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Modeling the Spray Deposition and Efficacy of Two Mineral Oil-based Products for the Control of California Red Scale *Aonidiella aurantii* (Maskell)

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Abstract

In this study we evaluated under laboratory conditions the efficacy of two petroleum-derived spray oils (PDSO) (Laincoil®, ~~Oil A,~~ and Sunspray Ultrafine®, ~~Oil B~~) applied at 1.5% concentration at five ~~water~~ volumes (0.5, 1, 2, 3 and 4 ml) against different stages of *Aonidiella aurantii* Maskell (Homoptera: Diaspididae). In parallel, we characterized the deposition pattern of treatments resulting of these five volumes and two PDSO. The objective was to model the characteristics of deposition and ~~the~~ efficacy as a function of the deposited volume in order to determine the optimum volume that should be applied in PDSO treatments against this pest. Different models that relate ~~the~~ efficacy as a function of the deposited volume have been obtained for both ~~two~~ PDSO and for the tested stages of *A.aurantii*. Results ~~reflected the optimum deposited volume for each oil and each stage, showing~~ showed that Sunspray Ultrafine® ~~Oil B~~ had higher efficacy and produced more but smaller impacts that Laincoil® ~~Oil A~~, which may indicate the influence of formulation on the efficacy of PDSO. We propose a methodology to evaluate the effect of PDSO on the spray deposition pattern and efficacy against various California red scale stages, thus providing a scientific basis for product comparison.

Key words: PDSO, coverage, spray volume, formulation

1. Introduction

Petroleum-derived spray oils (PDSO) have been used as crop protection products for over a hundred years; they were first applied in the 1920s (Ackerman, 1923; De Ong, 1926). Indeed, they have a good ecotoxicological profile and pests do not develop resistance. Furthermore, populations of beneficial arthropods are not severely affected because of the short-term residual activity of PDSO (Childers, 2002; Davidson, 1991; Nguyen et al., 2002; Riehl, 1981; Urbaneja et al., 2008).

California red scale *Aonidiella aurantii* (Maskell) (Homoptera: Diaspididae) (CRS), one of the pests with greater economic impact in worldwide citrus growing, has traditionally been controlled by organophosphate insecticides. However, the extensive and continuous use of these pesticides has caused environmental impact as well as resistance development in this pest (Bedford, 1998a, b; Grafton-Cardwell and Vehrs, 1995; Levitin and Cohen, 1988; Smith et al., 1997) ~~as well as environmental impact~~. PDSO show good efficacy against CRS and they are currently registered worldwide to control CRS in citrus and are commonly used in integrated pest management programs. In Spain, recommendations for PDSO application are based on a prescribed concentration, specifically 1.0 to 1.5% (MARM, 2010). However, information about volumes of water required depending on the quantity of plant canopy is not provided and is not regulated. This fact may lead to waste through overuse or ineffective control as a result of inadequate application.

The primary cause of mortality produced by PDSO is anoxia, i.e. suffocation by directly blocking the spiracles of scales (Kallianpur et al., 2002; Taverner, 2002). For this reason, high water volumes are presumed to be very important in order to completely cover the target insect (Gaskin et al., 2002).

Mineral oils are characterized by some parameters that might affect their efficacy such as

viscosity, gravity, unsulfonated residue, pour point, distillation temperature and n-paraffin carbon number (*n*C) (Agnello, 2002). PDSO normally contain a specific mineral oil as active ingredient (a.i.) mixed with a wide range of emulsifiers and surfactants. Although these coadjuvants probably do not directly affect the inherent oil toxicity, they have a great influence on the physico-chemical properties of the solution. It is widely known that these properties affect their droplet size spectrum (Bouse et al., 1990; Fraser and Eisenklam, 1956; Haq et al., 1983; Yates et al., 1983) and their deposition pattern (Salyani, 1988; Spillman, 1984; Zabkiewicz, 2007), affecting thereafter the PDSO wetting capacity, and consequently the plant-pest interaction and the efficacy (Agnello, 2002; Zabkiewicz, 2002).

