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Results of the Single Release Efficacy of the Predatory Mite *Neoseiulus californicus* (McGregor) against the Two-Spotted Spider Mite (*Tetranychus urticae* Koch) on a Hop Plantation

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Abstract: In 2015, we studied the efficiency of the predatory mite *Neoseiulus californicus* (McGregor) for suppression of the two-spotted spider mite *Tetranychus urticae* in a field experiment at a hop plantation. We randomly arranged four treatments in a three-block experiment. Fungicides were used in all treatments; insecticides were used in all treatments except the predatory mite treatment and acaricides were used in only two treatments. A single inundative release of the mite *N. californicus* was carried out on 4 July. On four different dates (10 June, 17 July, 29 July and 9 August), we counted the eggs and the mobile stages (larvae, nymphs and adults) of the two-spotted spider mite in all four treatments. In the treatment with the predatory mite, we established the fewest eggs and mobile stages of *T. urticae* 14 days after the release of the predator. The selected acaricides in our research acted in a primarily ovicidal manner, but we did not detect satisfactory effects on the mobile stages of the two-spotted spider mite. This result suggests the emergence of resistance of two-spotted spider mites to the acaricides hexythiazox and abamectin. Our research established comparable effects of the predatory mite *N. californicus* and acaricides, and further improvement of the efficiency would require release of the natural enemy into a hop plantation in mid-June, followed by a second release three weeks later. The costs of acaricide use in our experiment were from 12.7-fold (two sprayings of hexythiazox, and a single spraying with abamectin) to 17.8-fold (single treatments of hexythiazox and abamectin) lower than those of a single release of the biological control agent in question. The results of our study represent a starting point for future research, which could achieve satisfactory results in suppressing two-spotted spider mites on a hop plantation by repeated use of the predatory mite *N. californicus*.

Keywords: two-spotted spider mite; *Humulus lupulus*; predatory mite; biological control

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1. Introduction

Hop (*Humulus lupulus* L.) production is widespread in the countries of Middle Europe. Germany and the Czech Republic are among the leading global hop producers, while Slovenia was the fifth largest producer of hops in the world in 2016 [1]. Among the notable harmful pests that endanger hop production in the Northern Hemisphere are the damson-hop aphid (*Phorodon humuli*/Schrank/) and the two-spotted spider mite (*Tetranychus urticae* Koch) [2]. Both species of harmful pests have begun developing resistance due to the excessive use of insecticides and acaricides [2]. The two-spotted spider mite is among the most important harmful pests in the group of phytophagous mites, as it can develop and feed on more than 1100 species of host plants [3]. If more than 90 adults are found on a hop leaf [3], this mite can cause dire economic damage at harvest time.

Companion plants, which can increase the emergence of useful organisms in hop plantations, have been frequently mentioned as environmentally acceptable methods of hop protection [2]. Among the important natural enemies of two-spotted spider mites is the predatory mite *Neoseiulus californicus* (McGregor) [3], which is otherwise frequently used in vegetable plant protection [4,5]. Although studies exist in the literature on the importance of protective biological control [6] and inoculative release of predatory mites against two-spotted spider mites [7] in hop plantations, the scientific literature provides few data on the inundative release of the predatory mite *N. californicus* into plantations of this interesting field crop. According to [8], *N. californicus* was successful against two-spotted spider mites in hop rootstock. It has been said that the combination of *N. californicus* and *Phytoseiulus persimilis* can also be effective against two-spotted spider mites on hop [9].

This predatory mite species (*N. californicus*), which is present in Slovenia in the natural environment [10,11], can easily survive even when its main prey is scarce [12]. The results of studies to date provide evidence that *N. californicus* is resistant to some active substances in synthetic insecticides and acaricides [3]. This was the basis for our research, which aimed to study the efficiency of the predatory mite *N. californicus* against two-spotted spider mites with single release in combination with synthetic fungicides. In our work, we were interested in the compatibility of predatory mites and plant protection products, with a special emphasis on studying the possibility of introducing the predatory mite *N. californicus* to suppress two-spotted spider mites on hop.

2. Materials and Methods

2.1. Experimental Area and Use of Plant Protection Products

In 2015, we carried out the experiment on a hop plantation in the village of Kapla (46°14′49.76″ N; 15°1′11.91″ E; 303 m) on the outskirts of the Lower Savinja Valley. The experiment included 0.54 ha (plot with a length of 100 m and a width of 54 m) of a 7.33 ha hop plantation. The hop cultivar "Savinjski golding", which is placed among tall cultivars, was sown in 2002 at an inter-row distance of 2.4 m. The distance between plants in a row was 1.2 m. The experimental plot was divided into three blocks; within each block we randomly arranged four treatments, namely, the control treatment (spraying with fungicides and insecticides, without acaricides) (marked as Control in the figures), biological control (a single inundative release of the predatory mite and spraying with fungicides) (marked as Biological in the figures), Acaricides 1 (spraying with two acaricides (twice with one product and once with the other), fungicides and insecticides; indicated as Acaricides 1 in the figures) and Acaricides 2 (spraying with two acaricides (once with both preparations), fungicides and insecticides; indicated as Acaricides 2 in the figures). There were 160 hop plants in each treatment (plot with a length of 33.3 m and a width of 13.5 m). More detailed descriptions of the treatments, with the relevant spraying dates, are presented in Table 1. Table 2 presents a detailed list of the plant protection products used for protection against diseases and harmful insect pests in 2015. Decisions regarding which synthetic products would be used in our experiment were made according to the IOBC database [13], as we did not want to use products that would have an effect on the predatory mite in the biological control treatment.

