Factors influencing the efficacy of two organophosphate insecticides in controlling California red scale, Aonidiella aurantii (Maskell). A basis for reducing spray application volume in Mediterranean conditions.

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PEST MANAGEMENT SCIENCE

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Abstract

BACKGROUND: Because our society is seeking ways to lessen the environmental impact of agricultural activity, dose adjustment has become a key issue in current plant protection treatments with high spray application volumes, such as citrus plants. This work investigates, in field conditions, the factors affecting the efficacy of organophosphate insecticides against California red scale (CRS), Aonidiella aurantii (Maskell), when the delivery rate is decreased. Insecticide rate changes were induced by modifying the spray application volumes of two commercial organophosphate pesticides that are based on chlorpyrifos and chlorpyrifos-methyl.

RESULTS: Results showed that with increases in the spray volume, the coverage and the uniformity of deposition on the canopy increased, but final infestation depended neither on the spray application volume nor on the coverage. Furthermore, final infestation significantly depended on the pest pressure in the plot and the spray volume applied per unit volume of canopy ($l\ m^{-3}$ canopy). Moreover, we found that the final infestation was influenced by the efficiency of deposition in the applications that were carried out against the second-generation of CRS.

CONCLUSION: Because the spray application volume did not affect the final infestation, this research introduces the possibility that reducing the doses of current citrus organophosphate treatments may still allow effective plant protection in Mediterranean conditions.

Keywords: citrus; dose adjustment; chlorpyrifos; efficiency; environment

1 INTRODUCTION

California red scale (CRS), Aonidiella aurantii (Maskell) (Hemiptera: Diaspididae), is a key pest for global citrus production and is one of the most damaging pests in Spain.[1] The presence of CRS on the fruit skin causes cosmetic damage, resulting in the downgrading of the fruit in the packinghouse and dramatically decreasing its price, even when CRS populations are low. Moreover, heavy infestations reduce the vigour of trees and their fruit yield.[2-4]

Citrus growers use organophosphate pesticides to control CRS worldwide,[5-8] especially employing chlorpyrifos, due to its high efficacy.[9] However, excessive amounts of pesticide residue, associated with the overuse of these active materials, started to appear on the fruit in the last several years.[10, 11] The application of these pesticides can adversely affect the environment,[12] especially considering that organophosphate field treatments against CRS in citrus are normally applied with high volumes of water, because farmers intend to ensure coverage in a very dense and difficult to reach vegetation. These high volumes increase the risks of run-off and drift. Moreover, overuse also leads to an increase in pesticide resistance, causing these broad-spectrum insecticides lose their effectiveness and produce important economic losses, as has happened in South Africa,[13, 14] Israel,[15] California,[16] and Australia[17]. In Spain, one survey estimated susceptibility of CRS to chlorpyrifos and revealed important differences between orchards[18] but no further research has been conducted.

The spray application volume is the volume of spray solution per ground unit area that is emitted from the nozzles of the sprayer during a treatment. In Spain, as in many other countries, pesticide labels only indicate the allowed concentration of pesticide, but do not recommend an application volume. Reduction of the spray application volume results in the reduction of applied dose of pesticide per unit area because concentration is constant; but because this reduction affects not only the amount but also the distribution of pesticide in the canopy, we need to ensure that an adequate control of the pest is maintained. For instance, Salyani et al.[19] did not find a significant effect of the application volume on the amount of product deposited on the ground when it is expressed as a percentage of the application volume, but these researchers found significant effect of the application volume on the deposition when it is expressed as deposited amount of active ingredient (a.i.) per unit area. Thus, a reduction of the application volume would clearly decrease the negative risk of treatments.

It is also important to note that not all of the applied plant protection product reaches the intended target and that the distribution of the product on the plant's surface after the treatment plays an important role in the efficacy of the treatment. In this sense, it has been widely reported that the application volume can influence the penetration and deposition of the spray in the canopy. Several authors have concluded that lower spray volumes increase both the amount of deposition, due to runoff decrease, as well as deposition variability.[20-22] However, others have found that the higher the volume of spray, the higher the deposit, foliar coverage and uniformity of deposition.[23- 25] This phenomenon is particularly difficult to assess in the case of citrus because mature trees become taller, broader and denser as they grow, which can complicate penetration and uniformity of deposition.[26]

Few studies have analysed the effect of the spray application volume on the efficacy of pesticides used in citrus, and there has not been a conclusive result. Several authors have not found differences in control levels,[27-31] but others have.[32, 33] Even fewer works have studied the influence of the application volume on the efficacy and the distribution and coverage of the spray in the canopy at the same time. Salyani et al.[34] found that increasing the spray application volume did not have a significant effect on mean deposition or on the control of Phyllocoptruta oleivora Ashmead when applying different acaricides, but this approach did increase coverage uniformity. Chueca et al.[35] did not find an influence of spray application volume on the control of Coccus pseudomagnoliarum Kuwana (Hemiptera: Coccidae) using petroleum-derived spray oils, but they did find differences in coverage. Cunningham and Harden[36] found differences on both deposit patterns and efficacy that may be attributed to the spray volume in methidathion applications against CRS.

Undoubtedly, coverage and deposition uniformity have a practical significance on biological efficacy.[37] Coverage and deposition uniformity depend on the physico-chemical characteristics of the plant protection product. Efficacy depends not only on how and when the product is applied and how it reaches the arthropod to be controlled, but also on its biology (e.g., development stage, morphology) and its natural behaviour (e.g., location preferences, mobility, natural defences, tendency to aggregate in colonies). In the case of scales, which are sessile in many of their development stages, it has been stated that adequate control requires a threshold concentration of the appropriate pesticide on the surface of susceptible tissues.[38] In our opinion, divergences in the results reported in the literature demonstrate the need to make particular studies of each set of pests, pesticides, application methods and agricultural ecosystems to obtain conclusions for dose adjustments that could be reasonably generalised.

This work is aimed at providing scientific evidence for reducing pesticide dosage against CRS for citrus grown in Spain. This study examines the effect of the spray application volume on both the coverage and the biological efficacy against CRS of two organophosphate pesticides widely used in Spain, having chlorpyrifos and chlorpyrifos-methyl, respectively as an active ingredient. The intimate relationship between deposition and efficacy of these two organophosphate pesticides has already been demonstrated at laboratory level[39] and we have tried to relate these results to those obtained in the field. In this sense, minimum deposition needed to reach a certain level of control of CRS in the laboratory has been used to calculate the theoretical efficiency of such a deposition, which has been compared to the actual efficiency observed in the field.