Several researchers have attempted to determine how much volume should be applied in PDSO treatments against CRS, but the results were not conclusive and sometimes they were contradictory. Jeppson and Carman (1974) stated that a low volume field treatment (935 l/ha) did not successfully control CRS, probably due to a bad deposition in the canopy center. Riehl (1981) improved the efficacy of low volume treatments by adjusting the application equipment to reach this area in the tree center ~~of the tree~~, even decreasing concentration. This research also stated that efficacy depends on the pest stage ~~of the pest~~, in such a way that to control adult females, higher deposits (µg oil/cm^2) than those needed for young stages were necessary. Other authors also found differences in efficacy under field conditions related to oil deposit, depending more on volume than on concentration (Beattie et al., 2002; Grout and Stephen, 1993). In contrast, Grafton-Cardwell and Reagan (2005, 2006) found no difference in efficacy in several field trials conducted ~~made~~ with very high volumes (8000 and 15000 l/ha) varying simultaneously the concentration of oil. The lack of conclusive results is probably due to the existence of many factors under field conditions that can affect the efficacy of mineral oils treatments against CRS. These factors ~~which~~ are difficult to take into account altogether: the oil

formulation, the population level, the stage of scales, the size and shape of trees, the density of canopy, the type of sprayer used and its setup parameters, etc. Furthermore, each of these studies had different goals and authors provide data of different nature, depending on the factor of interest, and therefore results cannot be compared.

The objectives of this study were: (i) to characterize the deposition pattern of two PDSO applied in different volumes with constant concentration, (ii) to study the efficacy of these treatments on the different stages of CRS under laboratory conditions, and (iii) to model the efficacy as a function of the deposited volume. ~~Moreover,~~ We investigated if the two PSDO produced the same deposition and efficacy or if they followed different models, which would highlight the importance of the commercial product formulation on the pesticide distribution and pest control.

Material and Methods

Two experiments were carried out under laboratory conditions to test the effect of volume on (i) ~~1)~~ deposition characteristics, and (ii) ~~2)~~ efficacy of mineral oils-based treatments against different stages of CRS. In both experiments two of the most common PDSO in Spain were used: Laincoil®, an *n*C21 oil with a content of 83% w/v (Lainco, S.A., Barcelona, Spain), hereafter Oil A, and Sunspray Ultrafine®, an *n*C21 with a content of 85% w/v (Sun Oil Co., Antwerp, Belgium), hereafter Oil B. Both PDSO have an unsulfonated residue of 92%. They were used at the most common concentration in Spanish field applications against CRS, which is the maximum prescribed concentration of 1.5%. The spray volumes used for both experiments were 0.5, 1, 2, 3 and 4 ml. The maximum spray volume tested was 4 ml because the droplets coalesced at higher volumes, producing a surface of liquid that run-off from the target surface.

Applications were carried out ~~made~~ with a Potter Spray Tower fitted with its finest nozzle (internal diameter: 0.762 mm) (Burkard Scientific, Uxbridge, United Kingdom) (Potter, 1952). Pressure was fixed at 0.1 MPa.

The Potter Spray Tower was calibrated before each experiment. The volume of solution deposited per unit area (µl/cm^2) on the tower base ~~of the tower~~ for each spray volume was estimated by a series of tests. Different volumes of water were sprayed over Petri dishes of known area (63 cm^2). Petri dishes were weighed before and after the application using an analytical balance (XR 205 SM-DR, Precisa Instruments Ltd., Dietikon, Switzerland). Five replicates were used per volume tested. The average increase of weight produced by the deposition of droplets per unit area was measured. From these data, the amount of a.i. per unit area was estimated for each PDSO (Table 1).

Deposition Pattern

In order to study the deposition pattern, the five volumes were tested with two PDSO solutions (Oil A and Oil B), with 3 replicates per treatment.

White PVC-sheet 4.5 x 4.5 cm pieces were used as artificial collectors of the spray solution. PVC drop retention behavior is similar to that of citrus leaves (Mercader et al., 1995). Collectors were sprayed with the corresponding solution (water + PDSO), plus 2% of chelated iron (Sequestrene 138 Fe G-100, Syngenta Agro S.A., Madrid, Spain) as a dye to produce sufficient drop/background contrast for subsequent image analysis.

Collectors were then photographed and the images analyzed using the methodology described by Chueca et al., (2010). Three parameters were measured from each collector to describe the deposition: (i) coverage, expressed as percentage of area occupied by impinging droplets (henceforth impacts) against the total area (%); (ii) area of impacts, estimated by the mean of the sizes of all the impacts on the collector (mm^2); and (iii) number of impacts per unit area (No. of impacts/cm^2).

Efficacy Against CRS Stages.

Five volumes of both solutions (Oil A and Oil B) were tested as well as a control with just water. Experimental trials were conducted on lemons infested with CRS populations in different stages.

CRS-infested lemons were obtained from the rearing colonies of our institution (Centro de Agroingeniería, Instituto Valenciano de Investigaciones Agrarias). Rearing takes place in chambers with a temperature of 26 ± 3 ºC, 50 ± 5% relative humidity (RH) and continuous light, following the protocol developed by Pina (2006).