Table 1. Detailed description of treatments used in our survey.

Treatment	Description
Control treatment	Spraying with fungicides and insecticides, without acaricides
Biological control	Spraying with fungicides, without insecticides and acaricides, 4 July—inundative release of *N. californicus* (Californicus Breeding system 500)
Acaricides 1	Spraying with fungicides, insecticides and acaricides: Nissorun 10 WP—27 June, Vertimec Pro—3 July, Nissorun 10 WP—17 July
Acaricides 2	Spraying with fungicides, insecticides and acaricides: Vertimec Pro—3 July, Nissorun 10 WP—17 July

Table 2. List of insecticides, fungicides and acaricides used against the most important pests and diseases in hop plantations in 2015.

Disease/Insect	Product	Active Ingredient	Dose (kg/ha, L/ha)	Date of Spraying
Pseudoperonospora humuli	Delan 700 WG (SUPPLIER: BASF Ltd., Slovenia)	ditianone	0.6	20 June
	Delan 700 WG	ditianone	1.2	3 July
	Curzate R (supplier: Corteva Ltd., Slovenia)	cimoxanile and copper (copper oxychloride)	3	17 July
	Revus (Supplier: Syngenta Ltd., Slovenia)	mandipropamid	0.75	3 August
Phorodon humuli	* Kohinor SL 200 (supplier: Karsia Ltd., Slovenia)	imidacloprid	0.3	10 June
	Teppeki (supplier: Picount, Slovenia)	flonikamid	0.18	10, 20 June
Ostrinia nubilalis	Lepinox plus (supplier: Metrob Ltd., Slovenia)	*Bacillus thuringiensis* var. *kurstaki*	1	20 June
	* Karate 5 CS (supplier: Karsia Ltd., Slovenia)	lambda-cyhalothrin	0.25	3 August
Tetranychus urticae	Nissorun 10 WP (supplier: Karsia Ltd., Slovenia)	hexythiazox	1	27 June
	Vertimec Pro (supplier: Syngenta Ltd., Slovenia)	abamectin	1.25	3 July
	Nissorun 10 WP	hexythiazox	1	17 July

* sprayed only on plants in the control treatment group.

2.2. Release of N. californicus and Counting of the Two-Spotted Spider Mite Eggs and Mobile Stages

Two boxes of the product Californicus Breeding System 500 (producer: Biobest, Belgium; supplier: Kadmo, Croatia) were released on 4 July 2015. Each of the two boxes contained 500 bags, each of which contained approximately 100 mobile specimens. We hung one bag that contained larvae and adults on each hop guide at a height of 3 m. Thus, one plant had two bags. The treatment with biological protection included 480 hop plants, and we used 960 bags with predators.

The hop leaves were checked for the presence of two-spotted spider mites. For each treatment, we determined three sampling points. To avoid edge effects, the sampling points were set in the central parts of the plots. At these points, 10 leaves were collected at three different heights (h1: 0–2 m, h2: 2–4 m, h3: 4–6 m). In each treatment, we thus sampled 90 leaves. The collection of leaves was carried out on four dates (10 July, 17 July, 29 July and 9 August). The leaves were cut off from the hop plants in the morning and collected in paper bags. At the Slovenian Institute of Hop Research and Brewing in Žalec, we inspected both sides of each leaf with a stereomicroscope and counted the eggs and mobile forms of the two-spotted spider mite. We calculated the average number of eggs and mobile specimens (larvae, nymphs and adult mites) on a hop leaf.

2.3. Cost of Acaricides and Application of Predatory Mite N. californicus

We calculated the approximate costs of the application of acaricides compared with those of predator release. Costs are calculated per treatment area, i.e., 1.350 m^2. All values are presented in Table 3.

2.4. Assessment of Damage to Hop Cones

We assessed the damage due to sucking of two-spotted spider mites on hop cones during the technological maturity of the plants. The collection was carried out on 21 August. We collected 30 hop cones at heights of 2–4 m and 30 hop cones at heights of 4–6 m at each sampling point. At the height of 0–2 m, there were no hop cones or very few hop cones, so we did not sample them at this height. In accordance with [11], the hop cones were divided into four groups based on damage due to sucking of two-spotted spider mites:

1 = no damage or minimal damage (0–5% damaged hop cones); 2 = lesser extent of damage (5–20% damaged hop cones); 3 = moderate extent of damage (20–50% damaged hop cones); and 4 = high extent of damage (more than 50% damaged hop cones, visibly changed colour of a cone).

Table 3. Comparison of approximate costs of the application of acaricides and biological control agent single release.

