

Article

CitrusVol Validation for the Adjustment of Spray Volume in Treatments against *Tetranychus urticae* in Clementines

Alberto Fonte ¹, Cruz Garcerá ¹ , Alejandro Tena ² and Patricia Chueca ^{1,*}

¹ Centro de Agroingeniería, Instituto Valenciano de Investigaciones Agrarias (IVIA), CV-315, km 10.7, 46113 Moncada, Spain; fonte_alb@gva.es (A.F.); garcera_cru@gva.es (C.G.)

² Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias (IVIA), CV-315, km 10.7, 46113 Moncada, Spain; atena@ivia.es

* Correspondence: chueca_pat@gva.es; Tel.: +34-963-424-000

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Abstract: The optimization of the water volume used to apply pesticides with airblast sprayers is key to reducing water footprint, costs, operational time and drift of pesticides. This study evaluated a new tool (CitrusVol) that adjusts the spray volume to the characteristics of the vegetation and the pesticide used to control the two-spotted spider mite *Tetranychus urticae* in clementine trees. This mite is one of the main citrus pests because it damages fruit before harvest. For this aim, a total of 20 applications against *T. urticae* were evaluated during two consecutive years in seven commercial orchards. In these orchards, we evaluated: (i) the distribution of the spray in tree canopies, (ii) pest density before and after the treatment, and (iii) fruit damage at harvest when conventional volumes and volumes adjusted with CitrusVol were applied. On average, CitrusVol reduced 36% the water volume used to control *T. urticae* in the 20 applications. This reduction in the spray volume involved a decrease in the coverage in some parts of the canopy. However, *T. urticae* density and fruit damage at harvest were similar in trees treated with the adjusted volume calculated with CitrusVol and the volume used by the owners of the orchard. Therefore, the spray volume recommended by CitrusVol is adequate to control *T. urticae* in clementines.

Keywords: two-spotted spider mite; citrus; coverage; airblast sprayer; efficacy

1. Introduction

The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is one of the most important citrus pests in Mediterranean citrus [1,2]. Among the different citrus varieties, clementines (*Citrus clementina* Hort. ex Tan.) are the most heavily affected [3]. *Tetranychus urticae* colonies are preferably located on the underside of the citrus leaves, where they are protected with silk threads in summer. The mite feeds on the content of the epidermal and parenchyma cells of the leaf. This feeding causes chlorotic spots (yellow) and bulging in the upperside [4]. If the infestation is high, the tree can become defoliated. At the end of the summer and fall, the mite migrates from the leaves to the fruits where it can produce scars. This cosmetic damage is considered the main damage produced by *T. urticae* in clementines [3]. Although different biological control strategies have been tested during the last few years [2], acaricides are still widely used to control the mite in the Mediterranean basin [5]. *Tetranychus urticae*, however, is well-known for its ability to develop resistance to acaricides due to its high fertility, short life cycle, reproduction by arrhenotokous parthenogenesis, abundant food resources and high degree of polyphagy [6,7]. In addition, it has been demonstrated that tetranychid mites develop resistance faster than predatory phytoseiids [8], which aggravates the problem, leading to outbreaks of spider mite populations when pest management is not correctly conducted.

In citrus, plant protection products (PPP) are applied with airblast sprayers and high volumes of water to cover the dense vegetation of citrus trees [9]. Higher volumes of water are generally used for pests, such as *T. urticae*, that feed and develop in the underside of the leaves. However, the high volumes used to control these pests are not always supported by field studies as has been demonstrated by Garcerá et al. [10].

On the other hand, even under good agricultural practices, only 45% of the spray volume is deposited in target trees while the rest is deposited in adjacent trees or lost in the ground and the atmosphere as a result of drift, evaporation and/or runoff [11]. Therefore, the use of these high volume rates may increase the risk of run-off and drift. Moreover, overuse can also lead to an increase in pest resistance to PPP, reducing their efficacy [12].

The adjustment of the water volume is a way to make rational use of PPPs and optimize applications. In Spain, as in many other countries, the quantity of PPP to be used as recommended in labels is expressed by the concentration of pesticide. Therefore, a reduction of the spray application volume results in a reduction of applied dose of pesticide per unit area because concentration is constant. Nevertheless, this reduction affects both the amount and the distribution of pesticide in the canopy, and then it is necessary to ensure that an adequate control of the pest is maintained. In this sense, several studies have shown that reducing spray volume does not decrease the efficacy of the application against citrus pests with different biology: citrus rust mite *Phyllocoptura oleivora* (Ashmead) [13,14], citrus mealybug *Planococcus citri* Risso [15], California red scale *Aonidiella aurantii* Maskell [15,16], citricola scale *Coccus pseudomagnoliarum* (Kuwana) [17], Asian citrus psyllid *Diaphorina citri* Kuwayama and citrus leafminer *Phyllocnistis citrella* Stainton [18]. It has also been demonstrated that it is possible to reduce the volume of applications without affecting the control of fungal (citrus black spot caused by *Phyllosticta citricarpa* [19]) and bacterial (citrus canker (*Xanthomonas citri* subsp. *Citri* [20]) diseases.

The Valencian Institute of Agricultural Research (Instituto Valenciano de Investigaciones Agrarias, IVIA) has developed a tool (CitrusVol) to optimize the water volume of the applications with an airblast sprayer [21]. This tool adjusts the volume according to the characteristics of the vegetation (canopy volume, planting pattern, vegetation density and pruning level), the target pest and to the PPP. This tool is freely available online for citrus producers [22]. CitrusVol calculates the minimum volume necessary to deposit on the target organ of the citrus tree (leaf, trunk or fruit) to obtain the maximum efficacy. This minimum deposit was calculated from previous models determined under laboratory conditions [23,24]. The main objective of this work was to validate the CitrusVol tool for the control of the two-spotted spider mite in clementine trees under field conditions. For this aim, the efficacy of water volumes recommended by CitrusVol was compared with the efficacy of volumes used by farmers in seven orchards and during two consecutive years.

2. Materials and Methods

2.1. Orchards and Characterization of Vegetation

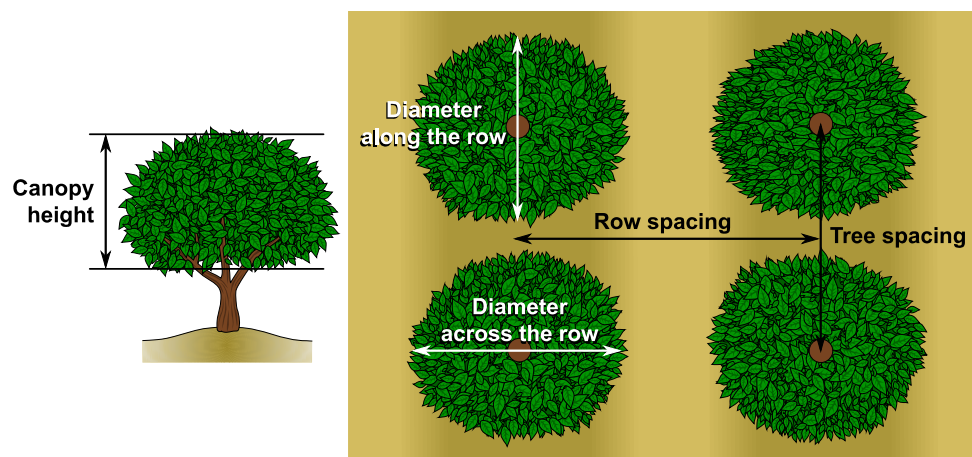
Assays were carried out in seven commercial clementine (*Citrus clementina* Hort. ex Tan.) orchards located in Valencia province (Spain) during two consecutive years (2016 and 2017) (Table 1). Six orchards were clementines of “Clemenules” variety and one was “Oronules” (Plot P2 in Table 1).

Table 1. Characteristics of trial orchards.

Plot	Location		Area (ha)	Planting Pattern ¹ (m)	Vegetation Density ² (m ² /m ³)	Year	Canopy Dimensions ³ (m)	Canopy Volume ⁴ (m ³)	Pruning Level
	Town	Geographic Coordinates							
P1	Chiva	39°26'32" N 0°33'23" W	1.6	6 × 3	13.56	2016 2017	2.51 × 4.33 × 3.08 2.60 × 4.03 × 3.05	17.53 16.73	Normal
P2	Aldaia	39°26'43" N 0°32'18" W	0.8	7 × 2	11.56	2016 2017	2.12 × 4.26 × 2.30 2.04 × 3.70 × 2.19	10.88 8.66	Normal
P3	Sagunto	39°39'14" N 0°18'26" W	1.7	6.5 × 5	10.36	2017	1.93 × 3.10 × 3.16	9.90	Severe
P4	Alfauir	38°56'46" N 0°14'15" W	2	5.5 × 5	14.55	2017	2.19 × 4.17 × 4.40	20.99	Normal
P5	Alfauir	38°56'56" N 0°14'32" W	2.2	6 × 2	13.46	2017	2.08 × 2.85 × 2.10	6.50	Normal
P6	Llíria	39°43'43" N 0°35'28" W	1	6.5 × 2.5	15.09	2017	2.54 × 3.56 × 2.57	12.16	Normal
P7	Llíria	39°43'58" N 0°35'33" W	2.7	6.5 × 3.5	17.94	2017	2.43 × 4.74 × 3.72	22.49	Normal

¹ Row spacing × tree spacing. ² Calculated considering the two sides of the leaves. ³ Canopy height × diameter across the row × diameter along the row. ⁴ Calculated considering citrus canopy as an ellipsoid.

