

Article

Evaluation of the Effectiveness of Spirotetramat on the Diaspine Scale *Parlatoria pergandii* in Citrus Orchards

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Abstract: The control of *Parlatoria pergandii* (Comstock, 1881) was studied in citrus orchards at Belksiri (Gharb area), Morocco. Three concentrations of Spirotetramat (T0 = 0 L/Ha, T1 = 0.625 L/Ha, T2 = 0.755 L/Ha, and T3 = 1 L/Ha) were applied to 4 ha of Valencia late orchard (each dose for 1 ha of citrus). The insecticidal of Spirotetramat was evaluated on two stages of larvae (L1 and L2) of *P. pergandii* and three stages of females, F1, F2, and F3. Similarly, the rates of parasitism by *Aphytis hispanicus* on the females of three different stages were monitored to evaluate the impact of Spirotetramat on this natural enemy. Results showed that Spirotetramat was more effective on larvae than females. Of the females, 26.04% were parasitized, 65.81% were inhibited, and only 8.15% were intact after the treatment period. Of the larvae, 79.73% were inhibited, and only 20.27% survived after the treatment period. Finally, our study highlights that all the tested concentrations of this product were effective on the population of *P. pergandii*. On the other hand, this product showed a less negative impact on the parasitized females with the low concentration (0.625 L/Ha) compared to the high concentrations of the pesticide (T2 = 0.755 L/Ha and T3 = 1 L/Ha), especially for the female stages F2 and F3. Moreover, the impact on the water table will be less severe with a low concentration.

Keywords: *Parlatoria pergandii*; parasitoid; pest control; Spirotetramat; Valencia late Morocco

1. Introduction

Citrus is one of the most essential fruit trees cultivated in the Mediterranean area [1,2]. In Morocco, the amount of land dedicated to citrus plantations is growing every year; the total annual production is ~3 million tons of citrus fruit. It is mainly produced in Souss, Berkane, Tadla and Haouz regions with a total surface area of 126.600 ha [3,4]. However, in addition to the complications of production and marketing, there are numerous pest species and diseases that are reducing the quantity and quality of citrus fruits [5,6].

To date, there are more than 155 weed species, 90 pest species, 34 diseases species, and 16 nematodes in citrus farming zones, mainly in the Mediterranean region from Turkey in the East to Morocco in the far West Nadine [7–9]. Among this diversity of

nuisances, the polyphagous *Parlatoria pergandii* (Comstock, 1881) (Hemiptera: Diaspididae) is one of the most widely-dispersed citrus pest species invading citrus worldwide and in Morocco [10,11]. Infestations caused by this insect pest can be documented by the occurrence of the scale-armor on citrus tree parts, including the leaves, stems, and fruits [12]. However, symptoms are mostly related to the severity of the infestation (density of pest on the host) and host immunity [13].

Infestations on the citrus trees, mainly on the stems and leaves, principally cause wilting and decrease the photosynthetic pigments and surfaces of the plants, leading to reductions in yields and revenues [14]. Generally, *P. pergandii* reduces the quantity and quality of citrus fruits produced. The latter decreases marketing opportunities, particularly in big markets, such as the European Union the USA where the exportation standards are more complex [15].

To control *P. pergandii*, many approaches, including chemical and eco-friendly methods, are used [16–18]. While in northern countries, sustainable methods are encouraged, pest management is profoundly dependent on chemical treatments in other southern countries [19]. In Morocco, before 2010, sustainable approaches were neglected and rudimentary [20]. However, with the newly imposed standards, mainly in terms of the use of chemical compounds, pesticides have become a problem because of the remaining residues on fruits. Moreover, the development of resistance to pesticides has negatively impacted production, harvesting, and exportation of citrus fruits [21].

The development cycle of *P. pergandii*, including the duration of each larval (L1 and L2), female (F1, F2, and F3), and male (pre-pupa, pupa, and adult) stage; fecundity; oviposition rate; and sex ratio are largely influenced by the host plant and temperature [22–24]. The life cycle lasts, on average, 56.28 days for lemons and only 45.33 days for squash. The fecundity is 86 larvae per female for lemons and 109 for squash [24]. On the other hand, the number of generations per year varies with climatic conditions—three generations in Morocco and Spain and three to four in Israel [23,25].