Date of Application	Treatment (1.350 m^2)		
	Acaricide 1	**Acaricide 2**	**Biological Control**
27 June	Nissorun 10 WP (0.14 kg)	/	/
3 July	Vertimec Pro (0.17 L)	Vertimec Pro (0.17 L)	/
4 July	/	/	Californicus Breeding System (2 boxes or 1.000 bags with 100 mobile stages/bag)
17 July	Nissorun 10 WP (0.14 kg)	Nissorun 10 WP (0.14 kg)	/
Approximate cost (EUR) *	48.21 EUR	34.31 EUR	610.98 EUR

* the prices of synthetic acaricides are calculated according to the price of one litre or kg of preparation per hectare (Nissorun 10 WP = 99.29 EUR/kg; Vertimec Pro = 119.98 EUR/L).

2.5. Weather Parameters

Weather parameters (average daily temperature (°C), average daily precipitation (mm), average daily relative humidity (R.h.)) were obtained from the Agrometeorological portal of Slovenia [14] for Ojstriška vas (46°14′25.47″ N; 15°1′13.77″ E, 314 m).

2.6. Statistical Analysis

The experimental results were statistically evaluated by the program Statgraphics Centurion XVI [15]. The data have been analysed using parametric analysis and were tested for normality by conducting Shapiro-Wilk's test and visual inspection of their histograms and box plots showed data approximately normally distrubuted. The differences between the average numbers of eggs/mobile stage and damaged hop cones were calculated by analysis of variance (ANOVA) and the Student-Neuman-Keuls test of multiple comparisons (ANOVA).

3. Results

3.1. Average Number of Two-Spotted Spider Mite (TSSM) Eggs Per Leaf

According to the general analysis of the results, the average number of TSSM eggs was significantly influenced by the date of evaluation (F = 30.36; df = 3; $p < 0.05$), height of sampling (F = 18.44; df = 2; $p < 0.05$), interaction between height of sampling and date of sampling (F = 6.97, df = 6; $p < 0.05$), interaction between treatment and date of evaluation (F = 4.73, df = 9, $p < 0.05$) and interaction between date of evaluation, treatment and height of evaluation (F = 1.66, df = 18, $p < 0.05$). We confirmed that there was no impact of treatment (F = 1, df = 3, $p = 0.3931$).

According to Figure 1, the average number of TSSM eggs was the lowest on 10 July in the "Acaricide 1" (0.5 ± 0.28 eggs/leaf) and "Acaricide 2" treatments (0.35 ± 0.15 eggs/leaf). On 29 July, the highest number of TSSM eggs was recorded for the control treatment (8.1 ± 1.82 eggs/leaf). All other values are presented in Figure 1.

On 10 July, a higher number of eggs/leaf was recorded in the biological treatment group between 0–2 m above the ground (8.53 ± 0.81 eggs/leaf). Regarding the second date of evaluation (17 July), the lowest average number of eggs was recorded in the biological control treatment group (at h1—3.4 ± 1.14 eggs/leaf; at h2—2.86 ± 1.24 eggs/leaf; at h3—3.4 ± 0.99 eggs/leaf). On the third date of evaluation, the highest number of eggs per leaf was detected 4−6 m above ground in almost all treatment groups, except for the "Acaricide 2" treatment. At the last date of sampling (evaluation), the number of eggs per leaf was the highest at 4−6 m above ground in all treatment groups. All detailed values are presented in Table 4.