2 MATERIAL AND METHODS

2.1 Experimental sites

Experiments were carried out in three commercial citrus orchards in Valencia (Spain) with previous CRS problems. Technicians in charge of the plots have not observed resistance problems in past years, and recognized that organophosphate insecticides still maintained the pest under acceptable levels. Characteristics of each plot are shown in Table 1.

It was observed that orange plots planted with the same cultivar had very different tree configurations, possibly due to the different pruning systems. Trees in plot B were much smaller and had almost uniform leaf density in the whole canopy. Trees in plot C were higher with most of the foliar mass on the external part of the canopy, with uneven vegetation and a lack of leaves in the interior being observed.

2.2 Description of the treatments and the sprayer

The experiments were conducted to study the effect of the spray application volume of two organophosphate insecticides on (1) coverage and (2) efficacy against CRS under field conditions. The chlorpyrifos-based product (CBP) was Dursban® 75 WG (a.i.: chlorpyrifos 750 g kg^{-1} WG) (Dow AgroSciences Ibérica, Madrid, Spain) and the chlorpyrifos-methyl-based product (CMBP) was Reldan® E (a.i.: chlorpyrifos-methyl 224 g l^{-1} EC) (Dow AgroSciences Ibérica, Madrid, Spain).

Both organophosphate pesticides, applied in the range of registered label concentrations at two application volumes, were compared with an untreated control, hereafter known as Control, and a positive control, called Standard. CBP treatments were applied at 1-1.25 g l^{-1} and CMBP at 3-4 ml l^{-1}. Organophosphate treatments were applied in the same manner at first and second generations of CRS. The Standard treatment involved the application of a mix of CBP (1 g l^{-1}) and a pyriproxyfen-based product (PBP) (0.75 ml l^{-1}) (Atominal® 10 EC, a.i.: 100 g l^{-1} EC. Sumimoto Chemical Co. Ltd., Tokyo, Japan) in the first generation of CRS, and a mix of CBP (1 g l^{-1}) and a petroleum-derived spray oil (PDSO) (10 ml l^{-1}) (Laincoil®, nC21 oil content: 8300 g m^{-3}; unsulfonated residue: 92%; density at 20 ºC: 820-860 kg m^{-3}; viscosity at 40 ºC: 1.438E-05 m^2 s^{-1}. Lainco, S.A., Barcelona, Spain) in the second generation. All of these treatments were applied at two spray application volumes. The heaviest application was deduced from a series of interviews with farmers and technicians. For this pest they use to apply around 5000 l ha^{-1}. The lowest was a 40% reduction, resulting in 3000 l ha^{-1}. In none of our experiments we observed runoff.

In plot A, both CBP and CMBP treatments were performed. In plot B, only the CBP treatments were tested, and in plot C, only the CMBP treatments were tested. Control and Standard treatments were applied in all three plots.

Organophosphate applications were always replicated ten times, and the others (Control and Standard) were replicated five times. Each replication consisted in the spraying of three adjoining trees in the same row.

Treatments were applied by means of a handgun hydraulic sprayer (mod. ML-65, Mañez y Lozano S.L., Alginet, Valencia, Spain) fitted with ceramic nozzles, operating at 3 Mpa in the manifold and with an opening angle of 28-30º.

The two application volumes, 3000 and 5000 $l\ ha^{-1}$, were applied with nozzles of different diameters, 1.5 mm and 1.8 mm, respectively, maintaining constant the pressure and the opening angle to change the spray time per tree as little as possible on each plot, regardless of the application volume. These nozzles were selected because in previous laboratory assay they show similar impact size spectrum collected on WSP. For 1.5 mm nozzle the number median diameter ($D_{n0.5}$) was 347 µm, the 10th percentile of the number-based diameter ($D_{n0.1}$) was 170 µm and the 90th percentile ($D_{n0.9}$) was 867 µm. For 1.8 mm nozzle the $D_{n0.5}$ was 353 µm, the $D_{n0.1}$ was 170 µm and the $D_{n0.9}$ was 847 µm.

Due to differences in tree spacing across plots, applied volume per tree was adjusted to obtain the desired application volume with each nozzle (Table 2). Consequently, the same application volume, expressed as a volume per ground unit area, involved different spray volumes applied per tree. Furthermore, the same volume also implied different spray volumes per canopy volume unit because the mean sizes of the canopies in each plot were different.

2.3 Application time

CRS may have 2-4 generations per year in Spanish citrus conditions.[40] Treatments against this pest are usually applied on the first and second generations. It is widely known that application time affects the efficacy of treatments because the sensitivity to pesticides is not the same for all stages, with the early stages (N1 and N2) being the most sensitive.[39, 41] All treatments were applied when the percentage of sensitive stages reached its maximum in each generation. Weekly samplings of leaves and twigs were performed, beginning at the end of March, to calculate the percentage of individuals in each stage. Counting and determination of the developmental stage was made by skilled operators. In the first generation, the maximum of sensitive stages in the population was assumed to coincide with the moment when they represented 60-70% of the total sample. In the second generation, we determined that the maximum of sensitive stages was reached when they represented 50-60% of the sample because the distribution of stages in the population is naturally less homogeneous in this generation.[4]

2.4 Spray distribution in the canopy

Spray distribution in the canopy was evaluated by estimating the coverage obtained after the applications. For this purpose, in three trees on each plot 30 pieces of 7.6 x 2.6 cm water sensitive paper (WSP) (TeeJet, Spraying Systems Co.) were distributed in the 15 zones of the canopy, which was arbitrarily divided. These zones resulted from dividing the canopy into three heights (Figure 1A) and five locations (Figure 1B). Locations 1-4 were located 50 cm from the edge of the canopy, whereas location 5 was in the centre of the tree. WSP were stapled to two randomly selected leaves.

We sprayed tap water without pesticides during these experiments. Once the trees were sprayed and the WSP dried, the pieces were collected and stored in dry conditions. In the laboratory, collectors were photographed, and these images were analysed with specific software (Matrox Inspector, version 2.2, Matrox, Dorval, Canada) following the methodology described by Chueca et al.[42] The images were taken with 20 pixels mm^{-1} resolution. Objects in the image constituted by one single pixel were considered to be noise and therefore removed. Therefore, impacts of less than 50 µm diameter were not detected. In each image, the programme detects all the impacts (deposited droplets produced by the spray over the collector) larger than 2.5E-03 mm^2 and later calculates the coverage, which is the percentage of the total surface covered by the impacts.