Aphytis hispanicus (Mercet, 1912) (Hymenoptera: Aphelinidae) is an important parasite of *P. pergandii* in Morocco [22–24]. They develop exclusively as primary ectoparasitoids of diaspine scaled insects [26]. The adult female pierces the shield of the scale with the ovipositor and lays one or several eggs on the body of the scaled insect [27].

Spirotetramat is a systemic insecticide listed in Group 23 of the Insecticide Resistance Action Committee. It is a compound that belongs to the chemical class of ketoenols [28]. It is active against piercing-sucking insects, by acting as an acetyl-CoA carboxylase (ACC) inhibitor, interrupting lipid biosynthesis in the insects [29,30]. In this study, we used field monitoring to investigate the adequate dose of Spirotetramat to control *P. pergandii* without affecting natural enemies of the pest in citrus orchards. More specifically, we studied the lowest effective dose of Spirotetramat on larvae and females regarding its impact on the parasitoids of *P. pergandii* inside citrus orchards.

2. Materials and Methods

2.1. Study Area

Fieldwork was conducted in the Belksiri northeast of the Kenitra province, situated at the Gharb plain, North of Morocco (Figure 1). Geographically, the study zone was at low altitude ranging between 300 and 500 m above sea level. The Gharb region is the largest agricultural area (600 km²) in Morocco [31]. This region is well known for the production of fruits and vegetables, including citrus, cereals, and vegetables due to the appropriate climate and soil properties [32]. The Gharb zone is characterized by a Mediterranean climate; annual precipitation ranges between 480 and 600 mm, and the average temperature is 27 °C during summer and 13 °C during winter.

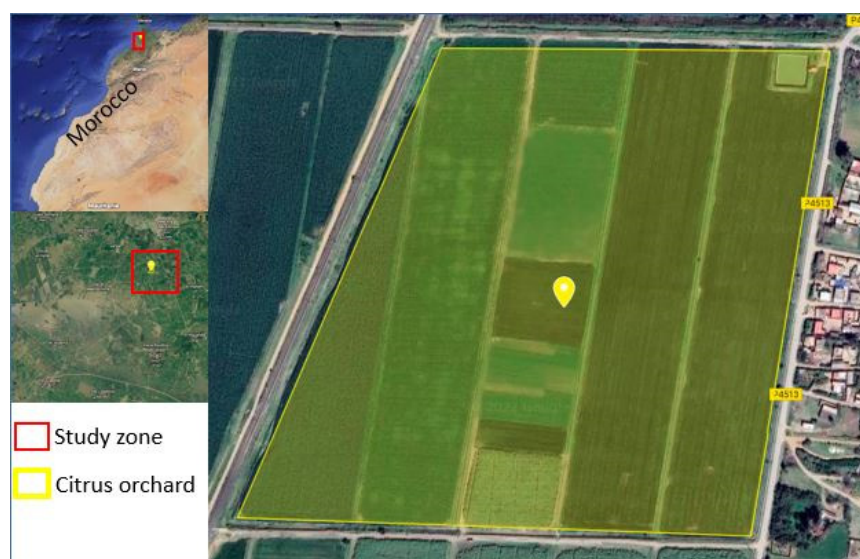


Figure 1. Location of the orchard used.

2.2. Sampling Design

To evaluate the effect of Spirotetramat on *P. pergandii*, an orchard of “Valencia Late” (*Citrus sinensis*) was selected and monitored because of its abundance in the area [32]. Spirotetramat was selected based on two reasons: (1) the pesticide is effective against a wide range of pests; (2) its low impacts on the environment and natural enemies. The orchard covers 4 ha with 25-year-old citrus trees. The orchard was divided into 4×1 ha small plots (Figure 2), and each plot was treated with a specific dose of the pesticide: (i) T0 = 0 L Spirotetramat (as a control experiment), (ii) T1 = 0.625 L, (iii) T2 = 0.755 L, and (iv) T3 = 1 L. In addition, the Teyme Eolo sprayer (Teyme Tecnologia Agricola, Girona, Spain), with its turbulent nozzle and exit diameter of 12 mm, delivered 1.55 L/min at a pressure of 20 bars. The towed sprayer delivers 2500 L of spray liquid per hectare, at a rate of 6 L of spray liquid for each tree, allowing us to wet all parts of the tree. (Figure 2). Concentrations were selected based mainly on the quantity of pesticide used regularly by local farmers.