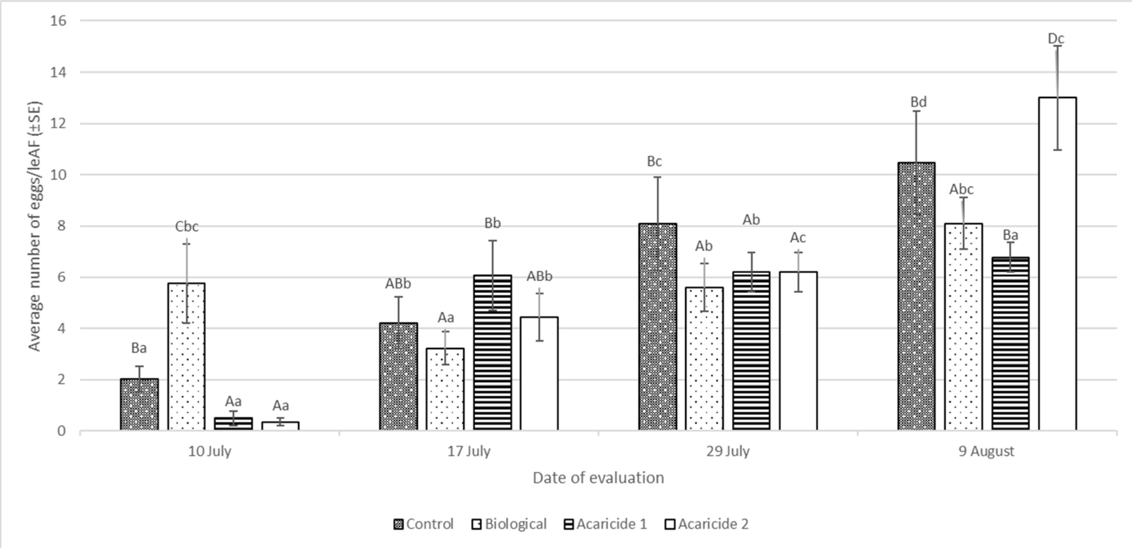

Figure 1. Average number of two-spotted spider mite eggs per leaf per treatment (for comparison between dates of evaluation within specific treatments (lowercase letters; df = 3, 359; for control treatment, F = 6.66, $p < 0.05$; for biological control treatment, F = 3.35, $p < 0.05$; for Acaricide 1, F = 4.20, $p < 0.05$; for Acaricide 2, F = 22.51, $p < 0.05$); for comparison between treatments on a specific date of evaluation (uppercase letters; df = 3,359; for 10 July, F = 9.23, $p < 0.05$; for 17 July, F = 1.35, $p = 0.2580$; for 29 July, F = 3.21, $p < 0.05$; for 9 August, F = 11, $p < 0.05$)).

Table 4. Average number of two-spotted spider mite eggs per leaf according to different sampling heights.

		Sampling Height		
		h1	**h2**	**h3**
10 July	Control	0.06 ± 0.02 Aa	0.83 ± 0.35 Aa	0.57 ± 0.41 Aa
	Biological	8.53 ± 3.82 Bc	2.93 ± 1.25 Bab	5.8 ± 2.29 Bab
	Acaricide 1	1.03 ± 0.83 Aa	0.23 ± 0.14 Aa	0.23 ± 0.14 Aa
	Acaricide 2	0.30 ± 0.20 Aa	0.53 ± 0.36 Aa	0.23 ± 0.16 Aa
17 July	Control	2.37 ± 0.91 Ab	4.93 ± 2.25 Ac	5.33 ± 1.84 Abb
	Biological	3.40 ± 1.14 ABab	2.87 ± 1.24 Aa	3.40 ± 0.99 Aa
	Acaricide 1	3.43 ± 1.50 Bb	4.27 ± 1.39 Ab	10.57 ± 3.49 Bc
	Acaricide 2	2.43 ± 1.35 Ab	3.73 ± 1.25 Ac	7.17 ± 2.03 Abc
29 July	Control	8.37 ± 3.66 Bd	4.63 ± 1.87 ABb	11.30 ± 3.60 Cc
	Biological	3.13 ± 0.75 Aa	5.27 ± 1.36 Bab	8.40 ± 2.36 Bb
	Acaricide 1	3.03 ± 0.97 Aa	7.36 ± 1.47 Cc	8.23 ± 1.36 Bb
	Acaricide 2	3.80 ± 0.93 Ac	3.07 ± 0.88 Ab	2.74 ± 0.69 Ab
9 August	Control	5.50 ± 1.25 Bc	7.00 ± 1.19 Abc	18.94 ± 5.51 Cd
	Biological	4.77 ± 1.28 Bab	6.03 ± 1.10 Ab	13.50 ± 2.26 Bc
	Acaricide 1	3.97 ± 0.76 ABb	7.90 ± 1.07 Bc	8.50 ± 0.98 Ab
	Acaricide 2	3.00 ± 0.72 Ab	16.06 ± 2.78 Cd	19.93 ± 4.95 Cd

(For comparison between dates of evaluation within specific treatment groups and sampling heights, lowercase letters; df = 3.119; for control treatment, h1, F = 1.51. $p = 0.2150$, h2, F = 2.52, $p = 0.0610$, h3, F = 4.97, $p < 0.05$; for biological control, h1, F = 1.37, $p = 0.2554$, h2, F = 1.71, $p = 0.1693$, h3, F = 4.44, $p < 0.05$; for Acaricide 1, h1, F = 1.46, $p = 0.2278$, h2, F = 9.39, $p < 0.05$, h3, F = 5.52, $p < 0.05$; for Acaricide 2, h1, F = 2.77, $p < 0.05$, h2, F = 2.77, $p < 0.05$, h3, F = 10.54, $p < 0.05$). (For comparison between treatments on specific evaluation dates at the same sampling height: uppercase letters, df = 3.119; 10 July, h1, F = 3.28, $p < 0.05$, h2, F = 3.26, $p < 0.05$, h3, F = 5.97, $p < 0.05$; for 17 July, h1, F = 7.38, $p < 0.05$, h2, F = 10.26, $p < 0.05$, h3, F = 16.37., $p < 0.05$; for 29 July, h1, F = 1.64, $p = 0.1838$, h2, F = 1.53, $p = 0.2107$, h3, F = 2.45, $p = 0.0672$; for 9 August, h1, F = 1.06, $p = 0.3698$, h2, F = 7.36, $p < 0.05$, h3, F = 1.84, $p < 0.05$).

3.2. Average Number of Two-Spotted Spider Mite (TSSM) Mobile Stages Per Leaf

According to the analysis of pooled results, the average number of mobile stages was influenced by the date of evaluation (F = 290.74, df = 3, $p < 0.05$), treatment (F = 31.76, df = 3, $p < 0.05$), height of sampling (F = 290.74; df = 3, $p = 0.06$), interaction between height of sampling and treatment (F = 5.07, df = 6, $p < 0.05$), interaction between height of sampling and date of evaluation (F = 13.10, df = 6, $p < 0.05$), interaction between date of evaluation and treatment (F = 29.93, df = 9, $p < 0.05$) and interaction between height of sampling, treatment and date of evaluation (F = 3.57, df = 18, $p < 0.05$). In general, the highest average number of two-spotted spider mites was recorded at the h3 sampling height (7.71 ± 1.13 mobile stage/leaf).