The size of the vegetation of each plot was estimated at the beginning of the season and after pruning. For this, 10 representative trees per plot were randomly selected and the height of the canopy, the diameter of the tree along the row, and the diameter across the row were measured (Figure 1).

**Figure 1.** Required parameters to obtain the adjusted volume in the CitrusVol calculator.

The volume of vegetation was calculated considering the tree canopy as an ellipsoid according to the Equation (1).

$$\text{Canopy volume (m}^3\text{)} = \frac{1}{6}\pi \times \text{canopy height (m)} \times \text{O across the row (m)} \times \text{O along the row (m)}, \quad (1)$$

In addition, vegetation density was estimated in three trees per plot. Given that they were commercial orchards, a whole tree was not defoliated, but different areas were defoliated in different trees. The defoliation zones were 18 and were the result of dividing the canopy into three heights (top, middle and bottom), two depths (inside and outside) and three widths (Figure 2). In each zone a 0.125 m³ cube was defoliated and the total amount of leaves was weighed. From each zone, a random sample of 30 leaves was weighed and digitized with the Food-Color Inspector software [25] to calculate the leaf area. In this way, the weight/leaf area ratio was determined, and the density of vegetation in each canopy zone was calculated. Averaging the value of each zone, the density of vegetation per tree

was obtained. In Plot 3, only two heights were considered due to the small size of the canopies and the severe pruning.

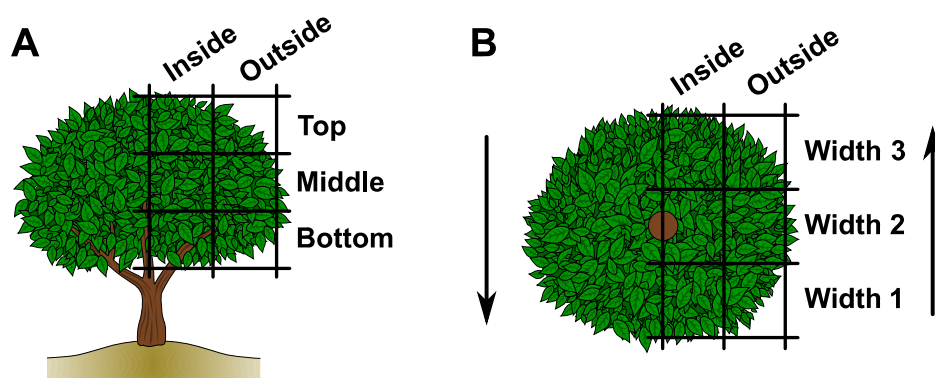


Figure 2. Quadrants of the canopy. (A) Side view and (B) top view of a standard citrus tree. Arrows indicate the forward sense of the sprayer.

2.2. Experimental Design

An experimental design of one factor (the spray volume) was carried out with two levels: (1) conventional volume (V_C), spray volume used by the farmer; and (2) adjusted volume (V_A), spray volume recommended by CitrusVol based on vegetation characteristics.

A total of 20 PPP applications against *T. urticae* were evaluated (five applications in 2016 and 15 in 2017). Each application was considered a replicate.

Each citrus orchard was divided into two blocks of similar size (between 0.4 and 1.35 ha, depending on the plot size). In one block, PPP were applied with the V_C and in the other with the V_A .

The response variables studied were: (1) the distribution of the spray in the tree canopy (percentage of coverage); (2) pest density measured as percentage of symptomatic leaves occupied by *T. urticae* seven, 14 and 21 days after application (DAA); and (3) fruit damage at harvest measured as the percentage of fruits with damage of *T. urticae*. Pest density and fruit damage is explained in detail in Sections 2.4 and 2.5.

2.3. Description of Sprayers and Plant Protection Products (PPP) Applications

To apply the PPP in the different plots, a total of five tractors and five airblast sprayers were used (Table 2). Previous to the PPP applications, the equipment was calibrated.

Table 2. Tractors and sprayers used in the applications.

Plot	Tractor	Airblast Sprayer	Tank Capacity (L)	Number of Nozzles
P1 P2	Landini REX 110 F	Ilemo-Hardi Arrow XF90	1500	38
P3	New Holland TN 95 FA	Mañez y Lozano Twister	2000	30
P4 P5	SAME Frutteto3 100 Classic	Fede Futur	1000	26
P6 ¹	Massey Ferguson 3655 F	Fede Futur	3000	26
	John Deere 5090 GF	Fede Futur	3000	26
	John Deere 5090 GF	Fede Futur	2000	26
P7	Massey Ferguson 3655 F	Fede Futur	3000	26

¹ In Plot 6, three combinations of tractor + sprayer were used in the three PPP applications chronologically: 10 July, 23 August and 14 September.

The operative characteristics in terms of working pressure, forward speed and air flow rate for the different PPP applications in each plot were established based on good agricultural practices [26]. These conditions were the same in each plot and for both treatments (V_A and V_C) except in the application of P7 (30 June 2017) in which a different pressure was used (Table 3). The water volume used in V_A applications was recommended by CitrusVol, selecting the following parameters in the web page [22]:

- Foliar density: Medium.
- Pruning level: Normal, except in P3 where was considered Severe (Table 1).
- Pest/disease: Tetranychid mites.
- Product: the active ingredient used (see Table 3).

In both treatments (V_A and V_C), the same trade and model of nozzles was used in each plot. To reduce the water volume in V_A applications different sizes of the nozzle model and different numbers of nozzles were used (Table 3). Furthermore, the spray cloud was adjusted to the vegetation in V_A . For this purpose, the nozzles were oriented and those that were not necessary were closed in order to reduce drift and product losses to the soil.

In both treatments (V_A and V_C) of each application, the same PPPs were used. The PPPs were selected by the technicians of the orchards. The concentration of the products was the recommended in the label (Table 3).

To determine the optimal moment to apply PPP against *T. urticae*, its density was monitored weekly or fortnightly (depending on the density) between June and September in the seven plots. The sampling protocol of the Spanish Citrus Integrated Pest Management guide [27] was followed. Some of the PPP applications were carried out before the treatment threshold was reached by decision of the technicians.

The meteorological conditions during the PPP applications are shown in the Appendix A (Table A1).

Table 3. Operative characteristics of plant protection products (PPP) applications.

Plot	Application Date	Pressure (bar)		Forward Speed (km/h)	Water Volume (L/ha)		Water Volume Reduction (%)	Air Flow Rate (m ³ /h)	Number of Open Nozzles		PPP Concentration (%)	PPP	Active Ingredient of PPP
		V _C	V _A		V _C	V _A			V _C	V _A			
P1	27 July 2016	8		1.32	4905	3255	33.64	55,342	38	30	0.100 0.040 0.023	Cal-Ex Movento 150 O-Teq Envidor	Abamectin Spirotetramat Spirodiclofen ¹
	11 October 2016	8		1.32	4905	3255	33.64	55,342	38	30	0.100	Cal-Ex	Abamectin ¹
	7 June 2017	8		1.32	4899	3487	28.82	74,894	38	30	0.267 0.100	Dursban 48 Stygma	Chlorpyrifos Abamectin ¹
	24 July 2017	8		1.32	4899	3153	35.64	74,894	38	30	0.100 0.023	Abasi EC Envidor	Abamectin Spirodiclofen ¹
	8 September 2017	8		1.32	4899	3153	35.64	74,894	38	30	0.400 0.100 0.023	Reldan E Dauparex Envidor	Chlorpyrifos-methyl Abamectin Spirodiclofen ¹
P2	22 June 2016	8		1.73	3215	2476	22.99	55,342	38	30	0.100 0.023	Cal-Ex Envidor	Abamectin Spirodiclofen ¹
	9 August 2016	8		1.32	4204	2800	33.40	55,342	38	28	0.100 0.040 0.023	Cal-Ex Movento 150 O-Teq Sento	Abamectin Spirotetramat Clofentazine ¹
	9 September 2016	8		1.73	3215	2476	22.99	55,342	38	30	0.100 0.023	Stygma/Cal-Ex Envidor	Abamectin Spirodiclofen ¹
	22 August 2017	8		1.32	4199	2070	50.70	74,894	38	30	0.100 0.050 0.023	Dauparex Movento 150 O-Teq Envidor	Abamectin Spirotetramat Spirodiclofen ¹
P3	23 June 2017	13		2.01	3395	1462	56.94	46,467	26	18	0.050 0.030	Movento 150 O-Teq Borneo	Spirotetramat Etoazole ¹
	3 August 2017	13		2.01	2950	1502	49.08	46,467	26	18	0.025	Envidor	Spirodiclofen ¹

Table 3. Cont.