Once the estimated coverage of each WSP was obtained, mean coverage of locations 1-4 was calculated for each height, thereby obtaining the value of the coverage at 50 cm depth. To study the effect of the spray application volume on the coverage at the different levels of height and depth, a one-way Analysis of Variance (ANOVA) was performed at each combination height and depth.

Because we determined that each WSP sampling zone in which the tree had been divided represented a certain percentage of the total volume of the canopy, to estimate the overall coverage in the trees with each application volume, we calculated what we call the Weighted Coverage (WC). We considered that each of the heights in the central part of the canopy represented 5% of the total volume, and those volumes corresponding to sampling locations at 50 cm depth accounted for 25% of the total volume in the upper and lower positions and the 35% at the Middle height.

Following this reasoning, the Weighted Coverage can be expressed by Equation 1:

$$WC\,(\%) = 0.05\,(\%Cov_{TC}+\%Cov_{MC}+\%Cov_{BC}) + 0.25\,(\%Cov_{T50}+\%Cov_{B50}) + 0.35\%Cov_{M50}, \quad \text{Equation 1}$$

where

$\%Cov_X$= Mean Coverage (%) of the zone X of the canopy

T/M/B= Height: Top / Middle / Bottom

C/50= Depth: Centre of the tree / 50 cm from the edge of the canopy

Finally, the significance of the spray application volume on the Weighted Coverage was studied by means of a one-way ANOVA.

2.5 Study of the biological efficacy of treatments

Efficacy of each treatment was evaluated by estimating the level of infestation prior to harvest using what we called the Infestation Index (II). This parameter was obtained by counting the scales present in 20 random fruits around the canopy of each tree. These fruits were taken from the central tree of each replicate using the outer trees as a guard between treatments.

The infestation of each fruit was evaluated according to a growing scale of seven levels of infestation intensity: 0 (without scales over the fruit), 1 (from 1 to 10 scales), 2 (from 11 to 20 scales), 3 (from 21 to 30 scales), 4 (from 31 to 40 scales), 5 (from 41 to 50 scales), 6 (from 51 to 100 scales) and 7 (more than 100 scales). Finally, the II of each tree was calculated taking into account the infestation intensity of each fruit and the frequency of each level (number of fruits in each level of infestation intensity), using the Townsend-Heuberger formula (Equation 2).[43]

$$Infestation\ Index\ (\%) = 100 \times \frac{\sum_{i=0}^{7} n_i \times v_i}{N \times V}, \qquad \text{Equation 2}$$

where

n_i= number of fruits in the i level of infestation intensity

v_i= i level of infestation intensity

N= total number of fruits evaluated in a tree (20)

V= maximum level of infestation (7)

The study of the biological efficacy of the treatment in each plot was performed using the following steps:

- Testing if the II of all the treatments was significantly different from the Control
- Testing if the organophosphate treatments differed from the Standard
- Studying if the spray application volume affected the II
- Determining which parameters affected II and if they were different for each treatment.

To test if the treatments were effective, we studied the II of all treatments, including the Standard, to determine if it significantly differed from that of the Control in each plot with the Dunnett's test.[44] A significant difference would indicate that they could be considered effective. Similarly, II of CBP and CMBP treatments were compared with II of the Standard to determine if they differed significantly. Again, the Dunnett's test was used.

Next, we studied the effect of the spray application volume on II. For this purpose, we performed three separate ANOVA tests of II using the spray application volume as a factor: one for the CBP treatment data, one for the CMBP treatment data and one for the Standard treatment data. Fisher's LSD test[45] was used for mean comparisons.

In all of these studies, the assumption of normal distribution of data was assessed using the Shapiro-Wilk test[46] and the assumption of homoscedasticity using the Levene's test[47].

In the next step, we tried to discover the parameters that had more influence on the Infestation Index. We used Multiple Linear Regression (MLR) to study the relationship between the II of the treatments (dependent variable) and the Infestation Index of the Control ($II_{CONTROL}$), the Weighted Coverage (%), the spray application volume ($l\ ha^{-1}$) and/or the water volume applied per volume unit of canopy ($l\ m^{-3}$) as independent variables.

In order to test if the relationship between the Infestation Index and the different parameters was affected by the products we included indicator variables in the regression model. An indicator variable is one that takes the values 0 or 1 to indicate the absence or presence of a categorical effect that may be expected to shift the outcome. When an indicator variable has n categories, only (n − 1) indicator variables are introduced in order to avoid multicolinearity. The category for which the indicator is not assigned is known as the base group.[48-49] In our case, it was the CBP treatment. We used I_{CMBP}, which took the value 1 for the experimental data corresponding to CMBP treatments and 0 otherwise. If I_{CMBP} or an interaction with I_{CMBP} is statistically significant, the relationship between the dependent variable and the independent variables for CMBP treatments is different from the rest of treatments. Similarly, we used I_{STAND} for assessing the influence of the Standard treatments, taking the value 1 for the experimental data corresponding to Standard treatments and 0 otherwise.

MLR analysis followed an iterative process in which all the experimental data were included. It started by including the independent variables, the two indicator variables and their interactions in the model. Then the variable with higher, non-significant p-value ($\alpha > 0.05$) was eliminated and the model recalculated until all variables present in the model had significant coefficients.

In all fitted models, all the assumptions of linear regression were checked. No outliers were identified.

2.6 Proposing a parameter to evaluate the quality of the treatments

This section is aimed at proposing a method for assessing the quality of the coverage by determining if the observed coverage in the experiments would be adequate to achieve a certain level of control of CRS. For this purpose, we defined a new parameter, called theoretical efficiency of deposition, based on the minimum deposit needed to achieve the maximum level of control of CRS in laboratory and evaluated if it had a statistical significant relationships with the Infestation Index.

2.6.1 Definition of the theoretical efficiency of deposition

In Garcerá et al.[39] the amount of deposited solution per unit surface of CBP and CMBP was related with coverage and mortality at different development stages of CRS. In these experiments, PVC collectors were used as artificial targets to measure coverage because their drop retention behaviour is similar to that of citrus leaves.[50]

In this work, we defined the theoretical efficiency of deposition (TE, %) in one of the arbitrary zones in which we divided the canopy, as the percentage of collectors that reached the minimum deposit that achieved the maximum efficacy in the above-mentioned work.