The highest numbers of mobile stages per leaf were recorded with "Acaricide 2" on all dates of evaluation. With the biological control treatment, the highest number of mobile stages was recorded at the beginning of evaluation on 10 July (Figure 2).

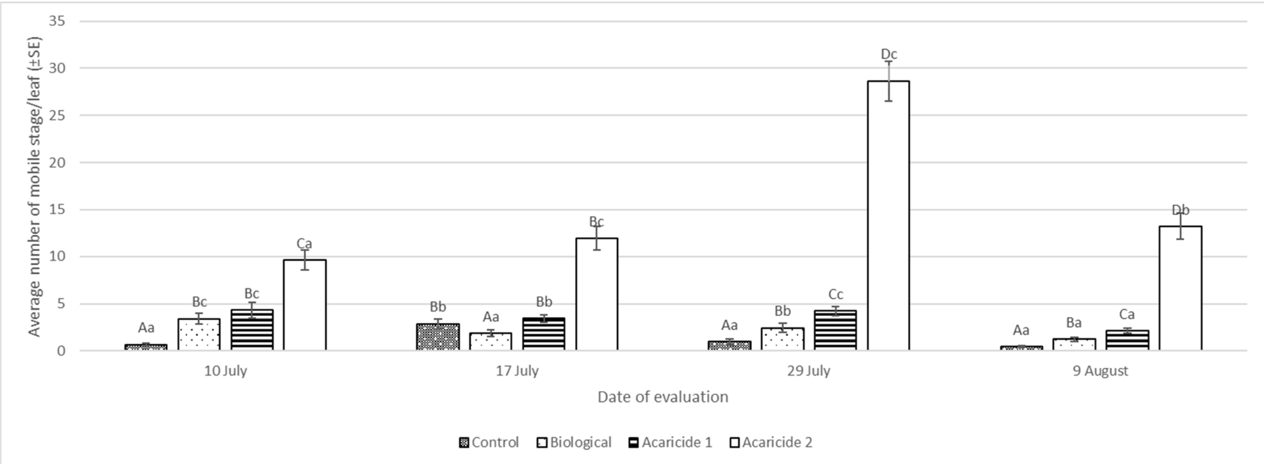

Figure 2. Average number of mobile stages of two-spotted spider mites per leaf (for comparison between dates of evaluation within specific treatment groups: lowercase letters; df = 3, 359; for control treatment, F = 25.23, $p < 0.05$; for biological control treatment, F = 37.25, $p < 0.05$; for Acaricide 1, F = 139.74, $p < 0.05$; for Acaricide 2, F = 67.73, $p < 0.05$). For comparison between treatments on a specific date of evaluation: uppercase letters; df = 3,359; for 10 July, F = 11.30, $p < 0.05$; for 17 July, F = 7.60, $p = 0.2580$; for 29 July, F = 3.23, $p < 0.05$; for 9 August, F = 44.98, $p < 0.05$).

According to Table 5 the population of two-spotted spider mites (mobile stage) was the highest on the last evaluation date (9 August) in all treatment groups at a given sampling height (h3). At the beginning of sampling (10 July), the number of mobile stages reached the highest values in the "Biological control" treatment group. All other values are presented in Table 5.

For comparison between dates of evaluation within specific treatment groups and sampling heights (lowercase letters; df = 3.119; for control treatment, h1, F = 6.94. $p < 0.05$, h2, F = 8.26, $p < 0.05$, h3, F = 11.22, $p < 0.05$; for biological control, h1, F = 13.97, $p < 0.05$, h2, F = 21.33, $p < 0.05$, h3, F = 15.61, $p < 0.05$; for Acaricide 1, h1, F = 22.33, $p < 0.05$, h2, F = 13.59, $p < 0.05$, h3, F = 5.52, $p < 0.05$; for Acaricide 2, h1, F = 6.77, $p < 0.05$, h2, F = 23.11, $p < 0.05$, h3, F = 18.54, $p < 0.05$). For comparison between treatments within specific dates of evaluation at the same sampling height (uppercase letters, df = 3.119; 10 July, h1, F = 3.26, $p = 0.100$, h2, F = 3.26, $p < 0.05$, h3, F = 12.04, $p < 0.05$; for 17 July, h1, F = 7.23, $p < 0.05$, h2, F = 10.26, $p < 0.05$, h3, F = 16.37, $p < 0.05$; for 29 July, h1, F = 1.64, $p = 0.1838$, h2, F = 1.53, $p = 0.2107$, h3, F = 2.45, $p = 0.0672$; for 9 August, h1, F = 6.67, $p = 0.3698$, h2, F = 45.36, $p < 0.05$, h3, F = 6.84, $p < 0.05$).

Table 5. Average number of two-spotted spider mite mobile stages per leaf according to different sampling heights.