Plot	Application Date	Pressure (bar)		Forward Speed (km/h)	Water Volume (L/ha)		Water Volume Reduction (%)	Air Flow Rate (m ³ /h)	Number of Open Nozzles		PPP Concentration (%)	PPP	Active Ingredient of PPP
		V _C	V _A		V _C	V _A			V _C	V _A			
P4	26 June 2017	10		1.96	4262	2998	29.66	69,385	26	18	0.060	Movento Gold	Spirotetramat
											0.035	Comanche Plus	Tebufenpyrad ¹
	18 September 2017	10		1.96	4262	2998	29.66	69,385	26	18	0.020	Envidor	Spirodiclofen ¹
P5	27 June 2017	10		1.96	3674	1811	50.71	69,385	24	16	0.060	Movento Gold	Spirotetramat
											0.035	Comanche Plus	Tebufenpyrad ¹
	19 September 2017	10		1.96	3674	1811	50.71	69,385	24	16	0.020	Envidor	Spirodiclofen ¹
P6	10 July 2017	10		1.24	4275	2567	39.95	82,644	26	22	0.100	Abasi EC	Abamectin ¹
	23 August 2017	11		1.26	3910	2493	36.24	82,644	22	22	0.100	Dauparex	Abamectin
											0.025	Envidor	Spirodiclofen ¹
	14 September 2017	9		1.05	4590	2514	45.23	77,512	24	22	0.100	Dauparex	Abamectin
											0.035	Comanche Plus	Tebufenpyrad ¹
P7	30 June 2017	11	8	1.42	3932	3290	16.33	82,644	26	26	0.067	Comanche Plus	Tebufenpyrad ¹
											0.050	Movento 150 O-Teq	Spirotetramat
	4 August 2017	10		1.43	3701	3246	12.29	82,644	26	26	0.100	Dauparex	Abamectin
											0.023	Envidor	Spirodiclofen ¹

V_C: conventional volume; V_A: adjusted volume; PPP: plant protection product. ¹ Main active ingredient for the application against two-spotted spider mite. It is the one that has been used in CitrusVol to calculate the adjusted volume.

2.4. Distribution of the Spray in the Tree Canopy

To evaluate the distribution of the spray in the tree canopy, the percentage of coverage in different parts of the vegetation (height, depth and side of the leaf) was estimated in one PPP application of each combination of plot and year. This resulted in nine evaluations; however, one more evaluation in P2 was carried out due to the significant change in the decision of V_C by the grower. Therefore, a total of 10 evaluations of the spray distribution were performed.

For this, before spraying, 72 water-sensitive papers (WSP) with a surface area of 76×26 mm (TeeJet, Spraying Systems Co. Wheaton, IL, USA) were placed per tree, in three trees per treatment (V_A and V_C). WSP were placed in the 18 zones in which the canopy was divided (Figure 2). Four WSPs were randomly placed in each zone: two in the upperside and two in the underside of the leaves. The airblast sprayer advanced spraying the trees from Width 1 to 3 on the side that the WSPs were placed, and from Width 3 to 1 on the side that there were no WSPs as shown in Figure 2.

Once the application was completed and the WSPs had dried, they were collected and kept in the proper conditions to transport them to the laboratory. After that, WSPs were digitized with a Canon EOS 700D camera (Canon Inc., Tokyo, Japan) under steady light conditions and were analyzed using a self-developed software based on Food-Color Inspector [25] to obtain the percentage of coverage. Previously the program was trained to assign some colours of the image as drops (blue) and other colours as background (yellow or green), then the program counts the number of pixels assigned as drops. Different training was used depending on the coloration of the WSPs, because when the ambient humidity was high the background of the WSP was green instead of yellow.

Coverage in each canopy zone (combination of height, depth and side of the leaf) was calculated as the average of the three widths of each zone.

The meteorological conditions during the coverage evaluations are shown in the Appendix A (Table A1).

2.5. Evaluation of *Tetranychus urticae* Density and Fruit Damage

2.5.1. *Tetranychus urticae* Density

Tetranychus urticae density was measured before and 7, 14 and 21 days after application (DAA), in both blocks of each plot in order to determine the effect of both volumes in its density levels. *Tetranychus urticae* density was measured as the percentage of occupied symptomatic leaves (yellowish marks). They were considered occupied when two or more adult females of *T. urticae* were presented in the leave. This method was selected because it is used to determine the economic threshold [28]. Forty trees were sampled in each block. In each tree, two symptomatic leaves from the middle zone of the canopy were sampled, one from the outside and another from the inside of the canopy. Trees of the contour of the plot and the rows of separation between treatment blocks were discarded.

2.5.2. Fruit Damage by *Tetranychus urticae*

Before harvest, the percentage of fruits with *T. urticae* damage was determined in each plot and in the two years of the experiment. In total, nine samplings were made (two in 2016 and seven in 2017).

For this, 10 random trees were sampled per treatment block and 40 fruits per tree were observed. In each space orientation (north, south, east and west), 10 fruits were sampled, half of them from the outside of the canopy and the other half from the inside. Three levels of damage were considered in each fruit: (1) without damage; (2) light damage (Figure 3A–C) or localized damage, mainly in the area of the apex or peduncle (sometimes at the point in contact with another fruit), this damage does not mean the fruit cannot be commercialised; and (3) severe damage (Figure 3D–F), that implies cull fruit, and can range from extensive damage in the apex and peduncle areas to damage to the entire clementine.

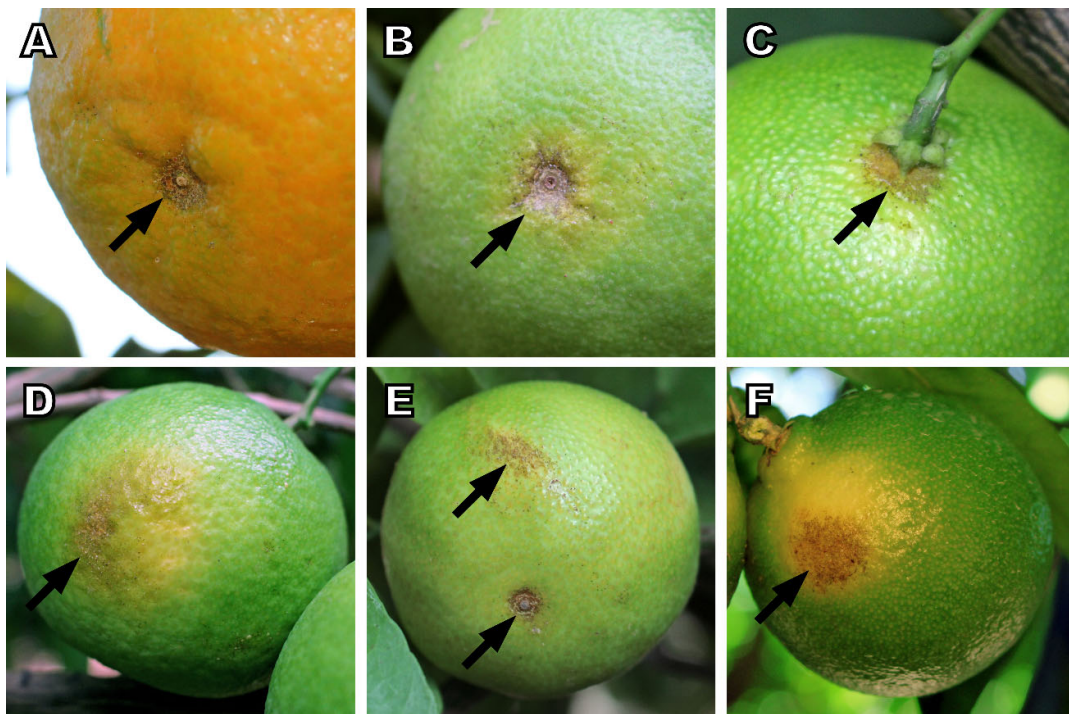


Figure 3. Fruits with damage caused by *Tetranychus urticae* (indicated by arrows). (A–C) Light damage and (D–F) severe damage.

2.6. Data Analysis

All statistical analyses were performed with Statgraphics Centurion XVI (Manugistics Inc., Rockville, MD, USA) and Microsoft Office Excel 2016 (Microsoft Corporation, Redmond, WA, USA).

As general considerations, in all multivariate analysis of variance (MANOVA) and one-way analysis of variance (ANOVA), it was verified that the assumptions of homoscedasticity (through the Levene test) and normality (normal probability plot of the residuals) were fulfilled.

When significant differences were found, the least significant difference (LSD) test was applied for the separation of the means. The level of confidence that was considered in all the analyses was 95%.

2.6.1. Percentage of Coverage

To study the effect of spray volume two analysis were carried out, one considering the whole three canopy and the other analysing each canopy zone.

For the whole canopy tree, a MANOVA was performed to study the effect of spray volume, height, depth and leaf side on the coverage.