It is important to remark that the proportion of individuals in a particular development stage varies during the season. In the first treatments, targeted to the first generation of CRS, younger states predominate. The minimum deposit to control the pest with both organophosphate products in this stage in laboratory trials was 1.01 µl cm^{-2}, which entailed 11% coverage on PVC for CBP and 22% for CMBP. These data were used to calculate TE in the first treatments (TE_1).

However, because there is not a clear predominance of young individuals and proportion of adult stages is higher in the second treatments, we used data concerning the efficacy on adults. Following our results, we concluded that the minimum necessary deposit increased to 3.41 µl cm^{-2} in treatments with CBP, which entailed 40% coverage on PVC, and a deposit of 4.72 µl cm^{-2} and 60% coverage in treatments with CMBP. These data were used to calculate TE in the second treatments (TE_2).

The methodology used to calculate TE in each plot is as follows. First, we estimated the coverage that would have been obtained on PVC from the data observed on WSP because laboratory experiments were performed on PVC. We applied Equation 3 (Garcerá et al., unpublished).

$$Coverage\ PVC\ (\%) = \frac{\ln\left(1 - \frac{\%\ Coverage\ WSP}{102}\right)}{-0.032} \qquad \text{Equation 3}$$

Next, taking into account all the collectors located in each zone of the canopy of all tested trees in a plot, we calculated the percentage of those collectors that reached the stated minimum coverage for each treatment (TE_1 and TE_2). Finally, a weighted TE for each generation (WTE_1 and WTE_2) and each product was calculated for the whole canopy by applying Equation 4.

$$WTE\ (\%) = 0.05\ (\%TE_{TC}+\%TE_{MC}+\%TE_{BC}) + 0.25\ (\%TE_{T50}+\%TE_{B50}) + 0.35\%TE_{M50}\ , \qquad \text{Equation 4}$$

where

$\%TE_X$= Theoretical Efficiency (%) of the zone X of the canopy

T/M/B= Height: Top / Middle / Bottom

C/50= Depth: Centre of the tree / 50 cm from the edge of the canopy

2.6.2 Testing the relationship between observed efficacy and theoretical efficiency of deposition

Multiple Linear Regression was employed to study relationships between the observed efficacy in the field, as represented by II, and the theoretical efficiency of the deposition, as represented by WTE_1 and WTE_2. For this purpose, we generated models in which II of the treatments in each plot were the independent variable, and the respective II of the Control, WTE_1, and WTE_2 were the dependent variables. We included II of the Control because we knew that it had an influence on efficacy.

Standard treatments were not included in the study since there is no information available about their TEs. Again, in all fitted models, the assumptions of linear regression were checked. No outliers were identified.

All statistical analysis performed in this work was considered at 95% confidence level and were carried out with Statgraphics® Plus version 5.1 (StatPoint Technologies Inc., Warrenton, Virginia, USA).

3 RESULTS

3.1 Analysis of spray distribution in the canopy

In plot A, despite a positive relationship between the spray application volume and coverage (Figure 2), only significant differences were found in the Middle height than can be attributed to applied volume (Table 3). .

In plot B, a positive relationship spray application volume-coverage was also observed except in the Middle height. However, differences of coverage due to application volume were only significant in the Top height and at 50 cm in depth. Figure 2 shows that the canopy reached the lowest coverage at the Top when applying 3000 l ha^{-1}. Coverage of the Middle height was already near saturation with the lowest volume. It is worth noting that coverage was close to 90-100% in all cases when applying 5000 l ha^{-1} of water, so we can assume that coverage by the formulations will be very high.

A positive relationship between spray application volume and coverage was also observed in plot C (Figure 2), but differences in coverage were only significant only at 50 cm in depth, both in the Middle and the Bottom of the canopy, and in the centre at Bottom height (Table 3). At the Bottom of the trees coverage increased almost to saturation.

Based on these results, plot A obtained a Weighted Coverage of 51.46 ± 7.95% when applying 3000 l ha^{-1} and 74.78 ± 7.94% when applying 5000 l ha^{-1} (mean ± SE), although this difference was not significant (F = 4.08; df: 1, 18; P = 0.0594). The result was also observed in plot B, which yielded a Weighted Coverage (mean ± SE) of 69.32 ± 5.55% for 3000 l ha^{-1} and 87.80 ± 4.76% for 5000 l ha^{-1}, which was not significantly different either (F = 6.39; df: 1, 5; P = 0.0648). In contrast, in plot C, Weighted Coverage achieved with each spray application volume differed significantly with values of 56.96 ± 6.06% for 3000 l ha^{-1} and 87.26 ± 0.32% for 5000 l ha^{-1} (F = 24.92; df: 1, 5; P = 0.0075). This can be due to the fact that plot C had double the apparent canopy volume of the other plots. Because the canopies were bigger, the surface to be wetted can be assumed to be also bigger and the differences of coverage can be higher than in the other plots.

3.2 Biological efficacy of the treatments

3.2.1 Efficacy of organophosphate treatments versus the Control and the Standard

The Infestation Index (mean ± SE) of Control was 58.86 ± 7.16% in plot A, 46.57 ± 6.77% in plot B and 37.57 ± 1.33% in plot C. Dunnett's test showed that the Standard treatments significantly decreased the II compared to the Control in all plots. In the treatments with CBP, the two spray application volumes tested significantly reduced the II compared to the Control, except those applied at 3000 l ha^{-1} in plot B. CMBP treatments IIs were not significantly different from that of the Control, except treatment at 5000 l ha^{-1} in plot C (Table 4). However, it is noteworthy that in the Control there appeared a greater number of fruits with high infestation levels (level 6-7) and treated trees had more fruits without scales or with an infestation of up to 10 scales per fruit (level 0-1) (data not shown).

When comparing organophosphate treatments against Standard ones, Dunnett's test found significant differences only in plot A. Moreover, IIs of organophosphate treatments in this plot were higher than those achieved in the other plots (Table 4).

3.2.2 Influence of the Spray application volume

There were no significant differences between the IIs of the organophosphate treatments applied at 3000 and 5000 l ha^{-1} (Figure 3). The only significant differences were found in the Standard treatment in plot A, where treatment at 3000 l ha^{-1} reached a significantly higher infestation than at 5000 l ha^{-1}. It is worth noting that the Infestation Index observed with both application volumes resulted in economically acceptable control of CRS, since after fruit conditioning in the packinghouse, technicians reported that there were not significant differences in the amount of downgraded fruits with respect to those coming from other orchards of the same exploitation.