		Sampling Height		
		h1	**h2**	**h3**
10 July	Control	3.83 ± 0.21 Ac	0.93 ± 0.37 Ba	1.33 ± 0.25 Ca
	Biological	3.70 ± 1.05 Cb	1.73 ± 0.72 Cab	3.23 ± 1.03 Dab
	Acaricide 1	1.93 ± 0.76 Bd	0.37 ± 0.17 Aa	0.63 ± 0.42 Ba
	Acaricide 2	0.73 ± 0.34 Aa	0.53 ± 0.16 Aa	0.20 ± 0.12 Aa
17 July	Control	3.60 ± 1.02 Ca	2.97 ± 0.95 Cb	3.70 ± 1.07 Bb
	Biological	1.86 ± 0.52 Ba	1.00 ± 0.28 Aa	2.80 ± 0.90 Aba
	Acaricide 1	1.63 ± 0.58 Ba	2.13 ± 1.26 Bb	3.60 ± 1.15 Bb
	Acaricide 2	0.46 ± 0.22 Aa	1.13 ± 0.39 Ab	2.10 ± 0.52 Ab
29 July	Control	4.90 ± 2.07 Cb	2.97 ± 1.04 BCb	5.07 ± 1.21 BCb
	Biological	3.23 ± 0.71 Bab	2.99 ± 0.55 Bb	4.17 ± 0.76 Bb
	Acaricide 1	2.53 ± 0.60 Aa	4.06 ± 0.71 Cc	6.10 ± 1.09 Cc
	Acaricide 2	2.60 ± 0.46 Ab	1.43 ± 0.32 Ab	2.43 ± 0.67 Ab
9 August	Control	9.70 ± 1.69 Bc	7.13 ± 1.13 Ac	12.00 ± 2.37 Ac
	Biological	10.76 ± 2.47 Bc	9.43 ± 1.37 Ac	15.66 ± 2.70 Abc
	Acaricide 1	19.60 ± 1.9.30 Cb	22.93 ± 3.02 Cd	43.33 ± 3.98 Cd
	Acaricide 2	8.37 ± 1.69 Ac	14.37 ± 2.05 B	17.00 ± 3.13 Bc

3.3. Evaluation of Damage Caused by Two-Spotted Spider Mites on Hop Cones

According to the analysis of pooled results, the per cent of damage caused by TSSM on hop cones was not influenced by sampling height (F = 0.09, $p = 0.9465$) or treatment (F = 1.00, $p = 0.9996$). The abundance of slightly damaged hop cones (index 1) was the highest for all treatments. When "biological control" treatment was applied, 23% of damaged hop cones were listed in group "index 2" at a sampling height of 4–6 m, while 11% of hop cones at a sampling height of 2–4 m were listed in "index 2".

Where "Acaricide 2" was applied, 78% of hop cones were organized into "index 1" at a 2–4 m sampling height, while only 47% of slightly damaged hop cones were recorded at a sampling height of 4–6 m. All other values are presented in Figure 3.