To evaluate the effect of each dose, after treatment (1 to 8 weeks) we sampled 200 leaves from late Valencia trees in each plot. Ten leaves/tree were collected randomly from the different directions (north, east, south, and west) of the tree, for a total of 20 trees/plot that belonged to a square block (3 repetitions were performed independently). We left 2 lines between the different plots treated with this systemic product. The collected leaves were transferred directly in polyethylene bags referenced to the laboratory for examination. The scaled insect stages on each leaf were determined and counted on both surfaces of the leaves by using a binocular microscope. Then, we counted the mortalities (M) and survivals (V) of *P. pergandii* at two larval stages (L1 and L2) and three female stages (F1, F2, and F3). In parallel, to evaluate the effect of Spirotetramat on natural enemies of *P. pergandii*, after each treatment, we counted the parasitism rates (P) by *Aphytis hispanicus* on the females at three different stages, F1, F2, and F3. *Aphytis hispanicus* is an important parasitoid of *P. pergandii* in the area and the Mediterranean in general.

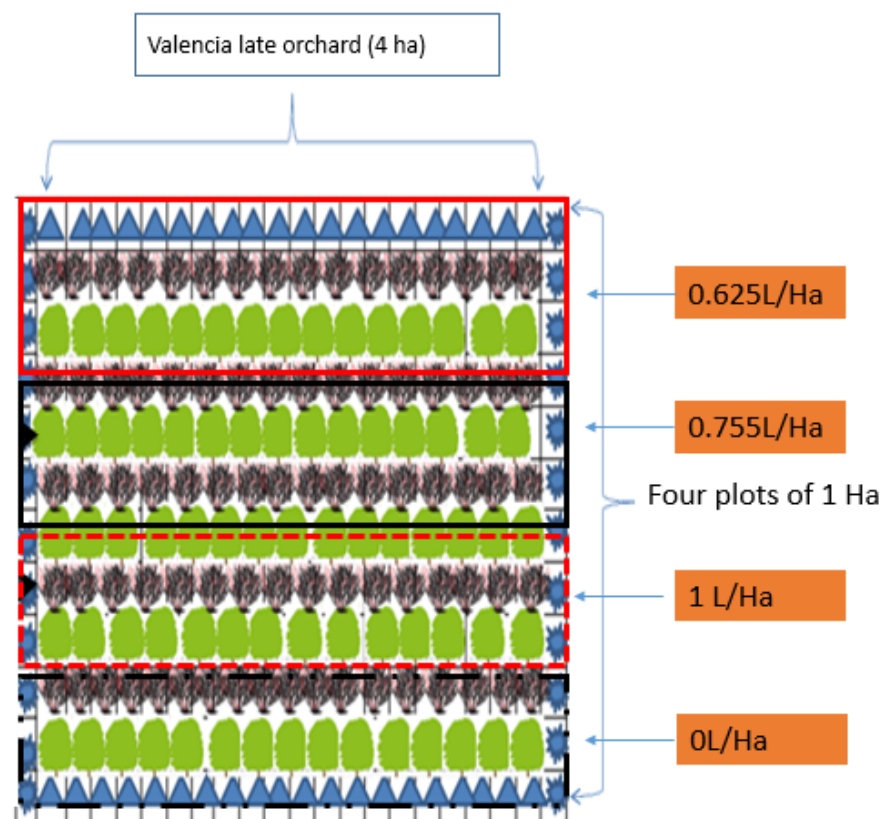


Figure 2. A model of the orchard we monitored (divided into four small plots of 1 ha) with the doses of Spirotetramat we used.

2.3. Statistics

Statistics were done using the Statgraphics Centurion software, version XVI.I for windows (Statgraphics Technologies, The Plains, VA, USA), and the results are given as sample size and mean \pm SD. Moreover, to evaluate the effectiveness of Spirotetramat, we calculated the survival (surviving specimens/total sampled specimens) and mortality (inhibited specimens/total sampled specimens) of *P. pergandii* for both larvae and females. In parallel, to evaluate the impact of the pesticide on natural enemies, we calculated the parasitism rate of *A. hispanicus* on females (parasitized females/total sampled females).