3.2.3 Factors that influence the Infestation Index

Table 5 shows the results of the regression analysis after eliminating variables with regression coefficients that were not significant. MLR analysis shows that a significant linear relationship (P_{MODEL}<0.0001; R-squared adjusted = 0.568; number of data points = 119) was found between IIs of treatments, II of the Control and the water volume applied per volume unit of canopy. It is important to note that neither the Weighted Coverage nor the spray application volume made a significant contribution to II in the MLR model (P_{WC}= 0.8876; $P_{SprayAppVol}$= 0.7205). Although the value of R-square is not high, this study was not aimed at deducting a predictive model for the Infestation Index but to find relationships between it and the other independent variables. Since P_{MODEL} is lower than 0.0001, we can conclude that we have found significant linear relationships between the Infestation Indexes of the treatments, the IIs of the Controls and the water volume applied per volume unit of canopy.

The significance and sign of the regression coefficients that appear in Table 5 indicate that the higher the $II_{CONTROL}$ in a plot, the higher the II of the treatments. Moreover, these findings also indicate that an increase in water volume applied per volume unit of canopy significantly decreased II. This suggests that this variable has more influence on II values than simply the spray application volumes. We have to consider that though the water volume applied per volume unit of canopy is calculated from the spray application volume, it also includes information about the tree density and average size of trees.

The analysis of the regression coefficients also notes that the IIs of Standard treatments depended less on the $II_{CONTROL}$ than the IIs of organophosphate treatments. By comparing CBP, CMBP and Standard treatments, it is noted that in CMBP treatments the influence of the volume on the II is lower than in CBP and Standard treatments, which may be because the product is less effective.

3.3 Proposing a parameter to evaluate the quality of the treatments

3.3.1 Assessment of the theoretical efficiency of deposition (TE)

3.3.1.1 CBP treatments

Applications in plot A at 3000 l ha^{-1} almost doubled TE in the outer canopy with respect to TE in the internal part of the tree (Figure 4). However, these differences disappeared in the treatments at 5000 l ha^{-1}. The lowest effect of the spray volume is observed in the Top of the canopy. The spray volume of 3000 l ha^{-1} stands out because the low values of TE in the Middle height of the centre of the canopy.

In plot B, all of the TE values were generally higher than in plot A. It is remarkable that in the Middle height both spray volumes reached 100% of TE and instead, when applying 3000 l ha^{-1} in the Top height and in the centre, only 33% of TE was obtained.

WTE increased in all cases by increasing the spray application volume (Table 6). The increase was between 40 and 50% in plot A in the treatments of first and second generation. In plot B, the values were in all cases higher, reaching 100% with 5000 l ha^{-1}.

3.3.1.2 CMBP treatments

In plot A, either inside or outside, at the Middle height the increase of spray volume increased TE, while in the Top part it had virtually no effect (Figure 5).

In plot C, a value of TE of almost 50% was reached in all zones when spraying 3000 l ha^{-1}. TE value increased to 100% by applying 5000 l ha^{-1}, except at the Top, where there was no increase.

As in CBP treatments, WTE increased in all cases by increasing the volume (Table 6). However, values were lower with CMBP, and the increase caused by the spray volume increase was not as high as with CBP.

3.3.2 Testing the relationship between field efficacy and theoretical efficiency of deposition

Table 7 shows the results of the regression after removing the variables whose regression coefficients were not significant. The significance and sign of the regression coefficients that appear in this table indicate again that the higher the $II_{CONTROL}$ in a plot, the higher the II of the treatments (P_{MODEL}<0.0001; R-squared adjusted = 0.35; number of data points = 79). Moreover, the regression coefficient of WTE_2 was also significant, but the coefficient of WTE_1 (P= 0.6601) was not.

4 DISCUSSION AND CONCLUSIONS

In general, we observed that the increase in the spray application volume increased mean coverage and distribution uniformity in the canopy. Differences of coverage were only statistically significant in certain cases and in different locations inside the canopy, which could be caused by differences in foliar density, canopy size and pruning.[38] The lowest values of coverage were always found at the Top of the canopy, showing that this was the most difficult area for the operators to treat. The highest levels of coverage were achieved at the Middle height, especially in plots B and C, probably because these parts of the canopy were easier to reach by the operators. As expected, due to the dense foliage, the inner canopy obtained lower coverage than the outer canopy, but this difference decreased when the highest application volume was applied. Similar results were observed using airblast sprayers.[20-22, 35]

It is important to state that, to not affect the experiment, coverage was measured by spraying only water without adding pesticides. Laboratory trials showed that for the same deposited volume, CBP and CMBP gave higher coverage than water[39]; therefore, it is possible that actual values of coverage produced by the applications were greater than the ones shown. Nevertheless, we believe that our results provide a good approximation of how the pesticides have been distributed in the canopy.

No significant differences in the biological efficacy against CRS were found between the two spray application volumes tested, although they differed by 40%: this finding was not shared by other authors. Differences among studies could be attributed to not only differences of shape, size and canopy density of trees, but also to the different active ingredients used and their different toxicities, modes of action and formulations. This difference should be noted because the results of different works are often compared in the literature without considering how the products actuate against the pest or how they are distributed in the tree. As Stover et al.[38] noted, biological efficacy is not only a consequence of the different sizes of the impacts and uniformity in the deposition.

Many authors found a positive relationship between the spray application volume and efficacy of control of scales. For instance, Cunningham and Harden,[36] who used the organophosphate methidathion that could be less effective than those employed in this work, as it has already been stated.[51] Similarly Beattie et al.[32], and Grout and Stephen[33] found differences in efficacy depending on the spray application volume, but they applied mineral oils that affect insects in a very different way. The routes of entry of organophosphates are through ingestion, absorption or inhalation and cause the inhibition of acetylcholinesterase. Mineral oils cause anoxia to the individuals by covering their respiration organs; therefore, their efficacy may depend more on coverage than on the deposited amount of product, even though both parameters are frequently related.[52- 54]

Conversely, Chueca et al.[35] did not find an influence of the application volume in the control of Coccus pseudomagnoliarum Kuwana, using mineral oils; neither did Grafton-Cardwell and Reagan[28, 29], using spirotetramat against CRS. In our opinion, this finding underlines not only the importance of the route of entry and the mode of action of the pesticide but also the biology of the target pest in all of these studies. Furthermore, this result highlights the difficulty of generalising results obtained with different plant protection products and under specific tree spacing and canopy architectures that produce different deposition and coverage distributions.

