by the fact that these stages are less protected because their shield is thinner compared to that of older stages as has been widely reported in literature (Asplanato and García-Marí, 2001; Busvine, 1971; Hernández-Penadés et al., 2004; Walker et al., 1990). Furthermore, this research shows that greater coverage does not necessarily result in greater efficacy. Experiments carried out in this work indicated that efficacy of the pesticides under study on adult stages of CRS reached a maximum value that was not surpassed by higher deposition of product. They also showed that this maximum differed for the two products.

In Spanish citrus growing areas, CRS may have 2-4 generations per year depending on climatic conditions. The first generation begins in May when temperatures are optimal for CRS development. At this time, there is a peak of crawlers in the population, so in this first generation the development stages of the insect are more or less homogenous. Therefore, it is recommended to control the pest at the beginning of its first generation because, in the succeeding generations, different stages coexist. Our experiments indicate that treatments carried out at this time should be set up in such a way that 11% coverage is attained on fruit for CBP solutions, or 22% coverage is attained for CMBP solutions. This probably implies the use of lower volumes of water than those that are usually sprayed. And thus, the amount of pesticide delivery could be reduced, decreasing both the environmental impact and the presence of residues in fruit. The next step of our experimentation will be devoted to validating if the proposed deposition attains the expected level of pest control in field conditions and to determine which operative conditions of the machinery (sprayer, pressure, speed, volume, nozzles, etc.) will be capable of producing such deposition in citrus orchards.

This study contributes to the understanding of how the spray volume is deposited and how it affects the efficacy of two organophosphate insecticides for controlling CRS. It shows the relevance of studying the relationship between efficacy of the pesticides against a pest and the deposition patterns of such products.

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Table 1. Estimated amount of active ingredient deposited per unit area (µg/cm^2) for the four volumes of solution and for each organophosphate pesticide sprayed with the Potter tower onto Petri dishes.

Fig. 1. Interaction between the factors deposited volume and product for coverage (A), mean impact area (B) and number of impacts per unit area (C)

Fig. 2. Interaction between the factors deposited volume and development stage for the effectiveness of Chlorpyrifos-based product (A) and Chlorpyrifos-methyl-based product (B)