We checked for normality and homogeneity of variance for all variables via Kolmogorov–Smirnov test. To assess the mortality of larvae of *P. pergandii*, we used the independent *t*-test, considering the two stages as unrelated parameters [33]. The mortality among females (three stages) was tested by one-way ANOVA. We analyzed separately, for both larvae and females, the relationship between Spirotetramat inhibition effectiveness (mortalities of *P. pergandii*) and survival (surviving specimens of *P. pergandii*) by simple regression (linear model) [34], and all variables with P values greater than 0.05 were considered non-significant.

To investigate the effectiveness of Spirotetramat, treatment doses as predictors of pest and parasitoid mortalities (response variables: with 0 (no mortalities) and 1 (at least one victim recorded)), a model with a binomial error structure and a logit link function was applied [35]. The model was applied for the totality of *P. pergandii* pests (all recorded specimens) and *A. hispanicus* parasitoids (all recorded parasitoids). In the case of parasitoids, the impact was reversed (the mortalities were considered as losses of natural enemies).

3. Results

3.1. Impact on Larvae

Among the 5115 recorded larvae (Figure 3), 79.73% ($n = 4078$) were inhibited, and only 20.27% ($n = 1037$) survived after the treatment period. Equally, during stage 1 (larvae 1),

2441 larvae were dead and 841 survived. Similarly, during stage 2 (larvae 2), 1637 larvae were eliminated and 196 survived. Surviving larvae were more common during stage 1 (Table 1). Mortalities were higher during stage 1. Mortalities and survival rates were uncorrelated during both stages (Figure 4).

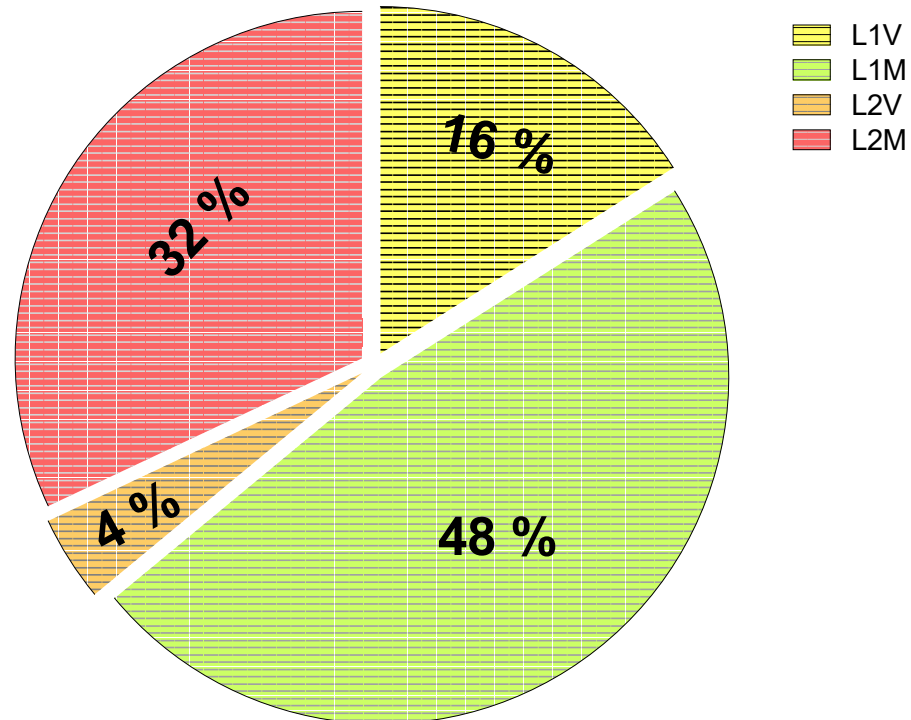


Figure 3. A comparison of mortalities (M) and survival rates (V) during two larvae stages (L1 and L2) of *Parlatoria pergandii* (L1V: surviving larvae of stage 1; L1M: dead larvae of stage 1; L2V: surviving larvae of stage 2; L2M: dead larvae of stage 2).