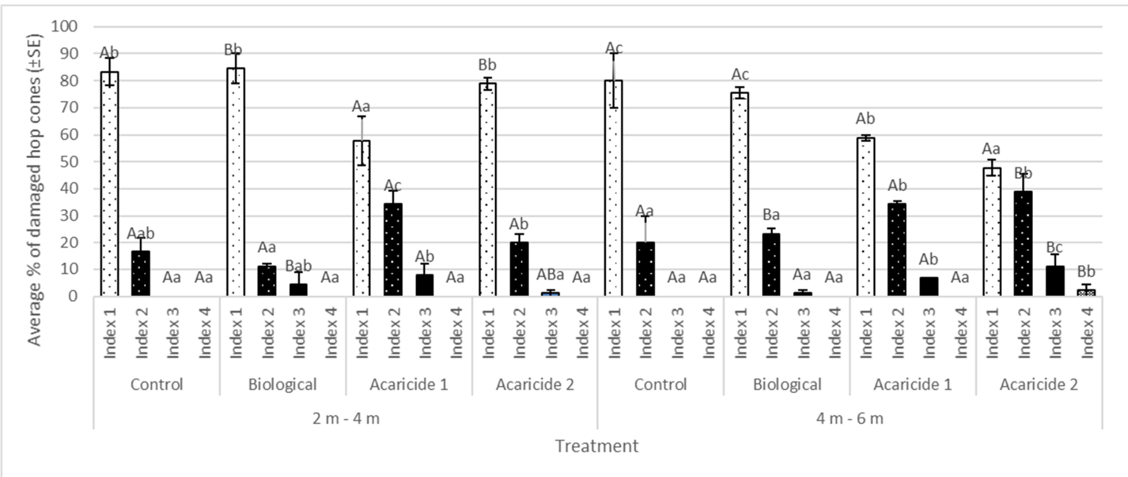

Figure 3. Average per cent of damaged hop cones according to sampling height and treatment (lowercase letters: for comparison at one sampling height, between treatments according to the same damage index (level of damage) (df = 3, 11; 2–4 m sampling height, index 1, F = 4.30, $p < 0.05$, index 2, F = 6.43, $p < 0.05$, index 3, F = 1.21, $p < 0.05$, index 4, no data available; 4–6 m, index 1, F = 12.29, $p < 0.05$, index 2, F = 6.46, $p < 0.05$, index 3, F = 7.41, $p < 0.05$, index 4, F = 12.12, $p < 0.05$); uppercase letters: for comparison between two sampling heights within the same damage index for a specific treatment (df = 1.5; control treatment, index 1, F = 0.09, $p = 0.7812$, index 2, F = 0.09, $p = 0.7812$, index 3 and 4, no data available; biological control, index 1, F = 2.21, $p = 0.2116$, index 2, F = 2.21, $p = 0.2116$, index 3, F = 0.53, $p = 0.5072$, index 4, no data available; Acaricide 1, index 1, F = 7.31, $p = 0.2116$, index 2, F = 7.21, $p = 0.3216$, index 3, F = 8.53, $p = 0.3072$, index 4, F = 10.70, $p < 0.05$ # Acaricide 2, index 1, F = 6.37, $p < 0.05$, index 2, F = 14.21, $p < 0.05$, index 3, F = 9.53, $p < 0.05$, index 4, F = 3.70, $p < 0.05$).

3.4. Weather Parameters

We obtained weather parameters from 4 July 2015 to 31 August 2015. The average daily temperatures in July ranged from 23.9 °C on 4 July to 16.7 °C on 31 July. The lowest average daily temperature in July was detected on 30 July (14.4 °C). In August 2015, the average daily temperature reached 20.61 °C, ranging from 17.6 °C on 1 August to 23.2 °C on 31 August. Due to the time period of our experiment, we had only 19 rainy days (more than 1 mm of rain per day). The highest R.h. (relative humidity) was achieved in the period from 24 July to 30 July, which is the same period during which the highest daily precipitation occurred. All other values are presented in Figure 4.

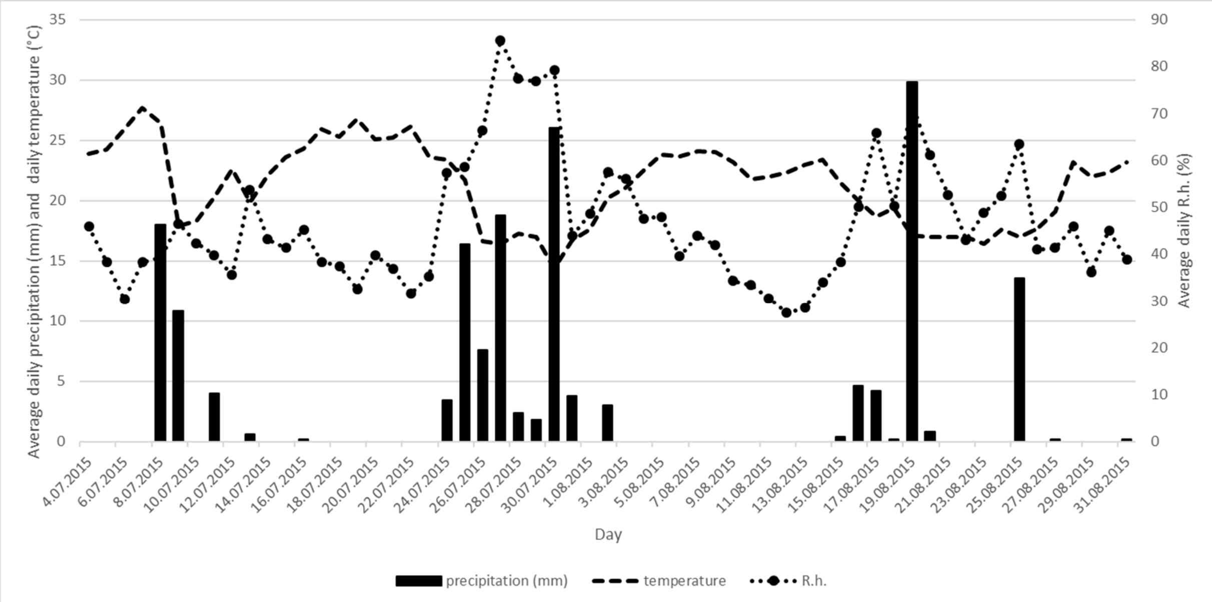

Figure 4. Average daily precipitation, average daily temperature and average daily R.h. (from 4 July 2015–31 August 2015).

4. Discussion

The purpose of our study was to investigate the efficiency of the indigenous predatory mite *N. californicus* [10,11] in a hop plantation, as allowed by the rules on biological plant protection [16]. Based on these rules, the use of native organisms is permitted for biological protection in Slovenia. *N. californicus* is also efficient in cases when two-spotted spider mites do not exceed the damage threshold [5], which influences the choice of a biological control agent.