The insect's life cycle was divided into four groups of stages, in a way that each group comprised various 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).

To infest the target lemons, clean lemons were partially covered by wax, leaving a clean surface (arena) of about 16 cm^2 where CRS developed. Lemons were big enough relative to the size of these arenas so that the arenas could be considered flat. These arenas were kept horizontal during and after spraying. ~~In the base of a box~~ A series of lemons infested with crawler-producing females from the colony was put in the base of a box with the area upward. ~~Over the arena of each lemon~~ A black paperboard tube ~~tubes~~ (10 cm high and 3 cm base diameter) was ~~were~~ put over the arena of each lemon. On the top of black paperboard tubes the waxed clean lemons were put with the areas upward. Fluorescent lights were placed over this set up to attract crawlers from the infested lemons to the clean lemons for 24 hours. After crawlers reached the “whitecap” stage, lemons with more than 50 fixed scales were removed and placed in a tray during a period long enough to allow the majority of individuals to reach one of the desired stages in which CRS life cycle had been divided for the trial. This period was of about 5 days for N1, 9 days for N2 and 15 days for N3 and PP, checking before the applications whether they had really reached the required stage.

Before the PDSO treatment, about 50 living individuals per lemon were circled with a permanent marker (Staedtler permanent Lumocolor, Staedtler, Germany). Ten days after treatment the circled individuals were turned over and the number of dead scales was recorded. N1, N2 and PP scales that had not matured to the next stage were considered as dead. N3 scales were considered dead when the body under the shield had a dry, thin and flat appearance. Percentage of mortality was calculated from these data.

The experimental design consisted of 48 treatments: six volumes x four stages x two PDSO. Each treatment was replicated 5 times, and one lemon was used for each replicate. Hence, 240 lemons were used. In each replicate, treatments were applied in a random order.

Data Analysis.

Multiple linear regression (MLR) was used to model the relationship between the volume deposited on the target, which will be referred to hereafter as variable *D* (deposited volume, µl solution/cm^2), and the parameters that characterize the deposition pattern (coverage, mean impact area and number of impacts per unit area). One MLR model was obtained for each parameter. Quadratic and cubic terms of the independent variable *D* were also taken into account. In order to study if both tested PDSO differ in their deposition pattern, an indicator variable called I_{OIL_A} was also considered. It takes the value one for the experimental data corresponding to Oil A and zero otherwise. Residues of the model were calculated and then Analysis of Variance (ANOVA) was performed on them using PDSO as the factor, in order to study the inclusion of the indicator variable in the regression model.

Regarding the mortality data obtained with infested lemons, Dunnett's test (Dunnett, 1985) was used to compare for each CRS stage and each spray volume the percentage of mortality in the control treatment (only water) versus the mortality of PDSO treatments. When significant

differences were found, efficacies were calculated using the Schneider-Orelli formula (Püntener, 1981). MLR was also applied to study the effect of *D*, PDSO and stage on efficacy. The above explained methodology was followed to study the inclusion of the quadratic term D^2 as well as the interaction between I_{OIL_A}, *D* and D^2. In order to assess whether there was a different response exhibited by any stage, the inclusion of three additional indicator variables were studied: I_{PP}, I_{N3} and I_{N2}, as well as their interactions with the rest of variables. Given that the number of variables was quite high in this case, stepwise MLR was used to identify those with a statistically significant effect on the efficacy. When the indicator variables were found significant, the model could be expressed as a set of equations that depended on the PDSO and the stage.

In all fitted models it was checked by means of a normal probability plot and a Shapiro-Wilks test (Shapiro-Wilk, 1965) that residuals followed approximately a normal distribution, and no outliers were identified. All MLR models were carried out with the software Statgraphics® Plus version 5.1 (StatPoint Technologies Inc., Warrenton, Virginia, USA).

Results

Deposition Pattern.

Equation 1 describes the effect of *D* on coverage. The coefficients of both independent variables, ~~*D* or D^2, in the model~~ were statistically significant (Table 2). The coefficient of the indicator variable I_{OIL_A} was not statistically significant (p=0.082) and it was not included in Equation 1.

~~When the inclusion of the dummy variable, I_{OIL_A}, was studied, factor "oil" did not significantly affect the residues of the model (F = 3.25; d.f.= 1, 29; p-value = 0.0822), so it was not included~~. These results indicated that equation 1 was valid for both PDSO assessed, with a coefficient of determination $R^2 = 0.864$.

$$\text{Coverage}\,(\%) = -0.410 + 22.722 \cdot D - 2.712 \cdot D^2 \quad (1)$$

The fitted equation is depicted in Figure 1A. It shows that in the tested range of volumes, the increase of coverage is very low for $D > 3.5$ µl/cm^2, reaching a maximum value of approximately 50%.