To study the effect of spray volume on coverage for each canopy zone (12 canopy zones combination of three heights, two depths and two sides of the leaf) an ANOVA was carried out for each canopy zone for each evaluation and considering all the evaluations.

2.6.2. Percentage of Symptomatic Leaves Occupied

To determine the effect of the spray volume on the percentage of symptomatic leaves occupied by *T. urticae* for the average of 20 PPP applications, an ANOVA was carried out for each sampling day at both depths of the canopy. It was necessary to transform the data with the square root of the arcsine for the normality hypothesis to be satisfied.

To study the effect of the spray volume on symptomatic leaves occupied of each application date per orchard a chi-square test with $p = 0.05$ was performed per each sampling day at both depths of the canopy.

2.6.3. Percentage of Fruits Damaged

The percentage of fruits damaged by *T. urticae* per canopy depth and level of damage for each orchard and year and considering all the orchards together was compared between treatments by using ANOVA. The arcsine square root transformation was applied to the data.

3. Results

3.1. Percentage of Coverage

In the analysis of the mean percentage of coverage considering the whole tree canopy, coverage was significantly higher for V_C (67.32%) than for V_A (54.56%) ($F = 40.67$; $df = 1, 231$; $p < 0.001$). When height, depth and leaf side were introduced as factors in the analysis, they affected the coverage through the interactions between them but not with the spray volume (Figure 4). The interaction between height and depth was significant ($F = 6.41$; $df = 2, 231$; $p = 0.002$). The outer part of the canopy obtained more coverage than the inner part, but in the outer part the higher coverage was obtained in the middle part followed by the bottom and finally the top part, meanwhile in the inside of the canopy the bottom part received higher coverage than the middle and the top part. The interaction between height and leaf side was also significant ($F = 3.79$; $df = 2, 231$; $p = 0.024$). The upper side of the leaf presented higher coverage than the underside, but in the bottom part of the canopy these differences were much higher than in the top and middle zone. Finally, the interaction between depth and leaf side was also significant ($F = 11.12$; $df = 1, 231$; $p = 0.001$). The differences in coverage between the upper side and underside of the leaves were higher inside the canopy than on the outside.

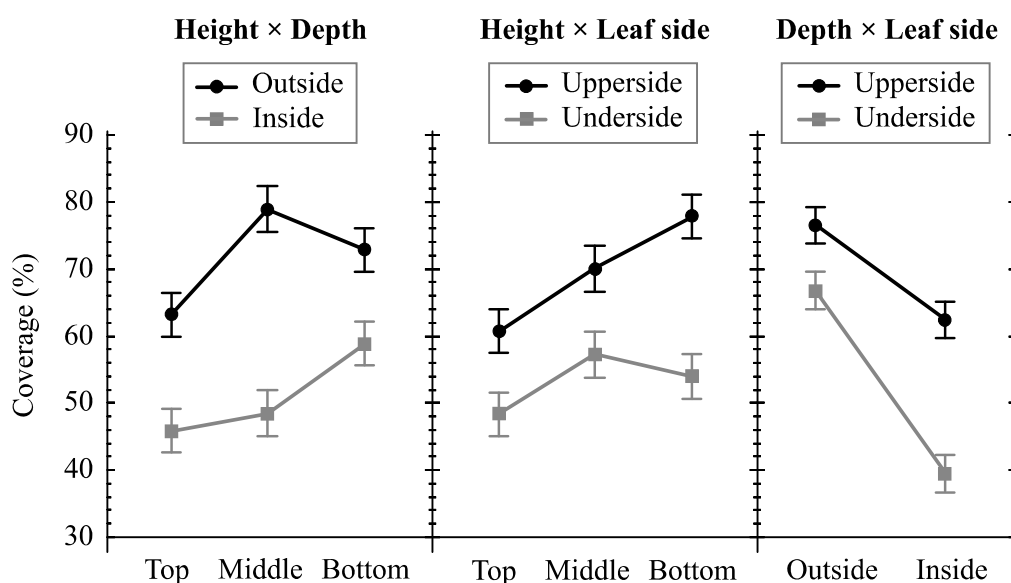


Figure 4. Interactions between height, depth and leaf side. Mean percentage of coverage and 95% Fisher's least significant difference (LSD) intervals.

In the analysis of the mean percentage of coverage in each of the 12 canopy zones studied (combination of three height, two depth and two side of the leaf), considering all the evaluations, it was observed that, in all zones the percentage of coverage was higher with the V_C in comparison with V_A , although the differences were only significant in four zones: Top × Outside × Upperside ($F = 9.03$; $df = 1, 19$; $p = 0.008$), Top × Outside × Underside ($F = 7.67$; $df = 1, 19$; $p = 0.013$), Top × Inside × Upperside ($F = 5.11$; $df = 1, 19$; $p = 0.036$) and Bottom × Inside × Underside ($F = 4.93$; $df = 1, 19$; $p = 0.04$) (Figure 5).

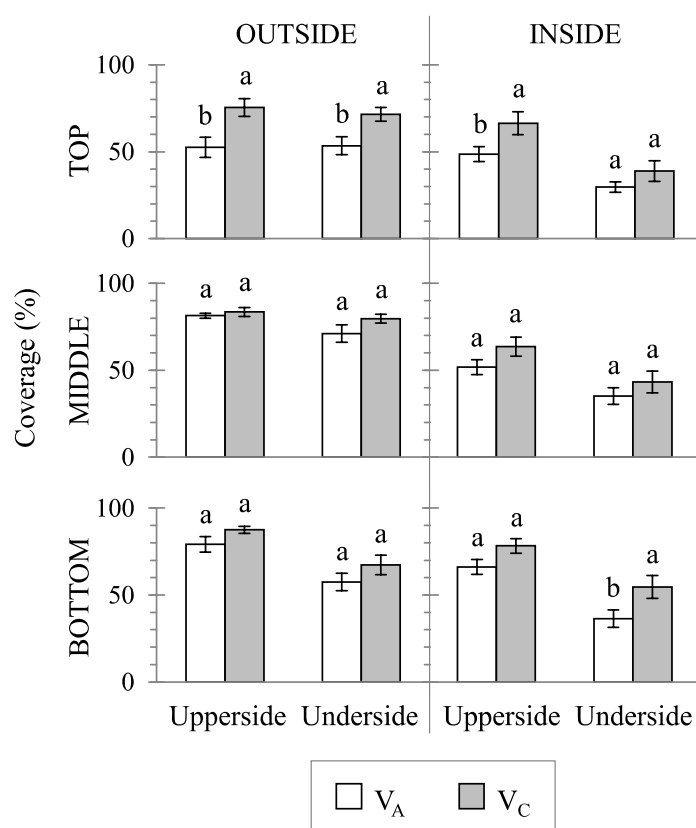


Figure 5. Mean (\pm standard error) coverage (%) per water volume (V_A : adjusted, V_C : conventional) at each height (top, middle, bottom) and depth (inside, outside) of the canopy, and side of the leaf (upperside, underside), of the 10 evaluations. Different letters above the bars within each height \times depth \times side combination indicate significant differences (LSD test, $p < 0.05$).

The results of coverage broken down for each of the 10 evaluations (combination of plot and year) and in the different canopy zones are shown in the Appendix A (Figure A1). It can be observed that coverage higher than 20% were obtained in all canopy zones except in Top \times Inside \times Underside for V_A in P1 (2017) (19.64%), Top \times Inside \times Underside for V_C in P2 (2016-1) (18.69%), Middle \times Inside \times Underside (15.53%) and Bottom \times Inside \times Underside (11.99%) for V_A in P2 (2017), Middle \times Inside \times Underside for both volumes in P5 (2017) (V_A : 17.31%; V_C : 16.93%) and Top \times Inside \times Underside for both volumes in P6 (2017) (V_A : 10.09%; V_C : 17.98%).

3.2. *Tetranychus urticae* Density

Before PPP applications, *Tetranychus urticae* density, measured as percentage of symptomatic leaves occupied by the mite, was similar in trees treated with the conventional and adjusted volumes on the outside of ($F = 0.71$; $df = 1, 39$; $p = 0.406$) and inside the canopy ($F = 0.28$; $df = 1, 39$; $p = 0.602$) (Figure 6).