At the time of the experiment, the weather conditions were favourable for the development of *N. californicus* populations since the development of this predatory mite is most intense in the temperature range of 15–35 °C [5]. Because the temperatures in the experimental period did not exceed 30 °C, we also recorded fewer specimens of the two-spotted spider mite, whose development is known to last 8–12 days under optimal conditions (30–32 °C) [17]. On the day of the release of the predator, the average daily temperature reached almost 24 °C, and 46% R.h. was recorded. The first counting of two-spotted spider mites was performed at a daily temperature of 18.3 °C and after two rainy days. We assume that the population of two-spotted spiders could be higher in no-rain conditions since previous studies [18] reported that the *T. urticae* population is restrained mainly by rainfall in the open field conditions. A third counting of the two-spotted spider mite population was also performed after five rainy days. On the day of release of the predator (4 July) and collecting cones (21 August), we recorded 20 days with R.h. values below 50%. It is known that the tested predatory mite is not sensitive to low R.h. [19].

The economic threshold depends upon the growth phases of hop [20]. At the beginning of June, we found 1–2 adult mites/leaf, while 5–10 adults per leaf can cause severe damage if they are found in mid-July. In the treatment that involved abamectin, we established ovicidal effects on two-spotted spider mites a week after spraying, while mobile specimens exhibited significantly fewer effects. The results indicate the emergence of resistance of harmful mites, which has been reported by some other studies [21,22]. Previous studies did not confirm the ovicidal effects of abamectin; rather, these studies mainly discussed the acaricidal mode of action of abamectin [23,24]. Ovicidal effects were also confirmed from the treatment in which we applied abamectin and hexythiazox a few days apart. It has been confirmed that hexythiazox acts as a growth inhibitor [25]. In our research, a hexythiazox-based acaricide was used in July, which was also demonstrated previously [26]. The selection of insecticides and fungicides in acaricides in our experiment was based on data demonstrating the influence of individual active substances on useful organisms obtained from the IOBC website [27], as our research primarily involved chemical substances that do not have effects or display little effect on predatory mites. The exception was the systemic insecticide imidacloprid, which we used in the control treatment against hop aphids, and it also displayed effects on two-spotted spider mites [28] and the predatory mite *N. californicus* [29]. The second application of the acaricide Nissorun in the Acaricide 2 treatment also did not prove to be an efficient measure for suppression of two-spotted spider mites, which additionally confirms the hypothesis that natural enemies of two-spotted spider mites have a role in suppression strategies targeting this harmful pest on hop plants.

The optimal effects of the predatory mite were recorded 14 days after release, when we detected the lowest number of eggs and mobile forms of the two-spotted spider mite. This observation can be attributed to the usual lifespan of this predator, which is 20 days [30]. Although the number of eggs of two-spotted spider mites per leaf slowly increased toward the end of the growth period, the number of mobile specimens in the treatment group with the predatory mite was lower than that on the plants treated with acaricides, which also suggests the emergence of resistance of two-spotted spider mites to the acaricides used [21]. In some studies [31,32], the use of spinosad, which is compatible with acaricides but it is effective also when is applied alone, are suggested against acaricide-resistant species of mites, and also against other pests of field crops.

We expected that the highest percentage of damaged hop cones due to two-spotted spider mites would be established under the treatment that involved biological control. Despite the favourable circumstances for the development of two-spotted spider mites, the damage to hop cones was very small. The share of damaged hop cones between the treatments did not show any statistically significant differences. More than two-thirds of hop cones in all treatments were undamaged or minimally damaged. Very damaged hop cones (more than 50% damaged hop cones) were found only in the Acaricides 2 treatment group, in which we also found the largest number of mobile specimens. Based on previous studies, fewer than 90 mites per hop leaf are tolerable at harvest time [3]; therefore, we cannot talk about differences between treatments.

According to our study, the number of mobile stages of two-spotted spider mites was influenced by light, as was also detected previously [33]. The highest number of two-spotted spider mites was detected on the top of the plants (4–6 m). Our findings, namely, that the number of eggs laid by two-spotted spider mites does not differ between different plant parts, can be related to the fact that eggs from two-spotted spider mites are laid in lower plant parts on purpose because their predator (*N. californicus*) usually feeds in the upper plant parts [34]. Therefore, eggs from two-spotted spider mites can be left uneaten.

According to the instructions of the supplier, the effects of the mite *N. californicus* are satisfactory for three weeks [30], and release has to be repeated. Due to the relatively late developmental stage of hop at the end of July, we did not conduct another release of the *N. californicus* mite in our study. The results of our research, in which we established comparable effects of the natural enemy and the chemical agents, suggest that the first release of the predatory mite *N. californicus* should be performed in mid-June, followed by the second release three weeks later, as the biological protection demonstrated in our study proved to be an efficient protective measure with unrealized potential [35].

According to the economic impact of the single release of the predatory mite in our experiment, we can confirm the findings from [36], as the costs of acaricide use in our experiment were from 12.7-fold (two sprayings of hexythiazox and a single spraying with abamectin) to 17.8-fold (single treatments of hexythiazox and abamectin) lower than those of the biological control agent in question.

5. Conclusions

Our research established comparable effects of the predatory mite *N. californicus* and acaricides in controlling two-spotted spider mites on hop. For further improvement of the efficiency of the predator, we suggest release of the natural enemy into hop plantations in mid-June, followed by a second release three weeks later. In this case, we are particularly interested in comparing the efficacy of predatory mites against *T. urticae* in single and multiple inundative releases.

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