Although the increase of the application volume from 3000 l ha^{-1} to 5000 l ha^{-1} produced an increase of coverage, it did not affect the Infestation Index in each plot in our experiments, it is important to note that the volume of pesticide applied per canopy volume unit significantly affected the Infestation Index. This effect was more evident in CBP treatments. This result may indicate that the differences between plots that caused differences in spray volume per canopy volume unit played a more important role for affecting Infestation Indexes than mere application volumes. This result underpins the idea that the influence of vegetation must be taken into consideration when recommending a dose. We propose that this information should be included in the label of the plant protection products.

We have shown that the Infestation Index in the Control trees was very different in each plot, indicating great differences in the level of initial infestation of each plot. We also demonstrated that this variable was strongly related to the efficacy of the treatments in each plot. This fact highlights the importance of the initial infestation level on the control achieved by organophosphate treatments against CRS.

We have proposed a method to assess the quality of the application by means of what we called theoretical efficiency of the treatment based in previous laboratory experiments. Our studies have shown that this parameter calculated in the second generation of CRS had a significant relationship with the final II value, thus proving its potential interest for further modelling of the efficacy of treatments. We are aware that the efficiency of field treatments is not necessarily the same that the one observed in laboratory (more adverse conditions in the field may reduce the survival expectance of the pest and, on the other hand, susceptibility of wild CRS populations to these pesticides could be lower); however our results suggest that some relationship between laboratory and field efficacies could be found.

Finally, this research shows that greater coverage did not result in greater efficacy, raising the possibility that the quantity of organophosphate pesticides used can be reduced through optimizing the spray application volume, based on the volume of the target canopy.

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Table 1. Characteristics of trial plots

Plot	Location: Town	Location: Geografic coordinates	Plant material	Row direction	Area (ha)	Tree spacing (m)	Canopy dimensions (m) (Height x Diameter 1 x Diameter 2)	Apparent canopy volume* (m^3 $tree^{-1}$)
A	El Puig	39º 37’ 7.25” N 0º 38’ 2.54” W	Clementine cv. Clemenvilla	East-West	0.66	5.6 x 5	2.8 x 3.8 x 3.8	17.65
B	Alzira	39º 9’ 7.84” N 0º 30’ 58.76” W	Orange cv. Navel	North-South	0.31	5.6 x 2.7	2.9 x 2.3 x 3.3	11.74
C	Chiva	39º 27’ 19.94” N 0º 25’ 7.94” W	Orange cv. Navel	North-South	0.52	6 x 5	3.2 x 4.4 x 4.3	32.21

* Considering citrus canopy as an ellipsoid

Table 2. Operative characteristics of treatments

Spray application volume (l ha^{-1})	Plot	Applied volume	
		l tree^{-1}	l m^{-3} canopy
3000 (nozzle 1.5)	A	8.4	0.47
	B	4.5	0.38
	C	9	0.28
5000 (nozzle 1.8)	A	14	0.79
	B	7.6	0.65
	C	15	0.46

Table 3. Significance of the spray application volume on coverage for each height and depth in each plot

Plot	Height	Depth	F	df	P
A	Top	50	0.18	1, 60	0.6758
		C	1.09	1, 17	0.3110
	Middle	50	12.87	1, 60	0.0007*
		C	11.83	1, 18	0.0031*
	Bottom	50	2.20	1, 59	0.1430
		C	2.64	1, 18	0.1223
B	Top	50	11.20	1, 19	0.0036*
		C	3.80	1, 5	0.1232
	Middle	50	0.00	1, 22	0.9918
		C	0.19	1, 5	0.6870
	Bottom	50	1.15	1, 23	0.2942
		C	4.20	1, 5	0.1097
C	Top	50	2.85	1, 22	0.1064
		C	0.01	1, 5	0.9090
	Middle	50	9.11	1, 23	0.0063*
		C	3.00	1, 5	0.1583
	Bottom	50	15.48	1, 23	0.0007*
		C	9.39	1, 5	0.0375*

*There are significant differences between spray application volumes (LSD test, $P<0.05$).

Table 4. Infestation Index (%) of each treatment in each plot (mean±SE). Significance of the differences of I.I. of CBP, CMBP and Standard applications with the Control and of CBP and CMBP applications with the Standard ones (Dunnett's test). Significance of spray application volume for each treatment in each plot (ANOVA & LSD test)

Spray application volume	Infestation Index (%)[1]						
	CBP		CMBP		Standard		
	A	B	A	C	A	B	C
3000 l ha^{-1}	40.92±4.35*,** a	30.07±5.06 a	46.93±4.90** a	26.75±4.09 a	18.86±2.54* a	15.86±3.79* a	16.28±1.73* a
5000 l ha^{-1}	39.21±3.62*,** a	21.78±2.37* a	47.57±2.93** a	24.14±3.21* a	10.14±2.37* b	17.14±2.78* a	13.57±3.79* a
F	0.09	2.20	0.01	0.26	6.28	0.07	0.42
df	1, 19	1, 19	1, 19	1, 18	1, 9	1, 9	1, 9
P	0.7652	0.1557	0.9116	0.6192	0.0366	0.7915	0.5331

*There are significant differences with the Control in the same plot (Dunnett's test, P<0.05)
**There are significant differences with the Standard in the same plot (Dunnett's test, P<0.05)
[1]Means within a column followed by a different letter are significantly different (LSD test, P < 0.05)

Table 5. MLR results: regression coefficients for II (%) as a function of $II_{CONTROL}$ (%), applied volume per unit canopy (l m^{-3}) and products (R2 = 0.586)

Parameter	Estimate	Standard Error	P
Constant	-16.557	6.842	0.0171
Volume (l m^{-3})	-16.377	6.875	0.0189
$II_{CONTROL}/10$	1.123	0.154	<0.0001
I_{STAND}[1]	33.999	11.691	0.0056
$(II_{CONTROL}/10)* I_{STAND}$[1]	-0.976	0.228	<0.0001
Volume (l m^{-3})* I_{CMBP}[2]	13.014	4.201	0.0025

[1] I_{STAND}= 1 for data obtained with Standard treatments, 0 otherwise

[2] I_{CMBP}= 1 for data obtained with CMBP treatments, 0 otherwise

Table 6. WTE (%) of treatments with CBP and CMBP against the first generation of CRS (predominance of N1&N2 stages, WTE_1) and the second generation (coexistence of a mix of stages, WTE_2) in plots A, B and C.