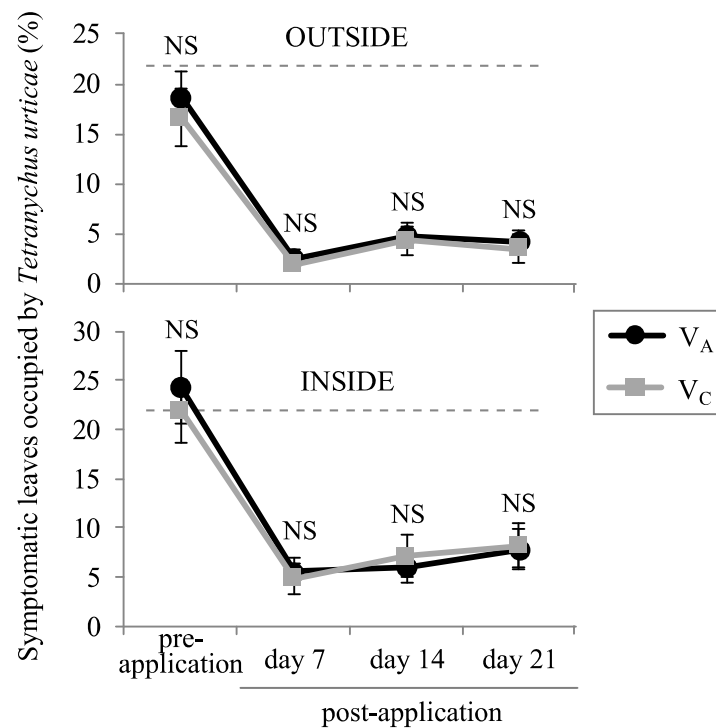


Figure 6. Symptomatic leaves occupied by *Tetranychus urticae* (%; mean \pm standard error) at two depths of the canopy (outside, inside) and per treatment (V_A: adjusted volume; V_C: conventional volume). The dotted line indicates the treatment threshold: 22% of symptomatic leaves occupied. NS stands for not statistically significant (LSD test, $p < 0.05$).

After PPP applications, *T. urticae* density decreased 7 DAA in all the orchards and in both treatments. 14 and 21 DAA, mite density remained constant or increased slightly (Figure 6). *T. urticae* density was similar between treatments on the outside of (7 DAA: $F = 0.38$; $df = 1, 39$; $p = 0.541$; 14 DAA: $F = 0.17$; $df = 1, 39$; $p = 0.683$; 21 DAA: $F = 0.67$; $df = 1, 37$; $p = 0.419$) and inside the canopy (7 DAA: $F = 0.08$; $df = 1, 39$; $p = 0.784$; 14 DAA: $F = 0.01$; $df = 1, 39$; $p = 0.924$; 21 DAA: $F < 0.001$; $df = 1, 37$; $p = 0.999$) throughout the assay. These results are presented by plot and year in the Appendix A (Figure A2) and it can be observed that in the majority of samplings of each application, no significant differences in the percentage of symptomatic leaves occupied by *T. urticae* between both spray volumes were found, and when significant differences were found, in some cases there was more spider mite in V_A and in others cases in V_C.

3.3. Fruit Damage by *Tetranychus urticae*

The mean percentage of fruits with light damage was similar in trees treated with the conventional and adjusted volume in the inside ($F = 0.55$; $df = 1, 17$; $p = 0.469$) and on the outside part of the canopy ($F = 0.1$; $df = 1, 17$; $p = 0.754$) (Figure 7). Similarly, the mean percentage of fruits with severe damage was similar in trees treated with the conventional and adjusted volume in the inside ($F = 0.01$; $df = 1, 17$; $p = 0.916$) and on the outside part of the canopy ($F < 0.001$; $df = 1, 17$; $p = 0.984$). These results are presented by plot and year in the Appendix A (Figure A3).

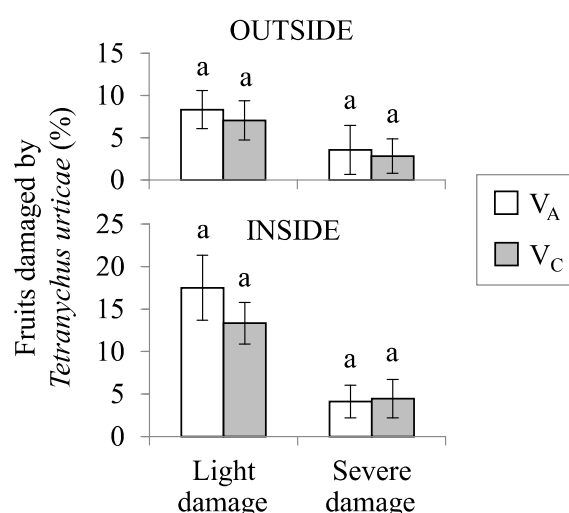


Figure 7. Fruits damaged by *Tetranychus urticae* (%), mean \pm standard error) at two depths of the canopy (outside, inside) and for both treatments (V_A: adjusted volume; V_C: conventional volume). Different letters above the bars indicate significant differences between V_A and V_C (LSD test, $p < 0.05$).

4. Discussion

CitrusVol reduced the spray volume used by citrus producers between 12.30% and 56.96%. On average, the reduction in seven orchards and two consecutive years was 35.71%. CitrusVol calculates the volume according to the amount of vegetation it is required to protect. This reduction in the spray volume involved a decrease in the coverage in some parts of the canopy. However, *T. urticae* density and fruit damage at harvest were similar in trees treated with the adjusted volume calculated with CitrusVol and the volume used by the owners of the orchard. Therefore, CitrusVol reduced the volume without affecting the efficacy of the applications. These results are based on seven orchards sampled during two consecutive years. This is in accordance with other authors who have not found differences in control levels when analysing the effect of the spray application rate on the efficacy of pesticides used in citrus [14,18,29–31]. Moreover, other authors who studied the influence of the application rate both on the efficacy and the distribution and coverage of the spray in the canopy at the same time, found that increasing the spray application rate did not have a significant effect on mean deposition or on the control of pest, but this approach did increase coverage uniformity [13,17].

Regardless of the spray volume used, coverage was higher on the outside of the canopy than on the inside at three heights and in both sides of the leaf. The top part of the canopy had less coverage than the middle and bottom zone. Similarly, the upperside of the leaves had more coverage than the underside. These results agree with those obtained by other authors using axial fan airblast sprayers [32–37] and with handgun hydraulic sprayers [10,37] and also indicate the necessity to improve the equipments to obtain more homogeneous coverage in all the canopy.

In general, PPP applications managed to control *T. urticae* in all the plots throughout the three weeks sampled. 21 DAA the percentage of symptomatic leaves occupied by *T. urticae* still remained below the treatment threshold. As exceptions, the applications in P1 (7 June 2017), in P3 (23 June 2017) and in P6 (10 June 2017) failed to control the spider mite independently of the volume. In these three applications, the active ingredients were abamectin, etoxazole and abamectin, respectively. Abamectin and spiroadiclofen were the most used acaricides in this study. Spider mite populations remained always below the threshold when spiroadiclofen was used. Wu et al. [38] found that the levels of resistance of *T. urticae* to etoxazole and abamectin are higher than to spiroadiclofen in hop crops. In addition, it has been demonstrated that abamectin resistance correlates with the number of applications [39]. In some of plots, abamectin had been commonly used the previous years (pers. observations). This

overuse of abamectine might explain why some treatments were not effective independently of the spray volume applied.

In general, the percentage of symptomatic leaves occupied by *T. urticae* was similar in the outside and inside part of the canopy before acaricides were sprayed. This result matches previous studies in clementines [40]. However, the percentage of fruits damaged by *T. urticae* was higher inside the canopy than outside. The lower coverage in the interior part of the canopy than on the exterior, independent of the volume, might explain this result. Further studies are necessary to reduce the percentage of fruits damaged in the interior part of the canopy.

When the dose expression in labels of PPP is mainly expressed as concentration, that is the case for vertical crops in EPPO (European and Mediterranean Plant Protection Organization) South of Europe zone [41], a reduction of spray volume implies a reduction of PPP. Also, it implies a reduction on the number of tank refills, and therefore a reduction of operational time and fuel consumption [21]. On the other hand, a reduction of spray volume entails a reduction in the losses of PPP to the environment (drift, soil, water) [42].

In conclusion, this work demonstrates that CitrusVol is an adequate tool for the recommendation of spray volumes to control *T. urticae* in clementines. It reduced the spray volume and the amount of PPP without affecting the efficacy of the applications. Consequently, CitrusVol provides a reduction of water footprint, PPP costs and operational time and the environmental impacts of pesticides.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Meteorological conditions during the PPP applications and coverage evaluations. The precipitation was 0 mm in all cases, except for P4 26 June 2017 V_A , where it was 0.02 mm. The meteorological conditions were taken from the weather stations of the Spanish SIAR (“Servicio de Información Agroclimática para el Regadío”, Agroclimatic Information Service for Irrigation) network [43].