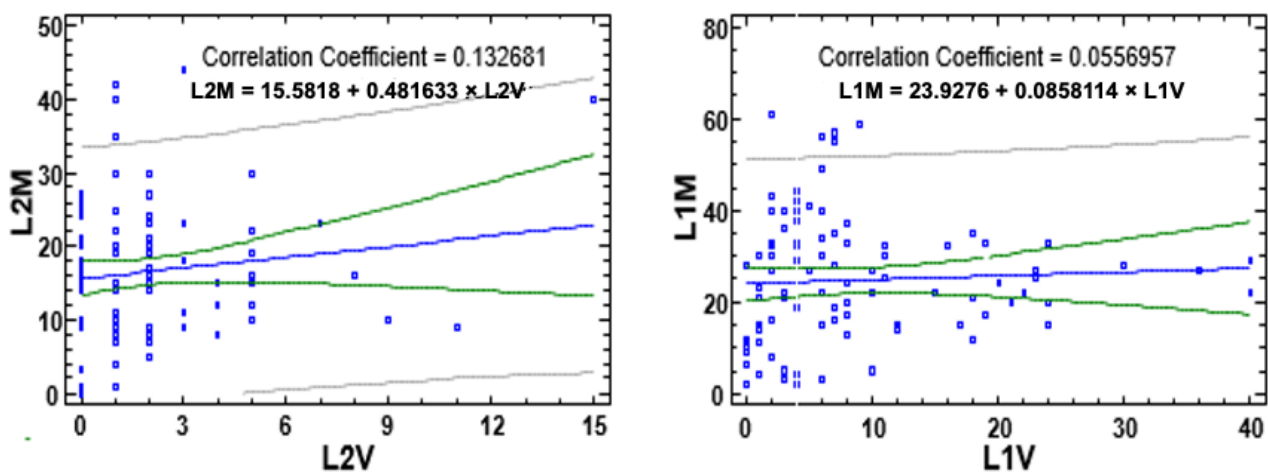


Figure 4. Analysis of the the survival rates of *Parlatoria pergandii* during stages larvae 1 and larvae 2.

In terms of the inhibition dose, the T2 of Spirotetramat was effective on larvae 1 with a mean of $29.37^a \pm 2.94$, whereas the dose T3 was effective on larvae 2 with an average value of $21.48^a \pm 1.82$ (Tables 2 and 3). On the contrary, the T1 was less effective at both larval stages. Moreover, the concentration of pesticide was correlated positively with the inhibition of larvae, and they were negatively correlated with insect survival.

Table 1. A comparison of mortalities (M) and survival (V) rates among different larval stages of *Parlatoria pergandii* treated with Spirotetramat.

Compared Parameters	<i>t</i>	df	<i>p</i> -Value
L1V-L1M	−10.223	98	0.000
L2V-L2M	−16.183	98	0.000
L1V-L2V	7.948	98	0.000
L1M-L2M	5.263	98	0.000

Table 2. The survival (V) and mortality (M) of two larval stages (L1 and L2) of *Parlatoria pergandii* treated with different concentrations of Spirotetramat (T0 = 0 L/ha, T1 = 0.625 L/ha, T2 = 0.755 L/ha, and T3 = 1 L/ha).

	L1V	L1M	L2V	L2M
T0	16.08 ^a ± 2.32	17.70 ^b ± 1.73	3.95 ^a ± 0.53	11.75 ^b ± 1.19
T1	5.04 ^b ± 0.68	26.08 ^{ab} ± 2.89	0.91 ^b ± 0.17	14.95 ^{ab} ± 1.19
T2	6.12 ^b ± 1.46	29.37 ^a ± 2.94	0.79 ^b ± 0.15	17.33 ^a ± 2.17
T3	6.92 ^b ± 1.30	25.37 ^{ab} ± 2.68	2.22 ^b ± 0.61	21.48 ^a ± 1.82

Values in the same column with different superscripts are significantly different ($p < 0.05$).

Table 3. Comparison of mortality (M), parasitism rates (P), and survival rates (V) of three female stages (F1, F2, and F3) of *Parlatoria pergandii* by one-way ANOVA during treatment dates.