Spray application volume (l ha^{-1})	CBP				CMBP			
	A		B		A		C	
	WTE_1	WTE_2	WTE_1	WTE_2	WTE_1	WTE_2	WTE_1	WTE_2
3000	65.00	50.91	95.00	68.33	57.27	36.82	82.50	35.00
5000	91.07	79.11	100.00	100.00	84.20	60.27	97.50	85.00

Table 7. Regression coefficients of the MLR for II (%) as a function of $II_{CONTROL}$ (%) and WTE (R2 = 0.367)

Parameter	Estimate	Standard Error	P
Constant	-6.142	8.722	0.4835
$II_{CONTROL}$	0.995	0.156	<0.0001
WTE_2	-0.146	0.064	0.0258

Figure 1. A) Side view of a standard tree. Distribution of WSP in height. B) Top view of a standard tree. Distribution of WSP at each height.

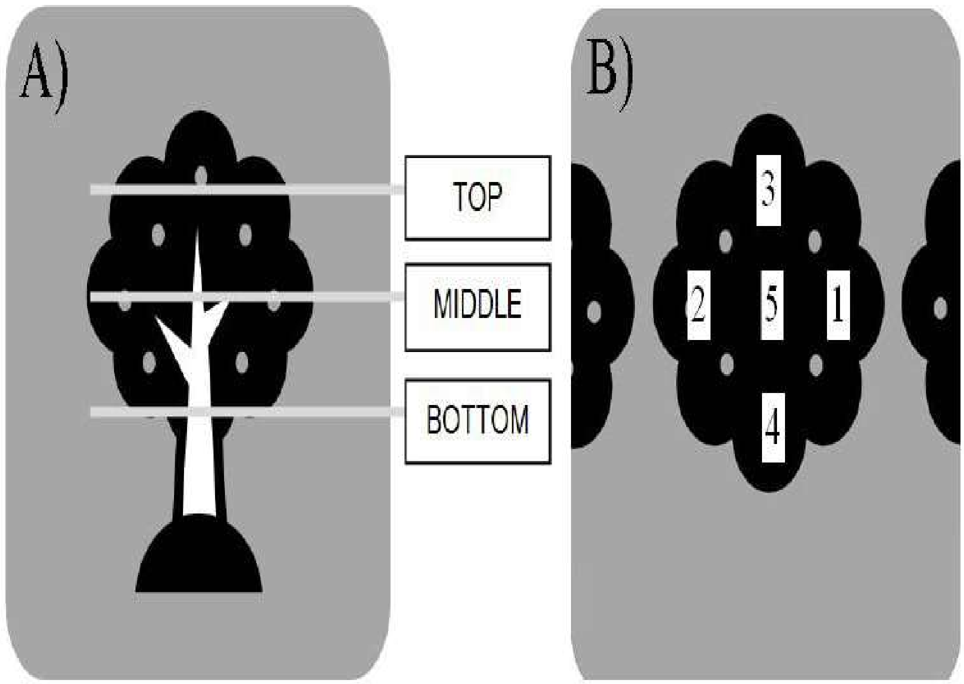

Figure 2. Coverage (%) at each height and depth (mean ± SE) in plots A, B and C. Means within each combination height x depth with different letters are significantly different (LSD test, P<0.05)

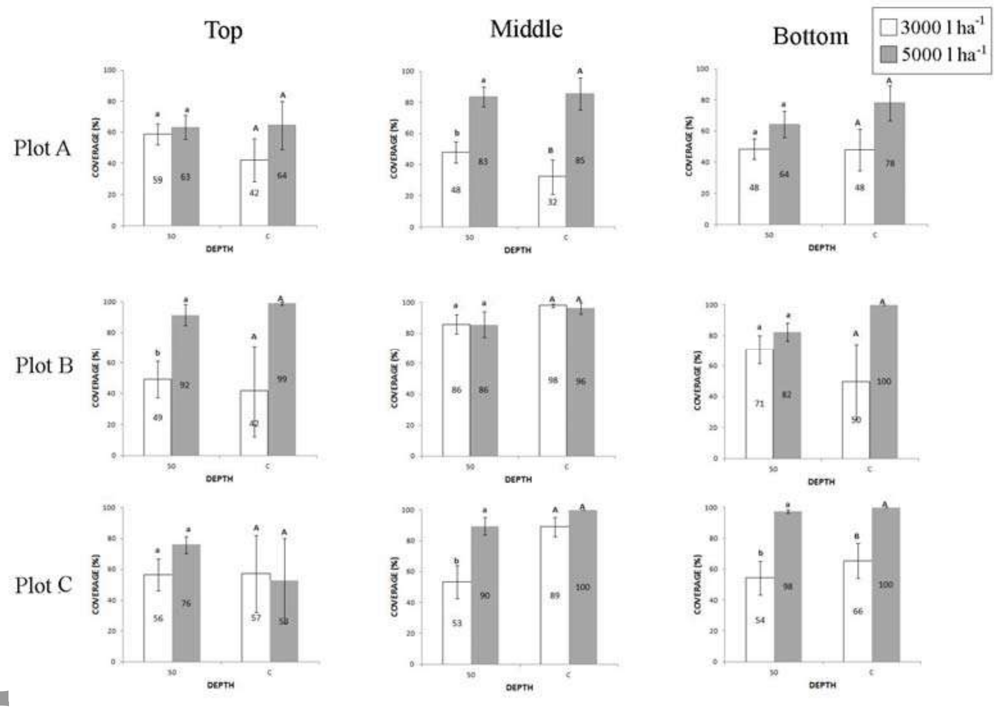

Figure 3. Infestation Index (%) of treatments prior to harvest (mean ± SE), for CBP, CMBP and Standard treatments for plot A, B and C respectively. Means within each combination product x plot with a different letter are significantly different (LSD test, $P<0.05$).