Plot	Coverage Evaluation	PPP Application	Date	Temperature (°C)		Relative Humidity (%)		Solar Irradiance (W/m ²)		Wind Speed (km/h)		Wind Direction	
				V_C	V_A	V_C	V_A	V_C	V_A	V_C	V_A	V_C	V_A
P1	P1 (2016)	●	27 May 2016	23	24	57	59	731	893	4	9	SE-S	E
			27 July 2016	25	27	63	67	337	474	2	5	NW-N-NE	N
	P1 (2017)	●	11 October 2016	25	20	31	59	678	456	5	8	W-SW	W
			7 June 2017	23	21	62	81	590	208	6	1	NE	NW-SE
			24 July 2017	27	26	53	73	364	263	3	2	SE-NE-E	NW-N-NE
8 September 2017	24	22	79	89	265	89	2	2	N-E	NW-W-SE			
P2	P2 (2016-2)	●	26 May 2016	22	22	66	68	811	609	7	7	E	E
	P2 (2016-1)		22 June 2016	25	24	51	58	730	575	5	3	E	SE
			9 August 2016	31	28	26	31	690	499	5	4	SE	SE
	P2 (2017)		9 September 2016	26	26	71	75	251	201	1	1	N	NW
P3	P3 (2017)	●	23 June 2017	29	27	50	50	712	353	5	1	SE	SE-E
			3 August 2017	27	26	55	56	204	196	7	2	N	NW-SW
P4	P4 (2017)	●	26 June 2017	24	30	77	54	241	846	1	6	SE-E	E-NE-N
			18 September 2017	28	23	27	48	732	293	3	4	SW-NW-W	S-SW-W
P5	P5 (2017)	●	27 June 2017	30	30	50	55	604	395	2	5	SE-E	E-NE
			19 September 2017	25	19	51	88	694	209	5	2	E-NE	SE-E
P6	P6 (2017)	●	10 July 2017	28	26	42	48	665	430	3	2	SE-S	S-SW
			23 August 2017	25	18	51	68	356	134	2	1	S	W-NE-W
			14 September 2017	27	20	44	71	470	231	6	2	NW-W	E-NE-NW
P7	P7 (2017)	●	30 June 2017	26	21	22	32	863	484	8	3	W	SW-NW
			4 August 2017	27	29	55	40	295	438	2	4	SE-N-SW	E-SE-S

V_C : conventional volume; V_A : adjusted volume; PPP: plant protection product.

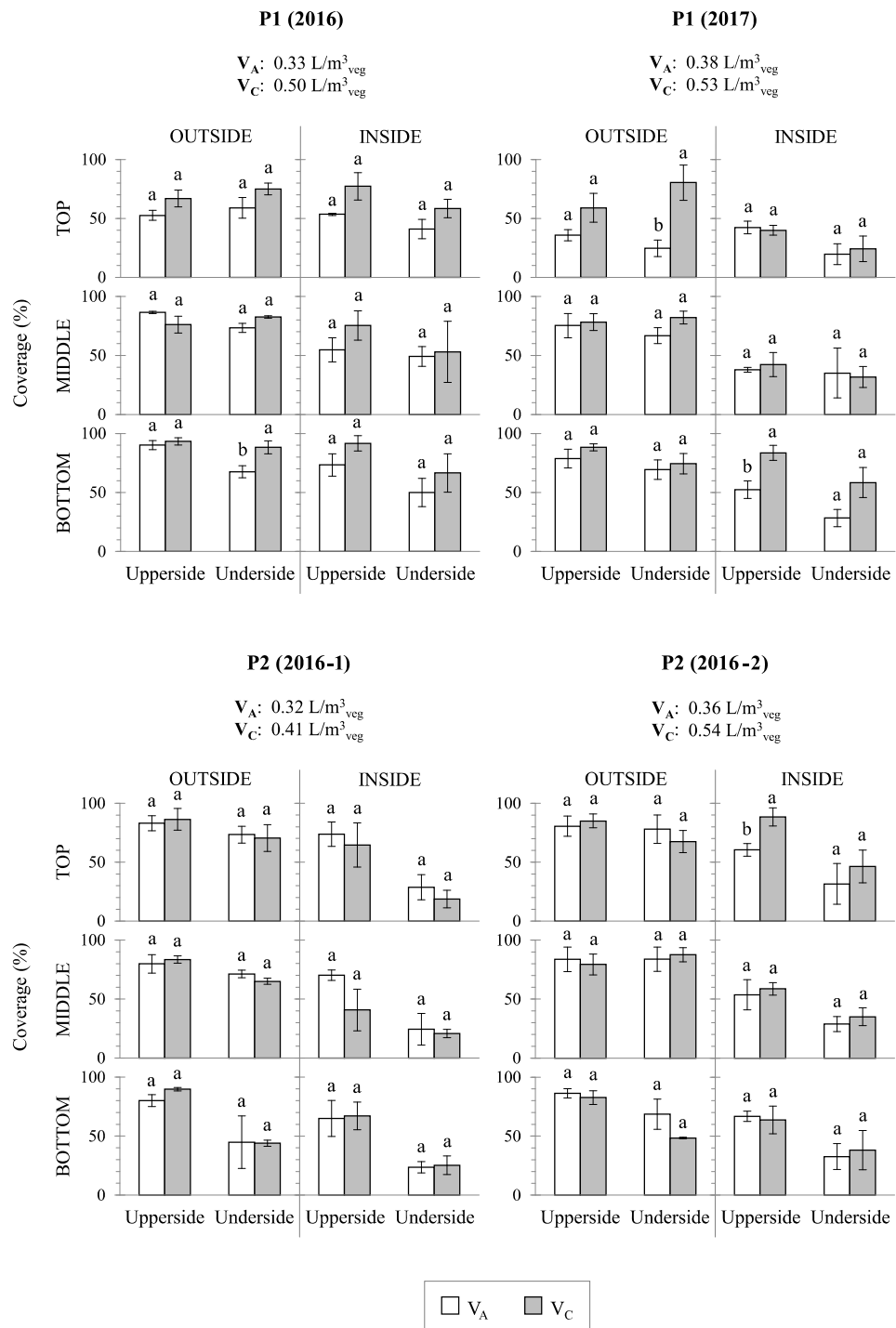


Figure A1. Cont.

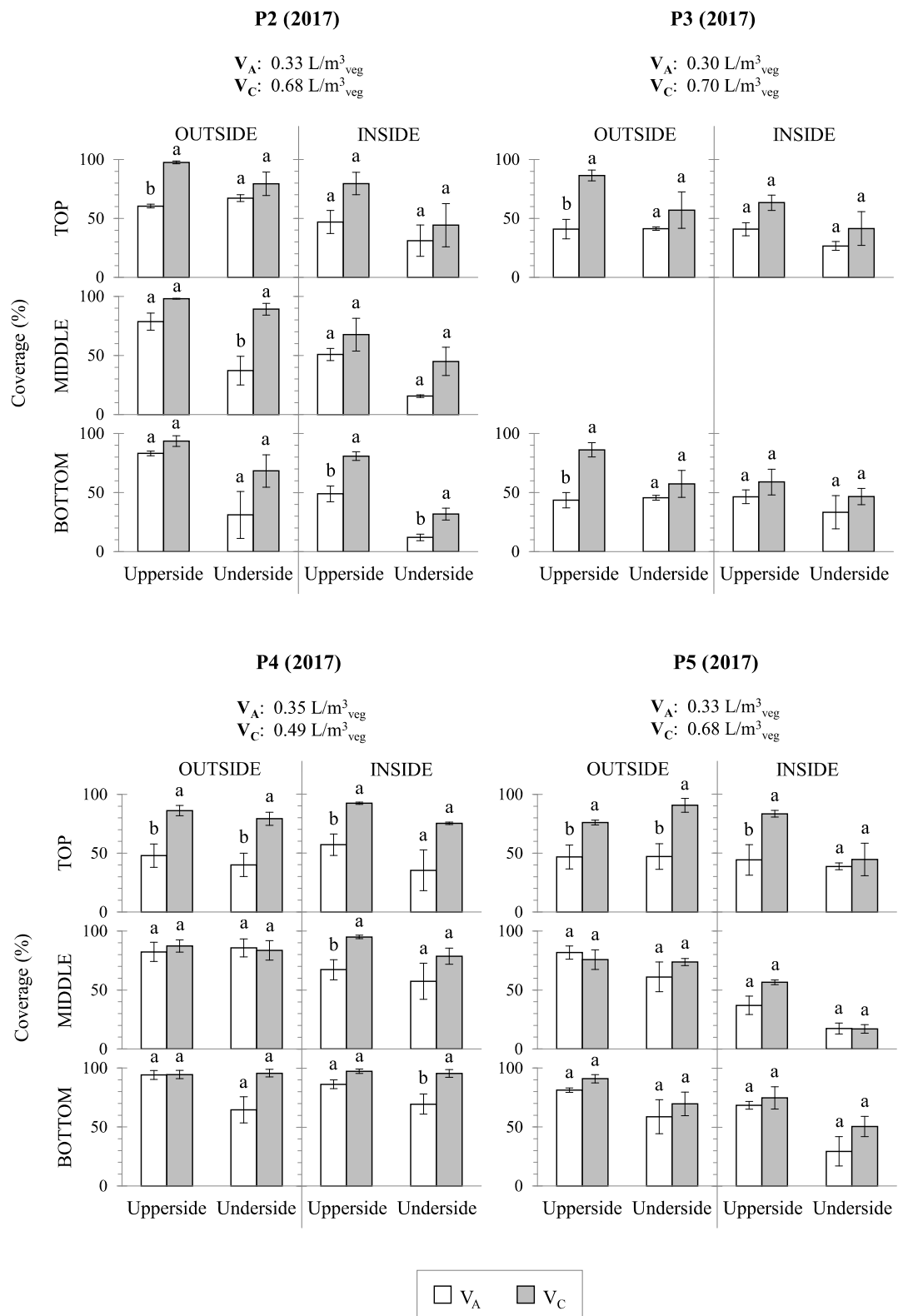


Figure A1. Cont.