		Sum of Squares	df	Mean Square	F	<i>p</i> -Value
F1V	Between Groups	61.365	3	20.455	5.476	0.002
	Within Groups	343.625	92	3.735		
	Total	404.990	95			
F2V	Between Groups	158.531	3	52.844	4.687	0.004
	Within Groups	1037.208	92	11.274		
	Total	1195.740	95			
F3V	Between Groups	125.531	3	41.844	1.974	0.123
	Within Groups	1950.208	92	21.198		
	Total	2075.740	95			
F1M	Between Groups	1766.365	3	588.788	8.181	0.000
	Within Groups	6621.375	92	71.971		
	Total	8387.740	95			
F2M	Between Groups	3655.031	3	1218.344	6.511	0.000
	Within Groups	17,213.958	92	187.108		
	Total	20,868.990	95			
F3M	Between Groups	11,303.865	3	3767.955	13.589	0.000
	Within Groups	25,509.292	92	277.275		
	Total	36,813.156	95			
F1P	Between Groups	618.698	3	206.233	19.971	0.000
	Within Groups	950.042	92	10.327		
	Total	1568.740	95			
F2P	Between Groups	78.781	3	26.260	0.382	0.767
	Within Groups	6332.208	92	68.828		
	Total	6410.990	95			
F3P	Between Groups	279.750	3	93.250	0.516	0.672
	Within Groups	16,634.750	92	180.813		
	Total	16,914.500	95			

3.2. Impact on Females

Among the 14,657 recorded females (Figure 5), 26.04% ($n = 3816$) were parasitized, 65.81% ($n = 9646$) were inhibited, while only 8.15% ($n = 1195$) were intact after the treatment period. In detail, during stage F1, only 1% ($n = 161$) survived; 10% ($n = 1526$) and 3% ($n = 421$) were inhibited and colonized by parasites, respectively. In addition, during

stage F2, 3% ($n = 373$) survived, 22% ($n = 3167$) were eliminated, and 9% ($n = 1343$) were parasitized. Equally, 4% ($n = 661$) survived, 34% ($n = 4953$) were inhibited, and 14% ($n = 2052$) were parasitized during stage F3.

In terms of a comparison among the stages, total surviving females and parasitism rates were higher during stage 3—4% and 14%, respectively (Figure 5). Inhibited females were higher in number during stages two and three. On the other hand, survival rates were variable for all periods (Table 3). Similarly, eliminated females (F1M, F2M, and F3M) were different during monitoring dates. Parasitism rates were variable only during stage F1.

With respect to T0 (untreated plot), all three doses of the systemic product were effective on surviving females at stages two and three (F2V and F3V). The same effect was produced for dead females of stages 1 and 2 (F1M and F2M); however, for dead females of stage 3 (F3M), the concentration 0.755 (T2) was most effective, with an average of $57.25^a \pm 5.07$. In addition, T1 ensured the higher parasitism rate at all three female stages (Tables 4 and 5). The average was $4.75^{ab} \pm 0.93$ for females of stage 1, $14.5^b \pm 0.99$ for females of stage 2, and $20.45^b \pm 1.22$ for females of stage 3.