Although there was no evidence of difference in the coverage produced by the two PDSO, they differed in the way that this coverage was achieved because the indicator variable I_{OIL_A} was statistically significant in the models obtained for the mean impact area and number of impacts per unit area (Tables 3 and 4). The coefficients of determination for these models were 0.823 and 0.750, respectively.

In the mean impact area model it was found that the relationship between deposited volume and mean impact area was linear, ~~and that residues differed significantly between PDSO, so the inclusion of the dummy variable in the model was studied~~. The regression coefficient of the variable $D \cdot I_{OIL\_A}$ was statistically significant (Table 3), which means that these responses could be described by two equations (2 and 3), one for each PDSO.

$$\text{mean area}_{OIL\_A}\,(\text{mm}^2) = 0.0046 + 0.026 \cdot D \quad (2)$$

$$\text{mean area}_{OIL\_B}\,(\text{mm}^2) = 0.0046 + 0.016 \cdot D \quad (3)$$

These models are depicted in Fig. 1B, showing that the slope for Oil A was significantly higher than for Oil B. This result suggests that increases of D resulted in a greater size of impacts for the Oil A applications.

Regarding the number of impacts per unit area, both PDSO showed an increasing trend between $D = 0.46$ and $D = 1$ µl/cm^2. However, the number of impacts decreased between $D = 2$ and $D = 3.4$ µl/cm^2. This was probably due to coalescence of droplets since the nozzle is static with respect to the target. In this case, the variable I_{OIL_A} was also statistically significant (Table 4),

212 which implies differences between the two PDSO. As a result, the fitted model can be described
213 by a different equation (4 and 5) for each PDSO. Oil B produced a higher number of impacts for
214 all volumes assessed. Taking together the results of both impact size and number, Oil B
215 generated smaller impacts but more numerous.

216 $$\left(\text{impacts}/\text{cm}^2\right)_{\text{OIL\_A}} = 310.633 + 647.683 \cdot D - 302.665 \cdot D^2 + 34.654 \cdot D^3 \quad (4)$$

217 $$\left(\text{impacts}/\text{cm}^2\right)_{\text{OIL\_B}} = 570.102 + 647.683 \cdot D - 302.665 \cdot D^2 + 34.654 \cdot D^3 \quad (5)$$

218 **Efficacy Against CRS Stages.**

219 N1, N2, N3 and PP mortalities resulting from both PDSO were significantly different from the
220 water control (Dunnett test, $P<0.05$), except in the lowest treatment with 0.46 µl solution/cm^2 of
221 Oil A. This resulted in negative values of efficacy (%) for this treatment when using the
222 Schneider-Orelli formula, as reflected in Fig. 2. The mortality percentages for water controls
223 were 12.00% (SE=2.00%) for N1, 11.33% (SE=2.40%) for N2, 7.42% (SE=2.63%) for N3 and
224 19.09% (SE=1.57%) for PP.

225 By means of stepwise MLR and after checking different alternative models, the best goodness-
226 of-fit was achieved with the model reflected in Table 5, resulting $R^2 = 0.826$. The quadratic term
227 D^2 was statistically significant as well as its interaction with several indicator variables
228 ($P<0.011$). This model can be expressed as five different equations (6-10), depending on the
229 PDSO and the stage (fitted curves in Fig. 2). ~~When the same model is used for different stages,~~
230 ~~it's because no differences in the residues were found~~.

231 a) OIL A

232 $$\%\,\text{Efficacy}_{\text{N1/N2}} = -10.099 + 41.613 \cdot D - 4.349 \cdot D^2 \quad (6)$$

233 $$\%\,\text{Efficacy}_{\text{N3}} = -10.099 + 41.613 \cdot D - 5.288 \cdot D^2 \quad (7)$$

$$\%\,\text{Efficacy}_{PP} = 11.886 + 17.047 \cdot D - 0.634 \cdot D^2 \quad (8)$$

b) OIL B

$$\%\,\text{Efficacy}_{N1/N2/N3} = -10.099 + 49.816 \cdot D - 6.087 \cdot D^2 \quad (9)$$

$$\%\,\text{Efficacy}_{PP} = 11.886 + 25.250 \cdot D - 2.373 \cdot D^2 \quad (10)$$

The efficacy of both PDSO against N1 and N2 was close to 90% for the highest tested volumes, and no significant differences were observed between those stages. The difference between the two PDSO depended on the amount of deposited volume required to reach the maximum efficacy. Oil A required a deposit close to 4 µl solution/cm^2 to reach 90% efficacy while Oil B needed a lower one, close to 3.5 µl solution/cm^2, and consequently, a lower coverage, to reach 92% efficacy.