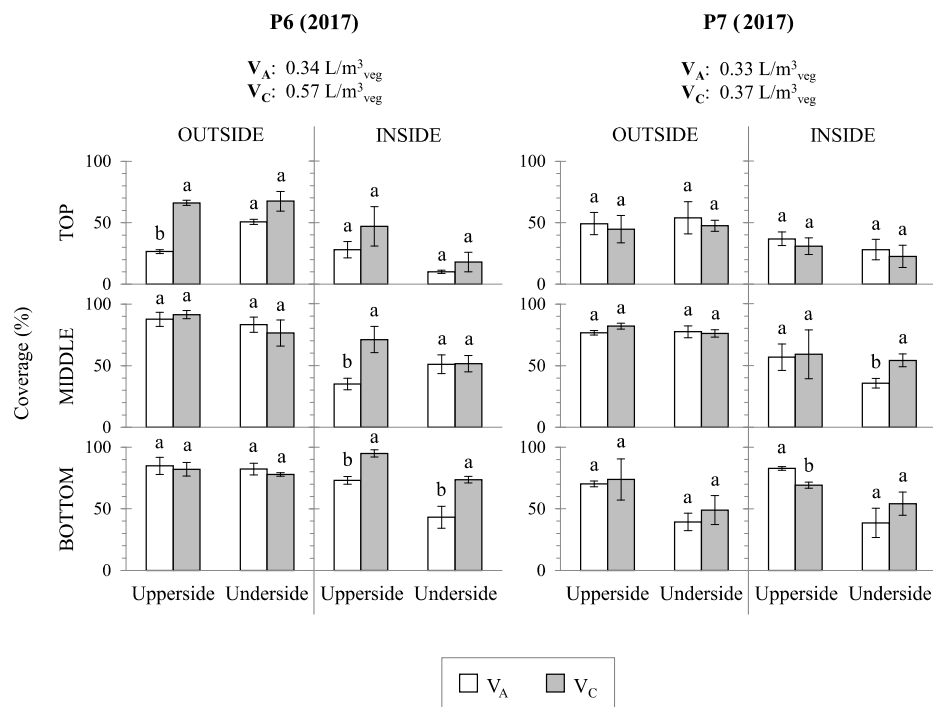


Figure A1. Mean (\pm standard error) coverage (%) per treatment (V_A : adjusted volume, V_C : conventional volume) at each height (top, middle, bottom) and depth (inside, outside) of the canopy, and side of the leaf (upperside, underside), in the different evaluations (combination of plot and year). The water volume is indicated in L/m³_{vegetation}. Different letters above the bars within each height \times depth \times side combination indicate significant differences (LSD test, $p < 0.05$).

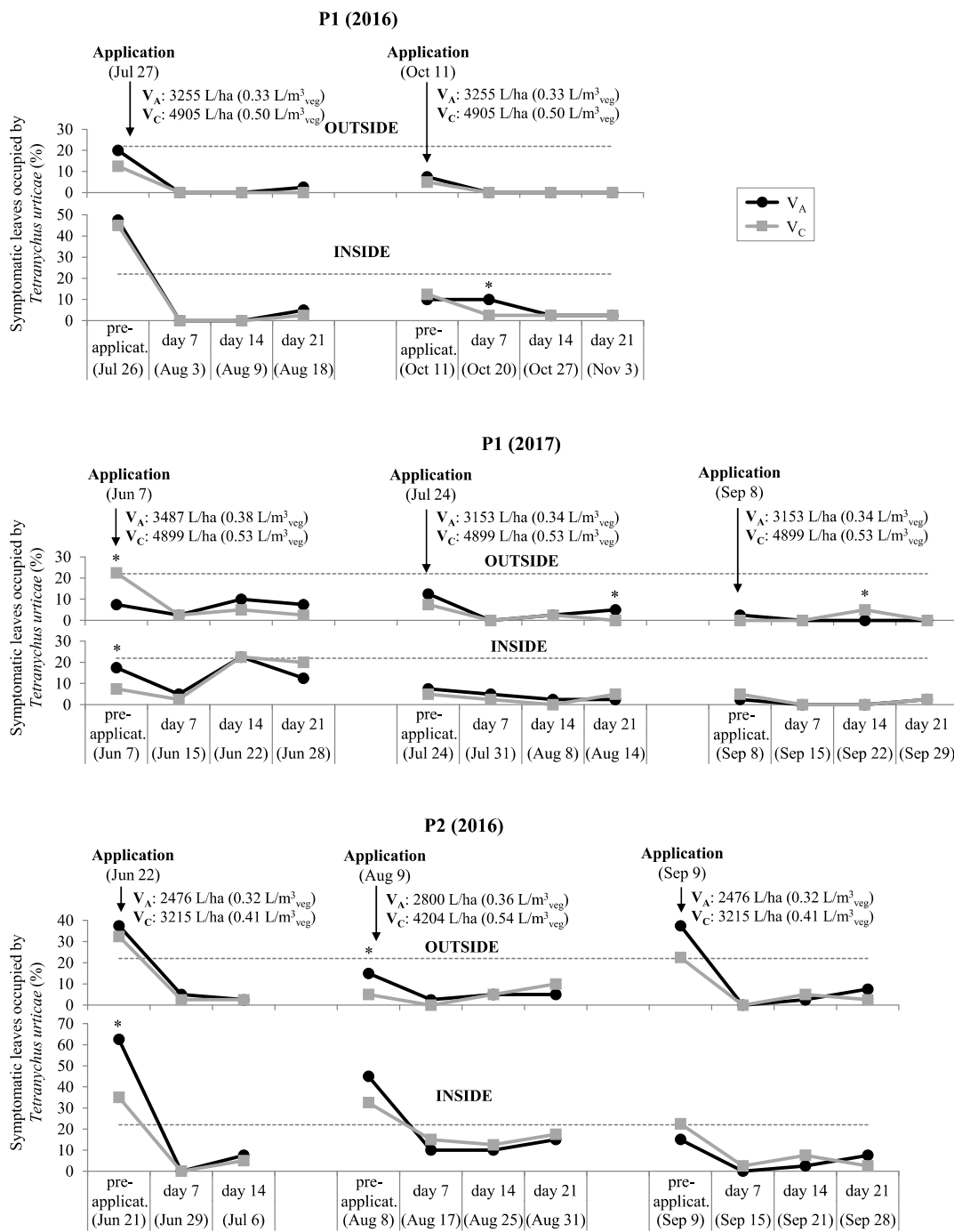


Figure A2. Cont.