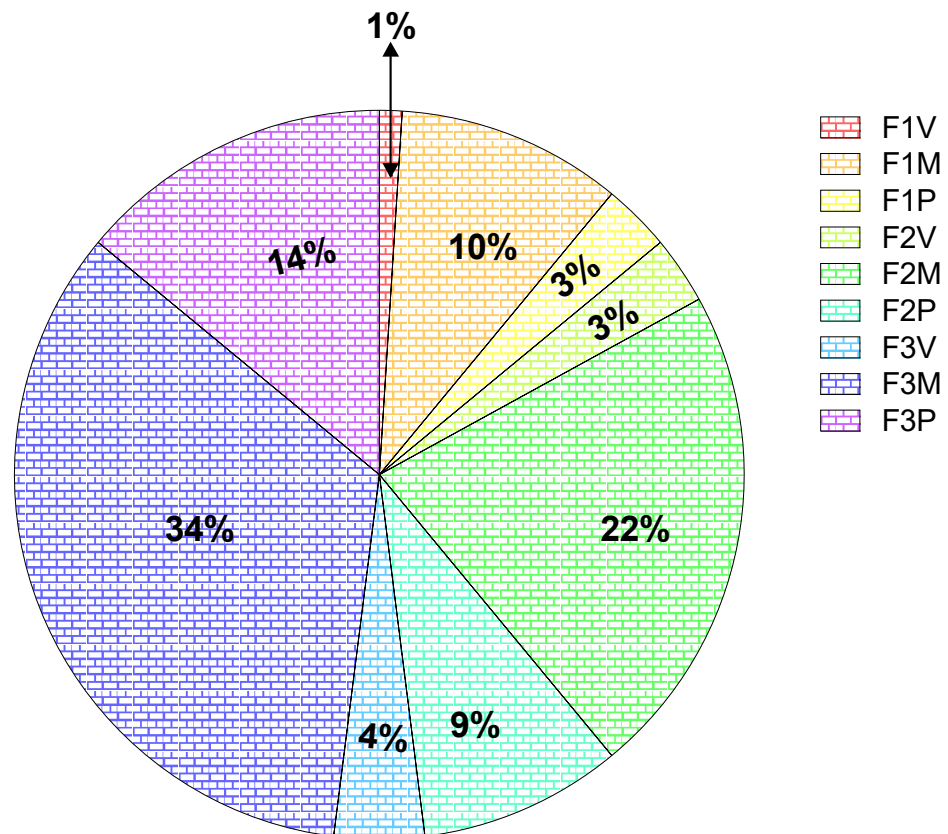


Figure 5. A comparison of mortalities (M), parasitism rates (P), and survival rates (V) during three female stages (F1V: survived females of stage 1; F1M: dead females of stage 1; F1P: parasitized females of stage 1; F2V: surviving females of stage 2; F2M: dead females of stage 2; F2P: parasitized females of stage 2; F3V: surviving females of stage 3; F3M: dead females of stage 3; F3P: parasitized females of stage 3).

Table 4. Survival (V), mortality (M), and parasitism (P) of *Parlatoria pergandii* females (F1, F2, and F3) treated with different concentrations of Spirotetramat (T0 = 0 L/ha, T1 = 0.625 L/ha, T2 = 0.755 L/ha, and T3 = 1 L/ha).

	F1V	F1M	F1P	F2V	F2M	F2P	F3V	F3M	F3P
T0	2.83 ^a ± 0.41	9.75 ^b ± 0.94	6.20 ^a ± 0.79	6.20 ^a ± 0.92	21.16 ^b ± 2.27	23.91 ^a ± 1.51	12.08 ^a ± 0.98	42.41 ^b ± 2.28	40.25 ^a ± 1.87
T1	1.95 ^{ab} ± 0.59	18.83 ^a ± 2.32	4.75 ^{ab} ± 0.93	3.66 ^b ± 0.77	37.16 ^a ± 2.21	14.5 ^b ± 0.99	4.5 ^b ± 0.50	52.25 ^{ab} ± 3.20	20.45 ^b ± 1.22
T2	1.16 ^{ab} ± 0.20	18.16 ^a ± 1.82	3.87 ^{ab} ± 0.87	2.58 ^b ± 0.31	40.20 ^a ± 3.49	8.79 ^c ± 0.69	4.58 ^b ± 0.53	57.25 ^a ± 5.07	13.45 ^c ± 1.23
T3	0.66 ^b ± 0.15	17.14 ^a ± 1.67	2.90 ^b ± 0.43	2.47 ^b ± 0.30	32.95 ^a ± 2.23	8.95 ^c ± 1.02	5.57 ^b ± 0.59	54.04 ^{ab} ± 4.41	11.57 ^c ± 1.11

Means that do not share a letter are significantly different.

Table 5. Effect of treatment doses (T0: 0 L/Ha Spirotetramat (as a witness experiment), T1 = 0.625 L/Ha, T2 = 0.755 L/Ha, and T3 = 1 L/Ha) on the mortalities of *Parlatoria pergandii*, and the parasitoid *Aphytis hispanicus*.