In the case of stage N3, it followed the same regression model as N1 and N2 in the experiments with Oil B. However, Oil A reached a lower efficacy against N3 for the higher volumes, with a maximum efficacy close to 70%. This result suggests that Oil B was more effective than Oil A against stage N3, since it reached a similar efficacy to that obtained for younger stages.

For both PDSO, efficacy against stage PP followed a different model and no relative maximum was reached. Thus, higher deposited volumes would become more effective against this stage. Generally, the efficacy on PP was lower than for the other stages at higher deposit levels.

Discussion

Various authors (Herron et al., 1995; Riehl and LaDue, 1952; Riehl et al., 1958; Riehl, 1981;) established that LD 95 for mineral oils ranges from 55 to 115 µg oil/cm^2 for CRS. The lowest value is similar to that obtained with the maximum deposited volume of 4.9 µl/cm^2 at concentration of 1.5% in our experiments (Table 1). Taking into account that collectors used in

this experiment behave similar to citrus leaves (Mercader et al., 1995) and higher volume applications will produce run-off, ~~we would expect that~~ the highest value of 115 µg oil/cm^2 reported in the literature would ~~could~~ only be attained in the field, under Spanish conditions, by increasing the oil concentration to more than 2.8 % which could potentially cause phytotoxicity, thus rendering it an unrealistic application. Because this work describes both the total deposition volume and the distribution of deposits, it also opens the possibility to relate the results obtained in laboratory to other reported studies, even with those conducted in field conditions. Consequently, one of the outcomes of our experiments is a more precise recommendation to Spanish citrus growers that is based on scientific evidence and has practical applications.

This study shows that younger stages of CRS were more susceptible than adult stages, which is consistent ~~according~~ with the literature (Riehl, 1981), however it proposes a new method to model the relationships between efficacy, developmental stage and deposited spray volume. Hence, this methodology could be used to determine the coverage necessary to be reached in field conditions to obtain the maximum efficacy. The maximum efficacy obtained under field conditions may differ from the maximum efficacy obtained under laboratory conditions because in laboratory it has not been taken into account the influence of uncontrolled ~~out-of-control~~ factors in real applications such as meteorological conditions, the resistance of the pest to the applied product, the lack of coverage uniformity on the tree canopy, etc.

Significant differences were found in deposition parameters and efficacy depending on the two particular PDSO employed in these experiments. The methodology proposed here can be useful to compare the efficacy of several commercial PDSO under laboratory conditions. This information, as well as their price could be of interest for citrus growers in order to choose the most convenient PDSO as well as for the manufacturers to improve product quality.

In our experiments, Oil A was somewhat less effective than Oil B in controlling CRS. However,

it is important to remark that mineral oils which are the base of both PDSO have similar unsulfonated residue and n-paraffin carbon number (*n*C), so the results may suggest that differences in deposition and efficacy could be due to other factors. We speculate that coadjuvants might play a significant role in these differences, since although total coverage was not significantly different, the resulting distribution of impacts was not the same: Oil B produced smaller but more numerous impacts than Oil A. Thus, more studies of commercial PDSO are needed because both spray distribution and efficacy are dependent on the commercial formulations, not only on the mineral oil on which they are based.

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Table 1. Estimated amount of active material deposited per unit area (µg/cm^2) (Mean and SEM*) for the five volumes of solution and for each PDSO sprayed with the Potter tower onto Petri dishes.

Table 2. Regression coefficients of the MLR equation for coverage as a function of *D* (deposited volume, µl solution/cm^2)

Table 3. Regression coefficients of the MLR equation for mean impact area as a function of *D* (deposited volume, µl solution/cm^2)

Table 4. Regression coefficients of the MLR equation for number of impacts per unit area as a function of *D* (deposited volume, µl solution/cm^2)

Table 5. Regression coefficients of the MLR equation for efficacy as a function of *D* (deposited volume, µl solution/cm^2)

Fig. 1. Experimental data and regression curves for coverage (A), mean impact area (B) and number of impacts per unit area (C) as a function of *D* (µl solution/cm^2) for each PDSO

Fig. 2. Experimental data and regression curves for efficacy (%) as a function of *D* (µl solution/cm^2) for Oil A (A) and Oil B (B)