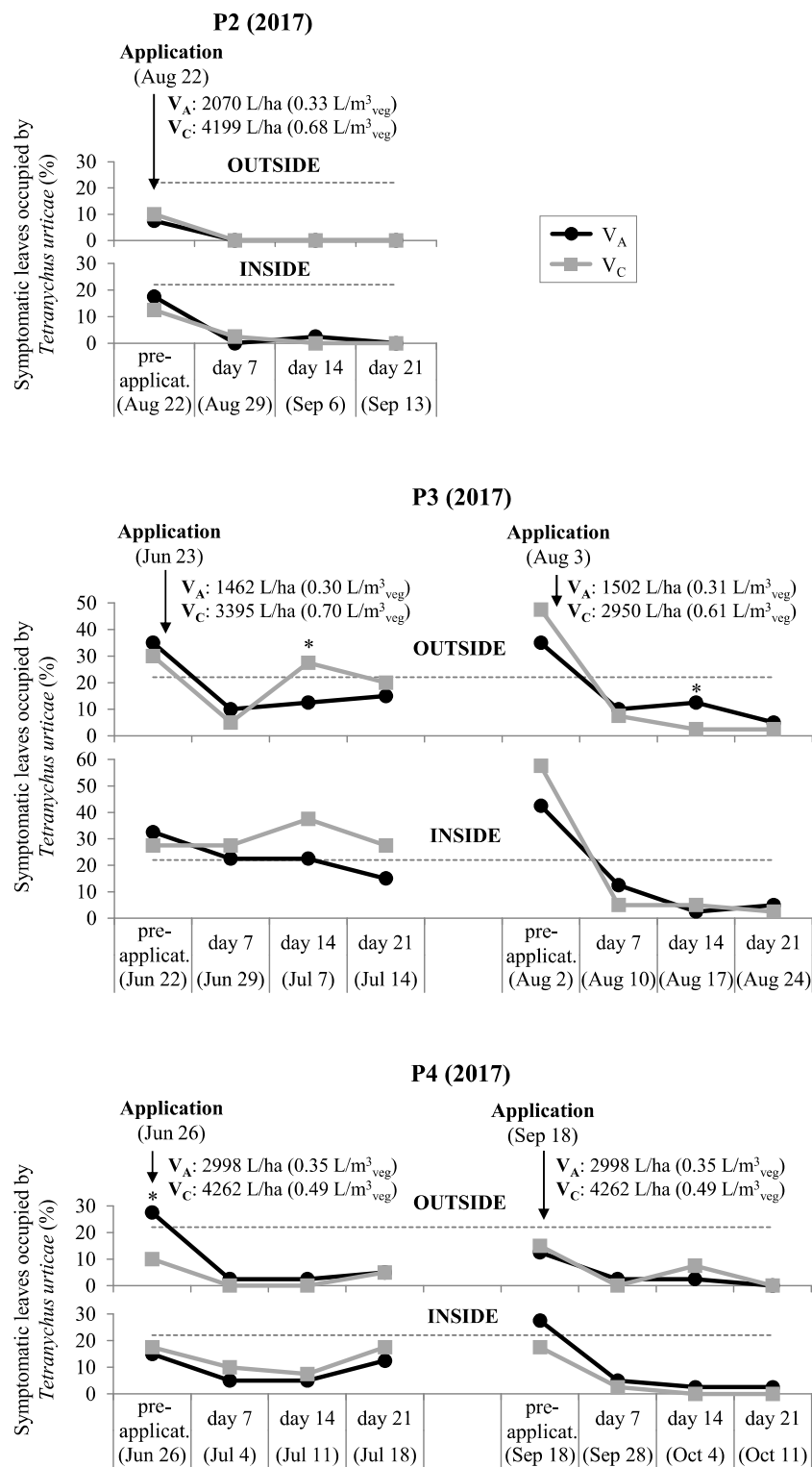


Figure A2. Cont.

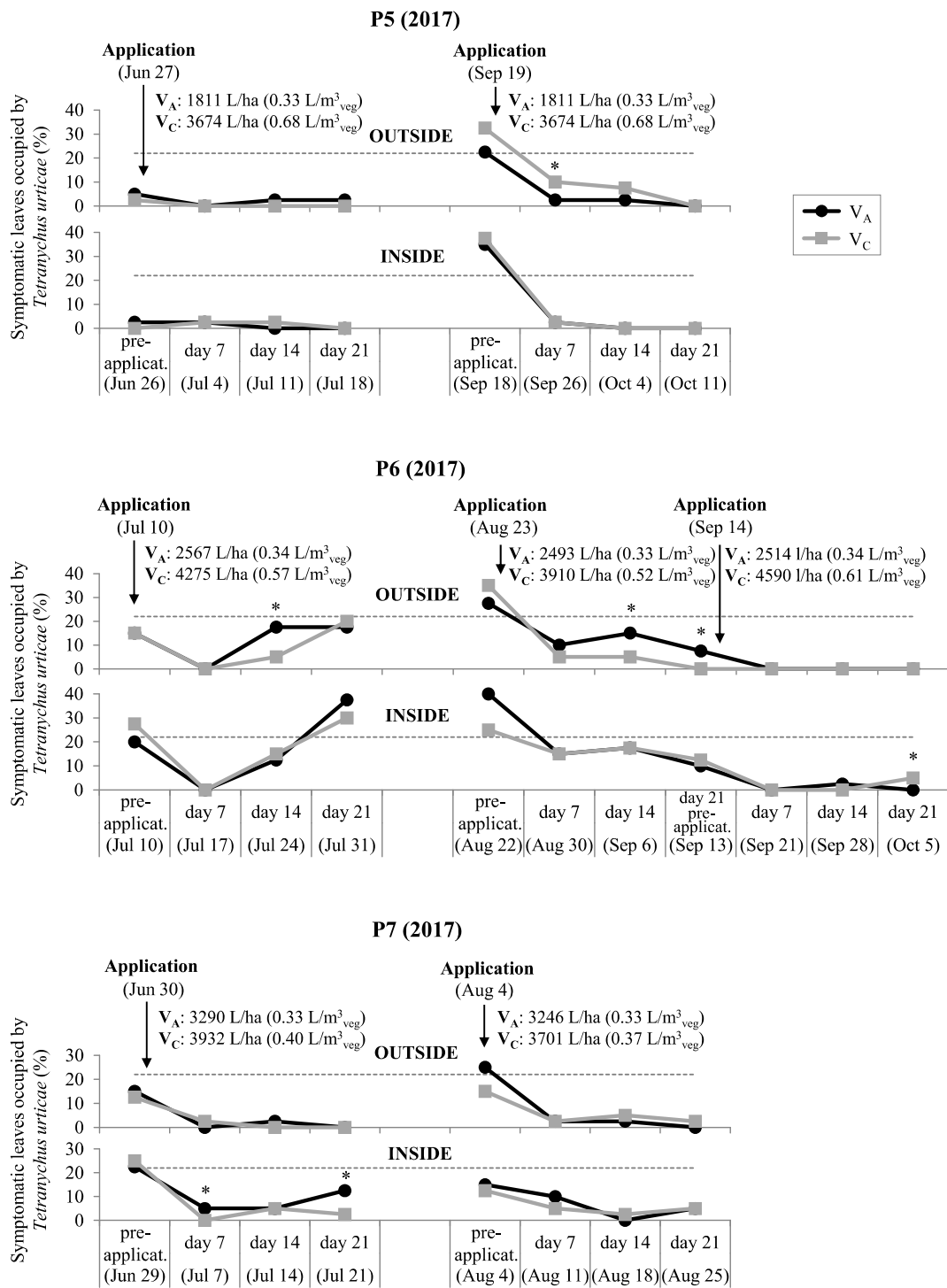


Figure A2. Symptomatic leaves occupied by *Tetranychus urticae* (% , mean) at two depths of the canopy (outside, inside), pre-application and after the applications (at 7, 14 and 21 DAA) with two different spray volumes (V_A : adjusted, V_C : conventional) and in different plots (P1–P7). The water volume is indicated in L/ha and L/m³_{vegetation}. The dotted line indicates the treatment threshold: 22% of symptomatic leaves occupied. The percentage of symptomatic leaves occupied by *Tetranychus urticae* in V_A and V_C treatments groups was compared with a chi-square test. The asterisk (*) indicates significant differences between volumes (chi-square test; $p = 0.05$).

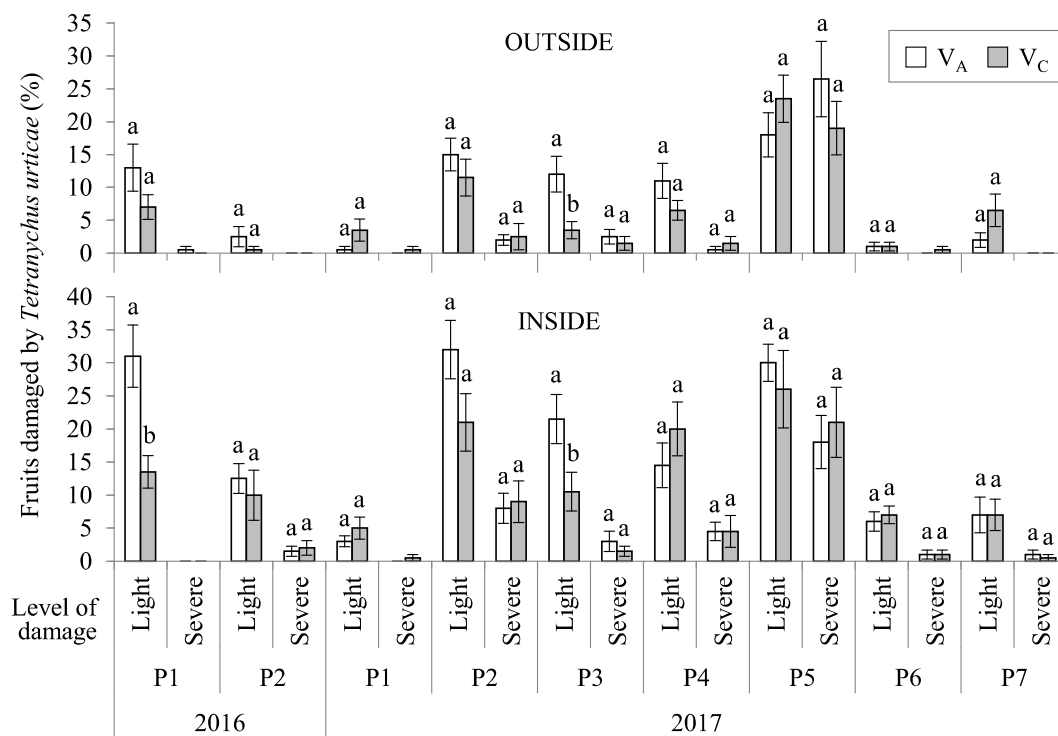


Figure A3. Fruits damaged by *Tetranychus urticae* (%; mean \pm standard error) per treatment (V_A : adjusted volume, V_C : conventional volume), at two depths of the canopy (outside, inside) and in different plots (P1–P7) and years (2016 and 2017). Different letters above the bars indicate significant differences (LSD test, $p < 0.05$).

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