		Estimate	Standard Error	Wald Test	p-Value
Survival	Intercept	−1.073	0.530	4.101	0.043
	T0	−0.118	0.038	9.523	0.002
	T1	0.063	0.028	4.991	0.055
	T2	−0.001	0.002	0.525	0.539
	T3	−0.136	0.563	0.561	0.056
	Scale	1.000	0.000		
Mortalities	Intercept	−1.046	0.480	5.212	0.065
	T0	−0.161	0.073	8.431	0.042
	T1	0.058	0.033	9.047	0.002
	T2	−0.051	0.012	10.321	0.001
	T3	−0.456	0.451	0.621	<0.001
	Scale	1.000	0.000		
Parasitoid Survival	Intercept	−1.064	0.690	5.654	0.007
	T0	−0.543	0.053	10.632	0.001
	T1	0.454	0.032	7.431	0.05
	T2	−0.034	0.0453	0.565	0.789
	T3	−0.136	0.563	0.674	0.796
	Scale	1.000	0.000		

4. Discussion and Conclusions

To our knowledge, this is the first study on the use of chemical treatment to control *P. pergandii*. Our main aim was to provide detailed data on the effectiveness of Spirotetramat regarding the pest and the secondary effect on the parasitoid *A. hispanicus*. These findings provided the first set of data related to the control of *P. pergandii* in citrus in Morocco and the entire Northwest African zone, which should be of great interest for the undertaking of new comparative studies and the adoption of sustainable doses to control the pest and protect the natural enemies.

Our results showed that in the larval stage the inhibition was nearly 80%, whereas in the adult stage the inhibition rate was lower. These results are similar to the results reported for other pests, including the citriculus mealybug *Pseudococcus cryptus* (Hempel), the spherical mealybug *Nipaecoccus viridis* (Newstead), the longtailed mealybug *P. longispinus* (Targioni-Tozzetti), *Planococcus citri* (Risso), the citrophilus mealybug *P. calceolariae* (Maskell), and the obscure mealybug *P. viburni* (Signoret) treated by different control methods and substances in the same area [36,37]. The lower mortality rates in females are mainly due to the vulnerability of larval stage [38]. Equally, females are characterized by their resistance toward chemicals, and this is considered among the issues of pesticide use in pest control [38,39].

In larval stages, the effectiveness of the concentrations was variable. T2 (0.755 L/Ha) was effective against larvae 1, and T3 (0.625 L/Ha) was effective on larvae 2. Increasing the amount of pesticide applied significantly increased the inhibition rate observed. However, the main goal was using the minimum pesticide concentration necessary to achieve effective control of *P. pergandii*. This approach will allow lower pesticide use and avoid the development of pesticide resistance. Consequently, less residues should remain on the fruits and in the soil [40,41].

Among the 14,657 recorded females, 26.04% were parasitized, 65.81% were inhibited, and only 8.15% were intact after the treatment period. In Algeria, similar results were mentioned for citrus: Spirotetramat controlled *P. pergandii* with encouraging results [42]. However, the effectiveness each concentration was variable depending on the insect stage. In fact, all three concentrations of the systemic product were effective on surviving females of stages two and three (F2V and F3V); the same was true for dead females of stages 1 and 2 (F1M and F2M); however, for dead females of stage 3 (F3M), the low concentration

0.625 (T1) was most effective. T1 ensured the highest parasitism rate for all three female stages. Therefore, 0.625 L/Ha is suggested to be less harmful to the environment and natural enemies. In Algeria, the same results were reported by [43]: all the doses used of Spirotetramat were effective on the population of *P. blanchardi* on date palms. Therefore, the choice of the right concentration remains a necessity in order to preserve the agroecosystem [44]. Generally, high quantities of pesticides allow one to control pests, but they negatively influence natural enemies of pests and the environment. On the other hand, the combination of natural enemies and low pesticide doses would allow effective and eco-friendly control of *P. pergandii*.

Our findings highlight four key messages: (1) data indicate that Spirotetramat was most effective on the larvae of *P. pergandii*; (2) the lowest dose was effective on different stages of female and had a low impact on the parasitoid *A. hispanicus*; (3) the combination of chemical measures and natural enemies will reduce the population of *P. pergandii* on citrus trees in the Gharb plain; (4) the use of small doses of the pesticide will reduce the impact on the environment and reduce the resistance of the insect. Given that Spirotetramat is an effective pesticide and is widely used, more studies on its effects on other populations of natural enemies and the residues remaining on fruits are needed to establish proper management strategies.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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