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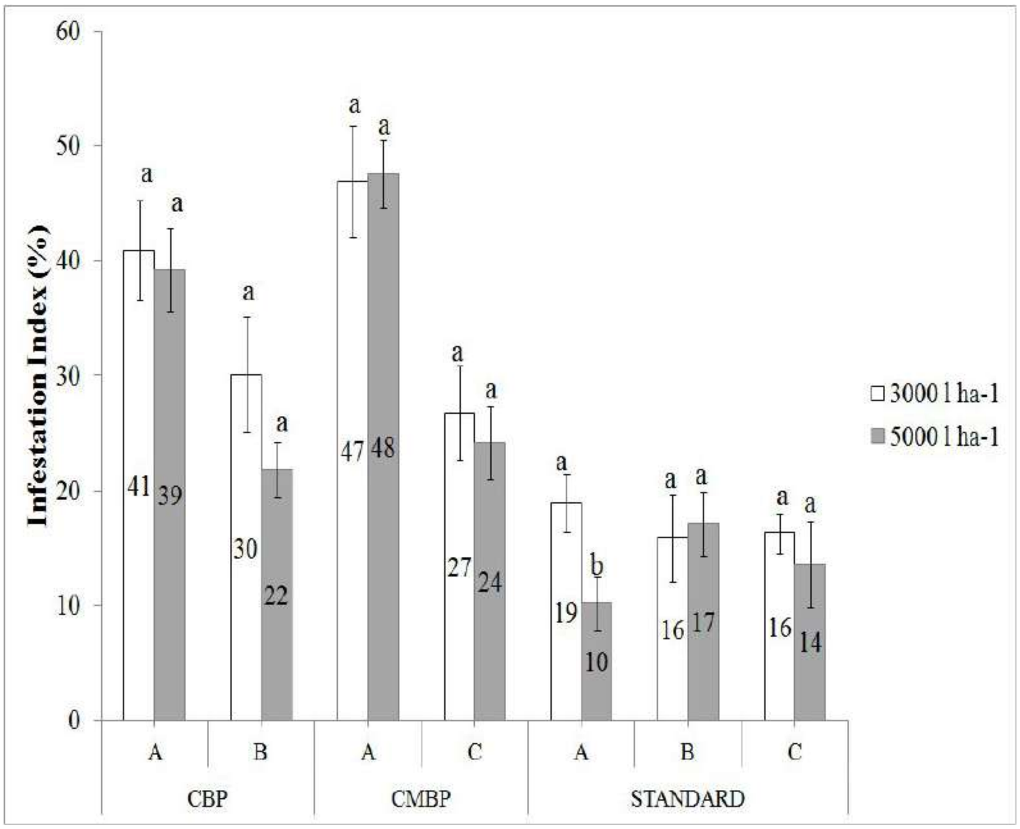

Figure 4. TE (%) of treatments with CBP against the first generation of CRS (predominance of N1/N2 stages, TE_1) and the second generation (coexistence of a stages mix, TE_2) for each height and depth in plots A and B.

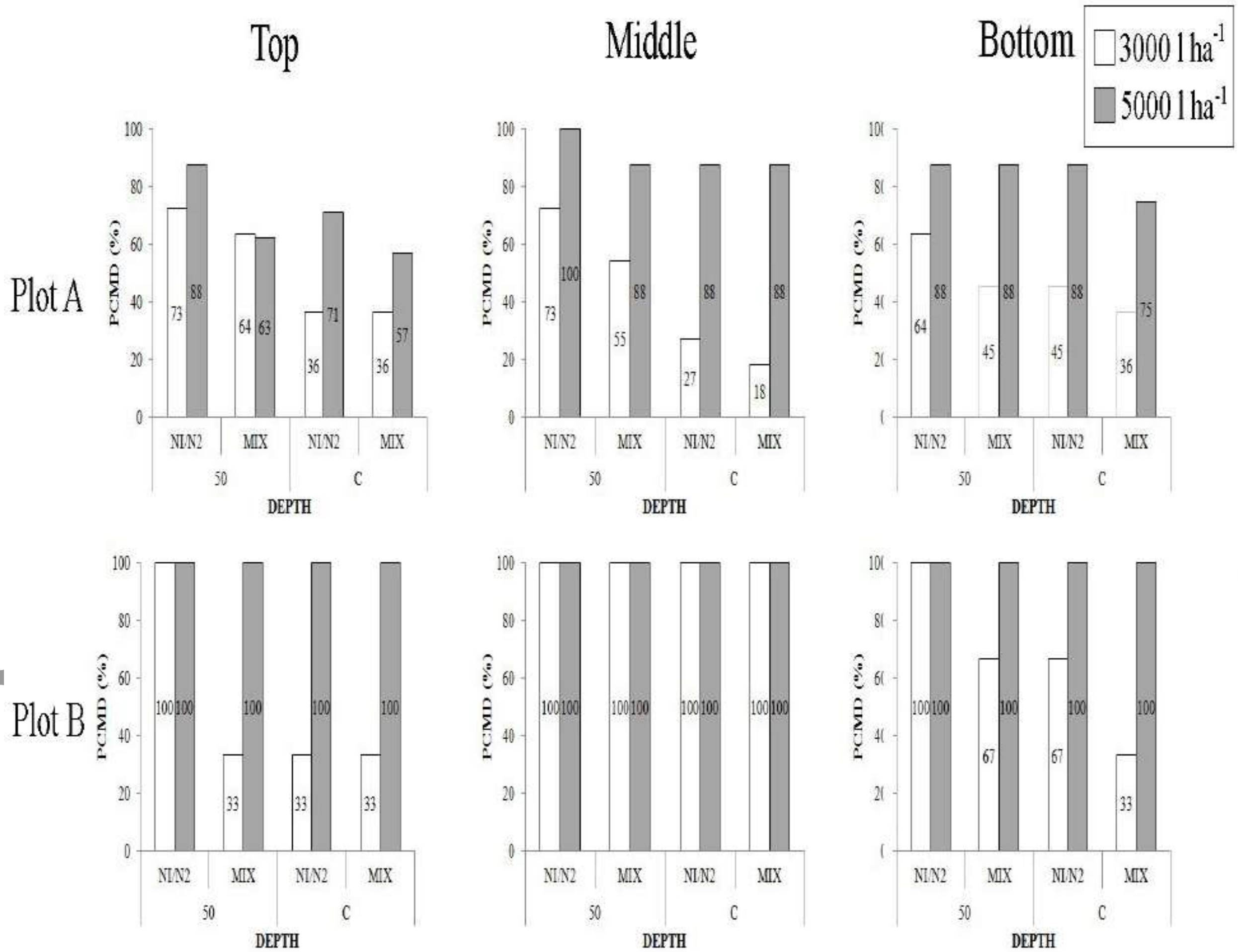

Figure 5. TE (%) of treatments with CMBP against the first generation of CRS (predominance of N1/N2 stages, TE_1) and the second generation (coexistence of a stages mix, TE_2) for each height and depth in plots A and C.